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Special Issue | May 2025

Pharmacotherapy of Energy Metabolism in Obesity

Issue Editor

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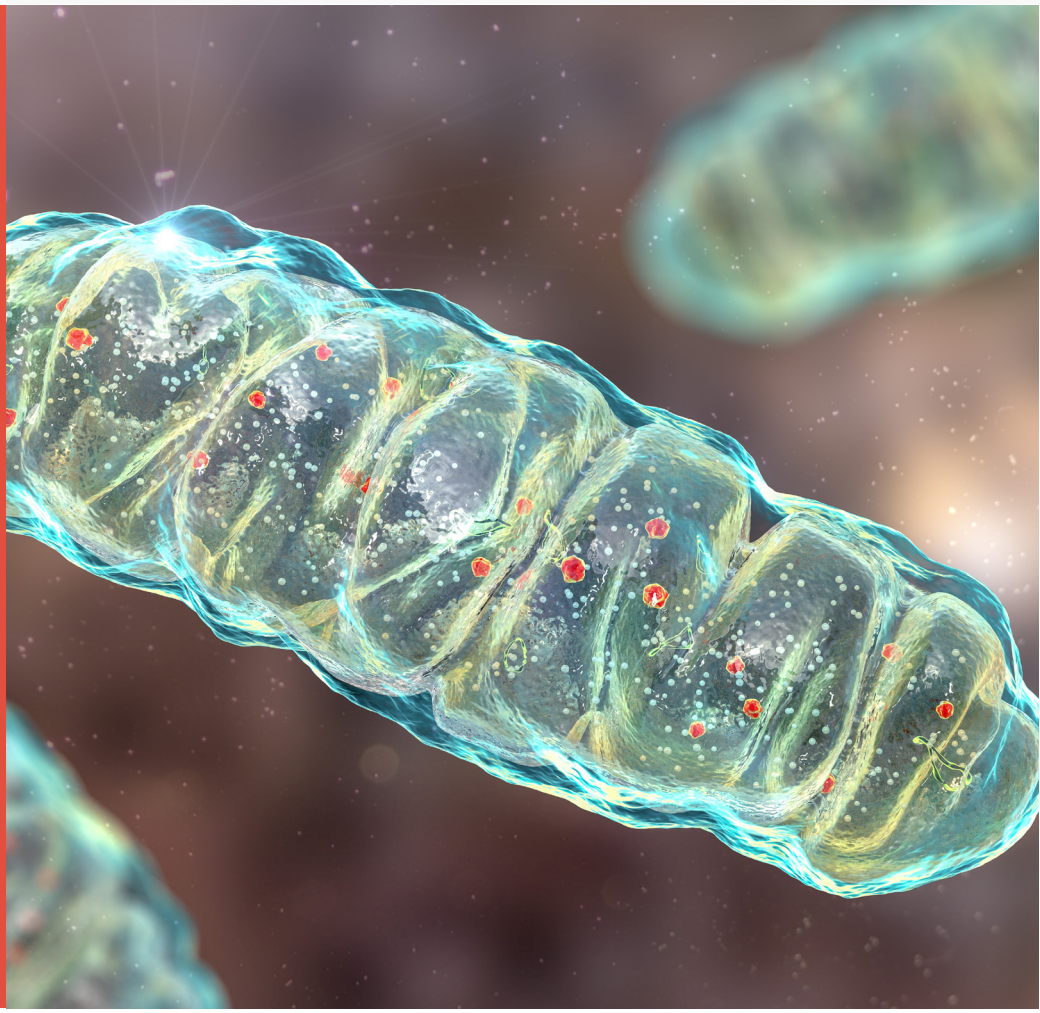

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Pharmacotherapy of Energy Metabolism in Obesity

ISSN 1482-1826
ISBN 978-2-8325-6300-7
DOI 10.3389/978-2-8325-6300-7

Obesity is a major risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD), driving widespread research into its underlying mechanisms. Key contributors—such as inflammation, oxidative stress, insulin resistance, and mitochondrial dysfunction—have been extensively studied, with growing attention to how obesity disrupts fuel metabolism, impacting carbohydrate and fatty acid oxidation in vital organs like the heart and liver. Understanding these metabolic disturbances presents a promising avenue for pharmacological intervention. This eBook compiles 12 papers from the Special Issue, Pharmacotherapy of Energy Metabolism in Obesity, exploring metabolic dysregulations in obesity-related diseases and pharmacotherapeutic strategies to address them. By bridging insights from preclinical and clinical research, this collection aims to advance our understanding of therapeutic targets and their implications for future treatments. We hope it serves as a valuable resource for researchers, clinicians, and the broader scientific community in the fight against obesity and its complications.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Special Issue Editorial
PUBLISHED 10 March 2025
DOI 10.3389/jpps.2025.14099

OPEN ACCESS

EDITED BY
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RECEIVED 21 November 2024
ACCEPTED 26 February 2025
PUBLISHED 10 March 2025

CITATION
Ussher JR (2025) Editorial: Pharmacotherapy of energy metabolism in obesity.
J. pharm. pharm. Sci. 28:14099.
doi: 10.3389/jpps.2025.14099

Editorial: Pharmacotherapy of energy metabolism in obesity

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KEYWORDS

obesity, type 2 diabetes, cardiovascular disease, energy metabolism, pharmacotherapy

Editorial on the Special Issue
Pharmacotherapy of energy metabolism in obesity

It is well established that body weight gain and increased adiposity during the progression of obesity increase one's risk for metabolic dysfunction associated steatotic liver disease (MASLD), type 2 diabetes (T2D), and cardiovascular disease (CVD). Furthermore, a plethora of evidence supports that obesity leads to several perturbations in energy metabolism, which are widely thought to contribute to the pathology of these obesity-associated cardiometabolic pathologies [1]. Accordingly, researchers have invested significant effort towards understanding the molecular alterations that are responsible for these perturbations in energy metabolism, as they may represent novel drug discovery targets to treat MASLD, T2D, and CVD due to underlying obesity.

With the increasing recognition that the adipose tissue itself is a complex endocrine organ that secretes several cytokines and adipokines that can influence metabolic homeostasis, understanding how intermittent fasting mediated weight loss impacts whole-body energy metabolism is an exciting area for potential drug discovery Vo et al. Although not as prevalent as in rodents, there is also excitement on whether brown fat metabolism can be influenced in obese humans to promote weight loss Prapaharan et al. In addition, numerous metabolomics studies have demonstrated that increases in circulating branched-chain amino acids (leucine, isoleucine, valine) are positively associated with T2D and CVD [2]. Whether targeting BCAA metabolism can reverse obesity-related cardiometabolic disease has engendered significant interest in the field, though which organ (i.e., skeletal muscle, heart, adipose tissue, etc.) BCAA metabolism is most relevant to manipulate is still an area of ongoing debate Abdualkader et al.

More recently, perturbations in ketone metabolism have also been shown to contribute to obesity-associated cardiometabolic diseases [3, 4]. One of the most intriguing questions within this realm pertains to whether pharmacological augmentation of hepatic ketogenesis can alleviate MASLD Kwon et al., as increased ketogenesis would result in an elevation of fatty acid oxidation and subsequent decrease in hepatic lipid accumulation. This is a highly relevant area for current drug discovery, since MASLD is a major cause for abnormal liver function tests and is a pathology that currently

has no approved therapies. Of interest, it is also gaining recognition that targeting energy metabolism within oxidative organs is not the only area for potential drug discovery, as the field of immunometabolism is one of the most rapidly expanding areas in obesity. This is due in part to increased adiposity often leading to a chronic low-grade inflammation that can contribute to T2D and CVD [5]. Whether perturbations in macrophage energy metabolism directly contribute to the chronic low-grade inflammation associated with obesity is unknown, and will be a key area for future interrogation that could lead to new exciting targets for drug development Wong et al.

Perturbations in energy metabolism are commonly observed in the myocardium during obesity/T2D, with several studies exploring whether targeting such perturbations can alleviate diabetic cardiomyopathy and/or heart failure [6]. Targets of interest include manipulating lipoprotein lipase activity to alleviate dyslipidemia and fatty acid supply to the myocardium Shang and Rodrigues, or manipulating the enzymatic machinery within cardiomyocytes to limit the accumulation of toxic lipids such as ceramides and diacylglycerols, thereby attenuating cardiac lipotoxicity Nakamura. One of the most robust metabolic perturbations in the myocardium during obesity/T2D is an impairment in glucose oxidation, with several studies illustrating that inhibition of the transcription factor, forkhead box O1, can restore glucose oxidation in the diabetic heart Shafaati and Gopal. There is also significant interest in understanding how acetylation of the enzymatic machinery controlling mitochondrial β-oxidation contributes to the elevations in myocardial fatty acid oxidation rates observed in obesity/T2D Ketema and Lopaschuk.

This special issue of the *Journal of Pharmacy and Pharmaceutical Sciences* features several topical review articles addressing these exciting areas of energy metabolism and how they have contributed to the potential development of new drugs for treating obesity-related cardiometabolic diseases. While targeting energy metabolism may prove fruitful towards alleviating MASLD, T2D, and/or CVD associated with obesity, there is also much excitement with the use of glucagon-like peptide-1 receptor (GLP-1R) agonists to directly treat obesity via decreasing appetite. Indeed, the significant weight loss resulting from GLP-1R agonist therapy has been shown to have salutary actions against cardiometabolic disease. As such, this special issue also addresses the expanding role of GLP-1R agonists to treat obesity, while contrasting the efficacy of these agents against that of bariatric surgery Morissette and Mulvihill. Furthermore, the prevalence of obesity is increasing in our adolescent population, and thus the potential pharmacotherapy of adolescent obesity will also be discussed Son. Taken together, an advanced understanding of the metabolic perturbations associated with obesity has the potential to provide several new drug targets for treating cardiometabolic diseases such as MASLD, T2D and CVD, while improving the quality of life for millions of individuals.

Author contributions

JRU wrote the editorial.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This article was supported by a Tier 2 Canada Research Chair to JRU.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 22 July 2024
DOI 10.3389/jpps.2024.13062

OPEN ACCESS

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RECEIVED 30 March 2024
ACCEPTED 05 July 2024
PUBLISHED 22 July 2024

CITATION
Vo N, Zhang Q and Sung H-K (2024), From fasting to fat reshaping: exploring the molecular pathways of intermittent fasting-induced adipose tissue remodeling.
J. Pharm. Pharm. Sci 27:13062.
doi: 10.3389/jpps.2024.13062

From fasting to fat reshaping: exploring the molecular pathways of intermittent fasting-induced adipose tissue remodeling

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Obesity, characterised by excessive fat accumulation, is a complex chronic condition that results from dysfunctional adipose tissue expansion due to prolonged calorie surplus. This leads to rapid adipocyte enlargement that exceeds the support capacity of the surrounding neurovascular network, resulting in increased hypoxia, inflammation, and insulin resistance. Intermittent fasting (IF), a dietary regimen that cycles between periods of fasting and eating, has emerged as an effective strategy to combat obesity and improve metabolic homeostasis by promoting healthy adipose tissue remodeling. However, the precise molecular and cellular mechanisms behind the metabolic improvements and remodeling of white adipose tissue (WAT) driven by IF remain elusive. This review aims to summarise and discuss the relationship between IF and adipose tissue remodeling and explore the potential mechanisms through which IF induces alterations in WAT. This includes several key structural changes, including angiogenesis and sympathetic innervation of WAT. We will also discuss the involvement of key signalling pathways, such as PI3K, SIRT, mTOR, and AMPK, which potentially play a crucial role in IF-mediated metabolic adaptations.

KEYWORDS

obesity, intermittent fasting, adipose tissue remodeling, angiogenesis, sympathetic innervation

Introduction

Obesity is a growing epidemic, impacting individuals and societies on a global scale. It is a complex chronic disease marked by the accumulation of excess body fat, or adiposity, which negatively affects an individual's health [1]. The impact of obesity extends beyond increased body weight and is linked with a myriad of cardiometabolic diseases, such as type 2 diabetes (T2D), hypertension, and atherosclerosis, in addition to respiratory diseases and certain types of cancer [2, 3]. White adipose tissue (WAT) is a critical endocrine organ implicated in the progression of obesity. WAT is associated with an extensive neurovascular network and contains a heterogeneous population of

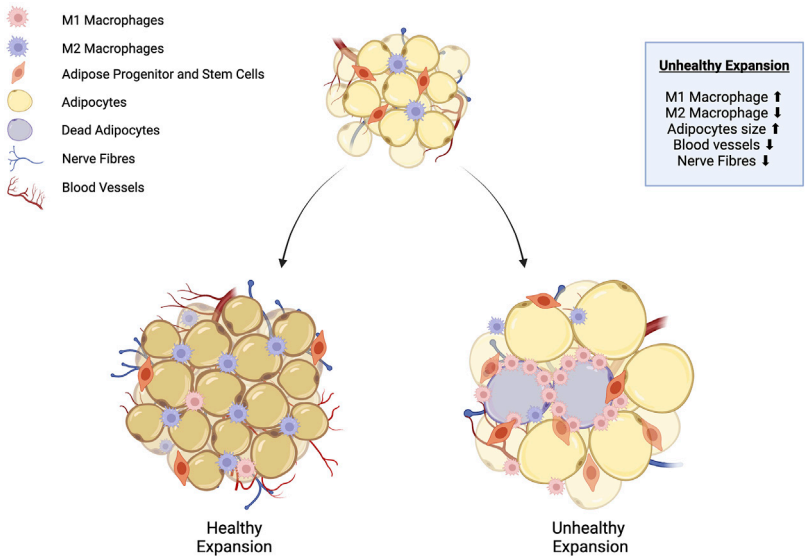

FIGURE 1
Adipose tissue remodeling: healthy vs. unhealthy WAT expansion. Healthy white adipose tissue (WAT) expansion is characterised by the proliferation of adipose stem and progenitor cells (ASPCs) into numerous small adipocytes. At the same time, anti-inflammatory M2 macrophages are recruited into the adipose tissue, and expansion is supported by the growth of the surrounding neurovascular architecture. Conversely, unhealthy WAT expansion is characterised by impaired differentiation of ASPCs into mature adipocytes. Lipids accumulate within existing adipocytes, causing them to progressively enlarge. Some adipocytes undergo apoptosis due to inadequate nutrient supply from blood vessels, which triggers the recruitment of pro-inflammatory M1 macrophages, leading to increased inflammation. Adapted from Choe et al. [5], licensed under CC BY 4.0. Created with BioRender.com.

cells, including mature adipocytes, adipose stem and progenitor cells (ASPCs) and vascular endothelial and immune cells [4]. These interactions contribute to the overall function of WAT in regulating whole-body metabolism and systemic homeostasis.

Adipose tissue remodeling is a biological process that involves changes in the morphology, cellular composition, and function of adipose tissue in response to physiological or pathological stimuli (Figure 1) [6]. A central component of the remodeling process is WAT expansion, which is characterised by two physiological processes: adipocyte hypertrophy, which is the increase in cell size, and hyperplasia, which leads to an increase in adipocyte cell number through adipogenesis [5]. The process of adipogenesis involves the differentiation of ASPCs into mature adipocytes and is controlled by a subset of regulatory signalling pathways [7].

It is also accompanied by the development of a supportive neurovascular architecture through signalling mechanisms such as the canonical vascular endothelial growth factor (VEGF)/VEGF Receptor 2 (VEGFR2). VEGF signalling is known to regulate angiogenesis, the growth of blood vessels, sympathetic innervation, and the extension and branching of sympathetic nerve fibres within adipose tissue [8]. Angiogenesis is critical for the delivery of essential nutrients, the removal of metabolic waste, the transport of adipokines, and the mobilisation of free fatty acids released during lipolysis [9]. Sympathetic innervation plays a significant role in the regulation of lipolysis, adipocyte proliferation, and thermogenesis [10–12].

A chronic imbalance between energy intake and expenditure can give rise to dysfunctional adipose tissue expansion, where adipocyte hypertrophy predominates and exceeds the capacity of the surrounding neurovascular network. This imbalance can result in hypoxia, triggering a cascade of inflammatory responses that ultimately contribute to the development of insulin resistance [13–15]. Thus, this highlights the considerable importance of adipose tissue remodeling in obesity, positioning it as a key target for therapeutic intervention.

Current strategies for managing obesity include dietary regimens, physical exercise, pharmacotherapy, and, in certain cases, bariatric surgery [16–19]. Among dietary approaches, calorie restriction (CR), which involves a decrease in total calories consumed, has been extensively studied and clinically implemented. CR is linked to numerous health benefits, including improvements in cardiovascular and metabolic health, cognitive function, and longevity [20–23]. However, maintaining weight loss through long-term adherence to a calorie-restricted regimen can be difficult, often resulting in poor compliance and subsequent weight regain once the regimen is discontinued [24, 25].

Given these challenges, intermittent fasting (IF) has emerged as a promising dietary strategy over the past decade. Unlike CR, it involves cycles of defined fasting and eating periods without

necessarily limiting the number of calories an individual consumes. Instead, IF restricts food consumption to certain hours of the day or specific days of the week. This regimen yields metabolic benefits similar to CR, including reduced body mass, improved adipose tissue inflammation and insulin sensitivity, and improved cardiometabolic health without reducing calorie intake [26–31]. Directly comparing the metabolic improvements between CR and IF requires additional studies, which are currently limited and ongoing [32–34].

IF encompasses a diverse range of dietary protocols that vary in the duration, frequency, and extent of calorie reduction during fasting periods. One notable strategy is alternate day fasting (ADF), which can be further categorised as complete ADF, involving zero calorie intake on fasting days, or modified ADF, which allows approximately 25% of an individual's daily calorie intake to be consumed during fasting periods. This adaptation mitigates some of the challenges associated with ADF and improves sustainability and adherence [35].

Periodic Fasting (PF) represents another form of IF, and it involves extended fasting periods or significantly reduced calorie intake interspersed with normal eating periods. Unlike ADF, PF typically involves longer fasting intervals of 2 days or more [29, 36]. The 5:2 IF regimen, one of the most recognised and popularised, allows unrestricted eating 5 days a week, with reduced calorie consumption on either two consecutive or non-consecutive days [37].

Time-restricted feeding (TRF) is a protocol that restricts food intake to a specific 8-h daily window, outside of which only water is allowed [38]. Recent clinical studies have highlighted that early TRF, with the window starting at 06:00 AM, further improves metabolic parameters such as fasting glucose and insulin sensitivity [39, 40]. These benefits are mediated, in part, by the synchronicity of TRF with our circadian rhythm. Ramadan fasting (RF) is a unique form of TRF practised by Muslims during the month of Ramadan, which involves restricted eating and drinking from dawn (Suhur) to sunset (Iftar) [41]. The duration of fasting depends on the time of year and geographical location. A recent meta-analysis by Fernando et al highlighted reductions in body weight and fat mass following RF, along with improvements in several metabolic markers such as fasting glucose and low-density lipoprotein levels [42, 43].

Despite the growing popularity and adoption of various IF protocols, the underlying biological mechanisms that facilitate these benefits remain to be elucidated. Existing research has demonstrated that IF promotes a range of metabolic adaptations, including enhanced lipolysis, β-oxidation, gut microbiota changes, and increased autophagy [44–48]. IF also significantly influences the browning of WAT, leading to the formation of beige adipocytes, which contain smaller lipid droplets and a higher mitochondrial density [26, 28, 49, 50]. This browning effect facilitates heat dissipation through respiratory uncoupling via uncoupling protein 1 (UCP1), a process known as WAT thermogenesis, which is critical in regulating energy expenditure and metabolic homeostasis [26, 28, 49, 50]. These processes contribute to the metabolic benefits observed with IF, including improved insulin sensitivity and glucose tolerance.

However, significant gaps persist in our understanding of the specific molecular pathways involved and the differential effects of IF on various tissues and organs. In this review, we aim to explore the signalling pathways, with a focus on adipose tissue that is influenced by IF and its downstream effects on adipose tissue remodeling. We also aim to identify key areas for future research to improve our current knowledge of the molecular targets that drive these metabolic adaptations. This understanding will set the stage for the development of pharmacological interventions that can mimic the beneficial effects of IF, potentially offering new strategies for the management of obesity.

Intermittent fasting and associated signalling pathways

Phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) pathways

The PI3K/Akt signalling pathway coordinates various anabolic processes that are essential for maintaining cell growth and proliferation, glucose and lipid metabolism, and autophagy. This pathway is crucial for insulin signal transduction and exerts beneficial effects on glucose homeostasis through the regulation of FOXO1 and GSK3β, mitochondrial biogenesis via mTORC1, and lipogenesis through the regulation of PPARγ and SREBP1-c [50].

Activation of PI3K/Akt signalling in adipocytes has been linked to improved insulin sensitivity and glucose tolerance, while also promoting lipogenesis over lipolysis *in vitro* and *in vivo* [51–54]. During the refeeding phase of IF in mice, there is an upregulation of CDC-like kinase 2 (CLK2) in brown adipose tissue, which contributes to the increased energy expenditure observed with IF. This upregulation may be regulated by insulin and PI3K signalling since treatment with a PI3K inhibitor has been shown to ameliorate the upregulation of CLK2 [55].

Although direct evidence connecting IF with PI3K/Akt signalling in WAT is still lacking, CR in mice has been shown to improve insulin sensitivity and promote lipid metabolism through Akt activation, particularly in the liver [56]. Catalpol, a naturally derived drug, has been shown to alleviate hepatic insulin resistance and improve glucose homeostasis in mice by stimulating AMP-activated protein kinase (AMPK) and PI3K/Akt signalling. Knockdown of AMPK in HepG2 cells was observed to prevent Akt phosphorylation and activation induced by Catalpol [57].

Furthermore, in a mouse model of diet-induced obesity (DIO), a 5-week ADF regimen mitigated obesity-induced remodeling of the atria. It also showed significant improvements in glucose tolerance and insulin sensitivity via SIRT3 and its downstream activation of AMPK and Akt [58]. Additionally, by modelling chronic myocardial ischaemia in rats, Katare et al demonstrated that long-term IF led to a substantial improvement in survival rates through activation of the BDNF/VEGF/PI3K signalling cascade in the heart, with a significant upregulation of phospho-Akt [59].

Conversely, the downregulation of PI3K/Akt signalling is also highlighted in specific circumstances. Butein, a phytochemical, can induce WAT beiging in DIO mice through inhibition of PI3K and downstream Akt signalling, leading to activation of PRDM4, a regulator of energy expenditure and thermogenesis [60]. A study of ADF in rats showed improvement in age-associated hypertrophy via downregulation of PI3K/Akt signalling in the heart's left ventricle [61]. Moreover, PI3K/Akt activation was also inhibited in the hippocampal region following IF, activating GSK3β and promoting neuronal differentiation in a mouse model of Alzheimer's disease [62]. Similarly, the hypothalamus showed reduced activation of PI3K/Akt/mTOR signalling following short-term fasting in rats [63].

IF facilitates the metabolic switch between catabolic and anabolic states in response to fasting and refeeding. This may result in the inhibition or activation of PI3K/Akt signalling. These findings illustrate the complex and context-dependent relationship between IF and PI3K/Akt signalling in various tissues, including adipose tissue, liver, heart, and brain. This dual regulation may explain how IF promotes insulin sensitivity while also stimulating catabolic processes such as WAT lipolysis and thermogenesis. Further research is essential to unravel the precise mechanisms of IF-induced modulation of the PI3K/Akt pathway in WAT.

Sirtuins (SIRTs) pathway

SIRTs are a family of nicotine adenine dinucleotide (NAD)+-dependent deacetylases that regulate numerous cellular processes, including metabolism, ageing, and oxidative stress [64, 65]. SIRTs catalyse the removal of acyl groups from target proteins and function as metabolic regulators in response to changes in NAD+ levels. The SIRT family consists of seven members, SIRT1 through SIRT7, each residing in specific subcellular compartments [64].

In individuals with obesity, SIRT6 expression in WAT was found to be significantly reduced [66]. Mice lacking SIRT6 in adipocytes, when subjected to a high-fat diet (HFD), manifested exacerbated insulin resistance and inflammation [66]. Similarly, adipocyte-specific SIRT6 knockout mice undergoing IF failed to show improvements in glucose homeostasis and insulin sensitivity. The absence of SIRT6 also led to reduced adipose browning and lower energy expenditure, suggesting that SIRT6 is a critical mediator of the metabolic improvements induced by IF [67].

Moreover, CR in rats and fasting in humans have been shown to upregulate SIRT1 expression in WAT [68, 69]. SIRT1 promotes WAT browning and increases energy expenditure by deacetylating PPARγ [70]. However, Boutant et al revealed that while SIRT1 overexpression leads to similar improvements in insulin sensitivity, it cannot replicate the effects of ADF on WAT metabolism, including increased mitochondrial respiration and distinct transcriptional alterations in epididymal WAT [71].

SIRT7 can also significantly affect lipid metabolism and thermogenesis [72, 73]. Yoshizawa et al found that whole-body and brown adipose tissue-specific SIRT7 knockout mice display augmented body temperature and energy expenditure along with increased UCP1 expression [73]. Furthermore, Tang et al demonstrated that IF can promote SIRT7 stability by regulating AMPK activity, thereby activating the GSK3β-SIRT7 axis in the context of enhancing the anti-tumour effects of chemotherapy [74]. These findings underscore the potential role of SIRTs in WAT metabolism and remodeling. Nevertheless, additional research is required to delineate the contributions of SIRT7 and other SIRTs within WAT.

Mammalian target of the rapamycin (mTOR) pathway

The mTOR signalling pathway is a critical regulator of autophagy, among its numerous roles in influencing growth, proliferation, glucose, and lipid metabolism [75]. Two functional protein complexes are involved in the regulation of mTOR signalling: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is known to directly phosphorylate and inhibit the activity of Unc-51-like kinase 1 (ULK1), thus negatively regulating autophagy [75].

The mTOR pathway is activated under conditions of excess calorie intake. This activation facilitates *de novo* lipogenesis by activating sterol regulatory element-binding protein 1 (SREBP1) while concurrently suppressing lipolysis by downregulating the expression of lipolytic enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [76, 77]. Moreover, activation of mTORC1 is associated with increased adipogenesis, which supports the storage of excess lipids [78]. Knock out of *raptor*, a component of mTORC1, in adipocytes results in increased insulin sensitivity, reduced fat mass, and elevated energy expenditure in mice. This is achieved through the beiging of WAT, as evidenced by the upregulation of UCP1 and other browning markers [79]. These observed metabolic benefits are similar to those promoted by IF,

highlighting the role of mTOR signalling in the regulation of WAT function and metabolism.

The inhibitory effects of IF on mTOR signalling in WAT have been largely unexplored. However, previous studies have shown the beneficial effects of IF and mTOR signalling in the heart. This is mediated through the intermittent activation of the transcription factor EB (TFEB), a positive regulator of autophagy [80]. Ma et al showed that IF can reduce the activation of mTOR, a negative regulator of TFEB, thereby stimulating TFEB and facilitating protective autophagic effects in a mouse model of desmin-related cardiomyopathy [81–83]. Notably, the phytochemical Acteoside can stimulate beige adipocyte formation *in vitro* via the mTORC1-TFEB signalling pathway, where upregulation of browning markers such as PGC-1α and UCP1 has been observed [84]. Whether IF can mediate autophagy and other positive effects on WAT remodeling via mTOR-TFEB signalling remains unknown and warrants further investigation.

AMPK as the upstream regulator of PI3K/Akt, SIRTs, and mTOR signalling

AMPK is a widely expressed serine/threonine kinase that functions as a metabolic switch, responding to changes in adenosine monophosphate (AMP)/adenosine triphosphate (ATP) levels within the cell. AMPK functions to restore energy balance by favouring catabolic processes that generate ATP while suppressing anabolic pathways [85]. AMPK is activated in response to various metabolic challenges, including exercise, cold exposure, and fasting [86–88]. Fasting can activate AMPK directly or indirectly, through the increased production of metabolic hormones such as catecholamines and adiponectin [86, 89, 90].

AMPK can activate PI3K/Akt signalling as demonstrated *in vitro* by treating differentiated 3T3-L1 adipocytes with AICAR, an AMPK agonist [91]. Impairments in AMPK and PI3K/Akt signalling, observed in obesity and T2D, may contribute to insulin resistance in humans [92, 93]. However, the relationship between AMPK and PI3K/Akt is complex and context-dependent, as activation of AMPK favours catabolic processes. Fasting-induced activation of AMPK can, in contrast, directly inhibit mTOR, leading to increased autophagy via ULK1 activation. This process also promotes catabolic pathways such as lipolysis and β-oxidation while suppressing adipogenesis and lipogenesis [94].

IF triggers significant fluctuations in cellular energy levels, providing a repeated stimulus that may give rise to the remodeling of WAT via activation of AMPK. Several studies have highlighted AMPK as a critical factor in regulating glucose and lipid metabolism, and driving thermogenesis via WAT browning [90, 95, 96]. Conversely, impairment in AMPK activity is associated with various metabolic disorders, including obesity and insulin resistance, type 2 diabetes and fatty liver disease [88, 90]. Although IF has recently been demonstrated to promote AMPK activation and remodeling in the hearts of rats, its role in facilitating IF-induced remodeling of WAT remains elusive [97].

Intermittent fasting and angiogenic remodeling

IF, similar to cold exposure and exercise, is a potent stimulus for promoting adipose tissue thermogenesis via the beiging of WAT [26, 49]. This is favourable in the context of obesity, where increasing energy expenditure may be one non-pharmacological approach to its management. IF is known to induce brown fat-like changes via adipose angiogenic remodeling. 2:1 IF (48-hour feeding, 24-hour fasting) under high-fat diet (HFD) conditions resulted in improved glucose tolerance, insulin sensitivity, and increased energy expenditure. These metabolic benefits are dependent on an IF-stimulated increase in VEGF-A expression, which leads to visceral WAT browning (upregulation of *Adrb3*, *Ppargc1a*, *Cidea*, *Ucp1*) via M2-like macrophage polarisation (upregulation of *Clec10a*, *Il10*, *Ym1*) [26]. IF can mediate browning in perigonadal and inguinal WAT (pWAT and iWAT, respectively), and higher energy expenditure was observed under both normal-chow and HFD-IF regimens [28].

The AMPK-SIRT1-PGC1α signalling axis may be a pivotal driver of VEGF-A-mediated angiogenic remodeling and browning in WAT in response to IF (Figure 2). This pathway's relevance was illustrated in HepG2 human hepatoma cells, where AICAR-stimulated AMPK activation resulted in increased VEGF-A production [98]. Glucose starvation for 4 h in HepG2 cells, *in vitro* conditions that mimic fasting, resulted in elevated phospho-AMPK and VEGF-A mRNA expression. Furthermore, pharmacological activation of SIRT1 led to significant metabolic improvements in DIO mice, along with improved vasculature and reduced fibrosis. In 3T3-L1 preadipocytes, SIRT1 activation was associated with the increased expression of angiogenic factors, including VEGF-A [99]. This provides evidence for SIRT1 being an upstream regulator of VEGF-A. Activation of AMPK is capable of phosphorylating and stimulating PGC-1α in skeletal muscle and WAT, an essential regulator of mitochondrial biogenesis and metabolism [100, 101]. PGC-1α activation is critical for VEGF-mediated angiogenesis in skeletal muscle, although its causative role in IF-induced WAT angiogenesis has yet to be established [102]. Nonetheless, *Ppargc1a* was significantly upregulated in WAT following IF in mice and may, therefore, be an upstream regulator of WAT angiogenesis [26]. The AMPK-SIRT1- PGC1α signalling axis was, in fact, directly activated by the anti-diabetic drug Canagliflozin in adipocytes *in vitro*, with downstream effects

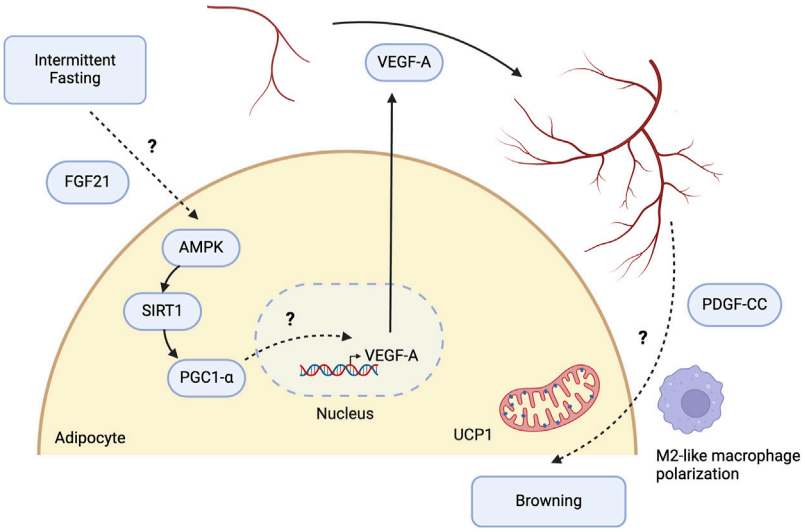

FIGURE 2
Potential molecular pathways involved in intermittent fasting-induced angiogenic remodeling of white adipose tissue. Intermittent fasting (IF) induces the expression of vascular endothelial growth factor A (VEGF-A) in white adipose tissue (WAT), promoting angiogenesis and browning. Liver-derived fibroblast growth factor 21 (FGF21) is indispensable for IF-mediated angiogenesis and browning in WAT. FGF21 upregulates the AMPK-SIRT1-PGC1-α signalling pathway and increases energy expenditure in adipocytes, highlighting FGF21 as one of the intermediary mechanisms between IF and AMPK activation. While PGC1-α regulates VEGF-A-mediated angiogenesis in skeletal muscle, its role in WAT remains to be determined. The mechanistic link between increased angiogenesis and WAT browning may involve endothelial cells releasing platelet-derived growth factor CC (PDGF-CC) in response to VEGF-A, leading to WAT browning. It may also be driven by alterations in immune cells, such as polarisation towards M2-like macrophages. However, the specific mechanisms remain to be elucidated. Created with BioRender.com.

including increased thermogenesis and energy expenditure [103]. This provides further support for the likely involvement of this pathway in IF. Although direct evidence linking IF to the activation of AMPK, SIRT1, and PGC-1α in WAT has yet to be shown, CR can activate this pathway in the heart and mitigate myocardial injury following reperfusion [104].

Fibroblast growth factor 21 (FGF21) also plays a crucial role in regulating VEGF-A expression. In mice with liver-specific knockout of FGF21 subjected to IF for 16 weeks, there was a lack of angiogenic growth and browning in WAT, as indicated by the reduced expression of browning markers such as *Ucp1*, *Ppargc1a*, and *Elovl6* [105]. Additionally, IF-mediated anti-inflammatory effects were lost following FGF21 knockout, as evidenced by an increase in pro-inflammatory M1-like macrophage markers. Since M2-like polarisation is essential for IF-induced browning, liver-derived FGF21 may serve as a critical upstream regulator in this process. Interestingly, Abu-Odeh et al highlighted that browning of iWAT following treatment with the β-adrenergic agonist CL-316,243, which mimics cold exposure, still occurred despite the liver-specific knockout of FGF21. These findings suggest that hepatic and adipocyte-derived FGF21 may have differing effects under various physiological stimuli, with liver-derived FGF21 being crucial for IF-induced browning and anti-inflammation, while adipocyte-derived FGF21 plays a key role in β-adrenergic receptor-mediated thermogenesis [106]. Nevertheless, Chau et al established that FGF21 increases energy expenditure through the phosphorylation and activation of AMPK, which in turn activates SIRT1 and PGC-1α both *in vitro* and *in vivo* [107]. Thus, IF may induce VEGF-A expression and angiogenic remodeling via an upstream pathway involving FGF21, AMPK, SIRT1, and PGC-1α. Further research is needed to confirm these mechanisms and to fully elucidate the role of FGF21 in IF-mediated angiogenesis.

The crosstalk between white adipocytes and endothelial cells is vital for angiogenesis and WAT browning. Seki et al investigated the effects of adrenergic activation induced by cold exposure and CL-316,243 treatment, which elicited a browning response similar to that observed with IF. Their research identified platelet-derived growth factor CC (PDGF-CC) as an endothelial-derived soluble factor activated by VEGF-A that promotes the differentiation of adipocyte progenitor cells towards a beige phenotype [108]. Furthermore, Seki et al demonstrated that deletion of VEGF receptor 1 (VEGFR1) in endothelial cells, a receptor that typically functions as a sink for VEGF-A and thus inhibits its function, results in WAT browning [109]. VEGF-A likely interacts with other receptors on endothelial cells besides VEGFR1, including VEGFR2 and neuropilin-2, indicating a complex network of potential signalling interactions between adipocytes and endothelial cells [110].

CR elicits a type 2 immune response similar to IF, mediating the browning of WAT [20]. Under CR conditions, SIRT1 levels were elevated in macrophages and eosinophils, a response likely driven by IL-4 [111]. In a contrasting study, J. Park et al transplanted adipose tissue overexpressing VEGF-A into DIO mice, offering a direct parallel to the IF-induced elevation of VEGF-A described by Kim et al [26, 112]. Following transplantation, angiogenic remodeling and the development of beige adipocytes were observed, independent of IL-4 [112]. Group 2 innate lymphoid cells (ILC2) have been extensively researched in adipose tissue for their role in fostering a type 2 inflammatory environment. The essential cytokines, IL-5 and IL-13 are critical for the activation of eosinophils and M2-like macrophages, thereby influencing WAT beiging and thermogenesis [113–115]. Given that VEGF-A does not directly induce the M2-like polarisation of macrophages necessary for the browning effects seen with IF, the interplay between endothelial cells, macrophages, and other immune cells, as well as proangiogenic factors will be important in determining the mechanism underlying IF-induced WAT browning.

Intermittent fasting and sympathetic innervation

Sympathetic innervation is a key regulator of lipolysis, allowing fat mobilisation from adipose tissue in response to the body's energy demands. In response to physiological stimuli, activation of the SNS drives the release of norepinephrine (NE) from local sympathetic nerves into the adipocyte microenvironment. This subsequently activates β-adrenergic G-protein coupled receptors expressed on adipocytes, including ADRB1, ADRB2, and ADRB3, resulting in the decoupling of the G_S protein and activation of adenylate cyclase (AC). This results in an increase in levels of intracellular cAMP, which activates protein kinase A (PKA) and culminates in a signalling cascade that phosphorylates key lipolytic enzymes such as HSL and perilipin A (PLIN1a) [116]. Activated HSL translocates to the lipid droplet monolayer, and ATGL is subsequently activated by the release of ABHD5 from activated PLIN1a, catalysing the hydrolysis of triglycerides [117].

Early evidence from Migliorini et al indicated increased sympathetic activity in epididymal WAT after 48 h of fasting, as evidenced by an increased NE turnover rate [118]. Additionally, 48-hour fasting has also been shown to stimulate sympathetic innervation, as evidenced by increased tyrosine hydroxylase (TH) protein content, in both epididymal and retroperitoneal WAT [119]. β-adrenergic activity can alter the expression of GLUT4 in WAT in response to short-term fasting and refeeding, thereby regulating glucose homeostasis [120]. It remains unclear whether IF utilises a similar mechanism to improve glucose homeostasis. IF also increases lipolysis through elevated phosphorylation of HSL and extracellular signal-regulated kinase (ERK), a kinase that activates lipolytic enzymes, including HSL [44]. The upregulation of *Adrb3* expression in mouse WAT under IF suggests that sympathetic activation of adrenergic receptors may initiate the downstream lipolytic cascade [26]. A similar mechanism is observed in humans, where propranolol-stimulated blockade of β-adrenergic receptors can mitigate fasting-induced lipolysis [121].

In contrast, Li et al elucidated an opposite effect of IF leading to reduced *Adrb3* expression in both BAT and WAT. Their study highlighted the importance of gut microbiota changes in driving WAT browning after every-other-day feeding (EODF), a regimen synonymous with ADF [49]. A separate study using proteomic analysis on fat pads from EODF-treated mice revealed a reduction in ADRB3 and lipolytic pathways, including reduced monoacylglycerol lipase, along with an upregulation in fatty acid synthesis pathways involving enzymes such as ATP-citrate lyase. This suggests a potential mechanism for energy conservation [122]. Given the evident reduction in calorie intake associated with EODF, the observed attenuation of lipolysis may be a metabolic adaptation compared to the isocaloric nature of the 2:1 IF regimen, which activates ADRB3 [26]. These findings underscore the importance of the specific type of IF intervention on WAT remodeling and its downstream functional effects.

The molecular mechanisms by which IF results in adaptive remodeling of the neural architecture of WAT, thereby promoting lipolysis and thermogenesis, remain to be investigated (Figure 3). Current evidence suggests that IF facilitates angiogenesis via upregulation of VEGF-A [26]. Separately, Zhao et al showed that VEGF-A overexpression in transgenic mice not only increases angiogenesis but also promotes sympathetic nerve growth [123]. VEGF-A overexpression leads to downstream activation of ADRB3 and phosphorylation of HSL, as well as increased expression of browning markers including UCP1 and PGC1- α. This highlights a potential mechanism by which IF may mediate lipolysis and browning via VEGF-A-induced sympathetic nerve growth [123].

Stimuli, such as exercise and cold exposure, which activate ADRB3 and confer metabolic benefits similar to those of IF, have been observed to stimulate both sympathetic innervation and angiogenesis in WAT. In addition to VEGF-A, several neurotrophic factors released in WAT regulate this remodeling process. Neuronal growth regulator 1 (NEGR1), a cell adhesion molecule that modulates neural innervation in the brain, is significantly associated with body mass index (BMI) in meta-analyses of genome-wide association studies [124, 125]. Exercise training for 10 weeks in C57BL/6 mice induced NEGR1 expression specifically from mature white and beige adipocytes in iWAT, leading to increased neurite growth. The increase in NEGR1 expression has also been observed in humans following treadmill training, with PRDM16, regulated by PPARγ, identified as the critical transcription factor governing NEGR1 expression [126].

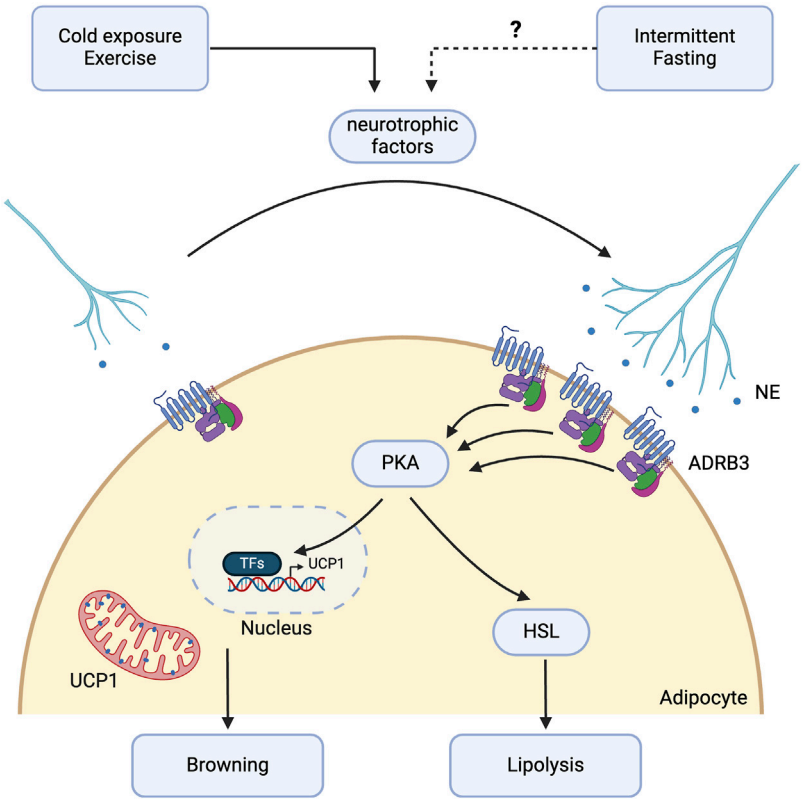

FIGURE 3
Potential molecular pathways involved in intermittent fasting-induced sympathetic innervation. Intermittent fasting (IF) may promote sympathetic activation in white adipose tissue (WAT) through the β-3 adrenergic signalling pathway. Although direct evidence showing IF's effect on sympathetic nerve growth in WAT is currently lacking, existing studies suggest that IF may promote this growth through VEGF-A or other neurotrophic factors such as neuronal growth regulator 1 (NEGR1), neurotrophin-3 (NT-3), neuregulin 4 (NRG4), nerve growth factor (NGF), and slit guidance ligand 3 (SLIT3), which are implicated in exercise or cold-induced sympathetic nerve growth. IF enhances β-3 adrenergic receptor expression and downstream lipolysis through activation of hormone-sensitive lipase (HSL). Sympathetic innervation further promotes the browning of WAT via the protein kinase A (PKA) intracellular pathway, increasing the expression of thermogenic genes such as uncoupling protein 1 (UCP1). Created with BioRender.com.

Neurotrophin-3 (NT-3) and neuregulin 4 (NRG4) are adipocyte-derived growth factors that mediate sympathetic nerve growth in WAT and promote beige fat formation under cold conditions [8, 10]. NT-3 binds to TrKC receptors in the sympathetic ganglia, while NRG4 functions through ErbB4 [127]. NRG4 can also directly stimulate browning in adipocytes *in vitro* [128]. Cold-induced production of nerve growth factor (NGF) by adipose eosinophils also stimulates neurite growth [129]. NGF, recognised as an adipokine, targets TrkA receptors in iWAT and is necessary for cold-stimulated beiging [130]. Increased NGF production is attributed to an increased accumulation of adipose eosinophils, driven by increased IL-5 secretion from ILC2s, which in turn is stimulated by IL-33 release from stromal cells in response to cold. Cold-induced sympathetic nerve growth is also mediated by the secretion of slit guidance ligand 3 (SLIT3) by M2-like macrophages, which bind to roundabout guidance receptor 1 (ROBO1) receptors on nerve fibres. The SLIT3-ROBO1 interaction promotes increased NE release, leading to the upregulation of the lipolytic and thermogenic pathways, marked by elevated PKA activity, phosphorylation of HSL, and increased UCP1 expression [131].

A plethora of neurotrophic factors regulate sympathetic nerve growth in WAT in response to exercise and cold exposure. However, whether these factors play a specific role in mediating IF-stimulated adipose tissue remodeling remains to be elucidated. IF is known to shift the immune cell landscape towards a type 2 inflammatory response with increased M2-like macrophage polarisation and eosinophils, as observed in aged mice subjected to IF [132]. This contrasts with the pro-inflammatory environment in obese adipose tissue, characterised by reduced ILC2 and eosinophil populations and a shift towards M1-like macrophages [133–135]. These immune alterations may contribute to the underlying mechanisms leading to reduced sympathetic innervation of WAT in obesity [136]. Furthermore, whether AMPK plays a role in regulating SNS

activation remains uncertain. Nevertheless, AMPK is known to directly regulate lipolysis through the phosphorylation of ATGL and HSL [137]. Additionally, the process of lipolysis itself leads to an increase in the AMP:ATP ratio, which may result in the activation of AMPK [138].

Discussion

Recent studies have established IF as an effective, economical, and practical strategy for weight management. Meta-analyses and umbrella reviews conducted in recent years highlight the efficacy of IF as a dietary intervention in improving health outcomes in obese or overweight participants, including reduced body weight and fat mass, favourable lipid profiles, reduced inflammatory markers, and improved fasting insulin as well as plasma glucose levels [139–143]. Beyond its efficacy in obese adult populations, IF may reduce BMI and the risk of cardiovascular disease in adolescents with obesity [144]. Despite the known metabolic health advantages associated with IF, compliance with a dietary regimen like IF can pose a challenge in the long term. However, adherence and feasibility can be maintained by implementing a personalised dietary regimen under the guidance of a professional dietitian and by building flexibility into the programme, allowing users to choose specific days of the week to fast or restrict calories [144].

To mediate the beneficial effects of IF on WAT remodeling and systemic metabolic homeostasis, IF may modulate a complex regulatory network involving signalling pathways such as PI3K/Akt, SIRTs, and mTOR. As AMPK is positioned as the central metabolic switch regulating the downstream pathways, activation of AMPK in response to IF may be integral in promoting a range of cellular processes, including lipolysis, β-oxidation, autophagy, and the browning of WAT. Nevertheless, both gain-of-function and loss-of-function studies of these intracellular pathways, especially in WAT, are necessary to validate their relevance in IF-mediated WAT remodeling. VEGF-A drives IF-induced angiogenesis in WAT, which may, in part, be regulated by the AMPK-SIRT1-PGC1α signalling axis. FGF21 has also emerged as an essential signalling factor upregulated by IF, which can activate AMPK and downstream PGC-1α [105]. VEGF-A, along with other neurotrophic factors, can promote sympathetic nerve growth, highlighting the close interplay observed in the remodeling of the neurovascular architecture of WAT. While IF can promote angiogenesis and sympathetic activation [26], its direct effect on sympathetic nerve growth has not been directly elucidated, with current evidence derived from studies on exercise [126] and cold-induced sympathetic nerve growth [8, 10, 130, 131] in WAT.

Additionally, the current mechanistic understanding of IF remains limited, particularly regarding its direct activation of pathways such as AMPK, PI3K/Akt, various SIRTs, and mTOR in WAT. The majority of the available evidence comes from studies in CR or from other tissues such as the heart [58, 59, 61, 80, 81, 83, 97], liver [56, 57], and brain [62, 63]. Furthermore, the variability between different IF regimens [26, 28, 49, 122] necessitates further investigation into their physiological effects and underlying mechanisms, which is crucial for standardising IF protocols for research and clinical applications. These differences also underscore the importance of thoroughly investigating both the benefits and downsides of IF. For example, ADF has been shown to exacerbate atherosclerosis in mice predisposed to the condition, and TRF increases hepatic insulin resistance in young rats with diet-induced obesity [145, 146]. Understanding these differences and potential adverse effects is essential for identifying appropriate target populations for IF.

It is also essential to consider the cyclical nature of IF with its fasting and refeeding cycles. The periodic activation and inhibition of these signalling pathways may be important in the development and administration of pharmacological mimetics. Intermittent administration of rapamycin can prolong the life of female C57BL/6 mice while negating any metabolic side effects such as impaired insulin sensitivity [147]. Similarly, periodic administration of metformin, an AMPK activator, showed metabolic improvements and weight loss without affecting the mortality of aged mice, despite its toxic side effects at the dosage used [148].

AMPK activators have demonstrated potential for promoting weight loss through the activation of AMPK, leading to the inhibition of fat synthesis and promotion of fat oxidation pathways. However, their clinical use has been predominantly focused on diabetes management rather than weight loss, possibly due to the wide range of targets affected by AMPK activators like metformin [149, 150]. Further research is needed to determine their specific mechanisms of action. The activity of AMPK is also highly dependent on several factors, such as intracellular ATP levels, dosage, and route of administration [151–153]. Additional studies are required to investigate whether AMPK's functional role in fat metabolism can be separated from its role in glucose regulation, thus supporting its potential as a standalone target for weight loss [154]. Several side effects associated with the use of AMPK activators, including gastrointestinal discomfort, headaches, and fatigue, may compromise adherence, effectiveness, and patient quality of life [153, 155].

In conclusion, IF holds great promise as a dietary intervention for weight loss and the improvement of metabolic health through its multifaceted effects on WAT remodeling. Addressing the gaps in our understanding of the molecular mechanisms driving these processes will enable the development of more targeted drug therapies that can replicate or even enhance the metabolic benefits of IF, potentially circumventing the need for significant dietary changes. Future studies should focus on standardising IF protocols, investigating the interplay between key signalling pathways, and exploring the potential of pharmacological mimetics or a combination of IF

and pharmacotherapy to provide a comprehensive approach to obesity management.

Author contributions

NV, QZ, and H-KS conceived and designed the research. All authors contributed to the article and approved the submitted version.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. H-KS is supported by grants from Canadian Institute of Health Research (CIHR, PJT-162083, PJT-190016), Natural Sciences and Engineering Research Council (NSERC, RGPIN-2016-06610) of Canada, Diabetes Canada (OG-3-23-5715-HS), Canada Foundation for Innovation (CFI, #40249), and Sun Life Financial New Investigator Award of Banting & Best Diabetes Centre (BBDC) of the University of Toronto. NV is supported by Novo-Nordisk Studentship from Banting and Best Diabetes Centre (BBDC) of the University of Toronto and Restracomp Master's Scholarship from The Hospital for Sick Children. QZ is supported by Doctoral Program from Chinese Scholarship Council (CSC202008340062).

Acknowledgments

We thank Dr. Jacques Togo and Dr. Bruno Rodrigues de Oliveira for their helpful suggestions during the writing of this review.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

T2D	Type 2 Diabetes
WAT	White Adipose Tissue
ASPCs	Adipose Stem and Progenitor Cells
CR	Calorie Restriction
IF	Intermittent Fasting
ADF	Alternate-Day Fasting
PF	Periodic Fasting
TRF	Time-Restricted Feeding
RF	Ramadan Fasting
UCP1	Uncoupling Protein 1
PI3K	Phosphatidylinositol 3-Kinase
Akt	Protein Kinase B
CLK2	CDC-like kinase 2
AMPK	AMP-activated protein kinase
DIO	Diet-Induced Obesity
SIRTs	Sirtuins
NAD+	Nicotinamide adenine dinucleotide
HFD	High-Fat Diet
mTOR	Mammalian Target of Rapamycin
mTORC1	mTOR Complex 1
mTORC2	mTOR Complex 2
ATGL	Adipose Triglyceride Lipase
HSL	Hormone-Sensitive Lipase
TFEB	Transcription Factor EB
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A
VEGFR2	Vascular Endothelial Growth Factor Receptor 2
FOXO1	Forkhead Box O1
GSK3β	Glycogen Synthase Kinase 3 Beta
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
SREBP1-c	Sterol Regulatory Element-Binding Protein 1-c
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ULK1	UNC-51-like kinase 1
pWAT	Perigonadal White Adipose Tissue
iWAT	Inguinal White Adipose Tissue
PGC-1α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
FGF21	Fibroblast Growth Factor 21
PDGF-CC	Platelet-Derived Growth Factor CC
VEGFR1	Vascular Endothelial Growth Factor Receptor 1
IL-4	Interleukin 4
ILC2	Group 2 Innate Lymphoid Cells
NE	Norepinephrine
ADRB1	Adrenergic Receptor Beta 1
ADRB2	Adrenergic Receptor Beta 2
ADRB3	Adrenergic Receptor Beta 3
AC	Adenylate Cyclase
PKA	Protein Kinase A
PLIN1a	Perilipin A
TH	Tyrosine Hydroxylase
ERK	Extracellular Signal-Regulated Kinase
GLUT4	Glucose Transporter Type 4
EODF	Every other day feeding
BMI	Body Mass Index
NEGR1	Neuronal Growth Regulator 1
NT-3	Neurotrophin-3
NRG4	Neuregulin-4
ErbB4	Erb-B2 Receptor Tyrosine Kinase 4
TrkC	Tropomyosin Receptor Kinase C
NGF	Nerve Growth Factor
TrkC	Tropomyosin Receptor Kinase A
SLIT3	Slit Guidance Ligand 3
ROBO1	Roundabout Guidance Receptor 1

CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 17 July 2024
DOI 10.3389/jpps.2024.13157

OPEN ACCESS

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RECEIVED 19 April 2024
ACCEPTED 01 July 2024
PUBLISHED 17 July 2024

CITATION
Prapaharan B, Lea M and Beaudry JL (2024), Weighing in on the role of brown adipose tissue for treatment of obesity. *J. Pharm. Pharm. Sci* 27:13157. doi: 10.3389/jpps.2024.13157

Weighing in on the role of brown adipose tissue for treatment of obesity

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Brown adipose tissue (BAT) activation is an emerging target for obesity treatments due to its thermogenic properties stemming from its ability to shuttle energy through uncoupling protein 1 (Ucp1). Recent rodent studies show how BAT and white adipose tissue (WAT) activity can be modulated to increase the expression of thermogenic proteins. Consequently, these alterations enable organisms to endure cold-temperatures and elevate energy expenditure, thereby promoting weight loss. In humans, BAT is less abundant in obese subjects and impacts of thermogenesis are less pronounced, bringing into question whether energy expending properties of BAT seen in rodents can be translated to human models. Our review will discuss pharmacological, hormonal, bioactive, sex-specific and environmental activators and inhibitors of BAT to determine the potential for BAT to act as a therapeutic strategy. We aim to address the feasibility of utilizing BAT modulators for weight reduction in obese individuals, as recent studies suggest that BAT's contributions to energy expenditure along with Ucp1-dependent and -independent pathways may or may not rectify energy imbalance characteristic of obesity.

KEYWORDS

obesity, energy expenditure, white adipose tissue, brown adipose tissue, weight loss

Introduction

Since the identification of metabolically active BAT in adult humans in 2009 [1], there has been a growing interest towards the idea of BAT activation as a therapeutic strategy to assist in treating obesity. Obesity, defined as excessive fat storage, is associated with increased risk of cardiovascular diseases and metabolic disorders including type 2 diabetes (T2D) and dyslipidemia. Consequently, finding a treatment to mitigate the development of obesity and its related diseases is crucial. Obesity is characterized by an energy imbalance, where energy intake exceeds energy expenditure (EE) [2]. Adipose tissue, a vital and dynamic organ, provides structural support but can be influenced by external and internal signals that affect its function. BAT has emerged as a potential therapeutic target for obesity due to the presence of uncoupling protein 1 (Ucp1), a thermogenic protein that boosts EE within the body [3]. Ucp1 is triggered by sympathetic activity and facilitates thermogenesis by redirecting

proton flow in the BAT inner mitochondrial membrane to generate heat rather than ATP [3]. Ucp1 can also be present in white adipose tissue (WAT), where it is involved in a process known as "browning". White adipocytes with Ucp1 develop a BAT-like phenotype including an increase in mitochondria, and formation of smaller lipid droplets within the cell. Enhancing BAT activity and browning in WAT could optimize EE, potentially restoring energy balance in obese individuals. To combat obesity by utilizing BAT activation mediated increased EE, it is essential to understand the mechanisms that govern BAT activation and inhibition, in both rodents and human models. Activators such as cold-exposure, thyroid hormones, and fish oil-derived omega-3 fatty acids promote BAT activity by upregulating the gene expression of Ucp1 and other browning-associated proteins [4–6]. Conversely, glucocorticoids, androgens, aldosterone, and high fat diet serve as BAT inactivators and hinder thermogenic function by dysregulating adipocytes and inflammation [7, 8]. It is imperative to study BAT in humans, which is known to vary in abundance, particularly in obese individuals [9]. Additionally, assessing the function of Ucp1 in BAT thermogenesis is also essential. In recent years, Ucp1 has been acknowledged as one of several thermogenic pathways in BAT, alongside newly identified Ucp1-independent pathways, specifically calcium and creatine substrate cycling [10, 11]. Consequently, the objective of this review is to compile preclinical and clinical evidence identifying pharmacological, hormonal, bioactive, and environmental factors that modulate BAT activity through both Ucp1-dependent and independent mechanisms. Endogenous factors that influence BAT activity and induce browning are analogous; however, the outcomes from exogenous factors and stimuli that modulate adipose tissue function and phenotype can differ [12]. Thus, the importance of browning in regulating EE, glucose, and lipid homeostasis should not be overlooked. Our primary focus is to review and discuss the potential of BAT as a viable therapeutic target for combating obesity, and present factors influencing BAT activation rather than WAT browning.

Activators of brown adipose tissue

Pharmacological activators

In both rodents and humans, BAT is activated by sympathetic nervous system (SNS) stimulation [13] through the release of norepinephrine and its non-selective agonism on β1, 2, and 3 adrenergic receptors (ADRs) on various BAT tissues [14]. Downstream activation of the βADRs increases the release of stored nutrients such as free fatty acids that upregulate Ucp1 in BAT and can also induce browning in WAT. Moreover, recent research indicates that Ucp1 is dispensable to increasing EE due to browning through the Ucp1 independent futile creatine cycling [15]. While stimulation by β3 agonists activates BAT in rodents [16, 17], there is conflicting evidence regarding which βADR subtype is the primary activator of human BAT [18–21]. In both rodents [22] and humans [23], BAT activation through cold exposure increases fatty acid uptake through the activation of βADRs. Obese and T2D individuals, who could benefit from increased EE through BAT activation, generally lack the presence of metabolically active BAT. Nevertheless, both rodent and human studies have investigated several pharmacological agents that activate BAT, through various mechanisms, including direct and indirect βADRs. However, these studies have shown differing levels of success. In the next section we will review BAT activators and discuss the relevance of the pathways involved.

Evidence in rodents

Various pharmacological activators have been studied in rodents to upregulate BAT activity for weight loss purpose. The activation of BAT results in the maintenance of thermal homeostasis and disposal of excess metabolites [1]. 2-4-dinitrophenol (DNP), a component of explosives, wood preservation solutions, and herbicides, was commercially used as a weight loss medication for 5 years in the 1930s [24]. While not a direct activator of BAT, DNP induces extreme increases in metabolic rate and thus weight loss through widespread mitochondrial uncoupling [24]. Interestingly, these mechanisms appear to be independent to Ucp1 pathways. DNP supplemented drinking water increased metabolic rate and induced fat and total body mass loss in diet-induced obese (DIO) female C57BL/6J mice held at thermoneutrality (30°C) after 9 weeks [25]. Surprisingly, DNP treatment decreased mRNA and protein levels of Ucp1, which could be reflective of reduced BAT function even in the face of increased EE.

Berberine (BBR), a plant derived anti-obesity medication, increases adipose triglyceride lipase (ATGL) expression and basal rates of TG lipolysis in adipose tissue [26]. Daily intraperitoneal (i.p.) BBR administration of 1.5 mg/kg for 6 weeks to obese C57BL/6 male mice increased BAT thermogenesis and EE [27]. Interestingly, this BBR treatment also increased BAT volume, glucose uptake, and *Ucp1* and *Prdm16* expression levels in these obese male mice [27]. Similarly, 4 weeks of BBR i.p. injections at a higher dose of 5 mg/kg/day in obese male C57BL/6J mice increased EE, lipid oxidation, BAT ^{18}F-FDG uptake, BAT mitochondrial content, UCP1 protein expression, and BAT transcription factor genes such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1-α*) that regulate energy metabolism [28]. This indicates that BBR can induce BAT activation through mechanisms other than direct βADR agonism. However, the direct pathways through which

BBR increases BAT remains unclear. Other plant derived agents include *Withania somnifera* extract (WSE), and withaferin A (WFA) a major constituent of WSE. Dietary supplementation of 0.5% WSE to a HFD for 10 weeks increased mRNA expression of genes involved in thermogenesis and mitochondrial biogenesis, and reduced lipid droplet size in BAT of DIO male C57BL/6J mice [29, 30]. Oral supplementation (1.5 mg/kg) of WFA for 7 days in DIO male C57BL/6J mice increased EE, UCP1 protein and mRNA levels in BAT, and phosphorylation of p38 and $ERK_{1/2}$ in WAT indicative of browning. Interestingly, WFA treated mice closely resembled the phenotypic results observed in the metabolically healthy low-fat diet-fed group [30]. While not providing direct evidence of BAT activation, these studies suggest that WSE and WSA may influence mitochondrial biogenesis and Ucp1 expression in both BAT and WAT.

Resveratrol, a polyphenol produced in the skin of fruits such as grapes and berries, has been investigated for its protective effects against weight gain [31, 37] and obesity related diseases. It has been extensively reviewed [32–35]. However, in brief, the changes in adipose tissue are suggested to act through WAT browning by promoting AMPK phosphorylation, which increases PGC1α and sirtuin 1 (SIRT1) production. This induction leads to mitochondrial biogenesis and browning, rather than direct BAT activation [36, 37]. This is supported by morphological changes in WAT to BAT-like lipid droplets, and increased BAT mass [38]. Alternately, resveratrol may adjust gut microbiota composition that improves intestinal barrier dysfunction and reduces inflammation [39]. While resveratrol supplementation offers benefits in improving metabolic conditions, there is little evidence supporting direct BAT activation. The interaction between resveratrol and adipose tissue appears to occur through WAT browning rather than BAT activation.

Several studies have evaluated βADR agonists to increase BAT activity in rodents. BAT in rodents expresses functional β3 and β1 ADR, with β3ADR now being well-established to directly upregulate BAT processes [40]. In obese female C57BL/6 (*ob*/*ob*) mice, oral administration of β3ADR agonists BRL 26830A, BRL 33725A, and BRL 35135A for 4 weeks showed a decrease in weight gain with no change in food intake, and BAT UCP1 protein expression [41].Wilson S et al reported that lean male Sprague-Dawley rats given single i.p injections of 5 mg/kg of BRL 26830A increased EE while simultaneously increasing lipid oxidation in BAT, thus resulting in a substantial decrease in BAT lipid droplet size [42]. In sedentary obese male Zucker rats, gastric cannula administration of BRL 35135 increased mitochondrial guanosine diphosphate (GDP) binding, an indicator of increased Ucp1 uncoupling, mitochondrial protein content, and reduced WAT mass compared to those that received either vehicle or an α_2 ADR agonist [43]. Both acute and chronic treatment with the selective β3ADR agonist ICI D7114 also suggest BAT activation in rats. In male Wistar rats, ICI D7114 increased EE and mitochondrial GDP binding in BAT suggesting BAT activation, however this was associated with an increase in heart rate [44]. As chronic tachycardia increases risk of cardiovascular events, a human safety trial is warranted [45]. To note, ICI D7114 had no effect on weight loss or changes in fat mass in male Sprague-Dawley rats [46]. Due to technological limitations in these studies, it is difficult to attribute metabolic changes to BAT activation alone.

Recently, there has been a shift towards investigating non-sympathomimetic methods for BAT activation, as sympathomimetics could inadvertently stimulate the central nervous system and non-target organs. Peroxisome proliferator-activated receptor γ (PPARγ) is a transcriptional regulator necessary for the maturation of brown adipocytes [47]. It promotes BAT adipocyte differentiation and lipid storage [48]. PPARγ agonists have been used in T2D treatment to improve insulin sensitization but can result in weight gain. Interestingly, PPARγ mediated increases in oxygen consumption and fatty acid oxidation have been repeatedly shown *in vitro* [49, 50]; however, these effects are not observed *in vivo*. In fact, opposite effects are seen in both humans [51] and rodents [52], where PPARγ activation increases lipid storage. While PPARγ agonism is an exciting concept for BAT activation *in vitro*, the inability to replicate increases in EE and lipid oxidation *in vivo* makes its use in treating metabolic complications beyond insulin sensitization unlikely.

Signalling from the angiotensin II (AngII) has regulatory effects on energy homeostasis and can act through PPARγ activation to promote adipocyte differentiation and insulin sensitivity. However the role of AngII signalling specifically in BAT is less clear [53]. Recently, the use of angiotensin type 2 receptor (AT2R) agonism has been explored as an alternate method of BAT activation [54]. C21, an AT2R agonist, was not protective against weight gain from HFD feeding in male C57BL/6J mice. However, C21-treated mice showed increased BAT mass and brown adipocyte differentiation despite being fed a HFD compared to mice fed a regular chow and treated with C21. While direct measures of BAT activity were not taken, protein levels of UCP1 and electron transport chain complexes I, II, III, and IV were upregulated with C21 treatment in HFD fed mice independent to changes in ATP synthase. This may suggest increased thermogenic capacity in an obese state, however further investigation is required to determine if AT2R agonism can improve metabolic outcomes such as glucose or lipid levels and if there is indeed BAT activation.

Metabokines produced from branch chain amino acid catabolism including 3-methyl-2-oxovaleric acid (MOVA), 5-oxyproline (5OP), and β-hydroxyisobutyric acid (BHIBA) significantly increase the expression of genes associated with BAT thermogenesis and lipid metabolism [55]. DIO mice treated with either MOVA, 5OP, or BHIBA via drinking water for 17 weeks showed increased EE with no changes in overall activity or food intake. Additionally, these metabokines

showed higher levels of mitochondrial biogenesis markers and ^{18}F-FDG uptake in BAT. MOVA and 5OP given together reduce fat mass by nearly 25% and improve glucose tolerance. The TCA cycle intermediate succinate is another potential metabolic signal of BAT activation [56]. Obese mice provided with succinate supplemented drinking water for 4 weeks had markedly decreased body weight, and improved glucose tolerance independent of changes in food intake. This response appears to be driven through Ucp1 mediated thermogenesis as *Ucp1* KO mice abolishes this positive effect. These results highlight the potential of non-sympathomimetic BAT activation.

Evidence in humans

Sympathomimetics are known to increase EE, however it is unclear if this is mediated by BAT activation in humans. Ephedrine, approved for treating hypotension, exerts its effects by inhibiting neuronal reuptake of norepinephrine allowing more time for it to act on postsynaptic βADRs [57]. Acute oral ingestion of 1 mg/kg body weight ephedrine in fasted healthy men increased perirenal BAT blood flow after 30 and 60 min in some participants [58]. This corresponded with an increase in both perirenal BAT and overall body temperature, circulating glucose and oxygen consumption by 19% after 60 min, and reduced RER after 150 min with no change in circulating non-esterified fatty acids (NEFAs) or glycerol levels. Both systolic blood pressure and heart rate increased over the duration of the experiment. A higher dose of 2.5 mg/kg body weight of ephedrine increased ^{18}F-FDG uptake into supraclavicular BAT in lean fasted men, but not in obese or placebo groups [19]. This also corresponded with increased BAT activation in lean but not obese participants and resulted in no change in core body temperature or plasma NEFA levels. Ephedrine also increased EE and circulating glucose, but also increased systolic blood pressure. In contrast to acute treatment, chronic treatment with 1.5 mg/kg ephedrine orally for 28 days in fasted metabolically healthy men results in no change in resting EE, respiratory exchange ratio (RER), and a decrease in systolic blood pressure and BAT activity at the end of treatment [59]. Cypess et al reported that lean male and females that received an intramuscular injection of 1 mg/kg ephedrine and subjected to cold exposure (14°C) maintained their temperature without shivering [60]. Both cold and ephedrine increased metabolic rate by 79 and 136 kcal/day, respectively, and decreased RER indicating more use of fatty acids as fuels, but no changes were observed between interventions. Systolic and diastolic blood pressure increased with cold and ephedrine treatment but only ephedrine increased heart rate [60]. Ephedrine elevated plasma glucose, NEFA, lactate, and insulin levels and no change in ^{18}F-FDG uptake compared to cold and placebo groups. Remarkably, this study included female participants and no sex-based differences were reported in response to ephedrine administration.

While the reasoning for using ephedrine as a BAT activator is logical, these results are conflicting and may suggest shifting focus to other approaches. With increases in EE and BAT activity, heart rate and blood pressure also increase with ephedrine. This makes the individual contribution to BAT difficult to discern as a systemic increase in blood flow and nonspecific βADR activation could account for much of this increase [18]. Additionally, the inability for ephedrine to stimulate BAT activity in obese individuals and its reduction in potency of BAT stimulation with chronic treatment make its use in treating obesity unlikely. It is also important to consider the extent of EE afforded by treatments that would lead to meaningful weight loss in obese individuals. Although ephedrine increased EE, this minimal increase may not be clinically relevant. Moreover, individuals with obesity did not respond to ephedrine for reasons unknown thereby rendering ephedrine an unlikely candidate to expend excess stored calories in humans.

Other approaches include direct βADR agonists that mimic SNS stimulation. Mirabegron, a β3ADR agonist used to treat overactive bladder [61], has been explored as a sympathomimetic BAT activator. Higher than the approved dose for hyperactive bladder, which is 50 mg, mirabegron shows promising effects to upregulate BAT activity. Healthy men selected with cold induced detectable BAT given an acute dose of 200 mg mirabegron showed an increase in BAT activity measured by ^{18}F-FDG uptake in all 12 participants [62]. Compared to placebo, there was higher resting metabolic rate, plasma glucose and NEFA, heart rate, and systolic blood pressure. However, these cardio stimulatory effects were lower compared to ephedrine [19, 60]. Interestingly, there were no changes in ^{18}F-FDG uptake in subcutaneous WAT, liver, or skeletal muscle, except for BAT that showed detectable changes in ^{18}F-FDG uptake with mirabegron treatment. A dose dependent effect on BAT activation was found when comparing between 50 and 200 mg mirabegron in healthy young men with cold activated detectable BAT [20]. Of note, only the 200 mg dose increased EE in these men with no changes in plasma glucose or insulin and increases in NEFA levels were dose dependent possibly through β3ADR mediated lipolysis. Heart rate and systolic blood pressure were higher with the 200 mg dose. 150 mg/day mirabegron treatment for 10 weeks increased UCP1 and CIDEA protein expression in subcutaneous WAT to a greater extent than cold treatment, however there was no change in PCG1α, a known indicator of mitochondrial biogenesis or mitochondrial DNA content, which is activated by βADR in rodents [63]. No change in heart rate or blood pressure were seen indicating that cardiovascular effects may only be an acute side effect. Chronic treatment with 100 mg mirabegron for 4 weeks increases BAT activity and volume in healthy women, particularly in those with initially lower BAT amount [64]. While there were acute changes with EE and RER, after 4 weeks there were no differences from baseline. At this lower

dose, heart rate and systolic blood pressure on day 1 were higher to a greater degree than in subjects from previous studies [20, 62, 63]. The increase in NEFA levels after chronic mirabegron treatment was decreased compared to day 1 and increased fasting plasma high density lipoprotein content after 4 weeks suggesting alterations to whole body lipid metabolism. In lower doses, mirabegron did not elicit any effects on BAT activation, however the higher 100–200 mg dose cause an increase in BAT activity. Similar results were shown with acute 50 and 200 mg mirabegron increased EE, however, only 200 mg increased BAT blood flow, oxidative metabolism, and ^{18}F-FDG uptake [18]. While β3ADR signalling is assumed to activate BAT, 50 mg dose showed no change in BAT activity. Similar to previous observations, higher doses of mirabegron increased heart rate and systolic blood pressure, which indicate nonselective βADR agonism by mirabegron and alternative βADR subtype activation of BAT [18]. Contrasting results have emerged regarding the abundance of βADR subtype in human BAT. β3ADR is reported as being the most abundant followed by with β2ADRs *in vivo* [65, 66]. Conversely, others report almost non-existent β3ADR mRNA expression *in vitro* in human BAT [67]. Differentiated human BAT adipocytes *in vitro* treated with formoterol, a highly selective β2ADR agonist, showed a similar increase in oxygen consumption rate (OCR) as norepinephrine, and higher than mirabegron [18]. Additionally, this effect was inhibited when formoterol was used in conjunction with a β2ADR antagonist. *In vivo*, formoterol has been shown to increase resting EE, and decrease RER with no change in heart rate or blood pressure in lean men, however no measures of BAT activity were performed [68]. β2ADRs have been shown to increase EE and rates of lipolysis in non-human models [69], however, if human BAT is indeed activated by β2ADR agonism, these alterations of EE and RER could potentially be mediated by BAT. However, these studies have yet to be explored.

Direct sympathomimetics effectively increase BAT EE but show limited efficacy in treating obesity by upregulating BAT thermogenesis. Mirabegron increases EE and BAT activity, but its cardio stimulatory effects could potentially be dangerous for certain populations. Notably, females and other target demographic of overweight and obese individuals who are already at a higher risk of hypertension and other cardiovascular complications.

Hormones

Evidence in rodents

Fibroblast growth factor 21

Fibroblast growth factor 21 (FGF21) is a hormone produced by the liver, which is involved in both glucose and lipid homeostasis [70, 71]. FGF21 is secreted during the fasting state and upon binding to FGF receptors (FGFR) and ß-klotho (KLB) on target tissue, increases expression of Pgc-1α, a key component of energy metabolism [72]. In turn, this increases fatty oxidation, glucose production and transcription of Ucp1 in adipose tissue to improve thermogenic capacity [73]. To date, the effects of FGF21 as a browning agent of WAT and BAT have been largely seen in rodents and are less evident in humans. In adult male Siberian hamsters, FGF21 treatment of 3 mg/kg for 7 days increased glucose and lipid uptake in interscapular BAT (iBAT) and glucose uptake in subcutaneous WAT (sWAT) and visceral WAT (vWAT) compared to pair-fed controls [74]. Treated mice experienced greater weight loss independent of food intake, increased EE, reduced RER, and increased *Ucp1* mRNA expression in iBAT, sWAT and vWAT [75]. Weight loss and browning effects of FGF21 persist in obesity as DIO male mice treated daily with FGF21 (1 mg/kg) for 2 weeks experience a 20% reduction in body weight without affecting food intake, and upregulated gene expression of *Pgc-1α*, and *Ucp1* in WAT and BAT compared to controls [76]. Similarly, HFD fed mice treated with 30 mg/d for 5–7 days see increased body temperature and greater EE [76]. The effectiveness of FGF21 also appears to be dependent on the presence of FGFR and KLB on adipocytes [77]. Paradoxically, obesity is associated with an increase in serum FGF21 levels and attributed to a reduction in FGFR and KLB expression on adipocytes [78, 79]. Overexpression of KLB in adipose tissues shows protection against DIO with increased *Ucp1* expression in WAT and lower plasma FGF21 levels compared to WT counterparts [80]. Whereas removing KLB from iBAT, iWAT and eWAT reduced *Ucp1* mRNA and protein expression and cold tolerance compared to WT control mice [78]. Therefore, in mice, FGF21 administration and receptor expression in adipose tissue seems to regulate EE and BAT activity.

Evidence in humans

Similar to rodents, both male and females that suffer from obesity exhibit higher levels of serum FGF21 compared to lean counterparts [1, 23], suggesting FGF21 resistance with increased levels of adiposity [79]. This increase in circulating FGF21 levels is generally attributed to a reduction in KLB expression in vWAT and sWAT [81]. Polymorphisms of the KLB gene are associated with obesity [82], further demonstrating the vital role of this receptor in facilitating FGF21 induced-weight loss. Clinical trials on FGF21 analogues demonstrate weight loss in obese individuals however causal relations with thermogenesis were not investigated. In a cohort of obese and T2D subjects (n = 38) FGF21 analogue LY2405319 modestly reduced weight in the high dose group (20 mg for 28 days) [83], but no measurements of food intake and EE were taken. More recently, the analog LL580 was found to have no effect on body weight in 64 obese participants across 12 weeks of treatment [84], but again no measures of thermogenesis were taken. Furthermore, safety profile of this analogue requires comprehensive evaluation as subjects in the trial developed serious adverse effects including lymphoma and respiratory failure [84]. More studies are needed

to assess the pharmacological efficacy of FGF21 administration in humans.

Irisin

Evidence in rodents

Irisin is a hormone secreted by skeletal muscle and adipose tissue [85]. Irisin is generated from the cleavage of FNDC5, a protein produced in response to transcriptional co-activator Pgc-1a [86]. Irisin released from the skeletal muscle into the bloodstream upon exercise stimulates WAT browning by increasing the expression of *Ucp1* encoding genes [87]. This process of irisin promoting Ucp1 expression happens downstream upon its binding to the adipocyte surface and is facilitated through the extracellular signal related kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) signalling pathways [88]. Previous research has identified how irisin can promote browning of white adipocytes in obese models however recent reports have critically examined the translation of research in rodents and potential of irisin in WAT in humans [89]. HFD fed mice given a daily dose of irisin (0.5 μg/g) for 2 weeks reduced body weight and significantly increased expression of browning genes including *Ucp1*, *Pgc-1α*, positive regulatory domain containing 16 (*Prdm16*), and transmembrane protein 26, which is a marker of beige cells in WAT [88]. However, no functional assessments were conducted to measure any impact on thermogenic capacity besides UCP1 protein content [88]. A significant limitation of recent research is a dearth of established reference values for endogenous irisin content in rodents or humans, which exhibit variable ranges spanning from 0.3 ng/mL as measured using mass spectrometry to 50–900 ng/mL measured using ELISA [89]. Although these findings support the proposition that irisin improves thermogenic capacity and promotes browning of WAT, methodological disparities in establishing irisin content severely limit the interpretation.

Evidence in humans

Zhang et al demonstrated that in mature human derived adipocytes, irisin treatment induces thermogenesis by upregulating *UCP1* expression in sWAT by upstream activation of the ERK and p38 MAPK pathways, without changing UCP1 or PRDM16 protein content in BAT derived adipocytes [90]. Due to inconsistencies in reporting endogenous irisin levels in humans as well (reviewed in 73), irisin function is indefinite. To note, these studies also do not report changes in *UCP1* expression in WAT of humans following exercise, which is known to stimulate irisin release [91]. Obesity is associated with a significant reduction in gene expression of *FNDC5*, irisin precursor, in muscle and both sWAT and vWAT [92]. However, no differences in muscle and WAT irisin levels have been found between obese and lean participants [93]. Furthermore, meta-analysis has failed to detect substantial evidence supporting a direct relation between irisin levels and disease development due to highly variable circulating irisin levels and absence of validated antibodies (reviewed in [89]). This coupled with limited subject numbers and methodological limitations, renders it uncertain whether irisin can adequately induce BAT activity or browning of WAT in obese humans.

Thyroid hormones (TH)

Evidence in rodents

Thyroid hormones (TH) are prominent modulators of metabolism in the body [94]. TH refers to the hormones thyroxine (T_4) and its active form triiodothyronine (T_3), produced and released by the thyroid gland. Exogenous intracerebroventricular delivery of T_3 increases SNS activity and expression of *Ucp1* mRNA in BAT of rats [95]. In both *ob/ob* and DIO mice, pharmacological activation using GC-1 (synthetic form of T_3) increased expression of thermogenic genes including *Ucp1, Prdm16, Cidea* in sWAT, but repressed the same genes in BAT [96]. Interestingly, levothyroxine (synthetic T_4) successfully promoted glucose uptake of BAT in DIO mice following cold exposure, showing conflicting impacts of TH on BAT [97]. HFD fed mice without functional thyroid receptors (TR) in the hypothalamic neurons ($TR^{hypo-/-}$) maintain thermogenic activity following cold exposure (4°C) but have reduced sympathetic activity in BAT and are more susceptible to DIO, highlighting how neuronal TH interactions regulate BAT function [98]. As TH mimetics continue to evolve, there is a growing interest in developing treatments that selectively targets the liver and adipose tissues to decrease ectopic lipid accumulation without adversely impacting the cardiovascular and bone mineralization systems. Some attempts have been made to pair the TH treatment with other peptides such as glucagon that would target BAT while simultaneously promoting liver action to offset hepatic steatosis, hyperglycemia and hyperlipidemia in DIO male mice [99]. The combo of glucagon/T3 produced no change in BAT gene expression profiles but triggered UCP1 gene and protein upregulation in iWAT, however, efficacy of combination was lower than T3 alone. Moreover, glucagon/T3 treatment in $Ucp1^{-/-}$ mice partially reduced changes in EE and RER, suggesting that other pathways besides UCP1 in the adipose tissues remain at play. Furthermore, increased bone turnover, cardiac volume, reduced fractional shortening and ejection fraction, suggest cardiovascular and bone thyrotoxicity of T3 treatment alone, which were mitigated in T3/glucagon combination [99]. Despite these beneficial effects in the mouse model, intensive investigation in humans is warranted as the glucagon receptor is less expressed in adult adipose tissue and the pharmacological amount required to induce enough

"beiging" of WAT to upregulate EE remains unknown (reviewed by [100]).

Evidence in humans

WAT from obese participants, serum T_4 levels positively correlated with mRNA levels of *UCP1, CIDEA* and *PRDM16* in both sWAT and vWAT from individuals with obesity [101]. Although research in obese populations is limited, studies conducted in hyperthyroid populations demonstrate that increased TH levels stimulate higher BAT activity, EE and fatty acid oxidation, as evidenced by lower RER levels [102]. Additionally, in euthyroid males, T_3 levels were inversely associated with BMI and positively associated with greater pericardial fat volume, implying a link between TH and lower BMI as well as greater thermogenic activity [103]. However, the potential adverse effects of TH therapy continue to be a concern. Achieving the desired effects on the liver and adipose tissue without any detrimental consequences on cardiovascular and bone health need further examinations.

Orexin

Evidence in rodents

Orexins (OX) are a group of neuropeptides, including Orexin-A and Orexin-B, generated in the hypothalamus. They were first discovered in 1998 in the lateral hypothalamic regions of the brain, which are associated with the regulation of feeding [104]. OX regulates sleep-wake cycle, arousal and is most importantly involved in the food-reward system by increasing motivation for palatable foods. In addition to these functions, OX is also required for brown adipocyte development. OX-null mice fed a HFD experience rapid weight gain, and OX-null neonates have reduced lipid accumulation and mitochondrial content [105]. These effects are prevented in offspring of OX-null mice given 3 injections of 30 mg/kg of Orexin-A [105]. *In vitro*, OX is necessary for brown adipocyte differentiation by stimulating adipogenesis via p38 MAPK [105]. OX is also involved in the thermogenic effect of bone morphogenetic protein 8B (BMP8B), a batokine released by mature brown adipocytes. BMP8B facilitates thermogenesis in part by inhibiting AMPK in the ventromedial nucleus of the hypothalamus, which increases OX expression in the lateral hypothalamus area [106]. As such, in OX-null mice, BMP8B treatment does not produce any thermogenic effects and in rats via inhibition of VGLUT2, glutamate transporters highly expressed in OX neurons, BMP8B treatment blunts thermogenic effects and decreases expression of UCP1 protein [106]. Overall, this suggests greater regulation of BAT activity through a hypothalamic network, where the influence of OX on BAT is dependent on AMPK activity.

Apart from OX, its receptor, orexin-receptor 1 (OXR1) is also integral to BAT function as an ablation of the receptor in mice leads to reductions in lipid stores in iBAT [105]. In HFD fed mice, inactivation of OXR1 in serotonergic neurons impairs energy homeostasis by reducing glucose uptake, *Ucp1* gene expression, mitochondria function and insulin sensitivity in BAT [107]. Therefore, OXR1 expression in the brain appears to play a protective role in maintaining peripheral glucose metabolism and BAT activity.

Evidence in humans

In humans, plasma levels of OX are inversely related to BMI, and significantly lower in obese and morbidly obese individuals compared to normal or overweight counterparts [108]. Moreover, higher serum levels of OX correlate to improved insulin sensitivity and lipid profile [109]. Expression of HRCTR1, the gene encoding OXR1, is found in human adipose tissue. Moreover, the gene is primarily expressed in vWAT rather than scWAT of non-obese males [109]. Investigating the role of OX on BAT activity in the neck and abdominal regions, Pino et al concluded that 100 nM of OX had no impact on BAT gene expression in differentiated cells, despite observing an inverse relationship between BMI and *OXR1* mRNA expression [110]. Additionally, treatment of 100 nM of OX had no impact on *UCP1*, *PPARGC1a*, or *OXR1* mRNA expression [110]. Hence, while studies suggest endogenous OX is greater in normal weight individuals, treatment using OX may not induce beneficial changes to BAT development that would increase thermogenic capacity.

Dietary

Fish oils

Evidence in rodents

Fish oils contain high levels of n-3 polyunsaturated fats (PUFAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that the body is unable to synthesize on its own. Fish oils are a well-documented activator of thermogenic activity in adipose tissue and n-3 PUFAs promote anti-inflammation and SNS activity [6]. The addition of EPA (36 g/kg) to HFD (45%) feeding in male C57BL/6J mice for 11 weeks increased UCP1 protein in BAT and other browning marker genes including *Prdm16*, *Pgc-1a* and *Fgf21*, whereas DHA (10 or 50 mg/kg) in HFD (60% fat) feeding over 8 weeks in male mice increased UCP1 content and anti-inflammatory macrophages in eWAT [111, 112]. In aging DIO mice, administration of DHA and EPA (683.4 mg/g, 46.7 mg EPA/g) restored UCP1 content, and increased presence of beneficial pro-resolving lipid mediators that are suggested to reduce age-related inflammation in BAT [113]. In obese mouse models, DHA increases Akt phosphorylation, downstream of the insulin receptor in eWAT and sWAT, demonstrating a positive effect on insulin signalling [112, 113]. Fish oil (DHA 25%, EPA 8%) may target the SNS by

activating β3ADR situated on the BAT in lean mice [114], but the effect of DHA and EPA to directly act on BAT in obese rodent models is unknown. The role of lipid mediators, derived from PUFAs present a new class of thermogenic regulators such as DHA derived lipid mediator, maresin 1 (MaR1) [115]. MaR1 promotes glucose uptake, enhances fatty acid oxidation, and upregulates anti-inflammatory and thermogenic gene expression in rodent brown adipocytes. Additionally, it induces M2 phenotype in macrophages and contributes to beige adipocyte remodelling in WAT of DIO mice [115]. In addition, the lipid mediator prostaglandin E_2 (PGE2), which is synthesized from arachidonic acid, n-6 PUFA, also induces beigeing phenotype in WAT [116]. These noteworthy effects showcase how fish oils through their derived lipid mediators may attenuate inflammation and adipocyte dysregulation in both WAT and BAT.

Evidence in humans

REDUCE-IT trial with icosapent ethyl, a highly purified EPA, reported improvement in hypertriglyceridemia and cardiovascular risk reduction [117]. In contrast, other studies showed no improvement in cardiovascular health with n-3 carboxylic acid [118], or a mixture of EPA and DHA [119] compared to corn oil placebo. The addition of 200 μM EPA to subcutaneous adipocytes derived from overweight female subjects resulted in an increase in *UCP1* and *PRDM16* and *CPT1* expression, indicating that EPA may promote fatty acid oxidation and thermogenesis in WAT [120]. These findings have been corroborated in sWAT adipocytes of lean females, suggesting browning by EPA may be independent of body mass [121]. Clinical studies in humans have not identified any direct relationships between EPA, DHA and BAT activity, however, consumption of DHA and EPA over 12 weeks improved insulin resistance in individuals with obesity by reducing fasting insulin levels independent of weight loss [122]. While fish oil can aid in reducing excess fat storage and increasing thermogenic capacity in human adipocytes *in vitro*, the lack of comprehensive *in vivo* studies limits translatability.

Bioactives

Evidence in rodents

There are a variety of bioactives that may induce browning of WAT or increase thermogenic capacity of BAT in obese rodent models. Quercetin, or onion peel extract (OPE) (50–150 μg/mL) induces browning in white adipocytes and increases mRNA expression of *Ucp1* dose-dependently [123]. The addition of OPEs to HFD fed mice increases fatty acid uptake and expression of thermogenic marker genes in WAT, without changes in body weight [123, 124]. Ginger activates Sirtuin 1 and PGC-1α signalling to induce thermogenic gene expression in both BAT and WAT of HFD mice, consequently reducing body weight and fat accumulation [125, 126]. Ginger capsules (500 mg/kg/d) have been reported to restore citric acid cycle metabolites altered by HFD (60% calories from fat) [126]. Similarly, allicin (garlic extract) induces browning of iWAT in HFD fed mouse models by increasing *Pgc-1α, Prdm16* and *Ucp1* expression [127]. Allicin regulates thermogenic gene expression in white adipocytes by increasing krupper-like factor 15, a transcription factor that regulates *Ucp1* expression via the ERK MAPK signalling pathway [127]. Curcumin and capsaicin are bioactives that have more substantial findings that appear to activate BAT in rodent models. A derivative of turmeric, curcumin (1%) reduces WAT inflammation, increases BAT mRNA expression of *Ucp1,* and improves thermogenic response following cold exposure in HFD (60% fat) fed male mice [128]. Similarly, pure capsaicin (0.01%) increases EE by acting on TRPV1 channels to increase *Ppar-α, Prdm16,* and *Pgc-1α* gene expression to facilitate browning of WAT affording protection against DIO in mice [129]. While these bioactives promote browning, their usage is restricted due to limited bioavailability and challenges in dosing [130–132].

Evidence in humans

In female participants with high adiposity, consumption of dried ginger extract (600 mg/d) for 3 months has no impact on EE [133]. Similarly, single consumption of a dried ginger powder capsule in males (1 g), failed to change body temperature [134]. Moreover, capsaicin administration did not elicit change in BAT EE or body weight in both lean and overweight subjects independent of or in conjunction with cold-exposure [135, 136]. Together, there is no strong evidence to suggest that these bioactives have any impact on enhancing BAT activity which may translate into body weight loss in humans.

Environmental

Cold temperature

Evidence in rodents

Cold exposure activates the SNS releasing norepinephrine that binds to β3ADRs, and subsequently activating downstream protein kinase A (PKA) leading to increased Ucp1 activity [3, 137]. HFD feeding impairs this mechanism by altering vagal nerve transmission and decreasing SNS activity [138]. Cold exposure can additionally sensitize transient receptor potential melastatin 8 (TRPM8) channels located on adipocytes to activate PKA signalling [137]. By activating TRPM8 with agonists such as menthol, HFD fed mice are protected against obesity and glucose intolerance [139]. Moreover, deletion of TRPM8 induces obesity and reduces fatty acid oxidation in mice housed in mild cold temperatures [140]. The activation of BAT in response to cold exposure can be attributed to various pathways and present different therapeutic strategy to sustain thermogenic activity.

Overview of Activators

Activators		Rodents	Humans
Pharmacological	Berberine	↑ BAT volume, activity, whole body EE, *Ucp1* in BAT	
	WSE	↑ Whole body EE, thermogenic mRNA	
	βADR Agonists	↑ BAT EE and mitochondria GDP binding	↑ ^{18}F-FDG uptake in BAT, whole body EE, NEFA, UCP1 in WAT
	Metabokines	↑ Thermogenic mRNA, whole body EE, ↓ body weight	
	Ephedrine		↑ Whole body EE, NEFA, plasma glucose, lactate, insulin, ↓ RER
	Resveratrol	↑ WAT browning and gut microbiota	
	PPARγ Agonists	↑ BAT adipocyte differentiation, lipid storage	↑ Lipid storage
	AT2R	↑ BAT Mass, adipocyte differentiation	
Hormones	FGF21	↑ Glucose uptake, whole body EE, ↓ body weight, RER	
	Irisin	↑ *Ucp1* in WAT, disputed	-
	Thyroid Hormone	↑ *Ucp1* and glucose uptake in BAT	Inversely associated with BMI
	Orexin	↑ Brown adipocyte differentiation, lipid storage	
Diet	Fish Oil	↑ UCP1and thermogenic mRNA in BAT and WAT, insulin signalling in DIO	↑ Thermogenic protein *in vitro* in human adipocytes
	OPE	↑ *Ucp1* in WAT in HFD fed mice	
	Allicin	↑ Thermogenic mRNA in WAT in HFD fed mice	
	Ginger	↑ Thermogenic mRNA in WAT, BAT in HFD fed mice	
	Capsaicin	↑ *Ucp1* in BAT and CIT	No change in EE or bodyweight in lean or overweight subjects
	Curcumin	↑ Thermogenic mRNA in WAT in DIO	
Environmental	Cold Exposure	Activate SNS to induce thermogenesis	↑ CIT in lean subjects

FIGURE 1
An overview of molecules that activate brown adipose tissue (BAT) or browning of white adipose tissue (WAT) and evidence to support in biological model of rodents or humans. *Note: the evidence summarized in each section represents data provided from both *in vitro* and *in vivo* models in rodents and humans. Abbreviations: Angiotensin type 2 receptor (AT2R), Beta-adrenergic receptor (βADR), Cold-induced thermogenesis (CIT), Diet induced obesity (DIO), Energy expenditure (EE), Fibroblast growth factor 21 (FGF21), Fludeoxyglucose (^{18}F-FDG), Guanosine diphosphate (GDP), High fat diet (HFD), Non-esterified fatty acid (NEFA), Onion peel extract (OPE), Peroxisome proliferator-activated receptor γ (PPARγ), Respiratory exchange ratio (RER), Sympathetic nervous system (SNS), *Withania somnifera* extract (WSE), Uncoupling Protein 1 (Ucp1). Image created in Biorender.

Evidence in humans

In a retroactive prospective study by Becher et al, across 52,000 patients imaged between 2009 and 2018 using ^{18}F-FDG PET/CT only 9.7% had detectable BAT [9]. Similarly, across 11,000 patients imaged in another study, only 8% had detectable BAT [141]. Controlling for an effect of cold exposure can only apply to a small cohort of individuals who have BAT, and so far, no RCTs have evaluated any effective changes to BAT activity which could lead to weight loss. Cold induced thermogenesis (CIT) is compromised in morbidly obese and obese subjects

compared to lean counterparts, suggesting that BAT activity is highly dependent on body fat ratio and overall weight [142–145]. Moreover, to measure the impact of casual cold-exposure in humans, young healthy males with a history of winter swimming in temperatures between 1°C and 9°C were assessed for CIT responses following intermittent thermal and cooling sessions compared to controls [146]. Winter swimmers have a greater response to CIT and overall lower core body temperature [146]. Control subjects had greater BAT glucose uptake at thermoneutrality suggesting that BAT is active to maintain core body temperature in adults [146]. Acute (1h) cold exposure for 7 days can reduce skeletal muscle shivering response and upregulate non-shivering thermogenesis in healthy adult males (aged 20–29 years old), however, this effect was not due to changes in whole body fuel selection [147]. These data demonstrate that routine cold-exposure influences thermogenesis and may therefore be exploited to rescue BAT activity in obese individuals. We have summarized BAT activators in Figure 1.

Inactivators of brown adipose tissue

Hormones

Glucocorticoids

Evidence in rodents

Glucocorticoids (GCs) are stress induced steroid hormones produced by the adrenal glands that cause rise in blood glucose levels and adipogenesis, ultimately aiming to maintain sufficient glucose supply to the brain [148]. GCs have been described as inhibitors of BAT and browning activity due to their nature to induce lipid accumulation at high concentrations [7]. In male Wistar rats, corticosterone (active GC in rodents) treatment promotes BAT remodeling towards a WAT phenotype (known as "whitening") and reduces *Ucp1* and *Prdm16* mRNA expression [149]. In HFD fed mice, GCs negatively regulate metabolism by inducing lipolysis and insulin resistance, and lead to greater adiposity by lowering EE and *Ucp1* expression in BAT [150, 150]. Antagonism of GC's increases PGC-1α content and sustains weight gain in DIO female mice indicating that blocking the effects of GCs may help to prevent unnecessary lipid spillover from the adipose tissues to peripheral tissues such as the liver [151]. GCs also enable whitening by regulating microRNAs (miRNA), which are small non-coding RNA segments that can change gene expression post-transcriptionally. miR-27b is highly expressed in WAT depots of HFD fed mice [152], and is potentiated by GC exposure to induce adipocyte whitening. Inhibiting miR-27b promotes browning by increasing UCP1 and PRDM16 content in WAT [153, 154]. β-adrenergic stimulation of BAT might effectively counteract the obesity-related effects of GCs and maintain thermogenic activity. In mice subjected to 4 weeks of cold exposure (13°C) and treated with corticosterone (50 μg/ml), BAT mass is preserved accompanied with an enhanced UCP1 protein expression compared to mice housed at room temperatures (22°C) [155]. The exact mechanisms behind how GCs induce whitening in BAT are not fully elucidated. However, Luijten et al demonstrated that GC-induced change in BAT lipid composition is not dependent on the Ucp1 signaling pathway [156].

Evidence in humans

In humans, excessive GC levels lead to adipose tissue expansion, impaired appetite and increased risk of diabetes [157]. Around 80% of GC users exhibit weight gain [158]. Patients with chronically excessive levels of GC, known as Cushing's syndrome (CS), are at greater risk for dyslipidemia, diabetes and obesity due to the impact of GC on lipid and glucose metabolism. Overweight subjects with CS have a negative correlation between cortisol and UCP1 protein compared to controls suggesting that excess GC reduces BAT function [159]. GCs in BAT of obese populations is less explored, however studies have demonstrated differences in acute and chronic GC treatment effects [160–162]. In healthy males, acute GC increases glucose uptake and EE of BAT under cold exposure. However, upon retrospective assessment of chronic GC treatment lasting 2 weeks, a reduction in BAT mass is observed, which correlates with decreased BAT activity [160]. Dosage of GC may also influence the effect on BAT as evident from reduced CIT following mild cold exposure (19°C) in healthy adults treated with a low dose of prednisone (15 mg/d) for 1 week. However, no such changes are observed with high dose prednisone (40 mg/d) for 1-week after cold-exposure (10°C) despite having greater EE [161, 163]. Increase in EE in high-dose prednisone treated males may be attributed to skeletal muscle calcium cycling as prednisone can stimulate calcium cycling genes [161]. While evidence regarding the influence of acute GC treatment in BAT function is diverse in humans, prolonged exposure to GC reduces thermogenic capacity and impairs BAT function. This may be due to differences in dosing and unstandardized treatment regimens.

Aldosterone

Evidence in rodents

Aldosterone is a mineralocorticoid hormone produced by the adrenal glands. Concerning BAT activity, aldosterone (100 nM) stimulation of the mineralocorticoid receptor (MR) downregulates *Ucp1* expression in brown adipocytes. This effect occurs through the inhibition of retinoid X receptor (RXR) and retinoic acid receptor (RAR) transcription factors located along the *Ucp1* gene [164]. Administration of mineralocorticoid receptor antagonists (MRA) including finerenone, have been successful in preserving thermogenic

markers by increasing UCP1, AMPK, and ATGL content in brown pre-adipocytes, indicating a greater fat mobilization and promotion of thermogenic pathways [165]. Similarly, in healthy and HFD fed mice, MRAs increase both iBAT density and thermogenic browning gene expression of *Ucp1*, *Prdm16*, *Pgc1*-α and *Cidea* [165–167]. In contrast, aldosterone deficiency in HFD fed mice neither prevent obesity nor alter insulin efficiency. However, it moderately alleviates white adipocyte dysfunction, as indicated by increased plasma adiponectin levels and reduced macrophage infiltration [168]. Collectively, these findings indicate that blocking MR activity may improve BAT thermogenic capacity, but no studies measure the direct impact of MRA treatment on the ability to withstand colder environments.

Evidence in humans

According to a study by Rossi et al, comprising of male (n = 56) and female (n = 44) obese/overweight patients with hypertension, there is a positive association between BMI and plasma aldosterone concentrations [169]. MR expression in sWAT and vWAT was greater in obese populations suggesting an increased risk for adipocyte inflammation [170], however this study only provided analysis in a sample size of 7, without direct assessment of adipocyte dysfunction or inflammation. In lean populations, MRA increased thermogenic activity indicated by ^{18}F-FDG/PET-CT imaging and BAT mass in supraclavicular regions compared to placebo control [171]. However, MRA treatment was only for 2 weeks and disproportionate sampling from males and females along with small sample size warrants further investigations [171].

Androgens

Evidence in rodents

Androgens, such as testosterone and dihydrotestosterone, are steroid hormones that play a role in the development of male reproductive system and characteristics. Several studies demonstrate an association between androgen activity and reduced thermogenic capacity. In healthy castrated male mice, WAT thermogenic capacity is high and sensitive to cold-induced browning [172], however, increasing concentrations of testosterone (10^{-9}–10^{-7} M) reduced *Ucp1* expression in differentiated adipocytes obtained from cervical, interscapular and auxiliary BAT in mice [173]. Androgen receptor knockout male mice fed either regular chow or HFD have reduced *Ucp1* gene expression and greater weight gain with vWAT accumulation, suggesting that androgen receptor signalling contributes to fatty acid uptake in adipose tissues [174, 175]. These studies provide conflicting literature on androgens and their receptor signaling, and therefore, more studies are needed to determine if androgen receptor agonism or antagonism at the level of the adipose tissue is needed to improve BAT function.

Evidence in humans

A longitudinal analysis by Gapstur et al showed reductions in testosterone levels with age and higher greater BMI and waist circumferences [176]. Studies in women with polycystic ovarian syndrome have reported conflicting results on a correlation between obesity and testosterone. Additionally, no association between testosterone and supraclavicular skin temperatures were noted [177–179]. Recent studies have found that CIT is greater in premenopausal compared to post-menopausal women, which could imply that estrogen is linked to improved thermogenic activity [180, 181]. Stronger evidence is required to determine the independent role of androgen signaling in human populations as results are inconclusive so far. BAT inactivators in rodents and humans are summarized in Figure 2.

Environmental inhibitors

Environmental pollutants

Evidence in rodents

In recent years, environmental pollutants such as pesticides, and air pollution have become associated with negative impacts on human health and have contributed to the development of obesity [182]. Pesticides such as deltamethrin pose harmful effects on brown adipocytes by reducing expression of *Ucp1* and cAMP activity. Interestingly, however, in male mice, deltamethrin had no impact on body composition and, at a low dose of 0.01 mg/kg/day, actually improved insulin sensitivity and energy expenditure [183]. Recently, 34 chemicals were identified due to their abundance in food product and packaging concluded that chlorpyrifos (CPF), a organophosphate pesticide commonly found in fruits, vegetables, grains, and meats was linked to reductions in *Ucp1* mRNA expression and mitochondrial respiration in immortalized brown adipocytes [184]. Concentrations of CPF as small as 1 pM reduced mitochondrial membrane potential, activity of complex IV in the electron transport chain, and RNA transcripts of genes involved in fatty acid oxidation [184]. In HFD fed mice, CPF increased weight gain and adiposity of WAT, reduced energy expenditure and inhibited diet-induced thermogenesis [184]. CPF also reduced cAMP activity in brown adipocytes, suggesting that CPF may also impair sympathetic activation of BAT thermogenesis [184]. Pesticides such as dichlorodiphenyltrichoroethane (DDT) have been previously associated with metabolic disruption and when exposed perinatally to female mice, lead to reduced energy expenditure, glucose intolerance and increased adiposity [185]. Additionally, when placed on a HFD, offspring develop insulin resistance and reduced *Ppargc1a* expression in BAT thus impairing thermogenesis [185]. Various other pesticides including permethrin and bifenthrin promote weight gain, insulin resistance, inflammation and lipid accumulation in

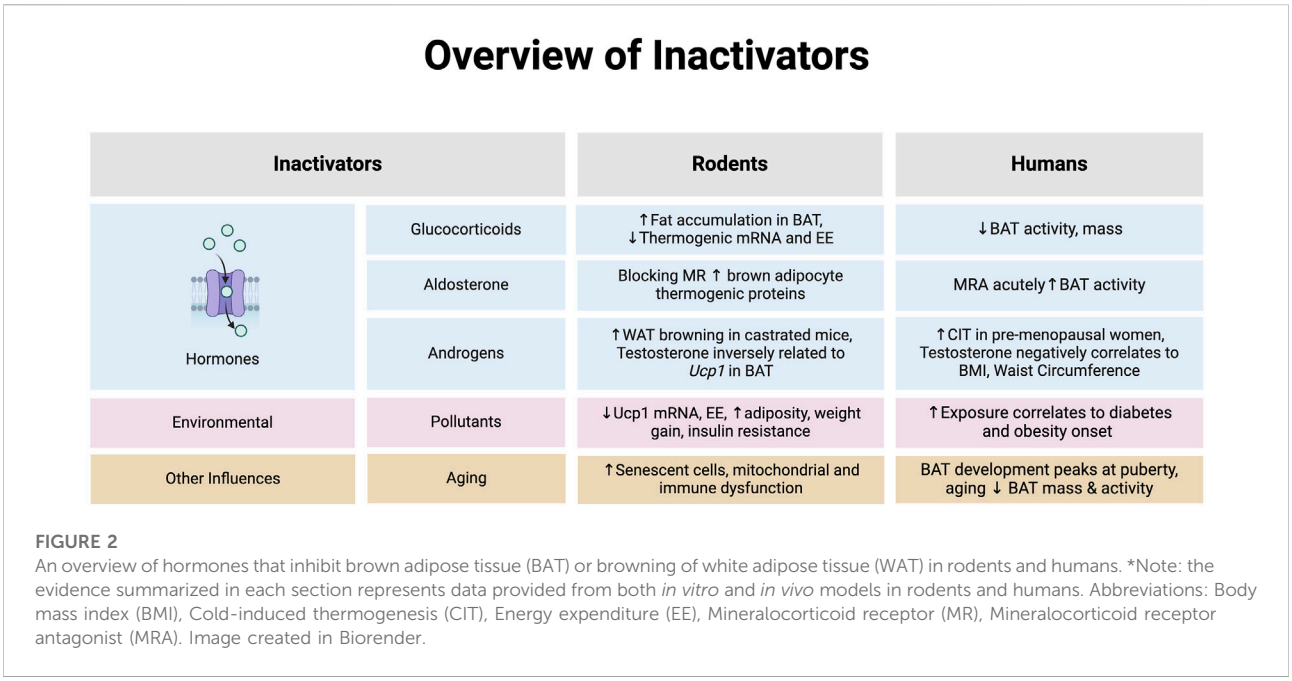

FIGURE 2
An overview of hormones that inhibit brown adipose tissue (BAT) or browning of white adipose tissue (WAT) in rodents and humans. *Note: the evidence summarized in each section represents data provided from both *in vitro* and *in vivo* models in rodents and humans. Abbreviations: Body mass index (BMI), Cold-induced thermogenesis (CIT), Energy expenditure (EE), Mineralocorticoid receptor (MR), Mineralocorticoid receptor antagonist (MRA). Image created in Biorender.

adipose tissue, however direct effects on BAT thermogenesis have yet to be determined [186]. Air pollutants also induce changes to BAT in male mice by increasing reactive oxygen species, reducing expression of UCP1 protein expression, and BAT mitochondrial area and count [187].

Evidence in humans

To date, studies have not investigated the impact of pesticides in human BAT; however, many studies have documented the association between pesticides and obesity. Research on farmers has confirmed greater incidences of diabetes and insulin resistance linked to routine exposure to organophosphate pesticides, including CPF [188–190]. Similarly, the use of pesticides has also been correlated with a greater risk of obesity among farmers in Thailand [191]. However, causality of these links has yet to be established.

Other influencers of BAT

Aging

Effects of aging on BAT is largely attributed to changes in immune and senescent cell activity as well as mitochondrial dysfunction. With aging, the immune system becomes more unregulated, and there is an increase in pro-inflammatory M1 macrophage activation within adipose tissue [192]. Additionally, there is an increased presence of senescent immune cells in BAT which damage adipose tissue by secreting inflammatory cytokines. Senescent immune cells reduce expression of thermogenic markers such as *Ucp1* and *Ppargc1a*, and also downregulate gene expression of adipose RNA binding motif 3 (RBM3) which prevents sympathetic innervation of the BAT [193]. Senescent T cells also promote BAT whitening in aging mice by secreting IFN- γ, which inhibits brown adipocyte differentiation [194]. Mitochondrial dysfunction increases with age and leads to a greater oxidative stress. Cui et al found that 20-month-old mice have less expression of BAT thermogenic genes including *Ucp1* and *Ppargc1a* in contrast to 6-week-old mice [195]. These changes in BAT were accompanied by a decrease in antioxidants, indicating higher age-related oxidative stress. Inducing oxidative stress using hydrogen peroxide in brown adipocytes also reduced the quantity and morphology of mitochondria; however, these effects were rescued by antioxidant treatments [195]. In humans, similar declines in BAT mass can be observed due to age, with some studies observing that the decline is more prevalent in males than females [9, 196]. Intriguingly, a puberty-related rise in BAT mass has been described in children over the age of 10, suggesting that BAT development peaks with sexual maturity and musculoskeletal development, coinciding with an increase in growth and sex-related hormones [197–199]. However, despite this rise, a decline in BAT activity persists into adulthood, likely attributed to greater body fat accumulation. The mechanisms underlying the regulation at the level of brown adipocytes remain unclear [200].

Biological sex and sex hormones

Sex based differences in BAT morphology and activity deserve attention. Although the majority of studies have

included males, there are notable differences in BAT function between males and females [201]. Female rats have been shown to have greater BAT mass, mitochondrial content, and *Ucp1* expression compared to male rats [202, 203]. PET/CT scanning has shown that females may have lower BAT volume but similar activity compared to male [204]. Conversely, other studies have shown that females have greater detectable BAT than males [205, 206]. Sex based differences in BAT presence are age-related, with higher BAT activity in females at younger ages. However, this difference becomes obsolete in post-menopausal women, indicating that changes in sex hormone levels may contribute to BAT function [201]. Recently, Blondin et al, [181] has demonstrated that BAT oxidative metabolism and glucose uptake is greater in premenopausal women in comparison to postmenopausal women but the change in BAT tissue radiodensity, which is an indirect lipid content in BAT was not altered between groups of women. Transcription of the batokine BMP8B, which aids in modulating BAT for thermogenesis, is promoted in female mice with estradiol 2 (E2, the main circulating form of estrogen) treatment, whereas ovariectomy drastically decreases BMP8B expression in female mice [207], and decreases thermogenic activity and *Ucp1* expression [208]. The mechanisms of estrogen induced increases in thermogenic activity may be through the activation of the estrogen receptor α leading to subsequent norepinephrine stimulated lipolysis. In contrast, testosterone levels lowers the rate of lipolysis [209]. Estrogen can also influence BAT activity via sympathetic nervous system stimulation to increase UCP1 protein expression and thermogenic activity [210]. The *in vivo* effects of androgens on BAT activity are less clear. Removal of testes, the primary location of testosterone production, increases UCP1 protein expression in BAT implying a blunting effect of testosterone on BAT thermogenic ability [211]. However, it is difficult to determine if changes in BAT function are directly mediated by testosterone signalling or by the conversion of testosterone to E2 in the tissue that may be modulating BAT activity [201]. It is important to consider the complexities and importance of sex-based genetic and hormonal influences on BAT function when investigating potential BAT activators to combat obesity, to meet the needs of all individuals who could benefit from these therapies. A comprehensive discussion of the effects of biological sex on BAT function, morphology, and growth has been discussed elsewhere [201, 212, 213].

Exercise

Evidence that exercise influences BAT activity is more prevalent in rodents than in humans. In obese male mice, 4 weeks of daily aerobic exercise increased BAT mass while also upregulating genes involved in glucose and lipid metabolism, however only serum glucose levels were reduced following training, whereas serum lipid and cholesterol were unchanged [214].

Exercise can also stimulate browning of WAT. Male Swiss rats that underwent 8 weeks of aerobic or resistance training [215], had significantly higher Ucp1 protein, *Ppargc1a,* and *Cidea* gene expression in both inguinal and retroperitoneal WAT [216]. Moreover, swim training in Sprague-Dawley rats fed a HFD demonstrate reductions in weight but does not alter the thermogenic profile in BAT. Instead, it promotes myogenic protein markers, suggesting that BAT may adopt a muscle-like oxidative function during exercise rather than a thermogenic function [216]. Conflicting evidence on the influence of exercise on BAT has emerged, as studies in humans fail to replicate findings seen in rodents. Recent research in sedentary humans demonstrates that exercise has no impact on BAT activation [217]. In the ACTIBATE trial, no changes in BAT volume or F-FDG uptake were identified across control, moderate-exercise, and vigorous-exercise groups following 24 weeks of combined endurance and resistance exercises [217]. Moreover, various studies in both male and female endurance athletes report lower BAT activity and volume compared to non-athletes counterparts, challenging the notion that BAT and exercise are complementary [218, 219].

Exercise also stimulates the release of hormones or activating factors, referred to as exerkines, that can influence BAT activity. Of note is AMPK, a key regulator of skeletal muscle metabolism. In relation to BAT, AMPK activation increases *Ucp1* expression in differentiated brown adipocytes derived from mice undergoing 4 weeks of voluntary aerobic training [220]. Exercise also improves efficiency of exerkines such as FGF-21 in obese mice by increasing the expression of KLB receptors in BAT [221]. However, not all exerkines affect BAT as seen with irisin. After acute swimming interventions, male mice show increased FDNC5 but no changes in *Ppargc1a*, *Ucp1* gene expression, or irisin protein levels in BAT [222]. In humans, the effects of exerkines are more varied. A recent study assessing 16 exerkines, including FGF-21, lactate, and irisin found that endurance activities increased FGF-21, reduced lactate, but failed to detect irisin or measure any changes [223]. This study, which had limitations due to its small sample size and inclusion of only female participants, concluded that exercise altered the circulation of exerkines other than irisin [223]. However, only changes in lactate levels were associated with changes in BAT volume [223]. Overall, exercise and BAT activity shows that this relationship is less reproducible in humans than in rodents. Although exercise and BAT thermogenesis have positive effects on energy expenditure and metabolism that result in weight loss, they work independently rather than in tandem. The impact of exercise likely outweighs any influence of BAT thermogenesis, as exercise demands a greater utilization of energy substrates and restricts blood flow to adipose tissue overall, whereas it stimulates or maintains muscle mass that makes up the majority of body weight as least in healthy humans.

Discussion

Is UCP1 indispensable in regulating BAT activity?

Ucp1 is a key protein involved in facilitating thermogenesis in BAT. However, evidence suggests that Ucp1 is only functional when it is activated. This is evident when mice with 50 times higher amount of Ucp1 are placed on a high fat/sucrose diet. Despite higher UCP1, animals experience weight gain at a similar rate to controls [224]. Only upon injection of β3ADR, mice with higher UCP1 display greater EE, indicating how activation of Ucp1 is key to producing any thermogenic effects. Similarly, *Ucp1*$^{-/-}$ and WT mice exhibit similar levels of EE and susceptibility to DIO at thermoneutrality. However, with noradrenaline treatment Ucp1 was activated in WT mice leading to greater EE [225]. This highlights that thermogenic activity in BAT does not occur ubiquitously and requires on-going activation of Ucp1. Therapeutic interventions must achieve sustained activation of Ucp1 for pharmacological efficacy. Current options capable of Ucp1 activation, such as βADR agonists, have inherent limitations as they increase heart rate and blood pressure [20].

On the other hand, Ucp1 is dispensable for thermogenesis evident from recent, studies that have identified non-shivering thermogenic processes that act independently of Ucp1 [10, 226], one of these being calcium cycling present in beige adipose tissue. Calcium (Ca^{2+}) is released and sequestrated by the sarco-endoplasmic reticulum (SERCA) through SERCA2b in beige adipocytes [10, 227]. This cycling process releases heat as a by-product and utilizes ATP to fuel SERCA2b uptake of Ca^{2+} into SERCA [10]. Ca^{2+} cycling is activated either by β-ADR stimulation [10] or cold-exposure [10, 228], and this process seems to be protective against DIO at thermoneutrality in both WT and *Ucp1*$^{-/-}$ mice [229].

Another UCP1-independent ATP-dependent mechanism of thermogenesis is creatine substrate futile cycling where phosphocreatine (PCr) is shuttled from the mitochondria towards the endoplasmic reticulum (ER) to be converted to ATP and creatine (Cr) by creatine kinase (CK) [11]. This cycle of ATP production through the PCr/CK cycle, which primarily takes place in beige adipocytes, provides fuel for calcium cycling, as SERCA2b present in the endoplasmic reticulum of cells can utilize ATP to take up Ca^{2+} by the support of cytosolic CK, leading to the dissipation of heat [11]. Kazek et al demonstrated that reductions in creatine induced by β-guanidinopropionic acid were linked to reductions in oxidative metabolism in both iWAT and BAT following β-3ADR agonist treatments [15]. Additionally, they found that among *Ucp1*$^{-/-}$ mice, those treated with β-GPA had reduced ability to maintain body temperature after cold exposure (4°C) compared to vehicle controls. The absence of creatine kinase B (Ckb), a key regulator of creatine cycling, in mice leads to decreases EE and increased risk of obesity [230]. This shows that in the absence of Ucp1, Cr cycling serves as a compensatory measure to maintain thermogenesis. This is corroborated by adipocyte-selective inactivation of Ckb that diminishes thermogenesis and predisposes to obesity [15]. Recently, demonstrated in mice with adipocyte-selective deletion of either Ucp1 or Ckb are euthermic which worsens cold intolerance making mice hypothermic. This suggests that thermogenic adipocytes use redundant, non-paralogous proteins to maintain body temperature [231]. Although further studies are required for comprehensive understanding, this suggests that while Ucp1 plays a significant role in thermogenesis, it is not indispensable.

The potential for BAT to mitigate obesity

It is important to consider the availability or "recruitability" of BAT and the amount it contributes to whole body EE when assessing its potential as a therapeutic strategy to manage obesity. We know that BAT is relatively less abundant in human adults, in both lean and obese populations compared to rodents [9]. Early studies in human BAT indicated that CIT (16°C) was significantly reduced in individuals that were obese compared to lean individuals [232]. More recently, when quantifying changes following CIT (16°C), lean males still seem to achieve greater BAT activity with a 17% increase in basal metabolic rate, whereas obese males only experienced an increase by 6% [144]. Among healthy males and females, following mild CIT (15.5°C), BAT only attributed to a modest 15–25 kcal/day increase in EE [233] and another study assessing EE following cold stimulus at 6°C found that BAT only contributed to a 10 ± 5 kcal/day increase in EE [234]. Additionally, the exact contribution of BAT activity to EE is questionable where cervio-thoracic muscles, as observed in PET-CT scans, have indicated greater impacts to cold-induced EE than BAT [234]. Interestingly, no linear relationship between BAT and whole-body EE has been indicated, and BAT contributions only represented 1% of the total whole-body changes in EE after CIT [234]. Although BAT can contribute towards whole body EE, which is especially apparent in rodents, the evidence thus far is insufficient to contribute weight reduction in obese human populations. It is also important to explore the benefits of BAT activation beyond solely increasing EE to aid in weight loss. BAT activation may provide protection against metabolic dysregulation such as hyperglycemia and hyperlipidemia associated with obesity. BAT activation using CL, 316243 (β3ADR agonist) over 10 weeks in *E3L and CETP* mice that closely resemble human lipoprotein metabolism robustly lowered plasma TG and total cholesterol levels while maintaining identical food intake [235]. These mice also showed significant reduction in atherosclerotic lesion size after 10 weeks of treatment further demonstrating the protective effects of highly functional BAT. Cold exposure increases glucose uptake in BAT [236, 237] even in fasted rats with low insulin levels [238] indicating sympathetic induced glucose uptake. While glucose is not thought to constitute much of the

thermogenic substrate, it may be used in the intracellular synthesis of triglycerides for thermogenesis [3]. In metabolically healthy men with functional BAT, cold exposure significantly increased glucose uptake into BAT, resting energy expenditure, and glucose and lipid oxidation [239]. However cold exposure has little effect in healthy individuals without detectable BAT even during cold exposure. Given that the prevalence of BAT is low, maximizing tissue activity is challenging and may not have an overwhelming effect on energy expenditure to offset obesity, and might not be responsive across all individuals and patient populations. Thus, we conclude that it is not yet adequately reported in humans that BAT is an effective therapeutic target to induce enough weight loss to protect against the adverse effects of obesity but may be a tissue that can detect and influence energy metabolism.

Author contributions

BP, ML, and JB contributed to writing, and editing of this manuscript and figure design.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. JB was supported in part by various funds, including CIHR Project Grant, NSERC-Discovery, Banting and Best Diabetes Centre; Novo Nordisk-BBDC New Investigator Award 2023–25, and Drucker Family Innovation Fund Grant 2023–24.

Acknowledgments

The authors would like to thank, Dr. Mihir Parikh who also contributed to the editing of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 28 June 2024
DOI 10.3389/jpps.2024.13040

OPEN ACCESS

EDITED BY
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RECEIVED 26 March 2024
ACCEPTED 19 June 2024
PUBLISHED 28 June 2024

CITATION
Abdualkader AM, Karwi QG, Lopaschuk GD and Al Batran R (2024), The role of branched-chain amino acids and their downstream metabolites in mediating insulin resistance. *J. Pharm. Pharm. Sci* 27:13040. doi: 10.3389/jpps.2024.13040

The role of branched-chain amino acids and their downstream metabolites in mediating insulin resistance

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Elevated levels of circulating branched-chain amino acids (BCAAs) and their associated metabolites have been strongly linked to insulin resistance and type 2 diabetes. Despite extensive research, the precise mechanisms linking increased BCAA levels with these conditions remain elusive. In this review, we highlight the key organs involved in maintaining BCAA homeostasis and discuss how obesity and insulin resistance disrupt the intricate interplay among these organs, thus affecting BCAA balance. Additionally, we outline recent research shedding light on the impact of tissue-specific or systemic modulation of BCAA metabolism on circulating BCAA levels, their metabolites, and insulin sensitivity, while also identifying specific knowledge gaps and areas requiring further investigation. Finally, we summarize the effects of BCAA supplementation or restriction on obesity and insulin sensitivity.

KEYWORDS

BCAAs, BCKAs, obesity, insulin resistance, type 2 diabetes

Introduction

Branched chain amino acids (BCAAs) are a group of three indispensable amino acids: leucine, isoleucine and valine. Together, they account for approximately 35% of the essential amino acids present in the human body. While the primary source of BCAAs is dietary intake [1], certain bacteria within the gut microbiome are capable of synthesizing them as well [2, 3]. However, the degree to which the gut microbiome produces BCAAs varies among individuals and is influenced by factors such as diet, gut microbiome composition, and overall health. Apart from serving as fundamental components in protein synthesis, BCAAs, especially leucine, play a critical role in stimulating protein synthesis through the activation of the mechanistic target of rapamycin (mTOR) signalling pathway [4]. Elevated plasma concentrations of BCAAs have been observed in both obese individuals and animal models of obesity [5–9]. Although plasma levels of other amino acid may also rise in obesity, the elevation in BCAAs are of particular interest

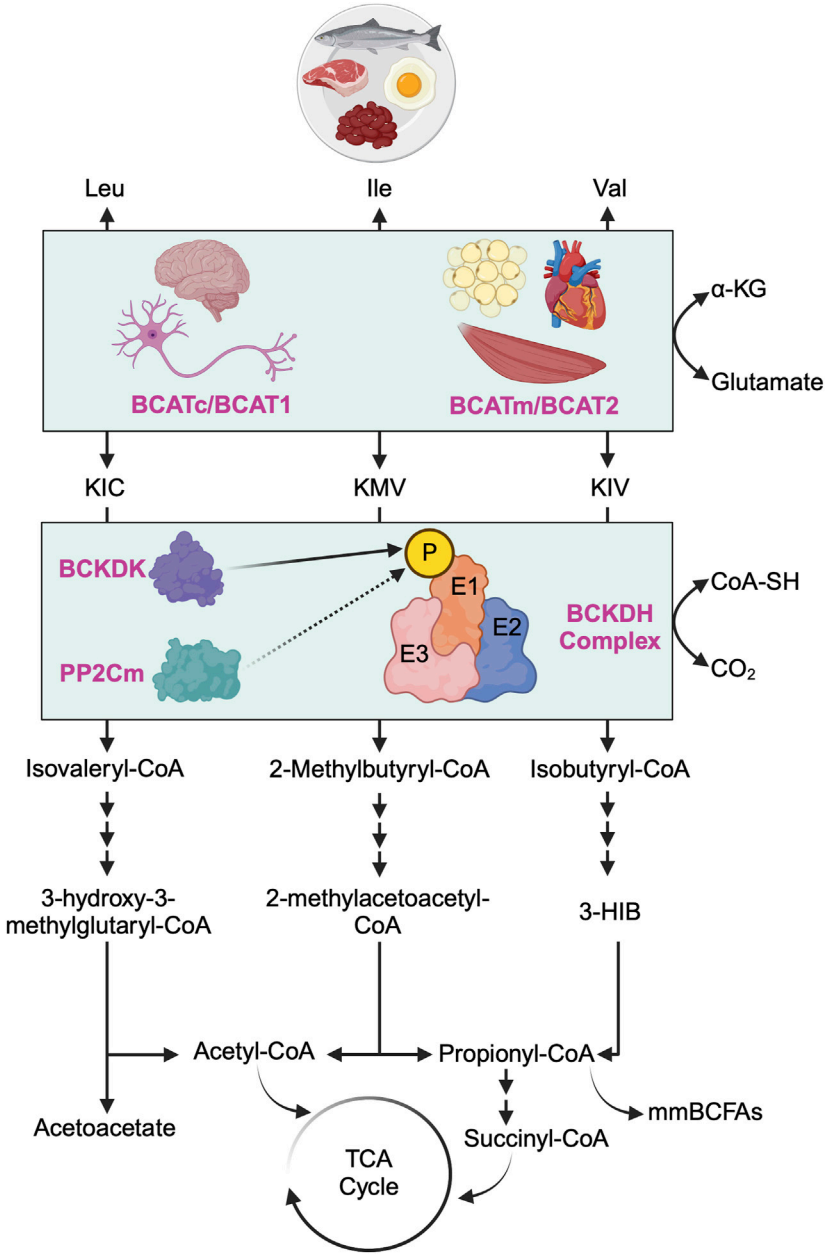

FIGURE 1
Overview of branched-chain amino acid catabolism pathway. The initial and shared step in the catabolism of all three branched-chain amino acids (BCAAs) - leucine (Leu), isoleucine (Ile), and valine (Val) - involves the reversible transamination of BCAAs to produce branched-chain alpha-ketoacids (BCKAs). Specifically, Leu yields α-ketoisocaproate (KIC), Ile yields α-keto-ß-methylvalerate (KMV), and Val yields α-ketovalerate (KIV). This transamination process is catalyzed by two distinct isoforms of branched-chain amino acid aminotransferase (BCAT): the cytosolic isoform (BCATc/BCAT1, encoded by the *Bcat1* gene), predominantly found in the central nervous system and peripheral nerves, and the mitochondrial isoform (BCATm/BCAT2, encoded by the *Bcat2* gene), primarily located in the mitochondria of most nonneuronal tissues. Subsequently, all three BCKAs (KIC, KMV, and KIV) undergo irreversible oxidative decarboxylation, facilitated by the branched-chain alpha-ketoacid dehydrogenase (BCKDH) complex, which serves as the rate-limiting enzyme in BCAA oxidation. The BCKDH complex comprises of three components: E1 (encoded by *Bckdha* and *Bckdhb* genes), E2 (encoded by *Dbt* gene), and E3 (encoded by *Dld* gene). The activity of the BCKDH complex is tightly regulated by BCKDH kinase (BCKDK), which phosphorylates E1 of the BCKDH complex and inhibits its activity (i.e., inhibiting BCAA oxidation), whereas protein phosphatase 2Cm (PP2Cm) dephosphorylates E1 of the BCKDH complex and activates its activity (i.e., activating BCAA oxidation). Post-decarboxylation, each BCKA follows a distinct metabolic pathway, generating acyl-CoA derivatives (isovaleryl-CoA from KIC, 2-methylbutyryl-CoA from KMV, and isobutyryl-CoA from KIV) and various downstream metabolites. These metabolites include critical metabolic intermediates for the TCA cycle, such as acetyl-CoA or succinyl-CoA, as well as acetoacetate, metabolic end products of Leu catabolism, 3-hydroxyisobutyrate (3-HIB), a downstream metabolite of Val that stimulates fatty acid uptake, and monomethyl branched-chain fatty acids (mmBCFAs), adipocyte-specific metabolites derived from mitochondrial BCAA catabolism, namely, propionyl-CoA.

because they appear to have unique effects in obesity-induced insulin resistance, and they are considered a major contributor to the pathology of type 2 diabetes (T2D) and coronary artery disease [10]. Numerous studies since the 1960s have consistently linked elevated plasma BCAAs with insulin resistance [7]. Furthermore, a landmark metabolomics profiling study even suggests that elevation in circulating BCAA levels can predict insulin resistance and T2D as much as 20 years prior to clinical presentation and a decade before any other known marker or test [11]. Interestingly, gastric bypass surgery in obese patients, which effectively lowers elevated BCAA levels, correlates with improved glucose homeostasis and enhanced insulin sensitivity [12]. Despite extensive research efforts, the underlying mechanisms by which elevated BCAA levels contribute to the development of insulin resistance and T2D remain unclear. In this Review, we highlight the major organs responsible for BCAA homeostasis. We then delve into how obesity and insulin resistance affect the communication between these organs, thereby influencing the maintenance of BCAA homeostasis. We also outline recent studies that sheds light on how modulating BCAA metabolism, either in a tissue-specific manner or at a whole-body level, impact circulating BCAA levels and their downstream metabolites, and the consequent effects on obesity and insulin resistance. We end by summarizing the effects of BCAA supplementation or restriction on obesity and insulin sensitivity.

Overview of BCAA catabolism

Plasma BCAA levels at the whole-body level are regulated by a delicate balance between input factors, such as dietary protein intake and proteolysis, and output factors, encompassing protein synthesis and oxidation. Insulin plays a pivotal role in maintaining this balance. Under normal and healthy conditions, insulin facilitates the cellular uptake of BCAAs while suppressing proteolysis, thus regulating plasma BCAA concentrations. However, in pathological states like insulin resistance, this regulatory mechanism may be disrupted. For instance, research on obese women has indicated that moderate obesity correlates with heightened proteolysis and impaired anti-proteolytic effects of insulin [13]. Another study suggested that the increased proteolysis observed in obesity and insulin resistance may be attributed to the compromised anti-proteolytic function of insulin [14]. The intricate regulatory relationship between insulin and BCAA metabolism has been extensively explored in previous literature reviews [15–17]. To facilitate a comprehensive understanding for the reader, we begin by providing essential information on BCAA catabolism and oxidation before delving into the role of BCAAs in mediating insulin resistance (Figure 1). The initial step in the BCAA catabolic pathway involves the reversible transamination of BCAAs catalyzed by branched-chain amino acid aminotransferase (BCAT). Notably, there exist two distinct isoforms of BCAT, namely, BCAT1 encoded by the cytosolic gene (*Bcat1*) and BCAT2 encoded by the mitochondrial gene (*Bcat2*). BCAT1 is the less common of the two isoforms and is primarily expressed in the cytoplasm, with a notable presence in the central and peripheral nervous systems [18, 19], while BCAT2 is the more ubiquitous isoform found in the mitochondria of most nonneuronal tissues, such as the heart, kidney, skeletal muscle and adipose tissue, excluding the liver [20, 21]. BCAT transfers the amino group from BCAAs to α-ketoglutarate, producing glutamate and the corresponding branched-chain α-keto acids (BCKAs): α-ketoisocaproate (KIC) from leucine, α-keto-β-methylvalerate (KMV) from isoleucine, and α-ketoisovalerate (KIV) from valine. This transamination reaction generates ammonia as a byproduct, particularly in the muscles. To remove excess ammonia, the muscle activates the alanine cycle (also known as the Cahill cycle), converting pyruvate to alanine by attaching the amino group from glutamate to pyruvate. Additionally, muscles convert glutamate and ammonia to glutamine as another means of ammonia detoxification. Both alanine and glutamine, as non-toxic carriers of ammonia, are transported to the liver, where the ammonia can be further processed and excreted [22, 23].

Following BCAAs transamination, the irreversible oxidative decarboxylation of BCKAs is catalyzed by the branched-chain α-ketoacid dehydrogenase (BCKDH) complex, serving as the rate-limiting step in BCAA oxidation. The BCKDH complex comprises three components: E1 (encoded by *Bckdha* and *Bckdhb* genes, functioning as a thiamine-dependent decarboxylase), E2 (encoded by *Dbt* gene, functioning as dihydrolipoyl transacylase), and E3 (encoded by *Dld* gene, functioning as dihydrolipoamide dehydrogenase) [17]. The activity of the BCKDH complex is tightly regulated by BCKDH kinase (BCKDK), which phosphorylates and inhibits the BCKDH complex, and protein phosphatase 2Cm (PP2Cm), responsible for dephosphorylating and activating the BCKDH complex [24]. After decarboxylation, each BCKA follows a distinct metabolic route, ultimately leading to the formation of either acetyl-CoA or succinyl-CoA for energy production in the tricarboxylic acid (TCA) cycle or other metabolic intermediates such as acetoacetate, 3-hydroxyisobutyrate (3-HIB) or monomethyl branched-chain fatty acids (mmBCFAs).

Major organs responsible for BCAA homeostasis

BCAA metabolism is an intricate process that relies on inter-organ communication to maintain BCAA homeostasis. Among the key contributors to the circulating pool of BCAAs, skeletal muscle emerges as a predominant site. Skeletal muscle plays a pivotal role in BCAA transamination, primarily owing to the substantial abundance of BCAT2 within the muscle and its considerable muscle mass [19]. Importantly, skeletal muscle

not only serves as a hub for BCAA transamination but also stands out as a major site for BCAA oxidation (accounting for 59% of whole-body BCAA oxidation) and protein synthesis (contributing to 24% of the total protein synthesis from BCAAs) [16]. In contrast, the liver does not engage in BCAA transamination or BCKA re-amination due to the lack of BCAT2 in hepatocytes [15]. Instead, owing to the liver's high BCKDH activity, BCKAs derived from BCAA transamination in extrahepatic tissues are transported to the liver, where they can serve as substrates for BCAA oxidation [15, 25]. While BCKDH complex activity is notably high in the liver and comparatively low in adipose tissue [19, 26], recent tracing studies in mice have uncovered brown adipose tissue as an additional significant site for BCAA oxidation, constituting 19% of whole-body BCAA oxidation, followed by the liver at 8% [27]. This observation has been further supported by another study that used positron emission tomography-computed tomography scans with a leucine-analogue tracer in mice and humans. The study concluded that, upon cold exposure, brown adipose tissue, but not white adipose tissue, significantly contributes to systemic BCAA clearance by enhancing BCAA uptake in this tissue compartment to generate heat through thermogenesis [28].

Numerous studies have demonstrated that inter-organ communication essential for maintaining BCAA homeostasis is disturbed in obesity and insulin resistance. For example, studies showed that in two different rodent models of obesity and insulin resistance (*ob/ob* mice and Zucker rats), the BCKDH activity is decreased in the liver [29–31]. Additionally, other studies have consistently revealed reductions in BCKDH complex expression or activity in white adipose tissue across various models of obesity and insulin resistance [32, 33]. Remarkably, transplanting white adipose tissue from wild-type mice into BCAT2 or PP2CM deficient mice has been found to lower circulating BCAA levels [32, 34], highlighting the pivotal role of adipose tissue in regulating BCAA levels systemically. In line with these findings, Neinast and colleagues uncovered that in *db/db* mice, a model of severe insulin resistance, BCAA oxidation is impaired in adipose tissues and liver and redirected towards skeletal muscle [27]. The same group also demonstrated that excess BCAA oxidation in skeletal muscle leads to the secretion of 3-HIB, a downstream metabolite of valine, which, in turn, stimulates muscle fatty acid uptake and lipid accumulation, thereby exacerbating insulin resistance [35]. Another group hypothesized that in obesity and insulin resistance, the accumulation of C3 and C5 acylcarnitines in muscle, which are by-products of BCAA catabolism and markers of incomplete fat oxidation, may contribute to insulin resistance [36, 37]. A recent hypothesis posits a direct association between BCKAs and insulin resistance, where exposure of muscle cells to high concentrations of BCKAs results in the inhibition of insulin-induced AKT phosphorylation (also known as protein kinase B) and glucose uptake [38], indicating a direct role of BCKAs in impairing insulin signalling. Finally, the classical mechanism linking elevated BCAA levels with insulin resistance involves chronic hyperactivation of mTORC1 and its downstream effector, ribosomal protein S6 kinase 1 (S6K1), also known as p70-S6K. This hyperactivation phosphorylates and inhibits insulin receptor substrate 1 (IRS-1), thus blunting insulin signalling and contributing to insulin resistance [39–41].

Indeed, the role of the gut microbiome in maintaining BCAA homeostasis was historically overlooked due to the complexity of the microbiome, technological limitations, and a traditional focus on host genetics and diet. However, recent advances in this domain have highlighted the microbiome's critical role in BCAA synthesis, regulation, and interaction with host metabolism. It is now evident that the gut microbiome contributes to the overall pool of BCAAs, potentially influencing the development of insulin resistance [42]. For example, a landmark study identified *Bacteroides vulgatus* and *Prevotella copri* as two key species of gut microbiome bacteria responsible for elevated BCAA biosynthesis and associated with insulin resistance in humans [3]. This study also demonstrated that *Prevotella copri* can induce insulin resistance, exacerbate glucose intolerance, and increase circulating levels of BCAAs in mice. Furthermore, a recent study demonstrated that feeding mice a variety of protein sources mirroring the composition of the Western diet exacerbates insulin resistance. This effect is attributed to an increase in gut microbial branched-chain fatty acids (BCFA) [43], a class of short-chain fatty acids produced in the gut through the proteolytic fermentation of BCAAs.

Nevertheless, in the subsequent sections, we will discuss and summarize the effects of modifying BCAA catabolism, either selectively in a tissue-specific manner (muscles, liver, adipose tissue, and heart) or systemically, on circulating BCAA levels and insulin sensitivity (Table 1)

Modulating BCAA catabolism to treat insulin resistance

Muscle: Skeletal muscle plays a crucial role in maintaining BCAA homeostasis, serving as the primary site for whole-body BCAA oxidation. In Zucker-fatty rats, BCKDH activity is elevated in skeletal muscle but reduced in the liver compared to Zucker-lean rats [29]. Similarly, Neinast and colleagues demonstrated that in *db/db* mice, but not in mice fed a high-fat diet for 14 weeks, BCAA oxidation is increased in skeletal muscle and decreased in the liver and adipose tissue [16, 27]. Furthermore, several studies have noted diminished BCAA oxidation in adipose tissues during obesity and insulin resistance [30, 32]. These collective observations from multiple research groups have led to the hypothesis that excess BCAA oxidation in skeletal muscle may contribute to insulin resistance. This may occur via two potential mechanisms: 1) through the overproduction of 3-HIB in muscle or 2) via the accumulation of acylcarnitines derived from muscle BCAA breakdown. In both

TABLE 1 The effects of modulating BCAA catabolism in various tissue compartments or systemically on insulin sensitivity in lean and obese animals.

Study design	Outcome	References
Muscle		
Muscle-specific *Bckdk* knockout mice fed a chow diet	• Increased muscle BCAA oxidation • Decreased plasma BCAA and BCKA levels only during fasting state • No change in glucose tolerance and insulin sensitivity	[44]
Muscle-specific *Bckdk* knockout mice fed an HFD or WD	• Decreased plasma BCAA levels • No change in glucose tolerance and insulin sensitivity	[44]
Muscle-specific *Dbt* knockout mice fed a chow diet	• Decreased muscle BCAA oxidation • No change in plasma BCAA levels during both fasting and refeeding states • No change in glucose tolerance and insulin sensitivity	[44]
Muscle-specific *Dbt* knockout mice fed an HFD	• No change in plasma BCAA levels during both fasting and refeeding states • No change in glucose tolerance and insulin sensitivity	[44]
Liver		
Overexpressing *Ppm1k* in the liver of Zucker fatty rats	• Increased liver BCAA oxidation • Decreased plasma BCAA levels • Improved glucose tolerance and insulin sensitivity	[45]
Liver-specific *Bckdk* knockout mice fed a chow or HFD	• Increased liver BCAA oxidation • No change in plasma BCAA levels during both fasting and refeeding states • No change in insulin sensitivity	[44]
Liver-specific *Dbt* knockout mice fed a chow or HFD	• Decreased liver BCAA oxidation • No change in plasma BCAA levels during both fasting and refeeding states • No change in insulin sensitivity	[44]
Muscle- and liver-specific *Bckdk* knockout mice fed an HFD	• Increased muscle and liver BCAA oxidation • No change in plasma BCAA levels during fasting state • No change in insulin sensitivity	[44]
Liver-specific *Bcat2* transgenic mice fed an HFD	• No change in plasma BCAA levels • Impaired glucose tolerance	[46]
Adipose Tissue		
BAT-specific *Bckdha* knockout mice fed an HFD	• Impaired BCAA clearance • Susceptible to HFD-induced obesity and insulin resistance • Impaired BAT BCAA and glucose oxidation	[28]
WAT-specific *Bcat2* knockout mice fed an HFD	• Increased plasma BCAA levels • Resistance to HFD-induced obesity and insulin resistance • BCKAs supplementation restore obesity and insulin resistance	[47]
Heart		
Heart-specific *Bcat2* knockout mice	• Decreased heart BCAA oxidation • Increased cardiac BCAAs and decreased BCKAs • Increased cardiac insulin sensitivity	[48]
Ppm1k knockout mice	• Decreased systemic BCAA oxidation • Increased plasma BCAA and BCKA levels • Sensitized the heart to ischemia-reperfusion injury	[49]
Ppm1k knockout mice	• Decreased systemic BCAA oxidation • Increased plasma BCAA and BCKA levels • Promoted heart failure	[50]
Systemic		
Bcat2 knockout mice fed an HFD	• Increased plasma BCAAs and decreased BCKAs • Improved glucose tolerance and insulin sensitivity • Increased energy expenditure	[51]

(Continued on following page)

TABLE 1 (*Continued*) The effects of modulating BCAA catabolism in various tissue compartments or systemically on insulin sensitivity in lean and obese animals.

Study design	Outcome	References
Bckdk knockout mice fed a chow diet	• Increased systemic BCAA oxidation • Decreased plasma BCAA and BCKA levels • No change in glucose tolerance	[27]
Ppm1k knockout mice fed a chow diet or HFD	• Decreased systemic BCAA oxidation • Increased plasma BCAA and BCKA levels • Improved glucose tolerance and insulin sensitivity	[52, 53]
Zucker fatty rats treated with LY3351337	• Increased plasma BCAA and glycine levels • Improved glucose tolerance and insulin sensitivity	[54]
Obese and insulin-resistant animals treated with Telmisartan	• Decreased plasma BCAA levels • Improved glucose tolerance and insulin sensitivity	[47]
Obese and insulin-resistant animals treated with BT2	• Increased systemic BCAA oxidation • Decreased plasma BCAA and BCKA levels • I mproved glucose tolerance and insulin sensitivity	[27, 44, 45, 55, 56]

HFD, high-fat diet; WD, western diet; BAT, brown adipose tissue; WAT, white adipose tissue; BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; *Bckdk*, branched-chain keto acid dehydrogenase kinase; *Dbt*, dihydrolipoamide branched-chain transacylase E2; *Ppm1k*, protein phosphatase, Mg^{2+}/Mn^{2+}. Dependent 1K; *Bcat2*, branched-chain amino acid transaminase 2; *Bckdha*, branched-chain keto acid dehydrogenase E1 subunit alpha; LY3351337, BCAT1 and BCAT2 inhibitor; Telmisartan, BCAT2 inhibitor; BT2, BCKDK, inhibitor.

scenarios, this would impair fatty acid oxidation and promote lipotoxicity [29, 37, 57]. To test this hypothesis, Blair et al [44] generated muscle-specific knockout mice lacking either the *Bckdk* gene, responsible for phosphorylating E1 of the BCKDH complex and inhibiting its activity, or the *Dbt* gene, crucial for BCAA oxidation as it encodes the E2 component of the BCKDH complex. Interestingly, their investigation revealed that augmenting muscle BCAA oxidation lowered plasma BCAA and BCKA levels only during the fasting state in muscle-specific *Bckdk* knockout mice fed a chow diet compared to their control littermates, human α-skeletal actin (HSA)-Cre mice. Conversely, diminishing muscle BCAA oxidation did not significantly alter plasma BCAA levels during both fasting and refeeding states in muscle-specific *Dbt* knockout mice fed a chow diet compared to HSA-Cre mice. However, the administration of a single bolus of BCAA resulted in impaired BCAA and BCKA clearance in muscle-specific *Dbt* knockout mice when compared to their controls. These findings indicate that manipulating muscle BCAA oxidation under healthy conditions impacts circulating BCAA levels predominantly during fasting.

To investigate whether modulating muscle BCAA oxidation impacts glucose homeostasis, muscle-specific *Bckdk* knockout mice were subjected to chronic feeding regimens of either a Western diet or a high-fat diet spanning from 4 up to 12 weeks, followed by assessment of insulin sensitivity and glucose handling using hyperinsulinemic-euglycemic clamp and glucose tolerance tests (GTT), respectively. Surprisingly, despite observing a reduction in plasma BCAAs and an increase in the 3-HIB/valine ratio during the fasted state in muscle-specific *Bckdk* knockout mice fed the obesogenic diet (Western diet or high-fat diet), this alteration did not manifest in changes in insulin sensitivity or glucose handling. Specifically, there were no discernible differences in euglycemic clamp and GTT outcomes between muscle-specific *Bckdk* knockout mice and their controls. Similarly, inhibiting muscle BCAA oxidation in muscle-specific *Dbt* knockout mice fed a high-fat diet did not affect insulin sensitivity during a euglycemic clamp or alter glucose handling during a GTT. Additionally, there were no significant changes observed in plasma BCAA levels or the 3-HIB/valine ratio in both fasted and refed states. These collective findings suggest that augmenting or diminishing muscle BCAA oxidation has no impact on whole-body insulin sensitivity in mice subjected to various obesogenic diets. While this study did not directly measure it, further exploration into the effects of modulating muscle BCAA oxidation on muscle insulin sensitivity itself would be intriguing. Moreover, investigating whether muscle acylcarnitine species, particularly C3 and C5, as well as BCKA levels play a role in improving or exacerbating muscle insulin sensitivity could provide valuable insights.

Liver: While the liver lacks the BCAT enzyme necessary for the conversion of BCAAs into BCKAs and *vice versa*, it remains a pivotal site for BCAA oxidation and protein synthesis, contributing up to 27% of whole-body BCAA incorporation into proteins [27]. As previously noted, multiple studies have demonstrated markedly elevated expression of liver BCKDK, which phosphorylates and inhibits BCKDH complex activity, in various models of obese and insulin-resistant rodents [29–31]. In an effort to understand whether the reduction in liver BCAA oxidation contributes to the development of insulin resistance, White and colleagues utilized adenovirus-mediated delivery of *Ppm1k*, the gene encoding PP2Cm (which dephosphorylates and activates the BCKDH complex), to specifically overexpress PP2Cm in the liver of Zucker fatty rats [45]. Their findings demonstrated that liver PP2Cm overexpression enhanced liver BCKDH activity, reduced circulating BCAAs, alleviated hepatic steatosis, and improved glucose tolerance and insulin sensitivity.

Interestingly, hepatic overexpression of PP2Cm increased the phosphorylation of ATP-citrate lyase (ACLY), a critical enzyme involved in lipid synthesis. This activation of ACLY subsequently stimulated *de novo* lipogenesis, thereby integrating BCAA metabolism with lipid metabolism.

On the contrary, manipulating liver BCAA oxidation levels through targeting either the *Bckdk* or *Dbt* gene in the liver, using an adeno-associated viral (AAV) vector carrying the Cre recombinase gene under the control of the thyroxine-binding globulin (TBG) promoter (AAV8-TBG-Cre) in *Bckdk* or *Dbt* floxed mice, did not influence circulating BCAA levels in either fasted or refed states, regardless of whether the mice were subjected to a chow or high-fat diet for 4–5 weeks [44]. Furthermore, neither enhancing nor suppressing liver BCAA oxidation affected whole-body insulin sensitivity in mice fed a normal chow or high-fat diet. Of note, augmenting both muscle and liver BCAA oxidation in mice, achieved by treating muscle-specific *Bckdk* knockout mice with AAV8-TBG-Cre to generate double knockout mice, also failed to impact whole-body insulin sensitivity in mice subjected to a high-fat diet for 6 weeks. This was observed despite a notable reduction in fasting plasma BCAAs and an increase in the 3-HIB/valine ratio in BCKDK double knockout mice compared to their control counterparts. Although the reasons for the discrepancy between the results regarding the manipulation of liver BCAA oxidation and insulin sensitivity in the mouse and rat studies remain unclear, it has been suggested that species differences may account for the contrasting outcomes [57]. Of note, a recent study revealed that mice lacking PP2Cm globally are protected against high-fat-diet-induced insulin resistance. Interestingly, this investigation also demonstrated that BCKAs selectively inhibits the mitochondrial pyruvate carrier (MPC) in hepatocytes, thus suppressing gluconeogenesis from pyruvate [52]. Nevertheless, further research is warranted to delineate the role of liver BCAA catabolism in insulin resistance.

Adipose tissue: Adipose tissue, traditionally viewed as a passive site for energy storage, is now recognized as a dynamic regulator impacting various aspects of whole-body metabolism, including BCAA catabolism. In conditions like obesity and insulin resistance, there is a notable suppression in the expression of nearly all enzymes responsible for BCAA catabolism, particularly within white adipose tissue [30, 32, 55, 58]. This decrease in BCAA catabolism is considered a significant contributor to the systemic elevation of BCAA levels during obesity and insulin resistance [33, 59]. Cross-tissue flux studies comparing lean and healthy individuals to insulin-sensitive or insulin-resistant obese subjects revealed negligible uptake of BCAAs from human abdominal subcutaneous white adipose tissue [33]. However, BCAA catabolic enzyme levels were markedly reduced in omental fat, a specific type of visceral fat, but not in subcutaneous white adipose tissue of obese individuals with metabolic syndrome compared to weight-matched healthy obese subjects. This finding suggests that alterations in BCAA catabolism in visceral white adipose tissue significantly contribute to the BCAA metabolic phenotype in individuals with insulin resistance. Furthermore, adipose tissue not only utilizes BCAAs to support adipocyte differentiation and lipogenesis [60], but it also has the capacity to release adipocyte-specific metabolites stemming from mitochondrial BCAA catabolism, such as mmBCFAs. These metabolites play a role in fueling *de novo* lipogenesis, with their levels being notably decreased in obese animals and increased during prolonged fasting [61]. Consequently, it is tempting to speculate that the reduced levels of plasma mmBCFAs observed in obesity may be attributed to decreased BCAA catabolism within this specific tissue compartment.

Adipose tissue, particularly brown adipose tissue, plays a significant role in utilizing BCAAs for thermogenesis during cold exposure in both mice and humans. This process contributes to systemic BCAA clearance by enhancing BCAA uptake via SLC25A44, a mitochondrial BCAA transporter [28]. Notably, BCAA clearance following oral administration of BCAAs is compromised in mice with targeted deletion of *Bckdha* in brown adipose tissue, the gene responsible for encoding the E1 component of the BCKDH complex and critical for BCAA oxidation. Moreover, brown adipose tissue-specific *Bckdha* knockout mice showed increased susceptibility to high-fat diet-induced obesity and insulin resistance, coupled with impaired glucose oxidation within brown adipose tissue. These findings underscore the critical role of intact BCAA oxidation in brown adipose tissue for systemic BCAA clearance and the amelioration of obesity and insulin resistance. Conversely, a recent study indicates that white adipose tissue-specific *Bcat2* knockout mice display resistance to high-fat diet-induced obesity and insulin resistance, attributed to enhanced browning and thermogenesis in white adipose tissue [47]. Intriguingly, the study also revealed that BCKAs inhibits white adipose tissue browning through the acetylation of the PR domain-containing protein 16 (PRDM16). Furthermore, supplementation of BCKAs in white adipose tissue-specific *Bcat2* knockout mice reverses these favorable effects, leading to the reinstatement of obesity and insulin resistance. These findings suggest that mitigating BCAA transamination into BCKAs in white adipose tissue, consequently affecting BCAA oxidation in this tissue compartment, is beneficial in attenuating obesity and insulin resistance.

One pivotal question emerges from the findings of these two studies: Why is the suppression of BCAA oxidation in brown adipose tissue detrimental rather than protective against insulin resistance, whereas its suppression in white adipose tissue appears to confer a protective effect? One potential explanation for this phenomenon is that inhibiting BCAA oxidation in brown adipose tissue not only raises BCAA levels but also elevates the level of BCKAs, thereby triggering insulin resistance. Conversely, targeting BCAT2 in white adipose tissue leads to increased BCAA levels while concurrently reducing BCKAs, thus mitigating insulin resistance. In support of this

hypothesis, mice with whole-body *Bcat2* deletion exhibit elevated plasma BCAAs and decreased BCKAs, yet remained protected from high-fat diet-induced obesity and insulin resistance [51]. Remarkably, this protection persists even in the presence of mTORC1 hyperactivation, as evidenced by the phosphorylation of mTOR downstream targets such as eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and S6K1 in the gastrocnemius of *Bcat2* knockout fasted mice. Collectively, these findings suggest that it may not be the mere elevation of circulating BCAAs *per se* that drives insulin resistance, but rather the accumulation of BCKAs that plays a crucial role in mediating insulin resistance. Given that skeletal muscle predominantly facilitates the conversion of BCAAs into BCKAs, it would be interesting to explore whether decreasing BCKAs, particularly in muscle tissue, by targeting BCAT2 yields outcomes akin to reducing BCKAs in white adipose tissue. Future studies are imperative to answer this question.

Heart: Decreased cardiac BCAA oxidation has been linked to the development of cardiac insulin resistance and impaired cardiac insulin signalling pathways [62]. Direct measurement of cardiac BCAA oxidation rates in isolated working mouse hearts demonstrated that these rates are decreased in a mouse model of high-fat diet-induced obesity [63]. This is associated with a decreased activity of Akt and glycogen synthase kinase-3β (GSK-3β) and cardiac insulin-stimulated glucose oxidation rates in obese mice [63]. Similarly, cardiac BCAA oxidation rates are also decreased in the failing heart, which is associated with impaired insulin signalling and insulin-stimulated glucose oxidation rates [64]. A whole-body PP2Cm deletion, a maneuver which decreases the activity of BCKDH complex and BCAA oxidation, is associated with decreased glucose oxidation by inhibiting pyruvate dehydrogenase (PDH) activity and increased vulnerability to myocardial ischemia/reperfusion injury [49].

Since impaired BCAA oxidation leads to the accumulation of BCAAs and BCKAs, it is difficult to ascertain whether BCAAs or BCKAs contribute to cardiac insulin resistance. Selective increasing cardiac BCKA levels abrogates insulin-stimulated cardiac glucose oxidation rates via inhibiting insulin signalling pathway *ex vivo* [48]. While BCKAs could be re-aminated to their correspondent BCAAs [65], it is unclear how fast this process is. In fact, we recently demonstrated that an acute increase in BCKA does not lead to a significant change in cardiac BCAA levels. Moreover, we recently developed a mouse model where we deleted BCAT2 in the heart to selectively increase cardiac BCAAs and decrease cardiac BCKAs [48]. The accumulation of cardiac BCAA levels in the *Bcat2* knockout hearts did not impact cardiac insulin sensitivity [48]. However, BCAT2 deletion enhances cardiac insulin signalling and insulin-stimulated glucose oxidation rates [48]. These findings demonstrate that it is BCKAs, not BCAAs, that influence cardiac insulin signalling. In further support of this, we recently showed that reducing cardiac BCKA levels by cardiac-specific deletion of *Bcat2* mitigates cardiac insulin resistance and enhances insulin-stimulated glucose oxidation rates in the failing heart [66]. This enhancement in cardiac glucose oxidation is mediated, at least in part, via enhancing mitochondrial Akt activity [66]. How BCKAs enhance insulin signalling in the heart remains an interesting scope for future investigations.

Systemic: At the whole-body level, the use of LY3351337 to inhibit both BCAT1 and BCAT2 in Zucker fatty rats results in increased circulating levels of BCAA and glycine [54], with the latter showing an inverse correlation with impaired glucose handling and T2D [67, 68]. This intervention significantly improves glucose tolerance and insulin sensitivity. Similarly, inhibition of BCAT2 with Telmisartan reduces circulating BCKA levels and body weight, leading to notable enhancements in glucose tolerance and insulin sensitivity in mice on a high-fat diet [47]. Furthermore, a plethora of studies utilizing various animal models of obesity and insulin resistance consistently demonstrates that treatment with the BCKDK inhibitor 3,6-dichlorobenzo[b] thiophene-2-carboxylic acid, commonly referred to as BT2, enhances BCAA oxidation, reduces circulating BCAA and BCKA levels, and notably improves glucose tolerance and insulin sensitivity [27, 44, 45, 55]. In a recent randomized and controlled clinical trial, T2D patients receiving sodium phenylbutyrate (4.8g per day), an FDA-approved drug for treating acute hyperammonemia that inhibits BCKDK and promotes BCAA oxidation, exhibited enhanced insulin sensitivity after just 2 weeks of treatment compared to the placebo group [69]. It is worth mentioning that enhancing systemic BCAA oxidation through global deletion of BCKDK in lean mice results in decreased circulating BCAA and BCKA levels [27]. However, this manipulation does not significantly impact glucose disposal in BCKDK knockout lean mice following oral glucose administration. Alternatively, suppressing whole-body BCAA oxidation in mice by deleting PP2Cm increases circulating BCAA and BCKA levels and enhances glucose tolerance and insulin sensitivity, regardless of the presence or absence of obesity and insulin resistance [52, 53].

One pivotal question emerges from these studies: Is it better to augment or reduce BCAA oxidation in obesity and insulin resistance? We argue that enhancing BCAA oxidation enhancing and mainly lowering BCAA and BCKA levels appears to be a more advantageous approach, given the adverse effects associated with suppressing BCAA oxidation and increasing BCAA and BCKA levels, such as ischemia-reperfusion injury and heart failure [49, 50]. Consequently, another fundamental question remains unanswered: Does systemic enhancement of BCAA oxidation alone alleviate insulin resistance, or is it the reduction in BCKAs that alleviates insulin resistance? We propose the latter based on the following evidence: **1)** While BT2 administration in animals dephosphorylates and activates the BCKDH complex in multiple organs, resulting in a systemic reduction in plasma BCAA and BCKA levels [45, 70], the magnitude of BCKA reduction appears more pronounced compared to BCAA reduction in *ob/ob* mice treated with BT2 over a period of 4–6 weeks [55]. **2)** Screening efforts aimed at discovering more potent BCKDK inhibitors led to the

TABLE 2 Preclinical studies demonstrating the impact of dietary BCAA supplementation or restriction on insulin sensitivity.

Study design	Outcome	References
BCAA Supplementation		
Wistar rats fed an HFD supplemented with BCAAs for 13 weeks	• Increased plasma BCAA levels • Impaired glucose tolerance and insulin sensitivity • Increased muscle C3 and C5 acylcarnitine levels	[36]
Obese mice subjected to exercise with or without BCAA supplementation for 12 weeks	• BCAA supplementation increased BCAA levels in WAT • BCAA supplementation impaired insulin sensitivity • Increased adiposity after BCAA supplementation	[75]
Ob/ob mice fed an isocaloric low-protein diet supplemented with BCAAs for 2 weeks	• Increased plasma BCAA and BCKA levels • Impaired glucose tolerance and insulin sensitivity • Increased plasma insulin levels	[55]
Mice fed an HFHS or HFD supplemented with BCAAs for 32 weeks	• Increased plasma BCAA and BCKA levels • No change in glucose tolerance and insulin sensitivity	[76]
Mice fed an HFD supplemented with valine for 15 weeks	• Impaired glucose tolerance and insulin sensitivity	[77]
BCAA Restriction		
Zucker-fatty rats fed an isocaloric BCAA-restricted LFD for 15 weeks	• Decreased plasma BCAA levels • No change in plasma BCKA levels • Improved muscle insulin sensitivity	[29]
Mice fed a BCAA-restricted WD for 12 weeks	• Reduced body weight and adiposity • Improved glucose tolerance and insulin sensitivity • Increased energy expenditure	[78]
Mice fed a low-protein or low-BCAA diet for 3 weeks	• Decreased plasma BCAA levels • Reduced body weight and adiposity • Improved glucose and pyruvate tolerance	[79]
Ob/ob mice fed an isocaloric low-protein diet for 4 weeks	• Decreased plasma AA levels • Decreased plasma BCAA and BCKA levels • Improved glucose tolerance and insulin sensitivity	[55]
Db/db mice fed diets lacking any individual BCAAs for 1 day	• Improved insulin sensitivity	[80]
Mice fed an isoleucine- or valine-restricted WD for 12 weeks	• Reduced body weight and adiposity • Improved glucose tolerance and hepatic insulin sensitivity	[81]
Mice fed an isoleucine-restricted diet for 14 weeks	• Reduced body weight and adiposity • Improved glucose tolerance and insulin sensitivity	[82]

LFD, low-fat diet; HFD, high-fat diet; HFHS, high-fat high-sucrose; WD, western diet; BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; AA, amino acid.

identification of thiophene PF-07208254 as an allosteric BCKDK inhibitor exhibiting superior potency to BT2 [56]. Both PF-07208254 and BT2 dephosphorylate BCKDH at the same site, resulting in diminished levels of BCKAs and improved glucose tolerance and insulin sensitivity. Intriguingly, structure-activity relationship studies have revealed thiazoles as BCKDK inhibitors with even greater potency than PF-07208254 and BT2. However, despite their ability to dephosphorylate BCKDH, thiazole inhibitors elevate BCKA levels and counteract the favorable effects of PF-07208254 and BT2 by increasing the proximity of BCKDK to BCKDH-E2. **3)** In individuals with maple syrup urine disease, the oxidation of BCAAs is hindered due to a deficiency in BCKDH enzyme. It's noteworthy that despite elevated plasma BCAA levels, these individuals do not typically experience insulin resistance [71–74].

Together, these observations further suggest that primarily reducing systemic levels of BCKAs may enhance insulin sensitivity. However, further research in this area is needed to thoroughly investigate and confirm this hypothesis.

The impact of dietary BCAA supplementation or restriction on insulin resistance

Numerous preclinical studies have indicated that supplementing with BCAAs worsens insulin resistance, while restricting their intake improves insulin sensitivity in various obese animal models (Table 2). Recent evidence further suggests that limiting dietary BCAAs could potentially improve health and longevity in male mice [83], whereas high BCAA consumption induces obesity and shortens lifespan in mice [84]. While many of these studies have treated all three BCAAs as having equivalent metabolic effects, emerging research indicates

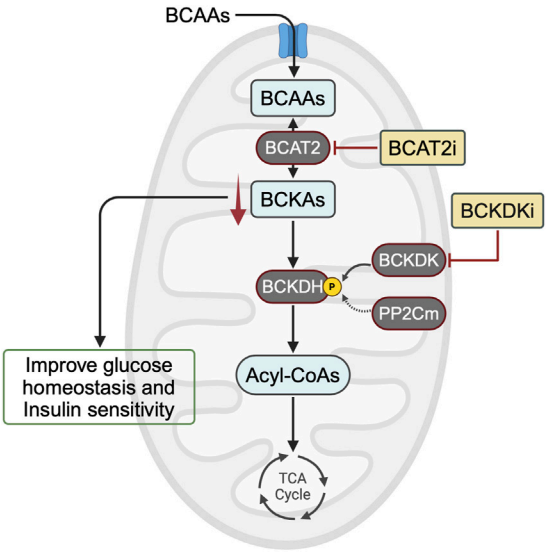

FIGURE 2
Potential targets for correcting alterations in BCAA metabolism to treat insulin resistance. In animal models of obesity and insulin resistance, mounting evidence suggests that BCKA accumulation, rather than BCAA accumulation, impairs glucose homeostasis and decreases insulin sensitivity. Therefore, one approach to lowering BCKA levels would be to inhibit BCAT2 or enhance BCAA oxidation activity using a BCKDK inhibitor. BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; BCAT2, branched-chain amino acid aminotransferase 2; BCKDH, branched-chain α-ketoacid dehydrogenase complex; BCKDK, BCKDH kinase; PP2Cm, protein phosphatase 2Cm; BCAT2i, BCAT2 inhibitor; BCKDKi, BCKDK inhibitor.

that each BCAA may exert unique influences on obesity and insulin sensitivity. For instance, Yu and colleagues demonstrated that restricting either isoleucine or valine, but not leucine, enhances glucose tolerance and hepatic insulin sensitivity in mice on a Western diet [81]. Intriguingly, reintroducing either isoleucine or all three BCAAs, but not leucine or valine alone, reverses these metabolic benefits. In another study, the same researchers found that lifelong isoleucine restriction increases lifespan and improves glucose homeostasis in both male and female mice [82]. Similarly, another group observed that valine supplementation in mice on a high-fat diet significantly impairs glucose tolerance and insulin sensitivity [77]. Likewise, a plethora of studies have illustrated that leucine supplementation yields various beneficial effects on glucose homeostasis across different mouse models of obesity and insulin resistance [85–91]. Notably, it is well-documented that leucine increases hypothalamic mTOR signalling while reducing food intake and body weight [92]. Collectively, these findings underscore that each of the individual BCAAs exerts distinct metabolic effects on obesity and insulin sensitivity. Furthermore, accumulating evidence suggests that the elevation of BCAA levels *per se* may not be the primary driver of insulin resistance [47, 48, 51], but rather their downstream metabolites (such as BCKAs, 3-HIB, and specific acylcarnitine species) that play a pivotal role in triggering the disease. Since each BCAA follows a distinct metabolic pathway after oxidation (Figure 1), this presents promising opportunities to selectively target either the isoleucine, valine, or both pathways to treat and prevent obesity and insulin resistance.

Discussion

While elevated plasma levels of BCAAs have consistently been linked to insulin resistance and T2D, recent evidence suggests that the direct implication of BCAAs themselves in insulin resistance may not be significant. Instead, emerging evidence suggests that the accumulation of their downstream metabolites, such as BCKAs, could play a crucial role in exacerbating insulin resistance. If elevated BCKA levels are indeed the main driver of insulin resistance, then lowering them can be accomplished through BCAT2 inhibition or BCKDK inhibition (Figure 2). Further research is needed to determine whether targeting these downstream metabolites of BCAAs could offer a promising avenue for treating and preventing obesity-induced insulin resistance and T2D.

Author contributions

RA and AA drafted the initial version of the manuscript. AA created the figures and tables. QK and GL revised the manuscript and contributed to the heart section. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This review was supported by a Seeding Grant from Diabetes Québec to RA. RA is a Research Scholar of the Fonds de Recherche du Québec - Santé (FRQS) and a New Investigator of the Kidney Research Scientist Core Education and National Training (KRESCENT).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

Figures 1, 2 was generated using BioRender.com.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Mini Review
PUBLISHED 30 September 2024
DOI 10.3389/jpps.2024.13375

OPEN ACCESS

EDITED BY
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RECEIVED 12 June 2024
ACCEPTED 13 September 2024
PUBLISHED 30 September 2024

CITATION
Kwon S, Jeyaratnam R and Kim K-H (2024) Targeting ketone body metabolism to treat fatty liver disease. *J. Pharm. Pharm. Sci* 27:13375. doi: 10.3389/jpps.2024.13375

Targeting ketone body metabolism to treat fatty liver disease

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a metabolic disorder marked by excessive accumulation of lipids within the liver. If untreated, this condition can progress to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma (HCC). Given the liver's pivotal role in glucose and fatty acid metabolism, disruptions in these processes are commonly observed in MASLD. Ketone bodies, crucial energy metabolites primarily produced in the liver, are also closely related to the progression of MASLD. Recent studies have demonstrated that disrupted ketogenesis not only accompanies MASLD, but may also play a causal role in its development and progression. Moreover, activation of the ketogenic pathway has been suggested as a promising strategy for reducing excessive hepatic fat accumulation. This review focuses on the regulation of ketogenesis in MASLD, emphasizing the significance of dietary and pharmacological interventions as potential therapeutic approaches to treat fatty liver disease.

KEYWORDS

MASLD, ketone bodies, ketogenesis, dietary interventions, pharmacological interventions

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a prevalent chronic liver disease [1, 2], globally affecting human health with an estimated prevalence of 32% [3]. This condition is characterized by increased fat accumulation within the liver, compromising its function. The prolonged accumulation of hepatic fat in MASLD can lead to severe conditions, such as metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma (HCC). This progression is driven by lipotoxicity, leading to increased hepatic oxidative stress and the development of MASH [4]. Concurrently, increases in free fatty acid uptake and oxidative stress activate resident liver macrophages, which promote inflammation through various signaling pathways, including Toll-like receptor (TLR) 4-mediated production of pro-inflammatory cytokines [5, 6]. As the liver attempts to repair itself amid heightened inflammation, fibrosis emerges, characterized by the accumulation of extracellular matrix proteins, tissue scarring and immune cell

infiltration [7, 8]. This persistent tissue scarring and immune activity eventually culminate into cirrhosis, marked by hepatocyte apoptosis [9] and impaired regenerative capacity [10]. Additionally, the elevated pro-inflammatory cytokine TNF has been associated with tumor promotion, as it stimulates hepatocyte proliferation, which can trigger the development of HCC [11]. As MASLD and its pathological progression arise from complex interactions of various factors affecting a broad spectrum of individuals, numerous studies have focused on elucidating the mechanisms driving the progression of this disease and developing effective therapeutic strategies.

Nevertheless, current treatment approaches for fatty liver disease, aside from lifestyle modifications such as weight management, dietary interventions, and exercise, are relatively limited. Insulin sensitizers, lipid-lowering medications, and antioxidants have been tested, but have not proven effective. Notably, drugs used for type 2 diabetes, such as metformin and sodium-glucose cotransporter-2 inhibitors (SGLT2i), have shown efficacy in treating fatty liver disease [12–14]. It remains unclear, though, whether the beneficial effects of these drugs on fatty liver disease are due to direct targeting of the liver function or are indirectly achieved through improved glucose homeostasis. Recently, the U.S. Food and Drug Administration approved resmetirom (Rezdiffra), a thyroid hormone receptor β (THRβ) agonist, as the first drug to directly target the liver for the treatment of MASH and moderate-to-advanced hepatic fibrosis. However, only 20%–30% of patients have shown improvement in key liver pathology indicators, and the long-term safety of resmetirom has not yet been assessed in clinical trials [15]. Therefore, the need to identify novel therapeutic targets for treating fatty liver disease remains a pressing and unmet challenge.

Dysregulated ketone body metabolism in fatty liver disease

Metabolic remodelling is a molecular and cellular hallmark in fatty liver diseases, which includes alterations in *de novo* lipogenesis, hepatic very-low-density lipoprotein secretion and lipoprotein metabolism, and gluconeogenesis [16]. Another notable change is the dysregulation of ketone body metabolism. In the early stage of fatty liver disease like simple steatosis, an increase in plasma ketone bodies is often observed as a result of the liver converting excessive fatty acids into ketone bodies to alleviate metabolic stress [17, 18]. However, as MASLD advances to more severe stages like MASH, levels of plasma ketone bodies in patients decrease [19]. This decline is attributed to impaired ketogenesis, a process of synthesizing water-soluble ketone bodies, such as β-hydroxybutyrate (BHB), acetoacetate (AcAc), and acetone, primarily in the liver, as fasting-induced ketosis is significantly reduced in humans with MASLD [20, 21]. In addition, the rate of ketogenesis, specifically the production of BHB and not AcAc, is negatively associated with the degree of hepatic triglyceride content [20]. Impaired ketogenesis in severe MASLD has also been consistently observed in both preclinical mouse models and humans [22, 23].

Ketone bodies are primarily generated in the liver during glucose-deprived conditions. Acetyl-CoA, mainly derived from fatty acids through beta-oxidation, undergoes a series of enzymatic reactions within the mitochondria. These reactions involve acetoacetyl-CoA thiolase (ACAT1), 3-hydroxy-3 methyglutaryl-CoA synthase 2 (HMGCS2) and HMG-CoA lyase (HMGCL), generating AcAc as a primary ketone body metabolite. AcAc is then further converted to BHB by β-hydroxybutyrate dehydrogenase (BDH1) [24–26]. Among these critical enzymes in the ketogenic pathways, HMGCS2 is notably implicated in dysregulated ketogenesis in fatty liver disease. In mice with high-fat diet (HFD)-induced MASLD, the fasting-induced increases in HMGCS2 transcript and protein are largely abolished [22]. Similarly, HMGCS2 expression is suppressed with more advanced steatotic stages, such as cirrhosis and HCC [27, 28].

Importantly, dysregulated ketogenesis is not simply an outcome but plays a causal role in the development of fatty liver disease. In infants, deficiencies in HMGCS2 or HMGCL lead to hepatomegaly and hepatic steatosis [29–31]. Consistently, postnatal mice lacking *Hmgcs2* gene spontaneously develop fatty liver disease [22, 32]. The impaired hepatic ketogenic conduit by Hmgcs2 ablation causes excessive accumulation of acetyl-CoA [32, 33]. This, in turn, enhances *de novo* lipogenesis, hepatic glucose production, and acetylation of mitochondrial proteins, which collectively contribute to steatosis and metabolic dysfunctions in the liver. In addition, altered hepatic ketogenesis and ketone body metabolism contribute to the progression of fatty liver disease by modulating inflammation and fibrosis. For instance, ketogenic insufficiency induced by antisense oligonucleotide (ASO)-mediated *Hmgcs2* knockdown in HFD-fed adult mice results in not only elevated hepatic triacylglycerol concentrations but also inflammation and injury with macrophage accumulation in the liver, characteristics of MASH [34–36]. Also, disturbance in hepatocyte-macrophage ketone body communication, specifically via AcAc (not BHB), leads to hepatic fibrosis by activating hepatic stellate cells [37]. Furthermore, hepatic deletion of monocarboxylate transporter 1 (MCT1, encoded by *Slc16a1*), one of the main transporters of ketone bodies [38], exacerbates hepatic steatosis in female mice [39], although it is unclear whether this aggravation of the fatty liver is mediated by impaired ketone body transport. Disruptions in key regulators of ketogenesis, including hormones such as insulin and glucagon and transcriptional regulators like PPARα and mTORC1 [40], also contribute to the development of fatty liver disease. For example, *PPARα* knockout mice, which exhibit impaired ketogenesis with decreased ketogenic enzymes, Hmgcs2 and Bdh1, develop hepatic steatosis [41–43]. Additionally, mTORC1, which suppresses Hmgcs2 expression and ketogenesis by inhibiting the transcriptional activity of PPARα [44], is frequently activated in fatty liver disease [45]. Collectively, these findings underscore the critical role of ketone body metabolism in MASLD development and progression.

Investigations into key enzymes and regulators, such as HMGCS2, BDH1, PPARα, and mTORC1, highlight the intricate interplay between ketone body metabolism and fatty liver disease.

Targeting ketone body metabolism to treat fatty liver disease

Ketone bodies primarily serve as alternative energy fuels in extrahepatic tissues - such as the heart, skeletal muscle, and brain - during various developmental and physiological conditions, including neonatal development, pregnancy, starvation, and exercise. Importantly, the multifaceted roles of ketone bodies in metabolic health have been extensively studied. They mediate cellular signaling via G-protein receptors (i.e., GPR41, GPR43 and GPR109A) and epigenetic gene regulation through post-translational modifications (PTMs), including histone modifications, such as lysine acetoacetylation and β-hydroxybytyrylation [46–48]. These mechanisms collectively exert anti-inflammatory, antioxidative and antifibrotic effects [49–53].

It is noteworthy that elevations in ketogenesis and the administration of ketone bodies can provide significant benefits against the development and progression of fatty liver disease, underscoring the substantial health implications of ketone bodies (Figure 1). Specifically, activating ketogenesis through *Hmgcs2* overexpression improves HFD-induced MASLD in mice and reduces lipid accumulation in HepG2 cells [22]. Concurrently, *Bdh1* overexpression in the liver ameliorates hepatic fibrosis, inflammation and apoptosis in *db/db* mice [54]. In addition, the exogenous administration of AcAc reduces hepatic fibrosis in mice fed a fibrogenic diet [37], while BHB supplementation lessens liver injury and exerts anti-inflammatory effects through the down-regulation of the NLRP3 inflammasome [55–57]. Similarly, dietary supplementation with ketone esters decreases MASLD and inflammation, along with a reduction in the expression of profibrotic and proinflammatory genes, such as *Col1a1* and *Pdgfb* [58, 59]. These findings emphasize the potential therapeutic avenues for addressing MASLD and its progression by targeting ketone body metabolism. There is growing interest in utilizing dietary and pharmacological interventions to enhance ketogenesis for treating hepatic steatosis and its progression, as detailed further below.

Dietary interventions

As ketogenesis has emerged as a potent target for MASLD treatment, dietary interventions that influence ketone body metabolism, such as nutritional interventions and fasting regimens, offer promising approaches for managing MASLD. Indeed, besides various positive effects on health and lifespan, nutritional interventions have demonstrated promising therapeutic impacts on MASLD with decreased hepatic triglyceride content in mice and reduced body fat and inflammation markers in humans [60, 61]. Notably, nutritional interventions, such as caloric restriction (10%–40% reduction) and ketogenic diets, effectively elevate blood ketone body levels and enhance their transport and utilization in both rodents and humans [62–65]. Specifically, caloric restriction, which entails a significant reduction in daily calorie intake, has been shown to decrease hepatic fat content [66], thereby reversing hepatic steatosis in obese rodents with metabolic diseases [60]. In MASLD patients, caloric restriction leads to reductions in fatty liver index and ALT values [67], indicating potential therapeutic benefits. Furthermore, the ketogenic diet, characterized by limited carbohydrate intake, stimulates the mobilization of fatty acids, leading to weight loss in humans and mice. It also effectively increases their blood ketone body levels while improving plasma glucose and triglycerides as well as insulin sensitivity in MASLD patients. A low-carbohydrate ketogenic diet significantly reduces intrahepatic triglyceride levels by 43.8% and alleviates hepatic inflammation and fibrosis in MASLD patients [65, 68, 69]. Consistently, ketogenic diets decrease the expression of genes involved in fatty acid synthesis while upregulating those involved in fatty acid oxidation [70–73]. These beneficial effects of ketogenic diets in the liver are mediated through hepatic fibroblast growth factor 21 (FGF21) as a regulator of the ketotic state [74, 75]. Together, these findings suggest that nutritional interventions are effective strategies for treating MASLD by promoting ketone body metabolism. However, some studies have noted that a ketogenic diet may induce hepatic steatosis, increase inflammation, and promote cellular senescence in mice [64, 76, 77]. Such discrepancies among different studies may potentially be attributed to variations in dietary composition, particularly the fat content, as well as differences in diet duration and the ages of subjects or participants. This underscores the need to carefully evaluate the potential adverse effects of ketogenic diets and understand their underlying mechanisms.

Fasting interventions, such as intermittent fasting and time-restricted feeding, which involve alternating periods of fasting and refeeding [78, 79], are effective in promoting cyclic ketogenesis, thereby potentially improving MASLD [80, 81]. Various intermittent fasting (IF) regimens, such as time-restricted feeding, alternate-day fasting, 2:1 IF, and 5:2 IF, have been shown to improve steatosis by downregulation of PPARγ, a transcription factor implicated in triglyceride homeostasis and activation of fatty acid oxidation via PPARα, in high-fat-fructose induced MASH rat models [82] and HFD-induced MASLD mice [81, 83–85]. Notably, IF also activates the hepatic autophagy-lysosome pathway, reducing hepatic lipid accumulation [84] while diminishing hepatic inflammation and fibrosis through decreased expression of IL-6 and TNFα, thereby mitigating MASH progression [81, 83, 84]. Furthermore, IF has proven effective in humans, reducing intrahepatic triglyceride content by 8.3% [86]. Collectively, these studies highlight that nutritional and fasting interventions can serve as effective therapeutic approaches for MASLD via activating ketogenesis.

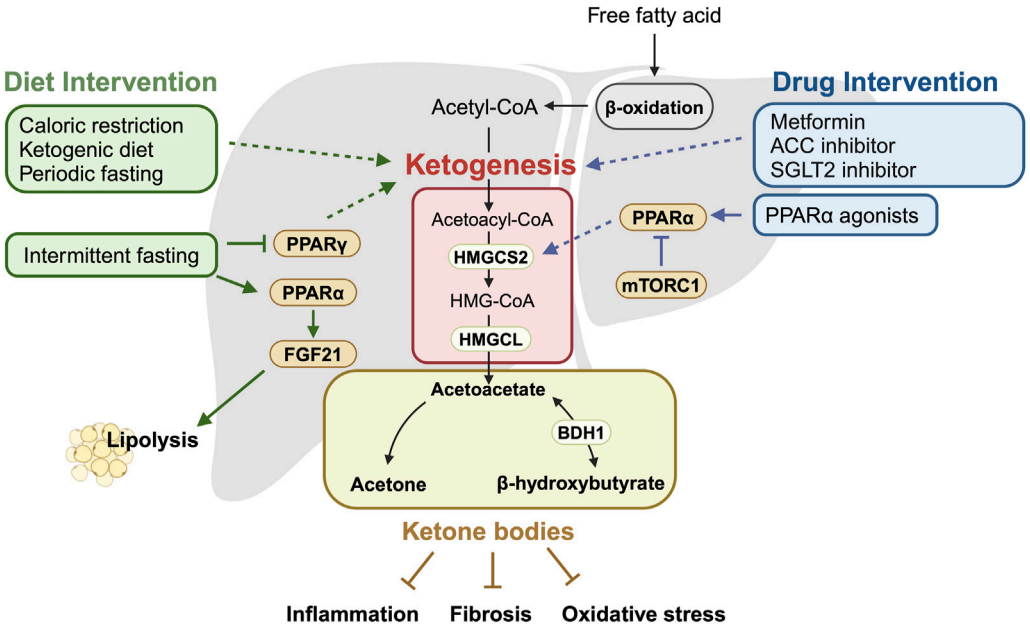

FIGURE 1
Summary of ketogenesis mechanisms in relation to dietary and pharmacological interventions. This schematic illustrates the key enzymes and pathways involved in ketogenesis and how they are modulated by dietary and pharmacological interventions. It highlights the impact of these interventions on the ketogenic pathway, leading to increased production of ketone bodies, which are crucial for managing MASLD. Activated ketogenesis helps reduce liver fat accumulation and inhibit fatty liver disease progression factors, such as inflammation, fibrosis, and oxidative stress. Solid arrows indicate the direction of regulatory effect, while dotted arrows represent effects that are known but not fully understood. The schematic was created with BioRender.com.

Pharmacological interventions

Several pharmacological candidates have shown potential for improving fatty liver disease outcomes by affecting ketone body metabolism. These include Metformin, PPARα agonist (Fibrates), ACC (Acetyl-CoA carboxylase) inhibitors and sodium-glucose cotransporter 2 (SGLT2) inhibitors [14, 87, 88].

Metformin (1, 1-dimethylbiguanide hydrochloride) has demonstrated potential in inhibiting the progression of MASLD. Clinical studies have indicated that metformin treatment in patients with MASLD improves liver function with reductions in hepatic fat accumulation and inflammation [89, 90]. By decreasing hepatic gluconeogenesis, metformin leads to reduced blood glucose levels, which in turn suppresses the activation of lipogenic pathways and promotes hepatic ketogenesis in rat liver [91]. It has also been shown that metformin induces fasting-mimicking metabolic modification, including ketogenesis, in humans [92]. However, the specific molecular mechanism by which metformin affects hepatic ketogenesis remains unclear, and it is unknown whether the metabolic therapeutic effects of metformin are mediated through ketone bodies.

PPARα agonists, such as fibrates, play a crucial role in regulating hepatic lipid metabolism. They have been shown to upregulate the expression of genes involved in fatty liver oxidation and lipoprotein metabolism, potentially contributing to increased ketogenesis [93, 94]. By enhancing these processes, fibrates could improve liver function and reduce hepatic fat accumulation in patients with fatty liver disease. Although fenofibrate has demonstrated efficacy in improving indicators of metabolic syndrome, blood sugar levels, and hepatic function tests in clinical investigations, it has not yielded significant improvement in liver histology, including steatosis score, inflammation grade, and fibrosis stage. To address these limitations, selective PPARα modulators like Pemafibrate have been developed, which offer improved efficacy and safety profiles. Specifically, Pemafibrate has been shown to ameliorate markers of liver inflammation and fibrosis in patients with MASLD [95, 96].

Acetyl-CoA carboxylase (ACC) is a pivotal enzyme in fatty acid synthesis, catalyzing the conversion of acetyl-CoA to malonyl-CoA, a crucial step in hepatic *de novo* lipogenesis. Owing to its central role in lipid metabolism, ACC has emerged as a promising target for therapeutic intervention in fatty liver disease. Numerous studies have demonstrated that inhibition of ACC can effectively reduce fatty acid synthesis and, consequently, decrease hepatic lipid accumulation [97]. For example, Firsocostat (GS-0976), a liver-targeted small molecule allosteric inhibitor of ACC1/2, improves MASH in both preclinical and clinical studies [98]. Additionally, another ACC1/2 inhibitor PF-05221304, either alone or in combination

with a DGAT2 (diacylglycerol O-acyltransferase 2) inhibitor, significantly reduces hepatic steatosis in patients with MASLD [99]. Furthermore, it has been shown that a small molecule IMA-1, which interrupts the arachidonate 12-lipoxygenase (ALOX12)-ACC1 interaction, decreases hepatic lipid accumulation and lowers inflammation and fibrosis in mice and macaques, addressing multiple key features of MASH [100]. Notably, a single oral dose of MK-4074, a liver-specific ACC1/2 inhibitor, increases plasma ketone bodies in mice and humans within 8 h [101], suggesting its strong ketogenic potential. Similarly, the observation that Firsocostat can increase BHB in non-hepatic cells further supports the conserved ketogenic action of ACC inhibition [102]. However, the implications of ketone bodies in ACC inhibitor-mediated hepatic protection have not been explored.

Another class of drugs that have shown promise in the context of ketogenesis and MASLD is the SGLT2 inhibitors, commonly used in the treatment of type 2 diabetes [103]. These drugs increase urinary excretion of glucose by the kidney, thereby reducing blood glucose levels. Beyond their primary use, SGLT2 inhibitors offer therapeutic benefits for MASLD by modulating key metabolic pathways. They promote lipolysis, stimulate mitochondrial biogenesis and autophagy, and reduce lipogenesis, oxidative stress, and fibrogenesis [104, 105]. Meta-analyses have also shown that SGLT2 inhibitors can reduce hepatic enzymes (e.g., ALT and AST), hepatic fat contents, and Fibrosis-4 (FIB-4) levels, suggesting they alleviate MASLD and its progression to MASH [106]. Notably, it is well known that treatment with SGLT2 inhibitors is associated with higher plasma ketone body levels in patients [104, 105]. While the exact mechanism linking SGLT2 inhibitors and ketogenesis is not fully understood [107], it has been suggested that a metabolic shift from glucose to fatty acids induced by SGLT2 inhibitors underlies ketogenesis [104]. Nevertheless, it remains unclear whether the salutary actions of SGLT2 inhibitors against MASLD are mediated by promoting ketogenesis or through SGLT2-independent actions, as observed in the failing heart [108]. Future studies are required to uncover the therapeutic mechanism of SGLT2 inhibitors for MASLD.

Additionally, Pimozide, which blocks skeletal muscle ketone oxidation, increases plasma ketone bodies and improves hyperglycemia [109], yet its effects on fatty liver disease remain unknown. Rapamycin, which inhibits mTORC1, the negative modulator of hepatic ketogenesis, also increases plasma ketone bodies [44]. However, due to its intricate actions in global metabolism and crosstalk with several pathways [110], targeting the mTOR pathway to treat fatty liver disease presents challenges. It is noteworthy that these pharmacological agents appear to promote ketogenesis indirectly, including through transcriptional activation and modulation of metabolic fluxes. The development of drug candidates that directly target ketogenic enzymes and their roles in treating fatty liver disease hold significant interest.

Discussion

In this review, we aim to summarize the current understanding of the potential role of ketogenesis as a critical player in the treatment of fatty liver disease, utilizing both dietary and drug interventions (Figure 1). The contributions of ketogenesis and ketone bodies in MASLD treatment are promising, yet further investigation is warranted to determine the extent to which the beneficial effects result from ketogenesis itself [22], the use of ketone bodies as fuel, or the cellular actions of ketone bodies as signaling molecules, or a combination of these processes [49]. In addition, careful consideration of several factors is required when evaluating treatment options that promote ketogenesis. For instance, ketoacidosis, a life-threatening complication of diabetes, has been reported as a potential side effect of both SGLT2 inhibitors [111] and ketogenic diets [112], though the underlying mechanisms are not fully understood. Furthermore, variations in the effects of ketogenic diets and intermittent fasting due to differences in sex and age have been observed [113, 114], as these factors are also known to impact ketone body metabolism [115]. Consequently, further investigation is essential to safely and effectively leverage ketone body metabolism for the treatment of fatty liver disease.

Author contributions

SK, RJ, and K-HK conceived and designed the research. All authors contributed to the article and approved the submitted version.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the End Diabetes Award from Diabetes Canada to K-HK (OG-3-22-5697-KK). He is also a recipient of the National New Investigator Award from the Heart and Stroke Foundation of Canada (HSFC) and the Early Career Award (ER22-17-236) from the Government of Ontario, Canada. SK was supported by the MITACS Elevate Fellowship (IT34864). RJ was supported by the Natural Sciences and Engineering Research Council of Canada - Undergraduate Student Research Award (NSERC-USRA).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Mini Review
PUBLISHED 26 June 2024
DOI 10.3389/jpps.2024.13210

OPEN ACCESS

EDITED BY
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RECEIVED 30 April 2024
ACCEPTED 14 June 2024
PUBLISHED 26 June 2024

CITATION
Wong A, Sun Q, Latif II and Karwi QG (2024), Metabolic flux in macrophages in obesity and type-2 diabetes. *J. Pharm. Pharm. Sci* 27:13210. doi: 10.3389/jpps.2024.13210

Metabolic flux in macrophages in obesity and type-2 diabetes

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Recent literature extensively investigates the crucial role of energy metabolism in determining the inflammatory response and polarization status of macrophages. This rapidly expanding area of research highlights the importance of understanding the link between energy metabolism and macrophage function. The metabolic pathways in macrophages are intricate and interdependent, and they can affect the polarization of macrophages. Previous studies suggested that glucose flux through cytosolic glycolysis is necessary to trigger pro-inflammatory phenotypes of macrophages, and fatty acid oxidation is crucial to support anti-inflammatory responses. However, recent studies demonstrated that this understanding is oversimplified and that the metabolic control of macrophage polarization is highly complex and not fully understood yet. How the metabolic flux through different metabolic pathways (glycolysis, glucose oxidation, fatty acid oxidation, ketone oxidation, and amino acid oxidation) is altered by obesity- and type 2 diabetes (T2D)-associated insulin resistance is also not fully defined. This mini-review focuses on the impact of insulin resistance in obesity and T2D on the metabolic flux through the main metabolic pathways in macrophages, which might be linked to changes in their inflammatory responses. We closely evaluated the experimental studies and methodologies used in the published research and highlighted priority research areas for future investigations.

KEYWORDS

macrophage, metabolism, obesity, type-2 diabetes, glucose, fatty acids, ketones, amino acids

Introduction

Obesity and type 2 diabetes (T2D) are conditions marked by insulin resistance and persistent low-grade inflammation [1–3]. Chronic tissue inflammation is now recognized as an essential characteristic of obesity and T2D, affecting insulin-target tissues such as adipose tissue, liver, muscle, and heart. The recruitment, accumulation, and activation of pro-inflammatory macrophages in metabolic tissues play important roles in driving this chronic low-grade inflammation. Although other types of immune cells also contribute to these inflammatory processes, macrophages are primary effector cells known to be closely associated with the development of cardiometabolic disease, including obesity and T2D

(see for [3–7] review). Macrophages are crucial immune cells involved in immune response [8] and play important roles in tissue repair and maintaining the body's homeostasis [9]. The inflammatory responses of macrophages are supported by different metabolic pathways (glycolysis, glucose oxidation, fatty acid oxidation, ketone oxidation, and amino acid oxidation) that adjust to their polarization state [10]. Importantly, the metabolic flux through different metabolic pathways could be influenced by the tissue microenvironment, the availability of oxidative substrates, and neurohormonal status. In addition, altered macrophage energy metabolism can impact tissue repair, inflammatory responses, and the severity of insulin resistance [2, 11–13].

Earlier studies suggested that pro-inflammatory (also called M1-like or classical) macrophages are highly glycolytic, while anti-inflammatory (also called M2-like or alternative) macrophages are highly oxidative [14–16]. However, this classification has been challenged as simplistic, as emerging evidence shows that macrophages have a very complex and dynamic metabolic profile that influences their activity. Importantly, "immunometabolism" has emerged as a prerequisite trigger of macrophage activation and phenotype. Nevertheless, minimal research has been steered to understand how the metabolic phenotype of macrophages influences disease progression [17]. Therefore, a better understanding of macrophage metabolism may shed an innovative light on the pathological basis of disease and lead to the future development of macrophage-targeted treatment approaches. In this mini-review, we discussed how carbon flux through the main metabolic pathways in macrophages is perturbed in obesity and T2D and how that influences the inflammatory response, activity and metabolic profile of macrophages.

Glycolysis

Insulin signalling is a key modulator of macrophage metabolism by regulating glucose uptake and oxidation [18, 19]. However, in obesity and T2D, the insulin signalling pathway is impaired, leading to insulin resistance in macrophages [18, 19]. Interestingly, this results in the upregulation of glycolysis and glucose uptake, which is associated with proinflammatory macrophage polarization [20]. This metabolic switch is crucial for the host defence mechanisms of macrophages, such as cytokine production and phagocytosis. Increased glucose influx in insulin-resistant macrophages is facilitated by upregulated glucose transporter 1 (GLUT1) expression [21, 22]. In high-fat diet (HFD) fed mice, the overexpression of GLUT1 in the pro-inflammatory macrophages also led to a hyperinflammatory state with the elevated secretion of inflammatory mediators and increased reactive oxygen species (ROS) production [23]. The metabolomic analysis also demonstrated an increased glucose uptake in the GLUT1 overexpressed macrophages enhances glucose flux through the pentose phosphate pathway, where glucose is utilized to generate NADPH for use in biosynthetic pathways and ROS production [23]. Upon activation, proinflammatory macrophages undergo a "respiratory burst," also called "glycolytic burst," driven by augmented NADPH oxidase activity to generate large amounts of ROS as a defence mechanism against pathogens [24]. To support redox balance, the glycolysis-PPP axis is triggered in response to M1 polarization, presumably to support the increased generation of NADPH for use by NADPH oxidase as well as glutathione production used by macrophages to protect from the excessive amounts of superoxide being produced [24]. This suggests that GLUT1-mediated glucose metabolism plays an important part in driving the pro-inflammatory state of macrophages in obesity and T2D (Figure 1). Consistent with that, genetic deletion of GLUT1 in bone marrow-derived macrophages (BMDMs) displays marked reductions in the classically activated pro-inflammatory markers and associated oxidative stress [25]. Inhibiting glycolysis or treating macrophages with an antioxidant (N-acetyl-cysteine) reversed GLUT1-mediated pro-inflammatory elevations [23].

Increased glucose availability in T2D-induced hyperglycemic conditions can also promote the formation of advanced glycation end products (AGEs) in macrophages [26, 27]. AGEs are pathogenic factors that trigger the activation of a number of signalling pathways in macrophages, including the NF- κB and the MAPK signalling pathways under hyperglycemia conditions [28, 29]. Enhanced AGEs in BMDMs increase interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) production [30]. AGEs also enhance the polarization of macrophages toward the pro-inflammatory state by inducing the expression of pro-inflammatory molecules in T2D [29, 31]. Hypoxia-inducible factor 1 subunit alpha (HIF1α) also plays a critical role in increasing glycolytic flux and abrogating oxidative metabolism (OXPHOS) in macrophages in obesity and T2D [32, 33]. To further support this, HIF-1α gene deletion in mice protected against HFD-induced adipose tissue inflammation and systemic insulin resistance [34].

Glucose oxidation

Increasing glucose uptake in macrophages by overexpressing GLUT1 enhances glucose flux through cytosolic glycolysis and mitochondrial glucose oxidation [23]. While these metabolic changes are associated with promoting the pro-inflammatory phenotype of macrophages, it is still unclear whether the increase in glycolysis and/or glucose oxidation are essential for promoting pro-inflammatory macrophages. Min et al. demonstrated that pyruvate dehydrogenase kinase (PDK), which inhibits the pyruvate dehydrogenase-mediated conversion of cytosolic pyruvate to mitochondrial acetyl-CoA, functions as a metabolic checkpoint in inflammatory macrophages [35]. Deletion of PDK2 and PDK4 completely abolishes the development of pro-inflammatory macrophages in HFD-induced insulin resistance [35]. Inhibition of macrophage glucose oxidation

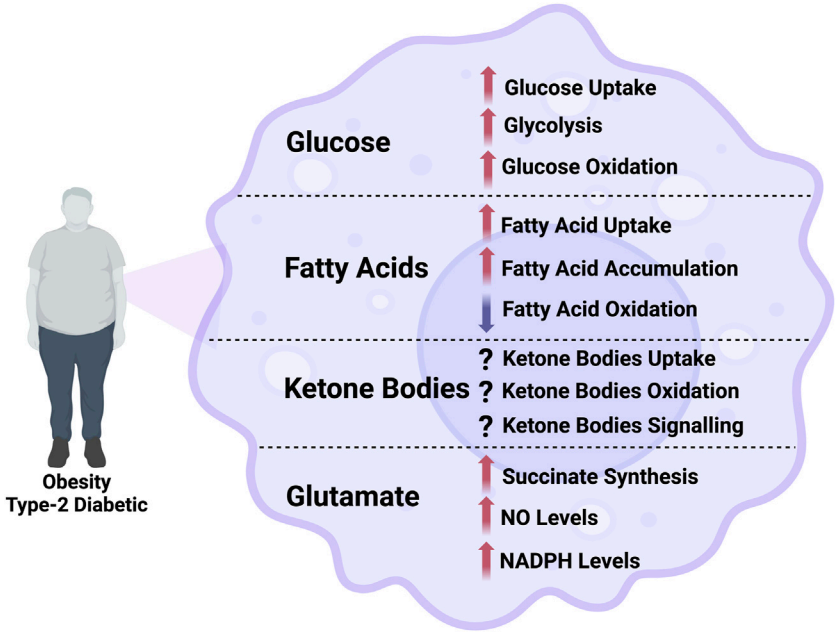

FIGURE 1
Illustration of the alterations in the flux through different metabolic pathways in macrophage in obesity and type-2 diabetes. NO, nitric oxide; NADPH, nicotinamide adenine dinucleotide phosphate.

is also associated with weight loss, reduced insulin resistance, and decreased adipose tissue inflammation [35]. Taken together, inhibiting macrophage glucose oxidation is a potential target to limit the severity of insulin resistance in obesity and T2D (Figure 1).

It has been suggested that the neurogenic locus notch homolog protein 1 (NOTCH1) signalling pathway contributes to the activation of mitochondrial glucose oxidation in obesity. For instance, Xu et al. showed an enhanced macrophage glucose oxidation in obesity that is mediated, at least in part, by increased recruitment of the NOTCH1 intracellular domain (NICD1) to nuclear and mitochondrial genes that encode respiratory chain components [36]. This effect also involved NOTCH-dependent induction of pyruvate dehydrogenase phosphatase 1 (Pdp1) expression, pyruvate dehydrogenase activity, and glucose flux to the tricarboxylic acid (TCA) cycle [36]. This enhancement of glucose oxidation is associated with augmented levels of mitochondrial DNA transcription in pro-inflammatory macrophages, thus causing enhanced mitochondrial ROS levels [36]. Therefore, glucose oxidation may be a target in macrophages to alleviate insulin resistance and inflammation induced by HFD.

Fatty acid oxidation

Obesity and T2D-induced lipid accumulation in adipose tissue are associated with elevated fatty acid uptake, increased macrophage infiltration, and decreased fatty acid oxidation [37, 38]. Moreover, increased lipolysis at the adipose tissue level has been linked to lipid-droplet accumulation in adipose tissue macrophages (ATMs) and obesity-induced inflammation [39, 40]. Studies have shown that macrophages develop a distinct phenotype in obesity exemplified by increased lysosomal acid type lipase, fatty acid receptor, ATP-binding cassette A1 expressions, and inflammatory cytokines (IL-1β and TNF-α) [41, 42]. In addition to acting as fuel for activated macrophages, excessive lipid intake is also shown to be a primary factor that causes pro-inflammatory macrophage polarization in obesity and T2D [43].

The fatty acid translocase CD36 binds to fatty acids and is important for fatty acid uptake at the myocardium, skeletal muscle, gastrointestinal tract, liver, and adipose tissue level [44–46]. In macrophages, CD36 primarily acts as a scavenger receptor, recognizing specific self and nonself molecular patterns and triggering internalization and inflammatory signalling pathways to eliminate pathogens and altered self components, such as apoptotic cells [47]. CD36 cooperates with toll-like receptor (TLR)-4 and −6 to trigger inflammatory responses to altered self-components oxidized LDL (ox-LDL) and amyloid-β [48]. CD36 also acts as a coreceptor with TLR2 and −6 in recognizing microbial diacylglycerides [49]. The deletion of CD36 in BMDMs displayed improved insulin signalling and reduced macrophage infiltration in adipose tissue [50, 51]. This may be attributed to the potential role of upregulated fatty acid uptake in mediating obesity-induced inflammation and insulin resistance (Figure 1), although this is yet to be directly

investigated. Recent studies have shown that CD36 is important in the mitochondrial metabolic switch from oxidative phosphorylation to superoxide production in response to ox-LDL and that mitochondrial reactive oxygen species positively correlate with macrophage CD36 expression [52].

Fatty acid oxidation occurs within the mitochondria, and several steps are required to activate the fatty acids and transport them into the mitochondria [53–55]. Cytosolic fatty acids are first esterified to fatty acyl-CoA (a process consuming two high-energy phosphate bonds as ATP is converted to AMP), followed by the transfer of the fatty acid moiety to carnitine via the action of carnitine palmitoyl-transferase 1 (CPT1) to form fatty acylcarnitine. CPT1, residing on the outer mitochondrial membrane, works collaboratively with carnitine acyltranslocase and carnitine palmitoyl-transferase 2 (CPT2), residing on the inner mitochondrial member, to transfer fatty acylcarnitine into the mitochondrial matrix, where it is converted back to fatty acyl-CoA. These acyl-CoAs then undergo β-oxidation to produce reduced equivalents (NADH and $FADH_2$) for the electron transport chain (ETC), as well as acetyl-CoA for the tricarboxylic acid (TCA) cycle. Malonyl-CoA can be generated by acetyl-CoA carboxylase (ACC) from cytosolic acetyl-CoA [56, 57]. Malonyl-CoA can be converted back to acetyl-CoA by malonyl-CoA decarboxylase (MCD) [58, 59]. While the role of ACC enzymes (ACC1 and ACC2) in macrophages is not fully defined, individual deletion of ACC1 or ACC2 in the myeloid lineage is a prerequisite for the function of highly proliferative T cells, but not for macrophages [60]. Recent studies have shown that ACC is required for the early metabolic switch to glycolysis and remodelling of the fatty acid metabolism in macrophages. Using mice with myeloid-specific deletion of both ACC isoforms, ACC deficiency impairs macrophage innate immune functions, including bacterial clearance [61]. Myeloid-specific deletion or pharmacological inhibition of ACC in mice attenuated LPS-induced expression of proinflammatory cytokines interleukin-6 (IL-6) and IL-1β. In contrast, pharmacological inhibition of ACC increased susceptibility to bacterial peritonitis in wild-type mice [61].

It has been shown that the overabundant influx of fatty acids largely shifts from fatty acid oxidation to triglyceride, phospholipid, and ceramide synthesis, contributing to macrophage lipotoxicity [62–64]. This also contributes to macrophage insulin resistance and the consequential promotion of mitochondrial dysfunction in macrophages [63]. While indirect evidence suggests that altered fatty acid metabolism influences macrophage activation in obesity and T2D [65], it is unknown whether macrophage fatty acid oxidation is upregulated or downregulated in obesity and T2D. Malandrino et al. reported that enhancing fatty acid oxidation in human ATMs reduces ROS, endoplasmic reticulum stress, and pro-inflammatory responses of macrophages [37]. In line with that, the deletion of macrophage carnitine palmitoyl transferase 1A (CPT1A) catalyzes the transfer of the long-chain acyl group in acyl-CoA ester to carnitine. This allows fatty acids to enter the mitochondrial matrix for oxidation and exacerbates the accumulation of diacylglycerols and triacylglycerols after palmitate treatment *in vitro* [66]. CPT1A deletion also increased pro-inflammatory signalling, cytokine expression and endoplasmic reticulum stress after palmitate treatment [66]. Consistent with that, decreasing triglyceride and free cholesterol levels in macrophages mitigates the activation of pro-inflammatory macrophages, supporting the link between lipid accumulation in these cells and the switch to the pro-inflammatory polarization state (Figure 1) [43]. The intermediates of the biosynthetic pathways for triacylglycerol or phospholipids can affect the inflammatory and insulin signalling pathways in different tissues. Saturated fatty acids are also precursors of sphingolipids; in particular, ceramides are strongly linked to insulin resistance and inflammation. TLR4 signalling can trigger ceramide biosynthesis, promoting insulin resistance by activating protein phosphatase 2A and protein kinase C-zeta, ultimately inhibiting Akt [67]. Ceramides may also activate the inflammasome, inducing IL-1β secretion in macrophages, which can blunt insulin signalling [68]. These results might suggest that enhancing macrophage fatty acid oxidation could reduce macrophage activation and mitigate insulin resistance in obesity and T2D. However, this needs to be directly investigated in future research.

Insulin-resistant adipocytes also release greater levels of fatty acids while activating ATMs, leading to an intensified cycle of inflammation through the indirect stimulation of the macrophage toll-like receptor 4 (TLR4) [69, 70]. This causes the initiation of the Jun N-terminal kinase and inhibitor of κB kinase (JNK/IKK- κB) pathways followed by inflammatory cascades [71]. Suganami et al. showed that coculturing obese mice-derived hypertrophied adipocytes and macrophages augments the production of TNF-α in macrophages [69]. The released TNF- α in turn promotes the secretion of FFAs and inflammatory changes in adipocytes [69]. To further support this, TLR4-deletion in BMDMs inhibits saturated FA-induced inflammation via inhibiting palmitate-induced activation of the JNK signalling pathway [72]. Therefore, this shift in fatty acid metabolism towards greater production of inflammatory lipids and levels of FFAs in ATMs exacerbate insulin resistance in obesity and T2D [73, 74]. Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme that produces cellular NADPH, which is required for cellular redox potential and the biosynthesis of fatty acids and cholesterol. Macrophage G6PD levels are increased in the adipose tissue of obese animals, and G6PD mRNA levels positively correlated with those of pro-inflammatory genes [75]. Lipopolysaccharide (LPS) and free fatty acids, which initiate pro-inflammatory signals, stimulated macrophage G6PD. Overexpression of macrophage G6PD potentiated the expression of pro-inflammatory and pro-oxidative genes responsible for the aggravation of insulin sensitivity in

adipocytes. Macrophage G6PD stimulates the p38 mitogen-activated protein kinase (MAPK) and NF-κB pathways, causing a vicious cycle of oxidative stress and pro-inflammatory cascade [75].

Macrophage infiltration to the myocardium also increases due to obesity and T2D-induced inflammation. Saturated fatty acids contribute to myocardial inflammation by inducing the release of inflammatory molecules through pattern recognition receptors (PRRs) in macrophages [71, 72, 76]. Enhanced fatty acid delivery to the heart in obesity and T2D causes uncoupling of fatty acid oxidation from adenosine diphosphate phosphorylation and promotes further mitochondrial dysfunction in cardiomyocytes [77–80]. The imbalance in cardiac lipid metabolism leads to the accumulation of ceramides and diacylglycerols in the hearts of obese and diabetic patients [77, 81–83]. Ceramides activate the NLRP3 inflammasome and further promote cardiac lipotoxicity in palmitate-exposed human cardiac cells and HFD-fed mice [84]. The interplay between altered cardiac fatty acid oxidation and macrophage infiltration into the myocardium in obesity and T2D is an interesting scope for future investigations.

Ketone oxidation

Ketone bodies are organic compounds mainly produced by the liver by breaking down fatty acid molecules. The three major ketone bodies are β-hydroxybutyrate (βOHB), acetoacetate (AcAc), and acetone. βOHB is first oxidized to AcAc by βOHB dehydrogenase, followed by conversion to acetoacetyl-CoA by succinyl-CoA:3 oxoacid-CoA transferase (SCOT). The end-product of ketone oxidation is acetyl-CoA, which has a similar fate as acetyl-CoA produced from fatty acid or glucose oxidation. While there is limited data regarding how ketone metabolism is regulated in macrophages and how it might be altered in obesity and T2D, a recent study using isotope tracking LC/MS untargeted metabolomics showed that macrophages could oxidize ketones with preferential utilization of AcAc compared to βOHB [85]. Preclinical studies have shown that enhancing circulating AcAc levels ameliorates diet-induced hepatic fibrosis, and this protective effect is abolished in macrophage-specific SCOT knock-out mice [85]. These findings suggest that increasing macrophage ketone oxidation plays a critical role in modifying the inflammatory responses of macrophages [85].

Furthermore, another study demonstrated that administration of βOHB increases the expression of IL-10 and arginase 1, markers of the inflammation-resolving state of macrophages and the resolution of damaged intestinal tissue in a mouse model of inflammatory bowel disease [86]. In addition to supporting macrophage energetics, βOHB could also influence macrophage activity by acting as a signalling molecule (see for [87] review). For instance, the inhibitory effect of βOHB on the NLRP3 inflammasome in BMDMs is mediating by acting as a ligand of macrophage GPR109A, a member of the hydrocarboxylic acid GPR sub-family expressed in adipose tissues (white and brown) and immune cells [88]. While these findings suggest that ketone bodies elicit a predominantly anti-inflammatory response, augmented circulating ketone levels in diabetic patients may trigger a pro-inflammatory response (Figure 1) [89–91]. Future studies are required to delineate whether this modulatory effect of ketones on macrophage function is mediated by enhancing increased macrophage ketone oxidation and/or via acting as a signalling molecule [87]. It would also be important to determine how modulating macrophage ketone oxidation affects macrophage function in obesity and T2D. Taken together, this encouraging emerging evidence suggests that ketone bodies have an inhibitory effect on the inflammatory response of macrophages, which might be beneficial against the low-grade chronic inflammation in obesity and T2D and its detrimental effects. However, it is yet to be determined whether ketone bodies modulate macrophage responses by serving as oxidative substrates to modulate macrophage ATP production or acting as signalling molecules.

Amino acid oxidation

The flux through different amino acid metabolic pathways changes according to macrophage phenotype. For instance, arginine is converted to nitric oxide (NO) via inducible NO synthase (iNOS) in pro-inflammatory M1-like macrophages [92, 93]. In contrast, it is converted to proline and polyamines via arginase-1 in inflammation-resolving M2-like macrophages (Figure 1) [94]. Glutamine is the most abundant amino acid in the body and acts as a main source of carbon and nitrogen for cells. While serum glutamine levels are lower in patients with obesity and diabetes [95], studies have demonstrated that glutamine metabolism is altered in macrophages on exposure to M1-or M2-polarizing agents. For instance, glutamine is channelled into the TCA cycle for synthesizing succinate in M1-like macrophages, leading to a marked intracellular accumulation of succinate, enhancing proinflammatory cytokine production [33]. On the contrary, glutamine is critical for acquiring the M2 polarization state. It is mostly converted to α-ketoglutarate in M2-like macrophages and enhances the production of key anti-inflammatory cytokines through its role in protein glycosylation (Figure 1) [96, 97]. Consistent with that, glutamine deprivation impairs the expression of M2-like macrophage markers *in vitro* [97]. Macrophages collected from obese insulin-resistant Zucker rats had a significantly lower NO production than those collected from lean control rats [98]. Interestingly, incubating macrophages from obese insulin-resistant Zucker rats with

glutamine increases NO production [98]. Since NO is produced using arginine as a precursor, these findings suggest crosstalk between arginine and glutamine metabolism in macrophages, highlighting the importance of differential use of amino acids to modulate macrophage responses in insulin resistance conditions. It is important to mention that the action of iNOS in converting arginine to NO depends on the availability of an important cofactor, NADPH. Macrophages can utilize glucose and glutamine to synthesize NADPH [99]. Macrophages collected from insulin-resistant rats have impaired NO production due to impaired synthesis of NADPH and less activation of iNOS in the absence of glutamine [98]. Therefore, suggesting that glutamate contributes to NADPH synthesis in insulin-resistant macrophages seems plausible.

Augmented levels of branched-chain amino acids (BCAAs), namely leucine, isoleucine, and valine, and their respective metabolites, namely branched-chain keto acids (BCKAs), have been linked with metabolic alterations, insulin resistance, and a predisposition to T2D [100]. It has been shown that BCAAs play a role in modulating inflammatory responses in immune cells. However, the data regarding whether high levels of BCAAs promote pro-inflammatory or anti-inflammatory immune cells are inconclusive. For instance, enhancing BCAA levels promotes oxidative stress, inflammation and human peripheral blood mononuclear cell migration [101]. In contrast, high levels of BCAA exert anti-inflammatory and anti-genotoxic activity in LPS-stimulated macrophages [102]. Whether these effects are mediated via enhancing BCAAs and BCKAs contributions to mitochondrial oxidative metabolism as fuel or via acting as signalling molecules in macrophages remains to be determined in future investigations.

Discussion

Recent studies have linked macrophage metabolic processes to their inflammatory behaviour. They demonstrated that macrophages could switch from promoting tissue protection to contributing to disease development by altering the flux through different metabolic pathways. Although macrophage insulin signalling is impaired in obesity and T2D, insulin-resistant macrophages have increased glucose uptake, glycolysis, and glucose oxidation. Macrophage fatty acid uptake is also increased, but it seems uncoupled to fatty acid oxidation, which is decreased in obesity and T2D. Instead, fatty acids are converted to triacylglycerol and ceramide accumulation, which contribute to lipotoxicity in insulin-resistant macrophages. Arginine and glutamate metabolism also have divergent effects in pro- and anti-inflammatory macrophages in obesity and T2D. Understanding the complex metabolic profile of different macrophage phenotypes will help characterize how different oxidative substrates could influence macrophage responses. In addition, understanding the metabolic reprogramming behind macrophage responses will help identify new avenues for therapeutic intervention to combat obesity and T2D.

Author contributions

All authors contributed to the study's conception and design. AW and QS performed a systematic review and critically appraised the literature. They wrote the first draft of the manuscript, and all authors commented on previous versions. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. QK is funded by a Janeway Foundation Research Grant and a Medical Research Fund Cox Award.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 16 July 2024
DOI 10.3389/jpps.2024.13199

OPEN ACCESS

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RECEIVED 28 April 2024
ACCEPTED 02 July 2024
PUBLISHED 16 July 2024

CITATION
Shang R and Rodrigues B (2024), Lipoprotein lipase as a target for obesity/diabetes related cardiovascular disease.
J. Pharm. Pharm. Sci 27:13199.
doi: 10.3389/jpps.2024.13199

Lipoprotein lipase as a target for obesity/diabetes related cardiovascular disease

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Worldwide, the prevalence of obesity and diabetes have increased, with heart disease being their leading cause of death. Traditionally, the management of obesity and diabetes has focused mainly on weight reduction and controlling high blood glucose. Unfortunately, despite these efforts, poor medication management predisposes these patients to heart failure. One instigator for the development of heart failure is how cardiac tissue utilizes different sources of fuel for energy. In this regard, the heart switches from using various substrates, to predominantly using fatty acids (FA). This transformation to using FA as an exclusive source of energy is helpful in the initial stages of the disease. However, over the progression of diabetes this has grave end results. This is because toxic by-products are produced by overuse of FA, which weaken heart function (heart disease). Lipoprotein lipase (LPL) is responsible for regulating FA delivery to the heart, and its function during diabetes has not been completely revealed. In this review, the mechanisms by which LPL regulates fuel utilization by the heart in control conditions and following diabetes will be discussed in an attempt to identify new targets for therapeutic intervention. Currently, as treatment options to directly target diabetic heart disease are scarce, research on LPL may assist in drug development that exclusively targets fuel utilization by the heart and lipid accumulation in macrophages to help delay, prevent, or treat cardiac failure, and provide long-term management of this condition during diabetes.

KEYWORDS

cardiomyopathy, lipoprotein, atherosclerosis, fatty acid metabolism, cardiomyocytes

Introduction

Continuous beating is a distinctive feature of the heart. As such, cardiomyocytes, which are responsible for this heart contraction, have a high requirement for energy and acquire it from several sources like fatty acids (FA) and glucose in addition to amino acids, lactate and ketones. Among these, the majority of ATP produced in the heart is made from glucose and FA through mitochondrial metabolism, with FA being the favored substrate. The heart is unable to synthesize FA and obtains it from other sources. These include a) release from adipose tissue triglyceride (TG) stores, b) endogenous TG within lipid droplets in the heart, and c) breakdown of circulating TG-rich lipoproteins to FA by

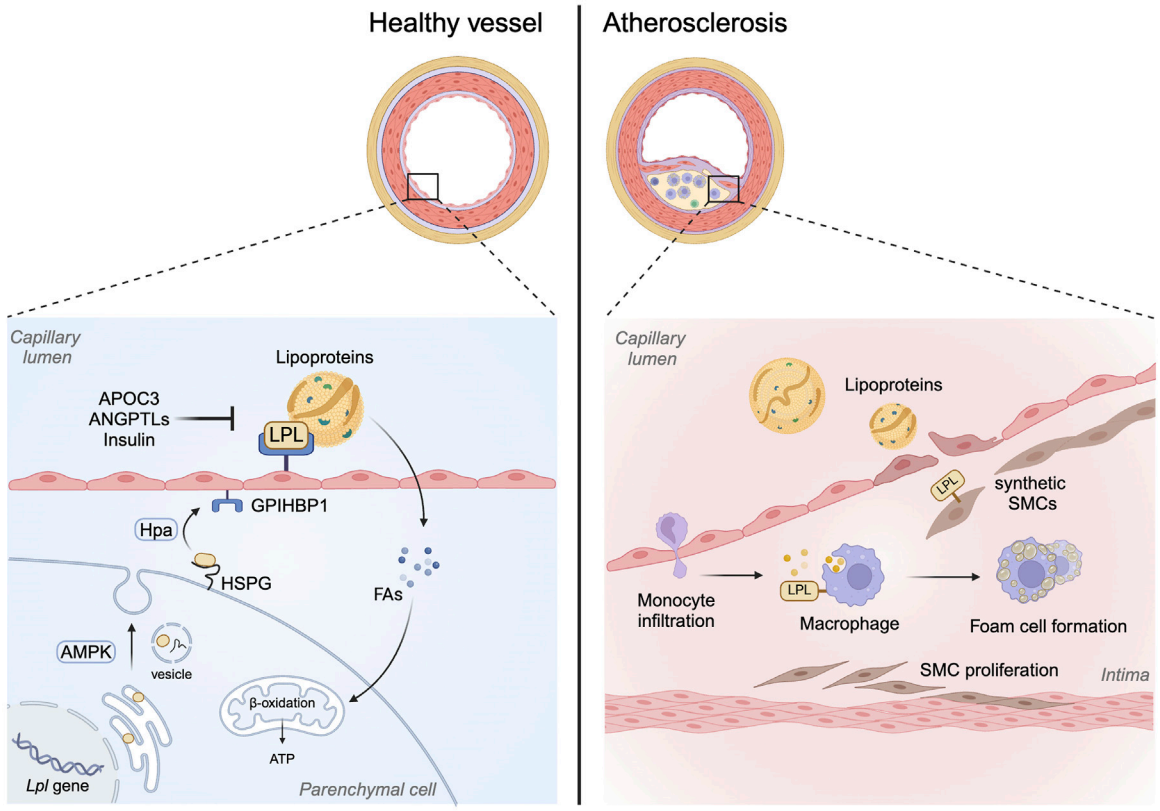

FIGURE 1
Role of LPL beyond its lipolytic action. LPL has traditionally been known to facilitate lipoprotein hydrolysis to release FA. This action of LPL normally occurs at the endothelial lining of the vasculature following the translocation of LPL from subjacent parenchymal cells to the apical side of endothelial cells. Posttranslationally, LPL activity can be regulated by a number of mechanisms including APOC3, ANGPTLs and insulin. On the other hand, in macrophages, the action of LPL is mainly to promote remnant cholesterol uptake, foam cell formation and plaque development in arteries. This bridging function of LPL in SMCs, especially synthetic cells that make up the plaque, may also contribute towards lipoprotein uptake and foam cell formation.

lipoprotein lipase (LPL) positioned at the endothelial cell (EC) surface of the coronary lumen. Of these, LPL-mediated breakdown of lipoproteins is suggested to be a major source of FA for cardiac energy generation. This review will cover the participation of LPL in FA delivery to the heart (for generation of energy) and adipose tissue (for storage as TG), and the consequences of its tissue mismanagement following diabetes. Specifically, we will focus on LPL function and dysfunction, and its contribution towards the development of both atherosclerosis and cardiomyopathy. It is hoped that by understanding LPL regulation and modification following diabetes, we can advance the clinical management of diabetic heart disease as it relates to FA metabolism.

Cardiac lipoprotein lipase—preamble

The breakdown of circulating TG in lipoproteins by LPL occurs in the vascular lumen. However, endothelial cells (EC) that line the lumen are incapable of producing LPL [1–3]. Using the heart (where LPL can be examined at different sites), it has been documented that this enzyme is made in cardiomyocytes before it is moved to the coronary lumen. Thus, immunogold labeling of LPL confirmed that in the heart, about 80% of LPL is present in cardiomyocytes, 18% is located at the capillary EC, and the remaining amount is located in the interstitial space (Figure 1) [4]. Related to its synthesis in cardiomyocytes, LPL has been reported to be produced as a monomer (inactive) in the endoplasmic reticulum. Enzyme activation follows dimerization, with subsequent cellular secretion [5, 6]. Recent evidence has suggested that monomeric LPL also shows enzyme activity [7]. Following its synthesis, there are two proteins that are important for LPL maturation (folding) and transport; lipase maturation factor and suppressor of lin-12-like protein 1 [8]. Intracellular vesicles then store LPL bound to syndecan-1 [9]. Vesicular movement to the cell surface permits LPL secretion onto heparan sulphate proteoglycan (HSPG) binding sites on the outer surface of cardiomyocytes [10]. Attachment of the positively charged LPL to HSPG is made possible by ionic binding to

the heparan sulfate (HS) side chains that is negatively charged. Location of LPL at this site serves as a rapidly accessible pool for the heart when it requires energy in the form of FAs [11, 12]. For its onward movement from the myocyte cell surface to the coronary lumen, LPL is released from HSPG, crosses the interstitial space and binds to glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) on the basolateral side of EC [13]. GPIHBP1 functions as a transporter, moving LPL from the basolateral side of EC to the capillary lumen [14–16]. At the lumen, GPIHBP1 can bind LPL and circulating lipoproteins to promote TG hydrolysis and supply FA to the underlying cardiomyocytes for energy production [16–18] (see detailed review [19]). Interestingly, the action of LPL on chylomicron (lipoprotein produced mainly in the gut) clearance is likely more substantial than its effect on VLDL (lipoprotein synthesised mainly in the liver) hydrolysis. Compared to VLDL, chylomicrons are larger, contain a greater amount of TG and have a better chance to interact with coronary LPL [20]. In addition, LPL breakdown of VLDL yields FA that require a FA transporter, CD36, for movement into cells, whereas its breakdown of chylomicrons produces a higher amount of FA that use a passive flip-flop and/or CD36-independent transport mechanisms [21]. A more recent study produced lipoproteins by administering radiolabelled ^{2}H-FA by gastric gavage. Following isolation and *i.v.* injection of the lipoproteins (d = 1.006 g/mL), a rapid (as quickly as 2 min) accumulation of radiolabelled FA within the cytosol and mitochondria of cardiomyocytes was observed, indicating that EC did not serve as a storehouse for FA as they traveled from the vascular lumen to the underlying cardiomyocytes [22]. Additionally, as a deficiency in CD36 did not modify the passage of lipoprotein FA into cardiomyocytes, the authors concluded that LPL derived FA can be taken up quickly by cardiomyocytes without the need for FA carriers [22]. Finally, when LPL-derived FA are compared to non-esterified fatty acid (NEFA) derived from adipose tissue, our data suggest that following fasting, NEFA plays a central role in energy generation [23], whereas LPL action also provides for lipid accumulation in the heart [24].

Posttranslational processes that regulate cardiac LPL

Of the multiple substrates that the heart can use as an energy source, FA is the preferred fuel. As such, intrinsic mechanisms have been developed by the heart to regulate delivery of this substrate, with LPL being a major player. Several mediators are present which modulate cardiac LPL. These include:

AMP-activated protein kinase (AMPK)

Upon a reduction in energy, AMPK is activated to stimulate energy producing pathways and turn off energy consuming pathways to restore the ATP/ADP ratio. Thus, AMPK is known to inhibit acetyl-CoA carboxylase, lower malonyl-CoA and increase the activity of carnitine palmitoyltransferase-1 to facilitate FA uptake and oxidation in the mitochondria [25, 26]. Stimulation of AMPK is also known to modulate FA uptake via CD36 [27]. Our laboratory has extensively published on the role of AMPK in LPL translocation to the vascular lumen [28]. Specifically, we reported that AMPK plays a role in vesicular formation and subsequent movement of LPL along the actin cytoskeleton in cardiomyocyte through activation of heat shock protein 25 [2]. Thus, physiological and pathological processes that change AMPK are known to impact coronary LPL activity. For instance, following overnight fasting, AMPK is activated to increase coronary LPL, that guarantees FA delivery to meet the energy demand in this nutrient deficient condition [29]. Using streptozotocin (STZ) to induce moderate diabetes in rats, we reported that like fasting, acute hypoinsulinemia stimulated AMPK phosphorylation, and resulted in an augmented coronary LPL activity. This enabled the heart to switch its substrate utilization to exclusively using LPL-derived FA [10]. With a higher dose of STZ to induce severe diabetes, these animals developed both hyperglycemia and severe hyperlipidemia with increased circulating FA [30], that are known to inhibit AMPK activation [31]. In hearts from these animals, LPL activity was reduced and an unregulated uptake of NEFA resulted in cardiac lipotoxicity and dysfunction [30, 32]. Overall, our data suggested that activation of AMPK is a significant contributor towards LPL movement and subsequent FA utilization. Thus, agents that are capable of increasing cardiac LPL activity through the AMPK pathway may be useful for preventing NEFA uptake and lipotoxicity following diabetes.

Heparanase (Hpa)

Hpa is an endo-β-glucuronidase that is produced in EC as an inactive latent protein (Hpa^{L}). Following its synthesis, it is secreted to be taken up by HSPG and stored in lysosomes [33, 34]. At this location, enzyme processing results in a 50-kDa polypeptide that is significantly more active (Hpa^{A}) than Hpa^{L} [35, 36]. Both forms of Hpa are stored within the EC until secreted in response to various stimuli. Related to its physiological functioning, Hpa has roles in embryonic development, wound healing and hair growth [37]. Studies from our lab was the first to identify a novel role of Hpa in cardiac metabolism. In this regard, we described how Hpa released cardiomyocyte LPL for subsequent transfer to the vascular lumen for FA generation [38]. In people living with Type 2 diabetes, plasma and urine levels of Hpa are increased [39, 40]. *In vitro* studies using EC established that acute incubation of these cells with high glucose had a robust influence on Hpa secretion [41]. Using an animal model of STZ-induced diabetes,

isolated hearts released significantly higher amounts of both forms of Hpa within the first 5 min, with Hpa^{L} secretion being greater than Hpa^{A} [42]. Related to Hpa^{A}, its heparan sulfate hydrolyzing ability would be capable of releasing myocyte surface-bound proteins including LPL. Intriguingly, enzymatically inactive Hpa^{L} is also able to initiate HSPG-clustering that activates p38 MAPK, Src, PI3K-Akt, and RhoA [38, 43–46]. These signalling pathways could then allow for replenishment of the cardiomyocyte pool of LPL that was released by Hpa^{A}. Overall, circulating Hpa has an important role in the communication between EC and cardiomyocytes to eventually supply FA to the heart. Intriguingly, unlike high glucose, when EC are exposed to increasing concentrations of palmitic acid, the nuclear content of Hpa was augmented [41]. Moreover, in recently published data from our lab, severe diabetes with concomitant hyperglycemia and hyperlipidemia reduced Hpa secretion from the isolated heart, a possible explanation for the lowered coronary LPL activity in these hearts [47]. Currently, whether manipulation of EC Hpa is capable of influencing cardiac metabolism following diabetes is unknown and should be investigated.

Heparan sulfate proteoglycans (HSPG)

To determine the contribution of HSPG to LPL transcytosis, LPL accumulation was determined following knock-out of GIPHBP1. In this condition, LPL in skeletal muscle and heart collected more at the basolateral side of EC as compared to the cardiomyocyte side suggesting that an HSPG gradient determines the direction of LPL flow from the underlying cardiomyocyte to the basolateral surface of EC [48]. At this location, collagen XVIII also acts as a reservoir for LPL [49].

GPIHBP1

LPL is expressed mainly in parenchymal cells like cardiomyocytes, whereas GPIHBP1 is located exclusively in capillary ECs. Interestingly, a comparable distribution of GPIHBP1 is described at both the luminal or abluminal sides of these cells [16, 50]. It should be noted that ECs that are part of large blood vessels, arterioles and venules do not display GPIHBP1 [16]. Regarding the binding of LPL to GPIHBP1, this occurs at a 1:1 ratio and with a higher affinity when compared to its binding to HSPG [51]. Structurally, as GPIHBP1 contains a GPI anchor, its release from the plasma membrane is achievable with phosphatidylinositol-specific phospholipase C that is known to digest this anchor [14]. It is the acidic domain of GPIHBP1 that can ionically attach LPL [52]. Given its defined role in the bidirectional translocation of LPL across the EC [16], and its ability to serve as a platform to promote lipoprotein-TG hydrolysis (it allows lipoproteins to stay bound/marginate to heart capillaries for several minutes [53, 54]), its absence in GPIHBP1 knockout mice causes robust hypertriglyceridemia even when these animals are fed a low-fat diet [16]. Similar effects are seen in patients with GPIHBP1 mutations [55]. More recent functions of GPIHBP1 include its ability to prevent the unfolding of LPL by angiopoietin-like protein 4 (ANGPTL4) [56–58]. Regarding its regulation, GPIHBP1 expression can be affected by fasting/refeeding [59]. Fasting augments cardiac GPIHBP1, and this effect can be overcome by refeeding [59]. Following diabetes, cardiac GPIHBP1 gene and protein expression also increase with an associated augmentation of coronary LPL activity [60]. Moreover, *in vitro* incubation of EC with high glucose also caused a rapid increase in GPIHBP1 mRNA and protein [50, 61]. Intriguingly, exposure of EC to Hpa^{L} or Hpa^{A} produced a significant increase in GPIHBP1 gene and protein [50]. Given that high glucose can stimulate the secretion of both forms of Hpa, this could be one mechanism by which the EC can increase GPIHBP1 to accelerate FA delivery to the cardiomyocytes after diabetes.

Angiopoietin-like proteins (ANGPTLs) regulation of LPL

ANGPTL 3, 4, and 8 are endogenous LPL antagonists. ANGPTL3 is exclusively expressed in the liver whereas ANGPTL4 and 8 are abundant in the liver, adipose tissue and muscle. One way by which fasting decreases LPL in the adipose tissue is that nutritional deprivation increases ANGPTL4 in adipose tissue. This is a positive effect as circulating TG are then diverted towards oxidative tissues for provision of energy [62].

Fatty acids (FA)

FA is known to affect LPL in multiple ways, including a) FA inhibition of LPL movement in the cardiomyocyte [63], b) FA suppression of Hpa secretion, thus reducing cardiomyocyte to EC transfer of LPL [41], c) FA detachment of vascular LPL for hepatic degradation [64], and d) FA inactivation of LPL, either directly [65] or through induction of ANGPTL4 [66–68]. With severe diabetes, animals developed hyperlipidemia that was associated with a reduction in heparin-releasable LPL activity in the heart [32]. This occurred in the absence of any change in LPL gene expression [69] suggesting that following diabetes, cardiac LPL activity is mainly modulated by post-translational mechanisms. In this regard, when RNA-seq was performed in diabetic hearts, of the more than fifteen hundred differentially expressed genes, the one that showed the greatest fold change (~25-fold increase) was ANGPTL4 [30]. Altogether, these results imply that circulating FA has the ability to supress vascular LPL by a host of mechanisms to prevent lipid overload of the heart.

Insulin

Changes in circulating insulin can affect LPL and this response varies with the tissues being studied [70]. Thus, a reduction in insulin after fasting decreases adipocyte LPL but enhances its activity in the heart [71], changes that occurred in the absence of LPL gene or total protein expression [29]. Consequently, the FA that are produced from lipoprotein-TG lipolysis by LPL are directed away from storage in the adipose tissue so that they can fulfill the metabolic demands of cardiomyocytes. As newly synthesized LPL can transfer from myocytes to the vascular EC within 30 min, an augmented vectorial movement of LPL could explain the rapid increase of coronary LPL following fasting [72]. Mechanistically, a reduction in insulin after fasting or STZ-induced diabetes decreases glucose uptake in the heart resulting in activation of AMPK [1, 73, 74] with stimulation of LPL translocation [2] (see detailed review [19]).

Apolipoproteins

Activators of LPL include Apolipoproteins (Apo) C-II and Apo A-V, whereas inhibitors include Apo C-III. Apo-CII is produced primarily in the liver and then incorporated into lipoproteins. On binding to LPL, it promotes conformational changes in the enzyme, allowing the catalytic site of LPL to interact with lipoproteins permitting their hydrolysis [75]. Apo A-V increases the activity of Apo C-II [76, 77].

Oscillations in cardiac LPL following diabetes and its impact on plasma triglycerides

In the clinical setting, plasma LPL activity is determined after infusion of heparin to release HSPG-bound LPL [12, 78]. The downside with this method is that the measured LPL represents enzyme that is released from a host of different tissues (heart, skeletal muscle, adipose tissue). Regarding tissue-specific detection of LPL following diabetes, adipose tissue and skeletal muscle show low levels of enzyme in homogenates [79], with virtually no information available on the cardiac content of this enzyme. Even if heart homogenates are used to determine LPL levels, this would only provide an estimation of total cardiac LPL and would not correctly reflect the enzymatically active LPL at the vascular lumen. Hence, studies in animals have provided the key source of information regarding LPL biology in the diabetic heart. Thus, acute insulin resistance following administration of dexamethasone [80, 81] or hyperglycemia and hypoinsulinemia in rats injected with 55 mg/kg STZ (D55) causes a significant increase of heparin-releasable LPL at the coronary lumen [10, 32, 82, 83]. This increase occurred due to a rapid filling of the unoccupied HSPG-binding sites [70, 82, 84] and was independent of changes in LPL gene and protein expression [82, 85]. Occupation of these empty HSPG-binding sites at the EC surface was mediated by enhanced translocation of LPL [29, 38, 41, 63, 69, 70, 86–91].

For a considerable period, it has been undecided whether the hypertriglyceridemia following diabetes was an outcome of augmented synthesis of VLDL-TG from the liver or a product of reduced clearance of lipoprotein-TG by LPL. In the moderately diabetic D55 animals with 2-fold increase in cardiac LPL activity, circulating TG and NEFA were maintained at levels that were comparable to control animals [30]. Willecke et al. using STZ-diabetic mice revealed that VLDL secretion remained unchanged in these mice [92]. Interestingly however, TG clearance was significantly reduced and was related to a reduction in skeletal muscle, cardiac, and brown adipose tissue LPL mRNA/activity, suggesting that LPL clearance of TG is the more important contributor.

In diabetes, an increase in circulating and intracellular TG are risk factors associated with atherosclerotic cardiovascular disease and cardiac lipotoxicity [93]. It is possible that in D55 heart, LPL derived FA are directed towards oxidative metabolism rather than storage (Figure 2) [30]. Intriguingly, similar to our observations in the moderately diabetic D55 heart, a modest overexpression of LPL in adipose tissue was associated with better glucose and insulin tolerance [94]. When these animals were provided a high fat diet, weight gain was not observed. In fact, dietary lipids did not accumulate in adipose tissue, and the animals displayed amplified energy expenditure. The authors proposed that a moderate increase in adipose LPL has favourable effects on total body energy metabolism. In contrast, we also observed a decline in LPL, both in animals infused with Intralipid [95] and with severe diabetes induced by injecting 100 mg/kg STZ (D100) [10, 30]. As the D100 diabetic animals exhibit elevated plasma FA, we concluded that LPL-mediated FA delivery would be redundant in these circumstances and is “turned off.” Additionally, this reduced cardiac LPL likely contributed to the robust increase in circulating TG. We have previously shown that when FA uptake by LPL action is augmented, this competes with NEFA uptake [23]. Thus, following severe diabetes, when cardiac LPL action is reduced, NEFA uptake and oxidation takes precedence over provision of FAs to the heart from circulating lipoproteins. This excessive supply of NEFA overwhelmed the mitochondrial capacity, leading to a mismatch between FA delivery and utilization, lipid metabolite build-up and cell death (Figure 2) [30]. Thus, approaches that maintain cardiac LPL would be a useful therapeutic approach to preventing cardiac pathology seen following diabetes that is poorly controlled.

Role of LPL in development of atherosclerosis

Atherosclerosis is defined as a thickening (and loss of elasticity) of the arterial intima as a consequence of lipid

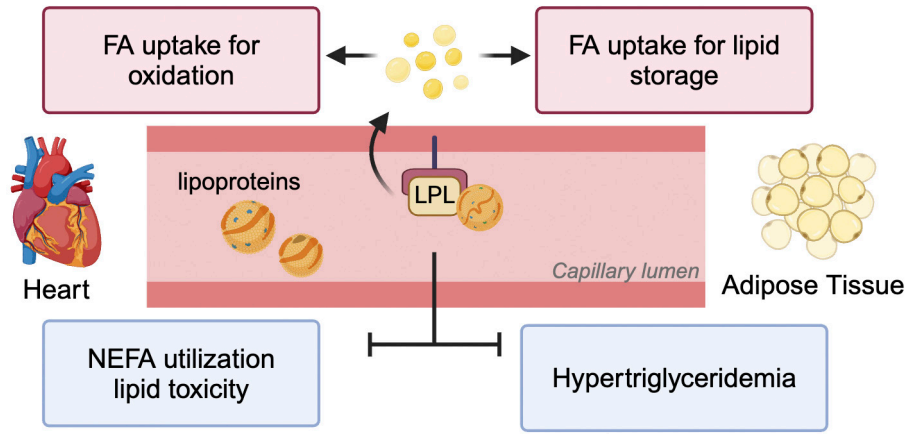

FIGURE 2
Impact of LPL-derived FA on heart and adipose tissue. LPL positioned at the vascular lumen is responsible for lipoprotein-TG break down to release FA for multiple purposes. These include uptake into the heart for generation of energy and adipose tissue for lipid storage. In pathological conditions like diabetes when LPL activity is reduced in both these tissues, the reduction in TG clearance results in hypertriglyceridemia. Additionally, the heart switches to using NEFA for oxidation leading to excessive TG accumulation and lipotoxicity.

accumulation [96]. During this condition, there is narrowing or obstruction of the vessel lumen and thinning of the vessel wall. In preclinical animal models, atherosclerosis is a progressive disease beginning with the development of fatty streaks and potentially leading to complicated atherosclerotic plaques that can rupture, set up thrombosis and occlude the lumen. The clinical manifestations of atherosclerosis are dependent on the site of lesion. Hence, its presence at the coronary arteries leads to angina pectoris and myocardial infarction, at the central nervous system it causes transient cerebral ischemia and stroke whereas its occurrence in the peripheral circulation elicits peripheral vascular disease [96]. The level of plasma cholesterol, and in particular LDL-associated cholesterol, is one of the main risk factors of atherosclerosis [97]. Following LDL infiltration and trapping in the arterial intima with potential oxidative modification, ox-LDL causes endothelial cells to express monocyte chemoattractant protein (MCP-1). MCP-1 attracts monocytes from the vessel lumen into the subendothelial space, one of the very early stages in the development of atherosclerosis. Modified LDL also promotes differentiation of monocytes into macrophages which avidly take up the ox-LDL. This accumulation transforms macrophages into lipid-rich foam cells, that are the hallmark of atherosclerosis [96]. Engorgement of foam cells with lipids causes release of cytokines, and eventually cell death. Macrophage/foam cell-released proteolytic enzymes (matrix metalloproteinases, MMPs) allows for smooth muscle cells from the adjacent media to migrate into the intima, proliferate and secrete fibrous connective tissue (i.e., collagen) and extracellular matrix (smooth muscle cells change their phenotype from contractile to synthetic cells). This makes the lesion harder and contributes to the formation of a fibrous cap (which includes a mixture of macrophages, lipid and cell debris which form a necrotic core). The expanding intima pushes against the endothelial wall of the intima and the fibrous cap is very susceptible to rupture. MMPs also cause a thinning of the fibrous cap with eventual cap destruction along with a host of other events like platelet aggregation and adhesion, thrombosis and clot formation. The rupture of such lesions is believed to be responsible for most cases of unstable angina and acute myocardial infarction. Dislodging of the clot blocks the artery near the plaque or in a more distal and narrower segment causing total or near total occlusion [96, 98].

LPL in macrophages

Given the contribution of LPL in supplying FAs to various tissues for storage and energy generation, in addition to its role in plasma lipoprotein clearance, the action of LPL is considered beneficial. Thus, the contribution of LPL towards the etiology of atherosclerosis is contentious. On the one hand, overexpression of LPL has been shown to protect against diet-induced atherosclerosis in $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice, established animal models to study atherosclerosis [99, 100]. This protective effect of LPL was linked to beneficial changes in plasma lipoproteins. On the other hand, this was not the case when LPL levels were manipulated in macrophages. Specific deletion of macrophage LPL (with no changes in total plasma LPL activity) significantly reduced atherosclerotic lesions in $Apoe^{-/-}$ mice, supporting an important role for macrophage LPL in atherosclerosis [101]. Similarly, overexpression of human LPL in rabbit macrophages accelerated atherosclerotic plaque development, and this

occurred in the absence of any changes in plasma lipids [102]. These studies reinforced a pro-atherogenic role for macrophage LPL. It is worth noting that although LPL has abundant expression in highly oxidative tissues, it is also detected in tissues like the kidneys, brain and macrophages where its binding properties are likely more important than its lipolytic activity [103]. Thus, the effect of macrophage LPL on atherosclerosis could be the result of its lipolytic activity and/or its ability to act as a receptor to assist remnant particle uptake. Related to the latter function of LPL, subsequent to lipolysis of lipoproteins, the remnant particles that detach from the endothelial GPIHBP1 platform have inactive LPL bound to them. It has been proposed that this inactive LPL may act as a hepatic receptor ligand to promote lipoprotein uptake [104–106]. When this process occurs in macrophages, LPL acted as a bridging molecule for remnant uptake to increase foam cell formation and lesion progression (Figure 1) [107].

LPL in smooth muscle cells (SMC)

The above discussion brings forward the contribution of macrophages towards foam cell formation and development of atherosclerosis. However, more recent studies have also implicated SMCs in foam cell formation, at least in humans [108]. Thus, in mouse models of atherosclerosis, the lipid milieu along with the resident inflammation recruits' monocytes across EC to initiate atherosclerosis [109]. In contrast, in human atherosclerosis, SMCs migration from the arterial media occurs before lipid accumulation and in fact SMC make up almost 50% of the foam cells in the plaque lesion [108]. It is possible that SMCs take up lipoprotein remnants through expression of scavenger receptors. However, LPL expression in SMC may also contribute to this mechanism. *In vitro*, SMC and macrophages synthesize LPL [110] which could act as a co-receptor to facilitate the binding of native and ox-LDL to HSPG (Figure 1) [111]. Interestingly, LPL has been detected in the fibrous cap of the atherosclerotic lesions [112]. Whether SMCs in the atherosclerotic lesion synthesize LPL *per se* or whether it is transferred from the macrophages is currently unknown. It should be noted that at least in the heart, the translocation of LPL has been reported from cells like the cardiomyocyte to endothelial cells [86]. In support, SMC interact with macrophages both directly and indirectly. For example, in the presence of macrophages, SMC increases their phagocytic activity by enhancing LPL and proteoglycans to promote lipoprotein uptake [113].

LPL as a therapeutic target

Given the importance of LPL in FA delivery to multiple tissues in addition to its contribution to atherosclerotic plaque development, there are a number of pharmaceutical approaches that have been attempted to lower cardiovascular risk by targeting LPL. These include:

Incretins

Oral glucose causes the release of gut hormones like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) that amplify glucose-induced insulin secretion in addition to acting via multiple mechanisms to influence blood glucose [114]. Drugs that mimic GLP-1 (e.g., Semaglutide) and/or GIP (e.g., Tirzepatide) are gaining in popularity not only due to their control of blood glucose but also due to their ability to promote weight loss [115, 116]. Intriguingly, cardiovascular outcome trials demonstrate that long-term use of GLP-1 receptor agonists reduce cardiovascular complications of diabetes [117] or obesity [118] by mechanisms that not completely understood. It has been proposed that incretins could offer cardioprotection via their influence on lipid metabolism. In this regard, GLP-1 has been shown to regulate secretion of lipoproteins and cholesterol metabolism [119, 120]. GIP, on the other hand, demonstrated a regulatory role in lipid metabolism that occurred partially via LPL activation. Thus, in cultured 3T3-L1 cells and human adipocytes, GIP stimulated LPL activity in a dose-dependent manner [121–123]. These results were supported by *in vivo* studies which reported that GIP accelerated chylomicron TG clearance in dogs [124]. Similarly, human studies revealed that GIP infusion significantly increased LPL action, where LPL-derived FA largely contributed to an increase in re-esterification rate and TG storage in adipose tissue of lean individuals [125]. However, unlike adipose tissue, the effects of GIP on cardiac LPL level remain unclear. Given the opposing mechanisms of LPL regulation between adipose tissue and the heart, it is possible that GIP lowers cardiac LPL activity. Interestingly, eliminating GIP receptor signaling protected the heart against experimental myocardial infarction, and this was associated with reduced phosphorylation of HSL and increased cardiac TG storage [126]. As intramuscular TG accumulation is predominantly regulated by the action of HSL and LPL [127], the contribution of cardiac LPL to the altered lipid accumulation and the cardioprotective phenotype of Gipr−/− mice following myocardial infarction would be interesting to study.

Apolipoprotein and angiopoietin-like protein inhibitors

Apolipoprotein C3 (APOC3) and angiopoietin-like protein (e.g. ANGPTL3) are known to inhibit LPL activity directly. Thus, a genetic reduction in *APOC3* increases LPL activity, reduces plasma TG and causes a decrease in coronary heart disease [128].

Related to targeting APOC3, two antisense oligonucleotides, Olezarsen and Volanesorsen, are currently in Phase 3 clinical trials to determine their therapeutic potential to lower circulating TG [129, 130]. Similar to APOC3, genetic variants in *ANGPTL3* impacts plasma TG [131]. As such, Evinacumab (human monoclonal antibody) is currently on the market to inhibit the action of APOC3 to lower TG levels [132].

Omega-3 fatty acids (icosapent ethyl)

Used in statin-treated patients with elevated TG (≥150 mg/dL), who are at high risk of cardiovascular events due to established cardiovascular disease, or diabetes, and at least one other cardiovascular risk factor. Mechanism of action not completely understood and likely multifactorial and includes a decreased production and accelerated clearance of triglycerides [133]. It decreases VLDL synthesis and secretion by a) reducing hepatic lipogenesis (synthesis of FA from acetyl CoA), b) increasing beta-oxidation of FA and c) inhibiting TG-synthesizing enzymes (e.g., DGAT). It increases VLDL clearance by a) augmenting LPL activity directly or b) indirectly by reducing APOC3, an inhibitor of LPL. It's a unique form of omega-3 fatty acid (eicosapentaenoic acid) that reduces VLDL-TG. Affects multiple atherosclerotic processes including endothelial function, oxidative stress, foam cell formation, inflammatory response (its anti-inflammatory), platelet aggregation and plaque rupture (it causes plaque regression) [134, 135].

Fibric acid derivatives

Also called fibrates (e.g., fenofibrate and bezafibrate). Function primarily as peroxisome proliferator-activated receptors (PPAR) agonists (stimulates the PPARα receptor), thereby increasing the oxidation of fatty acids in liver (decreases VLDL production) and striated muscle (also kidney and heart). Also increases LPL activity (increased catabolism of circulating TGs increases the rate of clearance of TG) by both transcription upregulation of LPL and down regulation of APOC3 (an inhibitor of LPL) [136, 137].

Statins

They are reversible, competitive inhibitors of HMG-CoA reductase. As a result, there is inhibition of intracellular cholesterol synthesis mainly in the liver. Because a precise amount of cholesterol is required in cells, on decrease of intracellular cholesterol, hepatocytes increase the expression of LDL receptors which then promote the extraction of LDL cholesterol from plasma secondarily. Are also known to influence LPL, especially in patients with Type 2 diabetes [138, 139]. These effects of statins occurred in a tissue specific manner, with an increased LPL production observed in skeletal muscle [140] and a decrease in LPL mass reported in macrophages [141].

Discussion

The enzyme LPL is essential for circulating TG clearance, FA delivery for both oxidation and storage, and for prompting lipoprotein uptake by acting as a receptor. Given these multiple functions, changes in LPL would be expected to have diverse consequences. For example, a decrease in adipose tissue LPL would impede lipoprotein clearance resulting in augmented plasma lipids. In the heart, reduction in LPL, as observed with severe diabetes, causes a switch in substrate utilization to predominantly NEFA. This overwhelms the mitochondrial oxidative capacity leading to TG storage and lipid toxicity. However, depletion of macrophage LPL demonstrated beneficial effects against the development of atherosclerosis. Thus, when attempting to modulate LPL levels, one should consider the tissue and cell type in addition to the disease entity. In this regard, tissue or cell-specific manipulation of LPL offers promise to overcome the cardiac complications associated with obesity and diabetes.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by an operating grant from the Canadian Institutes of Health Research [CIHR PJT-178134].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 19 April 2024
DOI 10.3389/jpps.2024.12568

OPEN ACCESS

EDITED BY
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RECEIVED 14 December 2023
ACCEPTED 09 April 2024
PUBLISHED 19 April 2024

CITATION
Nakamura M (2024), Lipotoxicity as a therapeutic target in obesity and diabetic cardiomyopathy.
J. Pharm. Pharm. Sci 27:12568.
doi: 10.3389/jpps.2024.12568

Lipotoxicity as a therapeutic target in obesity and diabetic cardiomyopathy

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Unhealthy sources of fats, ultra-processed foods with added sugars, and a sedentary lifestyle make humans more susceptible to developing overweight and obesity. While lipids constitute an integral component of the organism, excessive and abnormal lipid accumulation that exceeds the storage capacity of lipid droplets disrupts the intracellular composition of fatty acids and results in the release of deleterious lipid species, thereby giving rise to a pathological state termed lipotoxicity. This condition induces endoplasmic reticulum stress, mitochondrial dysfunction, inflammatory responses, and cell death. Recent advances in omics technologies and analytical methodologies and clinical research have provided novel insights into the mechanisms of lipotoxicity, including gut dysbiosis, epigenetic and epitranscriptomic modifications, dysfunction of lipid droplets, post-translational modifications, and altered membrane lipid composition. In this review, we discuss the recent knowledge on the mechanisms underlying the development of lipotoxicity and lipotoxic cardiometabolic disease in obesity, with a particular focus on lipotoxic and diabetic cardiomyopathy.

KEYWORDS

heart failure, lipotoxicity, obesity, diabetes, diabetic cardiomyopathy, lipotoxic cardiomyopathy, cardiometabolic disease, inflammation

Introduction

Lipid metabolism plays a pivotal role in diverse physiological processes in the heart [1]. Obesity, defined as an increased body mass index (BMI) resulting from abnormal or excessive fat accumulation, is a disease that impairs health. Obesity perturbs lipid metabolism across nearly all tissues, giving rise to ectopic and excessive lipid accumulation, where lipids become toxic, termed lipotoxicity. Overweight and obesity are commonly linked to cardiac diseases, including cardiac hypertrophy, remodeling, and cardiomyopathy [2, 3]. By focusing on the intricate pathways that govern lipid metabolism, there is potential for therapeutic interventions to improve cardiovascular outcomes in patients with cardiovascular disease (CVD) [4–6].

The population with overweight or obesity is increasing worldwide [7]. Based on the NCD Risk Factor Collaboration, 2 billion adults (39% of the world's adult population) were estimated to be overweight and 671 million (12% of the world's adult population) of whom had obesity. In the United States, the prevalence of being either overweight or obese

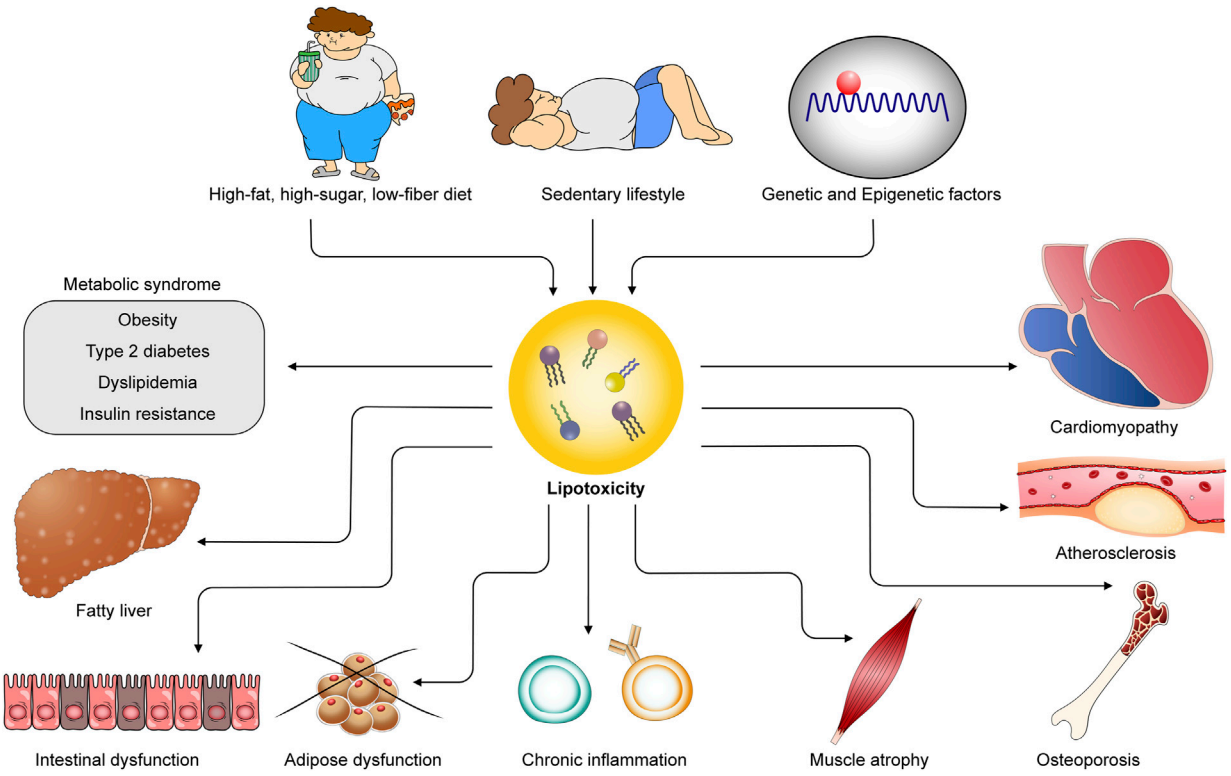

FIGURE 1
Obesogenic environments and genetic factors contribute to lipotoxicity, leading to cardiometabolic diseases. Unhealthy diets, sedentary lifestyles, and genetic and epigenetic influences provoke excess lipid accumulation in both adipose and non-adipose tissues, giving rise to lipotoxicity. Lipotoxicity underlies the pathogenesis of diverse cardiometabolic diseases, including metabolic syndrome, cardiomyopathy, atherosclerosis, fatty liver disease, intestinal dysfunction, adipose dysfunction, chronic inflammation, and osteoporosis.

is 36.8% in children and adolescents and the age-adjusted prevalence of overweight or obesity is 71.2% in adults [8]. The clinical diagnosis of obesity is made with a BMI ≥30 kg/m^2 or ≥27.5 kg/m^2 for people of Asian origin, calculated as weight in kilograms divided by height in meters squared. Individuals who are overweight or obesity are more prone to developing hypertension, dyslipidemia, type 2 diabetes, fatty liver disease, osteoarthritis, cancers, obstructive sleep apnea, and CVD (Figure 1). Every 5 kg/m^2 increase in BMI is associated with 41% increased risk for the development of heart failure [8]. However, according to the fact sheets provided by the World Health Organization, obesity is not merely a risk factor, but rather classified as a disease[1]. Obesity is linked to higher mortality rate [9]. All-cause mortality is lowest at about 22.5–25 kg/m^2 of BMI and higher degrees of obesity are associated with progressively premature mortality with a reduced median survival by 8–10 years at class 3 obesity, defined as a BMI 40 to <45 kg/m^2, due mainly to CVD [10]. The data presented strongly support the notion that targeting obesity should be a priority in order to prevent cardiometabolic disease.

The pathogenesis of obesity is complex and multifactorial (Figure 2). The traditional studies that search genetic variants for obesity susceptibility with a combination of genetically altered mouse models ("The human obesity gene map") unveiled the relationship between single-gene mutations and obesity, as well as elucidated certain causal links between them [11] (Figure 2C). Genome-wide association studies over the past two decades have identified a number of genetic loci associated with obesity and estimated that common genetic variants may account for >20% of the variation in BMI [12]. Monogenic obesity, which arises from chromosomal deletions or single-gene defects, is typically a rare condition that follows a Mendelian pattern of inheritance. It is characterized by early-onset and severe obesity. Conversely, polygenic obesity, also referred to as common obesity, is attributed to the influence of multiple polymorphisms. Each genetic locus is considered to have a small effect on the susceptibility to obesity. However, recent studies on gene discovery have uncovered a striking similarity in the

1 https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

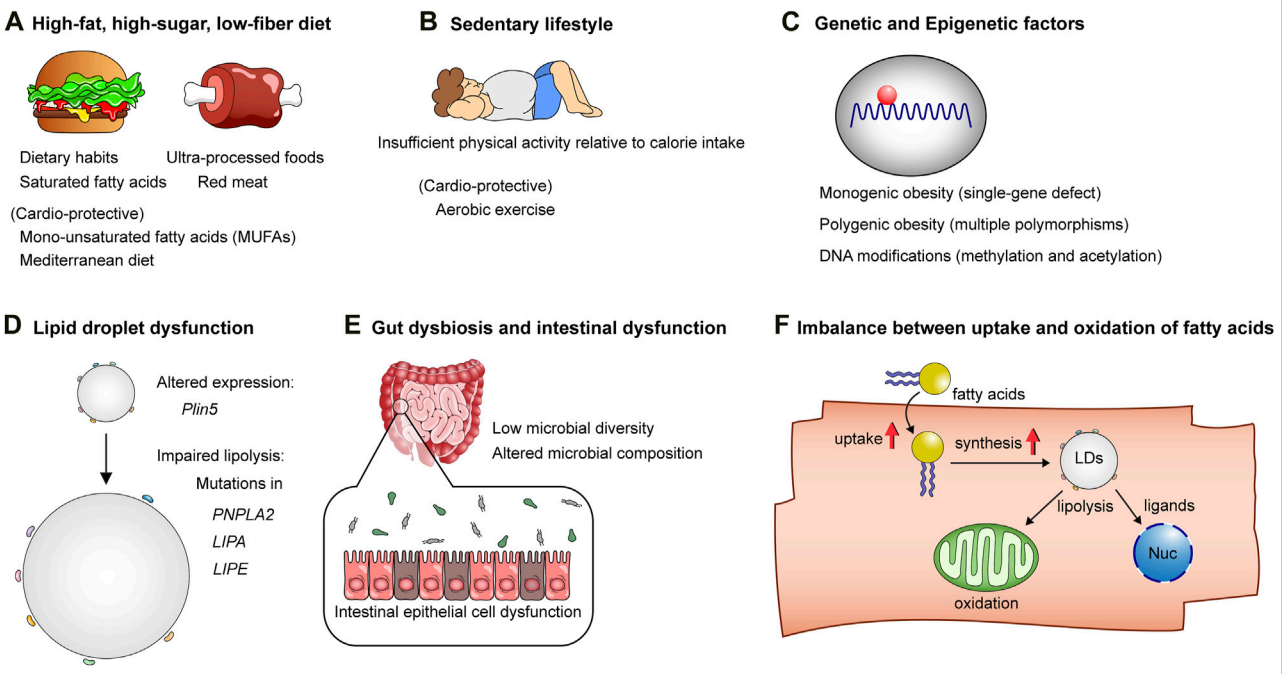

FIGURE 2
The mechanisms underlying obesity and lipotoxicity. **(A)** High-fat, high-sugar, low-fiber diets: Unhealthy dietary habits and patterns, characterized by saturated fatty acids, ultra-processed foods, and red meat, contribute to the onset of obesity and cellular lipotoxicity, while diets rich in mono-unsaturated fatty acids (MUFAs) and Mediterranean diets are considered healthy alternatives, counteracting the lipotoxic effects of unhealthy dietary patterns. **(B)** Sedentary lifestyle: Insufficient physical activity relative to calorie intake predisposes individuals to obesity. **(C)** Genetic factors and DNA modifications: These factors influence susceptibility to obesity and lipotoxicity-related cardiometabolic diseases. **(D)** The biology of lipid droplets: Lipid droplets play a pivotal role in maintaining the homeostasis of lipid metabolism. Mutations or altered expression of genes related to lipid droplet formation or lipolysis can result in lipotoxicity. **(E)** Intestinal epithelial cell dysfunction, low diversity of gut microbiota, and altered microbial composition affect host lipid metabolism, thereby contributing to obesity and lipotoxic tissue injuries. **(F)** Increased fatty acid uptake and lipid droplet synthesis without adequate facilitation of fatty acid oxidation in mitochondria, as well as ligand-dependent and -independent transcriptional activation in the nucleus, result in excessive lipid accumulation in cells, leading to lipotoxicity.

underlying biological mechanisms between monogenic and polygenic obesity (reviewed in Ref. [13]). These studies have unequivocally demonstrated the critical role of genetics in contributing the variation in BMI. Future investigation is necessary to elucidate how specific genetic loci and their interactions impact biological processes, ultimately leading to an increased susceptibility to obesity.

In the general population, environmental factors, collectively known as the "obesogenic environment," are the primary contributors to overweight and obesity (Figures 2A, B). These factors encompass a range of elements, including dietary habits, levels of physical activity, income, and education. More specifically, the composition of diets, dietary patterns, fat compositions in diets, and the calorie balance between calorie intake and expenditure all play significant roles in determining an individual's weight status. Fat is an essential component of dietary nutrients, alongside protein, carbohydrate, vitamin, and mineral. Fat provides the highest calories per Gram, with 9 calories per Gram, compared to carbohydrate and protein which provide 4 calories per Gram. Dietary fats consist of saturated, unsaturated, and polyunsaturated fatty acids. Saturated fatty acids are generally regarded as unhealthy fats, while polyunsaturated fatty acids are considered to be healthier fats. A double-blind, parallel-group, randomized trial (LIPOGAIN) showed that 7-week overconsumption of muffins high in saturated fatty acids leads to significant increases in hepatic and visceral fat storage compared with overconsumption of those high in n-6 polyunsaturated fatty acids (PUFAs), despite both groups experiencing similar weight gain in healthy individuals [14]. Randomized controlled trials and meta-analysis suggest that replacing dietary saturated fats with unsaturated fats, especially polyunsaturated vegetable oils, reduces serum cholesterol by 15–20% and CVD by ~30% [15–17]. Further, while there is no universally accepted definition for ultra-processed foods [18], population-based prospective cohort studies and meta-analyses have consistently shown an association between the consumption of ultra-processed foods and the development of diabetes [19], obesity, and CVD [20] and all-cause mortality [21–23]. Collectively, these studies provide compelling evidence that both environmental and genomic factors contribute to the susceptibility of humans to overweight and obesity, which disrupt the homeostasis of lipid

metabolism, consequently giving rise to deleterious effects on cardiometabolic health.

In this review, we will discuss the intricate interactions between lipotoxicity and cardiomyopathy in the context of obesity. This review will provide a comprehensive overview of the current understanding of factors associated with obesity, the impact of obesity on lipid metabolism, the mechanisms underlying abnormal and excessive lipid accumulation in cells that induces cellular toxicity and cardiomyopathy, as well as potential therapeutic approaches targeting lipotoxicity for CVD.

Lipid metabolism in health

Lipids are a diverse group of organic compounds, including fats, oils, hormones, waxes, and cell membrane components. Fats are a specific type of lipids primarily composed of triesters formed from fatty acids and glycerol. Dietary fats supply humans with essential source of fatty acids, which act as cellular membrane components, molecules of energy storage, substrates of adenosine triphosphate (ATP) production in mitochondria, and signaling molecules. Dietary fats, predominantly composed of triglycerides, undergo digestion by lipase to generate fatty acids and monoglycerides in the intestinal lumen. These components are then absorbed by enterocytes, where they are re-esterification into triglycerides and packaged into chylomicrons. Chylomicrons are subsequently secreted into the lymphatic system and transported through the large vessels.

Circulating fatty acids are taken up by various organs, including skeletal muscle, heart, and liver. When nutrients are abundant, fats are primarily stored in adipocytes within adipose tissue for long-term storage, while the liver serves as short-term storage. During fasting, adipose tissue releases fatty acids to be used by peripheral tissues. Additionally, brown and beige adipose tissues, specialized forms of adipose tissue, utilize lipids to generate heat and maintain body temperature. Adipocytes can store large amounts of lipids in the form of lipid droplets, preventing abnormal lipid accumulation in other tissues. However, when lipids exceed the storage capacity of lipid droplets within adipocytes, such as in obesity and diabetes, excessive lipids lead to ectopic intracellular lipid deposition in other tissues. Consequently, adipose tissue dysfunction is closely linked to obesity and other cardiometabolic disorders, including diabetes, insulin resistance, and CVD. In the heart, an oxidative tissue, lipids are stored similarly in the form of lipid droplets as a means to sequester toxic lipids. This mechanism serves as a form of cardioprotection, as demonstrated by experiments conducted on rodents. The transgenic mice that overexpress diacylglycerol acyltransferase 1 (DGAT1), an enzyme that catalyzes the conversion of diglycerides and fatty acyl-CoA to triglycerides, in cardiomyocytes using α-myocyte heavy chain promoter doubled the myocardial triglyceride content but reduced the levels of ceramides and diglycerides, which are considered as toxic lipids, with normal contractile function [24]. Overexpression of long-chain acyl-CoA synthetase (ACS) in cardiomyocytes leads to lipotoxic cardiomyopathy in mice, a phenotype similar to diabetic cardiomyopathy [25]. However, the detrimental effect was rescued when these mice were crossed with DGAT1 transgenic mice [24]. These findings suggest that triglycerides may not be inherently toxic, and lipid droplets formation actually serves as a protective mechanism for the heart. On the other hand, cardiomyocytes generate ATP by oxidizing long-chain fatty acids (LCFAs) in mitochondria, with a significant portion of these LCFAs being derived from lipid droplets [26]. This highlights the crucial role of lipid droplets biology in maintaining the balance of lipid metabolism and overall homeostasis.

Lipid droplets as a physiological lipid storage organelle

In a state of nutrient surplus, lipids are stored in intracellular organelles, called lipid droplets, which are composed of a hydrophobic core of neutral lipids, mainly triglycerides and cholesteryl esters, encircled by a phospholipid monolayer with integral and peripheral proteins [27]. Lipid droplets are primarily formed in adipocytes of adipose tissues during fed conditions. However, they can also be found in essentially every cell type of other tissues to protect the cells from lipid-induced toxicity by buffering excessive amounts of toxic lipids (Figure 2D). Depending on the status of cellular metabolism, lipid droplets are assembled on the endoplasmic reticulum (ER) through a series of processes. Lipid droplets are generated; 1) from neutral lipids, most commonly triglycerides made by DGAT1/2 and sterol esters made by acyl-CoA: cholesterol *O*-acyltransferases (ACAT1/2); 2) lipid droplet budding facilitated by ER membrane phospholipid composition and some proteins, such as fat storage-inducing transmembrane proteins (FIT1/2), Pln1 (mammalian perilipins), and Seipin; 3) growth and maturation of lipid droplets by droplet-droplet fusion or transfer of triglyceride to lipid droplets; and 4) targeting of integral and peripheral proteins to lipid droplets. High-confident proximity labelling approach has identified around 150 proteins on the lipid droplet monolayer [27–29]. Among them, the perilipin (PLIN) protein family has been extensively studied as a key regulator of hydrolysis to facilitate the release of fatty acids for ATP production.

Lipid droplets dynamically, structurally, and functionally interact with other cellular organelles, including mitochondria, peroxisomes, and lysosomes, playing a crucial role in facilitating diverse functions of lipid droplets [27, 30]. The interaction with mitochondria could provide sites for trafficking of fatty acids hydrolyzed from lipid droplets to mitochondria for ATP production via β-oxidation in response to starvation in mouse

embryonic fibroblasts (MEFs) [31]. This interaction also provides protection to mitochondria in MEFs by promoting triglyceride synthesis through DGAT1 and reducing fatty acid incorporation into other toxic lipid species, including acylcarnitines, preventing their exposure to mitochondria [32]. A recent study demonstrated that efficient lipid droplets-to-mitochondria fatty acid trafficking and β-oxidation require Ser155 phosphorylation of PLIN5 and its direct interaction with the mitochondrial fatty acid transport protein 4 (FATP4) during myoblast starvation [33]. The interaction between lipid droplets and mitochondria has been observed to decrease in failing human hearts compared to donor hearts, as assessed by transmission electron microscopy [34], which may potentially contribute to the reduced fatty acid utilization in mitochondria in failing hearts. In brown adipocytes, as opposed to other cell types, peri-lipid droplet mitochondria increase pyruvate oxidation and ATP synthesis capacity with reduced β-oxidation capacity, which contributes to promoting triglyceride synthesis and lipid droplet expansion [35]. Lipid droplets also interact with peroxisomes, membrane-enclosed organelles that carry out oxidation reactions, including fatty acids β-oxidation [30]. A recent study [36] demonstrated that the lipid droplets and peroxisome network mediates the longevity effect of dietary mono-unsaturated fatty acids (MUFAs), rich in the Mediterranean diet that is linked with increased human lifespan and decreased CVD [37, 38]. Papsdorf et al. used *Caenorhabditis elegans* with a combination of genetics and lipidomics analyses and found that MUFAs upregulate the numbers of lipid droplets and peroxisomes with decreased lipid oxidation in the intestinal cells [36]. This contributes to decreasing lipid membrane damage and preserving membrane integrity during ageing, thereby driving lifespan extension. Additionally, lipid droplets interact with lysosomes via perilipin 2/3 (PLIN2/3) and chaperone-mediated autophagy machinery [30], which promotes lipid droplet and neutral lipid turnover with elevated levels of adipose triglyceride lipase (ATGL) and autophagy proteins during starvation [39]. These findings strongly indicate that the biology of lipid droplets plays a central and pivotal role in maintaining lipid metabolism.

It has been reported that lipid droplets can also be formed directly from the inner nuclear membrane, serving as a site for lipid storage in high-fat conditions in yeast cells [40]. This process is regulated by Seipin through detection of phosphatidic acid and diglyceride enrichment at the inner nuclear membrane [40]. Nuclear lipid droplets may also contribute to nuclear envelope expansion and regulation of gene expression by sequestering transcription factors on lipid droplets, including Opi, an ER-associated transcription factor, which may affect cellular lipid metabolism [40]. In contrast, another study demonstrated that nuclear lipid droplets in hepatocytes are derived from lipoprotein precursors present in the ER membrane in a Seipin-independent manner [41], indicating species and cell-type specific mechanisms of nuclear lipid droplets formation. Nuclear lipid droplets appear functionally distinct from cytoplasmic lipid droplets, although their precise role remains largely unknown.

Depending on cellular metabolism, demands, and nutrient availability, such as nutrient deprivation, esterified lipids stored in lipid droplets within adipose tissue undergo hydrolysis via lipolysis, a catabolic process of lipid droplets, or lipophagy, a specific form of autophagy that selectively degrades cytoplasmic lipid droplets, to liberate fatty acids and sterols into the bloodstream [42]. Lipolysis is stimulated by the binding of sympathetic nervous system-mediated catecholamine to β-adrenergic G-protein-coupled receptors [43]. Fatty acids released from lipid droplets serve as a crucial resource for diverse cellular processes in peripheral tissues, including ATP production, membrane biogenesis during periods of high demand for membranes, and acting as mediators of signaling pathways [27]. Lipolysis is regulated by three major enzymes; ATGL [also known as Patatin-like phospholipase domain-containing protein 2 (PNPLA2)], a rate-limiting enzyme that catalyzes the hydrolysis of triglycerides to diglycerides; abhydrolase domain containing 5 (ABHD5, also known as CGI-58), an essential coactivator of ATGL; and hormone-sensitive lipase (HSL), an enzyme that mediates hydrolysis of diglycerides. Monoacylglycerol lipase (MGL) hydrolyzes monoglycerides to generate glycerol and fatty acids. Lipophagy, also known as acid lipolysis, is another form of intracellular pathway responsible for the triglycerides degradation that takes place in lysosomes. Lysosomal acid lipase (LAL) is an enzyme essential for the hydrolysis of triglycerides and cholesteryl esters within lysosomes. LAL deficiency is an autosomal recessive disease caused by mutations in the *LIPA* gene. Wolman's disease is a severe disorder characterized by dyslipidemia, severe hepatosteatosis, hepatosplenomegaly, and premature death during infancy. Cholesteryl ester storage disease (CESD) is a less severe disorder that manifests dyslipidemia, atherosclerosis, and coronary artery disease due to enhanced foam-cell formation [44]. GWAS identified single nucleotide polymorphisms in the *LIPA* gene that associate with coronary artery disease [45, 46].

Compared to the knowledge of lipid droplets in adipose tissue and liver, the pathophysiological role of lipolysis in the heart is limited [47]. LCFAs released from lipid droplets act as the primary fuel source for oxidative ATP generation, ligands for nuclear hormone receptors, and substrates for synthesis of membrane lipids in cardiomyocytes. Mutations in either *PNPLA2* or *ABHD5* gene in humans cause neutral lipid storage disease with myopathy (NLSDM), characterized by systemic accumulation of triglycerides in lipid droplets [48], which often requires heart transplantation due to severe cardiomyopathy. Homozygous frameshift mutations in the *LIPE* gene encoding HSL impair triglyceride catabolism, although the clinical manifestations are less pronounced than that of NLSDM. Patients with defective HSL display

dyslipidemia, systemic insulin resistance, diabetes, and hepatic steatosis (partial lipodystrophy) [49]. Failing hearts, primarily due to non-ischemic cardiomyopathy, exhibit a 0.64-fold reduction in mRNA expression of *Plin 5*, the encoded protein preferentially expressed in highly oxidative tissues such as the heart, compared to donor hearts [34]. Systemic deletion of *Plin 5* results in the absence of myocardial lipid droplets and diminishes myocardial triglyceride levels, exacerbating age-related cardiomyopathy due to increased oxidative stress, a condition mitigated by antioxidant therapy with *N*-acetylcysteine (NAC) [50]. Conversely, overexpression of PLIN5 in cardiomyocytes via the α-MHC promoter results in enlarged and increased lipid droplets with massive triglyceride accumulation (3.5-fold increase) in the heart. This occurs through direct inhibition of ATGL-mediated lipolysis [51, 52], leading to concentric hypertrophy with preserved systolic function in 4-month-old male mice fed a normal chow diet [53]. PLIN5 cardiac-specific transgenic (cTg) mice show exacerbated cardiac hypertrophy and reduced systolic function when fed a HFD [54]. However, intriguingly, PLIN5 cTg mice display resistance to developing obesity and glucose intolerance under a HFD, presumably due to enhanced β-adrenergic signaling in adipose tissue, compared to WT mice. The role of MGL in the heart has not been thoroughly investigated. Pharmacological blockade of MGL with JZL184 increases systemic levels of 2-arachidonoylglycerol (2-AG), a class of signaling lipids called endocannabinoids. This increase enhances the myocardial recruitment of neutrophils and monocytes through the upregulation of neutrophil recruiting chemokines, CXCL1 and CXCL2, after myocardial infarction, thereby exacerbating inflammation, infarct size, and cardiac dysfunction in mice [55]. These results suggest the role of MGL in the myeloid cell recruitment from the bone marrow to the heart and subsequent cardiac inflammation via regulating endocannabinoid catabolism. These findings highlight the pathophysiological importance of effectively regulating lipid droplets in lipid metabolism and overall cardiometabolic health.

The impact of gut microbiota on host lipid metabolism and obesity

The gastrointestinal (GI) tract is the digestive system, which facilitates the movement of food and liquids through peristalsis. It also plays a crucial role in the digestion and absorption of catabolized nutrients in the intestine, such as amino acids derived from proteins, simple sugars (glucose, fructose, and sucrose) derived from carbohydrates, and fatty acids and glycerol derived from fats. In addition to enzymatic digestion, the GI tract harbors a diverse community of microorganisms, called gut microbiota, which not only helps digestion of food but also modulates the effects of dietary nutrients on host physiology and disease [56, 57] (Figure 2E). Genomic analysis of fecal samples from obese and lean twins demonstrated the significant association between the diversity of gut microbiota and obese and lean phenotypes in humans [58]. Transplantation of fecal microbiota from obese humans increases body mass and adiposity in mice fed a low-fat high-fiber diet than those from lean humans [59]. Importantly, transplantation of fecal microbiota from lean humans increase fermentation of short-chain fatty acids and decrease branched-chain amino acids metabolism compared to those from obese humans in mice. The low diversity of gut microbiota is associated with long-term dietary habits characterized by low consumption of fruits, vegetables, and fishery products in obese or overweight subjects [60]. Additionally, in the same study, dietary intervention aimed at reducing calorie intake for weight loss have been shown to improve gut microbial diversity, particularly in individuals with initially low microbial diversity, and clinical phenotypes such as insulin resistance and elevated blood triglycerides and high-sensitivity C-reactive protein levels, especially in individuals with initially high microbial diversity [60]. These clinical studies provide clear evidence of the significant relationship between gut microbiota and the cardiometabolic health of the host human.

Dietary fats impact gut microbiota population, which in turn plays fundamental roles in host lipid metabolism (Figure 2E). Various diseases develop by microbial dysbiosis, where the gut microbial communities are imbalanced or lack diversity without regard to the presence or absence of harmful or beneficial microbes [61, 62]. A recent study demonstrated the effects of different fatty acid compositions in dietary fats on gut microbiota composition in humans. Schoeler et al. showed that individuals consuming lower amounts of saturated fatty acids exhibit higher microbial diversity compared to those consuming higher amounts of saturated fatty acids, with no significant association observed between the amount of MUFAs or PUFAs consumption and microbial diversity [63]. In addition, the study showed a negative correlation between gut microbial diversity and the degree of liver steatosis, as determined by magnetic resonance imaging, in both obese and lean individuals, alongside a positive correlation between dietary intake of saturated fatty acids and MUFA and the fatty liver index, only in obese individulas [63]. It is known that individuals with metabolic syndrome, including obesity and type 2 diabetes, exhibit altered gut microbial features [64]. Two large-scale studies have identified unique gut microbiome and serum metabolome features as risk factors to develop ischemic heart disease in humans. These features include decreases in gut microbial density and serum levels of short-chain fatty acids (SCFAs) as well as an increase in the production of branched-chain amino acids [65, 66], the changes commonly observed in individuals with obesity and diabetes [64]. On the contrary, the Mediterranean diet, characterized by a low intake of animal-derived foods and a high intake of plant-based fatty acids, was associated with a higher capacity of host gut microbiota to produce SCFAs and a lower risk of cardiometabolic disease in humans [67]. These data suggest that SCFAs produced by the gut

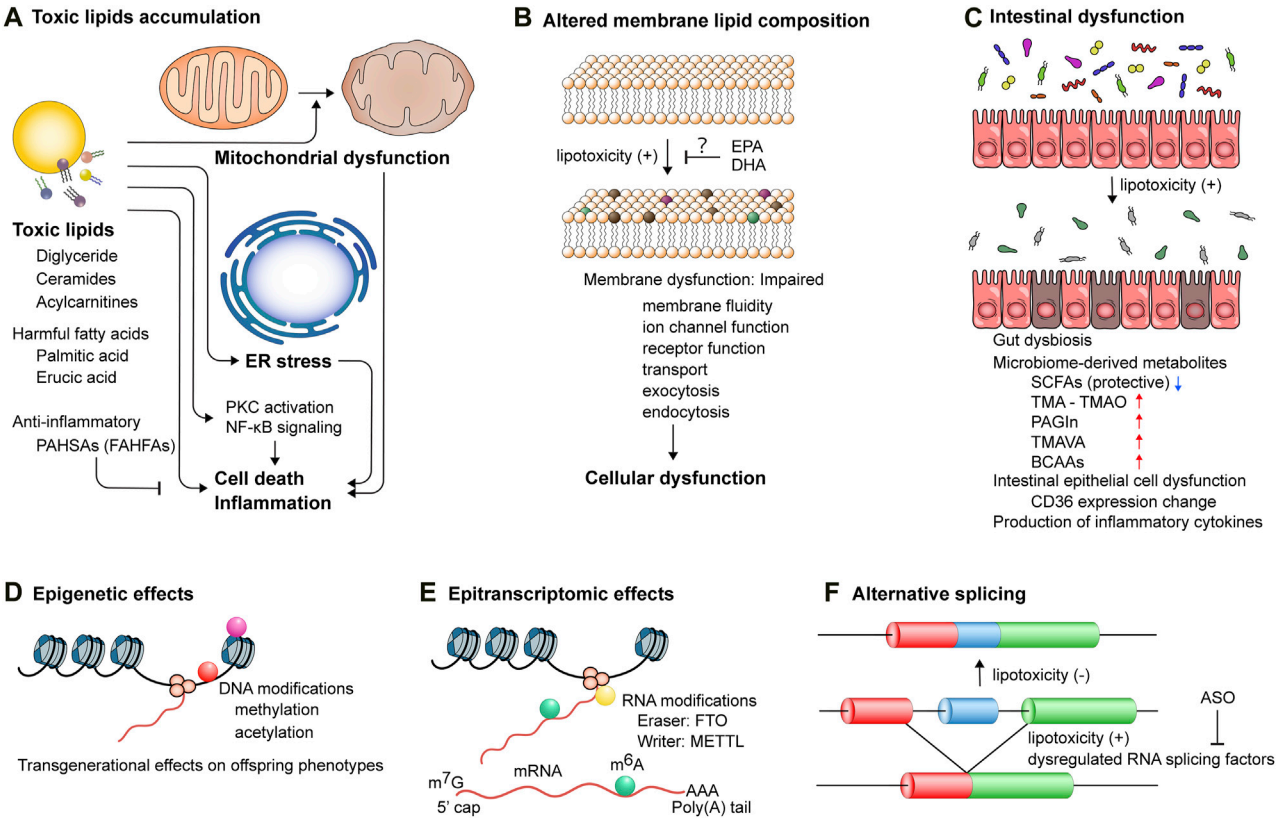

FIGURE 3
The mechanisms underlying lipotoxicity-induced cellular and tissue dysfunction. **(A)** Accumulation of toxic lipids triggers mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and activation of protein kinase c (PKC) and NF-κB signaling pathways, leading to cell death and inflammation. PAHSAs, palmitic acid esters of hydroxy stearic acids; FAHFAs, branched fatty acid esters of hydroxy fatty acids. **(B)** Lipotoxicity perturbs membrane lipid composition, which impairs membrane functionality, including membrane fluidity, ion channel function, receptor function, transport, exocytosis, and endocytosis, thereby inducing cellular dysfunction. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. **(C)** Intestinal epithelial cell dysfunction and gut dysbiosis induce lipotoxicity and also augment the production of microbiome-derived metabolites, such as trimethylamine (TMA), phenylacetylglutamine (PAGln), NNN-trimethyl-5-aminovaleric acid (TMAVA), and branched chain amino acids (BCAAs), while reducing the production of short-chain fatty acids (SCFAs). **(D)** DNA modifications, including methylation and acetylation, exert transgenerational effects on offspring cardiometabolic phenotypes. Lifestyle interventions of parents can potentially reverse these modifications. **(E)** Epitranscriptomic modifications of post-transcriptional mRNA regulate gene expression. Aberrant deposition of RNA modifications, such as N^6-methyladenosin (m^6A), mediated by dysregulated proteins, such as fat mass and obesity associated (FTO) and methyltransferase-like (METTL), is implicated in the pathogenesis of cardiometabolic diseases. **(F)** Toxic lipid overload induces alternative splicing events, which can be modulated by antisense oligonucleotides (ASOs) targeting pre-mRNA splicing.

microbiota may play a significant role in the interplay between gut microbiota and cardiometabolic health [68] (Figure 3).

In addition to SCFAs, certain gut-derived metabolites have been demonstrated to correlate with host cardiometabolic health (Figure 3C). Gut microbiota-derived trimethylamine (TMA) is absorbed in the intestine and oxidized in the host liver to form trimethylamine *N*-oxide (TMAO). The impact of elevated circulating TMAO levels on the development of CVD, particularly atherosclerosis, has been identified in humans [69] as well as its effects on platelet hyperactivation in human platelets, incident risk for thrombotic events in humans and a carotid artery injury mouse model, and mortality in human subjects [70, 71]. A recent study using cryopreserved human heart specimens revealed a substantial accumulation of TMAO in the myocardium of ischemic cardiomyopathy (fold change = 2.2) compared to donor myocardium [72]. It is noteworthy that this distinction attained statistical significance only in the male myocardium of ischemic cardiomyopathy (fold change = 1.95) and dilated cardiomyopathy (fold change = 1.96) compared to male donor hearts. Conversely, the female myocardium displayed no significant difference between cardiomyopathy and donor hearts [72], indicating a sex-specific metabolic regulatory mechanism in the gut-heart axis. Dietary choline and L-carnitine, abundant in red meat and containing a trimethylamine structure, alter gut microbial composition, significantly increasing TMA synthesis and circulating TMAO levels in mice [73]. A recent study showed that a HFD impairs mitochondrial bioenergetics in the host colonic epithelium,

leading to increased luminal bioavailability of oxygen and nitrate. This alteration in turn amplifies respiration-dependent choline catabolism by E. *Coli*, thereby enhancing host circulating TMAO levels [74]. This study emphasizes the significance of colonocyte dysfunction induced by a HFD. Furthermore, through untargeted metabolomics, phenylacetylglutamine (PAGIn) has been identified as a gut microbiota-derived metabolite that activates platelets through G-protein coupled receptors, including α2A, α2B, and β2-adrenergic receptors, with its circulating levels positively associated with thrombosis risk, CVD, and major adverse cardiovascular events in humans [75]. In another study, untargeted metabolomics analysis using prospective heart failure cohort samples revealed that N,N,N-trimethyl-5-aminovaleric acid (TMAVA), derived from trimethyllysine through the gut microbiota, is significantly elevated in hypertensive individuals, with its plasma levels positively associated with incident cardiac death [76]. TMAVA treatment exacerbated 12-week HFD-induced cardiac hypertrophy and dysfunction in mice by inhibiting fatty acid oxidation through the reduction of carnitine metabolism and subsequently increasing myocardial lipid accumulation [76]. These findings indicate that the Western dietary habit, characterized by excess ingestion of red meat and a high-fat, low-fiber diet, increases the risk of CVD, including atherosclerosis and lipotoxic cardiomyopathy, in part through alterations of gut microbial compositions and gut microbiota-derived metabolites.

Moreover, a multi-omics study demonstrated that increased fecal carbohydrate metabolism, particularly monosaccharides, in the gut microbiome contributes to the development of insulin resistance, accompanied by an increase in inflammatory cytokines [77]. This is consistent with a previous finding that excessive monosaccharides promote ectopic lipid accumulation and low-grade inflammation [78–80]. The metabolic reprogramming of immune cells, their dysregulation, and the concomitant changes in cytokine production in the intestinal epithelium in response to a HFD feeding play crucial roles in the development of chronic low-grade inflammation in obesity and diabetes (reviewed in Ref. [81]). Using T cell-specific Myd88 knockout mice, which exhibit defective T follicular helper cells, it has been demonstrated that T cell-dependent immunity protects mice from diet-induced obesity by reducing lipid absorption through a secreted microbial molecule-mediated regulation of host *CD36* gene expression [82]. Another recent study revealed that dietary sugar induces gut dysbiosis, eliminating T helper (Th) 17 cells. The Th17 cells regulate lipid absorption across the intestinal epithelium by suppressing *CD36* expression in an IL-17-dependent manner. Consequently, the elimination of intestinal Th17 cells exacerbates the development of metabolic syndrome induced by a high-fat, high-sugar diet in mice [83]. Furthermore, integrated multi-omics analyses have revealed the associations between the usage of drugs, including statins and metformin, and diversity of the host gut microbiota in humans [84]. The data suggests that certain kinds of medication may have the potential to influence cardiometabolic disease by modulating the population of gut microbiota. Additionally, there is an association between host genetic factors and microbial diversity. However, it is crucial to note that environmental factors, particularly dietary habits, have a more significant impact on gut microbial diversity [85]. In summary, these studies provide compelling evidence that diets high in saturated fatty acids and sugar, and low in fiber, lead to a reduction in gut microbial diversity and density, which in turn produce more harmful and fewer beneficial fatty acid metabolites and alter immune cell population and function. Consequently, these alterations contribute to the development of cardiometabolic disease through impaired lipid metabolism and inflammation.

Molecular mechanisms by which excessive lipid accumulation leads to lipotoxicity

Lipids constitute essential components of normal cellular biology; however, their excessive accumulation within cells can give rise to toxicity. Accumulated toxic lipids induce ER stress [86], oxidative stress, and mitochondrial dysfunction, accompanied by impaired autophagy and mitophagy [87], thereby causing cell death and inflammation [2]. It is noteworthy that the impact of free fatty acids on cellular functions varies, with not all being uniformly detrimental or beneficial at comparable levels. Circulating free fatty acids are elevated in obesity and diabetes, concomitant with increased myocardial fatty acid uptake and decreased glucose uptake. Imaging tools, such as positron emission tomography and magnetic resonance spectroscopy, enable the visualization and quantification of changes in human myocardial substrate and energy metabolism associated with metabolic syndrome (reviewed in Ref. [88]). Here we discuss the recent studies that provide insight into the molecular mechanisms as to how the storage of lipids is facilitated beyond physiological levels and how lipids become toxic in cells and tissues.

Imbalance between fatty acid uptake/storage and utilization

When nutrients are abundant, such as during feeding and in metabolic syndrome, cardiomyocytes increase the uptake of fatty acids, storing them for later use. Prolonged high-fat diet (HFD) consumption leads to the development of cardiac hypertrophy and contractile dysfunction in mice, a phenotype similar to diabetic cardiomyopathy [89]. A recent study demonstrated that palmitic acid, but not oleic acid, activates GSK-3α, a

serine/threonine protein kinase, in the nucleus in cardiomyocytes, which phosphorylates peroxisome proliferator-activated receptor α (PPARα) at Ser280, located in the ligand binding domain [90]. This phosphorylation reaction stimulates fatty acid uptake and storage, but not β-oxidation, leading to an imbalance between lipid uptake and utilization in cardiomyocytes. Initially, this signaling manifests as physiological to facilitate the storage of energy source; however, due to the limited capacity for lipid droplet storage in the heart, persistent activation of this pathway, as observed in prolonged exposure to a HFD, induces myocardial ectopic lipid accumulation, resulting in diabetic cardiomyopathy in mice [90]. These findings suggest that 1) GSK-3α senses the availability of fatty acids, particularly saturated fatty acids, stimulating lipid uptake and storage through biased activation of PPARα, and that 2) the lipid storage signaling depends on the saturation level of fatty acids [90] (Figure 2F). In line with this finding, increasing fatty acid oxidation by acetyl coenzyme A carboxylase 2 (ACC2) deletion specifically in cardiomyocytes prevents HFD-induced diabetic cardiomyopathy in mice, indicating that increasing fatty acid oxidation alone does not impair cardiac function. This beneficial effect is, in part, mediated by the upregulation of parkin-mediated mitophagy, contributing to the restoration of mitochondrial function [91]. These findings underscore the significance of maintaining a balance between lipid uptake and utilization in the heart, serving as a preventive measure against diabetic cardiomyopathy.

Toxic lipid accumulation

Intramyocardial lipids that exceed the capacity for storage and utilization can be toxic (Figure 3A). It is important to note that the pathogenesis of diabetic cardiomyopathy is multifactorial, including toxic lipid accumulation and toxic glucose metabolites. For example, metabolic intermediates of glycolytic pathways (e.g., dihydroxyacetone phosphate, methylglyoxal, and glucose-6 phosphate) either generate advanced glycation end products (AGEs) or facilitate the biosynthesis of glycoproteins (protein O-GlcNAcylation), which leads to the excessive accumulation of NADPH [2, 92, 93]. Among these multifactorial components, cardiac steatosis stands out as a clinical hallmark of diabetic cardiomyopathy and serves as an independent predictor of diastolic dysfunction [94, 95]. Importantly, cardiac steatosis precedes the onset of diabetes and the manifestation of systolic dysfunction [96]. Several lines of transgenic mice, especially those overexpressing long-chain acyl-CoA synthetase in cardiomyocytes [25], fatty acid transport protein 1 [97], lipoprotein lipase (LpL) [98], or PPARα [99] using the α-myosin heavy chain promoter, exhibited similar phenotypes to diabetic cardiomyopathy. These include excessive myocardial lipid accumulation, cardiac hypertrophy, and contractile dysfunction, leading to premature death. These rodent studies provide convincing evidence that increased ectopic lipid accumulation in the heart is sufficient to induce lipotoxic or diabetic cardiomyopathy independently of systemic metabolism. A recent study using hyperpolarized ^{31}P pyruvate tracer with ^{1}H and ^{13}C in noninvasive magnetic resonance spectroscopy analysis has confirmed increased myocardial lipid accumulation and impaired pyruvate metabolism and energetics in the heart of diabetic patients with diastolic dysfunction [100]. It is noteworthy that the size and number of lipid droplets may be a surrogate marker of lipotoxicity since neither lipid droplet nor triglyceride *per se* appears harmful in cells. It is considered that lipid droplets serve as a mechanism for sequestrating toxic lipids away from other organelles, including mitochondria and ER. The primary questions center on precisely defining the identities of toxic lipids and elucidating their roles in cellular function. Lipid metabolism intermediates, such as diglycerides, ceramides, and acylcarnitines, have been documented as toxic lipids and extensively reviewed elsewhere [2, 101] (Figure 3A).

Briefly, diglyceride is a lipid metabolite that acts as a second messenger to activate protein kinase C (PKC) by binding to the C1B domain. PKC phosphorylates a plethora of cellular substrates, including insulin receptor substrate 1, calcium channel, titin, and nuclear factor-κB (NF-κB) subunits, which leads to insulin resistance, inflammation, oxidative stress, Ca^{2+} overload, and cell death, thereby giving rise to the development of cardiac hypertrophy and dysfunction [102]. Ceramides are a family of lipid composed of sphingosine covalently linked to a fatty acid. Ceramide production is increased by various kinds of cellular stresses by three major pathways: *de novo* synthesis, sphingomyelin hydrolysis, and the salvage pathway [103]. Ceramides induce insulin resistance by inhibiting Akt signaling in mice [104] and activate the mitochondrial apoptotic pathway via increased membrane permeability [105, 106]. Lipotoxicity in LpL transgenic mouse heart was ameliorated by pharmacological inhibition of *de novo* ceramide biosynthesis with myriocin or crossing with genetic haploinsufficiency of LCB1 mice, a subunit of serine palmitoyltransferase (SPT). This was evidenced by the amelioration of cardiac dysfunction and the prolonged survival rate, implying that ceramide accumulation can be cardiotoxic, thereby contributing to the pathogenesis of lipotoxic cardiomyopathy [107]. Intraperitoneal injection of a SPT I inhibitor, myriocin, reduced intramyocardial levels of ceramides as well as diglycerides in mice, which was associated with improved cardiac glycolysis rates, as assessed by an isolated working heart perfusion model, in the setting of diet-induced obesity [108]. Acylcarnitines are intermediate oxidative metabolites composed of a fatty acid esterified to a carnitine molecule, synthesized by the enzymes carnitine palmitoyltransferase 1 and carnitine palmitoyltransferase 2. Acylcarnitines serve as carriers to transport long-chain fatty acids (LCFAs) across the mitochondrial membrane. Accumulation of acylcarnitine through the inhibition of

DGAT1 induces mitochondrial uncoupling and dysfunction, indicating the protective role of DGAT1-mediated lipid droplet biosynthesis, possibly by acting as a buffer against lipotoxic acylcarnitines [32]. A metabolic profiling of blood samples obtained simultaneously via invasive catheterization from the aortic root and coronary sinus in patients with severe aortic stenosis and hypertrophic cardiomyopathy revealed myocardial accumulation of long-chain acylcarnitines, presumably due to suppressed fatty acid oxidation, in the heart with aortic stenosis [109]. Additionally, circulating levels of long-chain acylcarnitines were independently associated with measures of maladaptive left ventricular remodeling in patients with severe aortic stenosis [110].

The amounts of free fatty acids, the degree of fatty acid saturation, and the cellular fatty acid compositions also represent critical factors in determining toxicity. In a recent unbiased analysis encompassing 61 structurally diverse fatty acids, the integration of transcriptomics, lipidomics, cell morphological features, and functional profiling has identified a subset of 20 fatty acids that could be toxic to β-cells [111]. Among these fatty acids, the well-known toxic fatty acid, palmitic acid, was included, serving as a substrate for the *de novo* synthesis of ceramides. Consistent with prior reports, oleic acid was identified as a protective fatty acid. Among 20 toxic fatty acids, 12 fatty acids were MUFAs, including erucic acid [111]. It is important to note that MUFAs are generally regarded as non-toxic or healthy fatty acids. Erucic acid was found to associate with decreased membrane fluidity, ER stress, and cell death. The data presented indicates that relying solely on traditional criteria based on saturations categories (saturated, monounsaturated, and polyunsaturated fatty acids) does not adequately define the lipotoxic potential of fatty acids. Piccolis et al. conducted transcriptomic, lipidomics, and proteomics analyses with genome-wide short hairpin RNA (shRNA) screening and have demonstrated that palmitate, a saturated fatty acid, increases saturated glycerolipids and ER stress in human leukemia cells, where di-saturated glycerolipids play a central role in lipotoxicity [112]. Furthermore, genome-wide shRNA screen identified more than 350 genes as genetic modifiers of lipotoxicity. Among these genes, the deletion of ER-localized glycerol-3-phosphate acyltransferase (GPAT) or the putative E3 ubiquitin ligase RNF213 was found to be sufficient to protect cells from lipotoxicity [112], indicating that these proteins are critical downstream targets of lipotoxicity.

It is noteworthy that fatty acids act not only as membrane components and energy substrates but also possess anti-inflammatory and anti-diabetic properties. Recently, branched fatty acid esters of hydroxy fatty acids (FAHFAs) have been identified as endogenous lipids in mammals [113], exhibiting unique biological functions [114]. Various FAHFAs display distinct biological activities, with some demonstrating anti-inflammatory and anti-diabetic effects in both murine models and humans [113, 115] (reviewed in Ref. [116]). Levels of palmitic acid esters of hydroxy stearic acids (PAHSAs), a subfamily of FAHFAs, are reduced in the serum and adipose tissue of individuals with insulin resistance [113, 117]. Long-term administration of PAHSAs improves insulin sensitivity and glucose tolerance in mice, partly by activating the G protein-coupled receptor 40 (GPR40), a long-chain fatty acid receptor [117]. Furthermore, PAHSAs decrease β-cell inflammation in mice by attenuating ER stress through a glucagon-like peptide 1 receptor (GLP-1R) and mitogen-activated protein kinase signaling [118]. A recent study has identified ATGL as a biosynthetic enzyme for FAHFAs by esterifying an FHA with a fatty acid from triglyceride or diglyceride [119], underscoring the significance of transacylase activity in ATGL alongside its lipase function. Considering the detrimental effects of functional knockdown of ATGL on cardiac morphology and function, the potential beneficial effects arising from ATGL-mediated release of FAHFAs may hold significance in understanding cardiac physiology. Given the anti-inflammatory properties of FAHFAs, there emerges a critical need to elucidate the functional roles of both endogenously produced and exogenously administered FAHFAs in cardiac pathologies in the context of obesity.

Altered membrane lipid composition

Lipids constitute the main component of cellular membranes, thereby regulating biological processes through modulation of membrane properties (such as membrane fluidity, ion channel and receptor function, and transport), as well as influencing the processes of exocytosis and endocytosis of molecules [120]. Altered membrane lipid composition and function result in cellular dysfunction (Figure 3B). For example, docosahexaenoic acid (DHA), a PUFA, in glycerophospholipids (GPLs) reduce membrane bending rigidity, contributing to functional endocytosis. In contrast, lipids with long and saturated fatty acids (e.g., sphingolipids) induce thicker and less fluid membranes [121, 122]. The distinct effects of eicosapentaenoic acid (EPA) and DHA treatment on atherosclerosis, membrane oxidation, membrane lipid dynamics and fluidity, as well as downstream lipid metabolite function, have been reviewed in Ref. [123]. A recent study unveiled that increased lipid saturation and exogenous saturated fatty acid overload rigidify the nuclear envelope and ER membranes, thereby fostering nuclear envelope rupture [124]. This paper showed that lipid acyl chain unsaturation with balanced lipid saturation is required for nuclear pore complex integrity and nucleocytoplasmic transport. In this regard, lipid droplets can buffer saturated membrane lipids to preserve nuclear envelope architecture. Collectively, these findings indicate that an increase in fatty acid saturation leads to changes in membrane lipid composition, triggering ER stress and the pathway of cell death.

Epigenetic effects of lipotoxicity

Maternal and paternal obesity, prolonged exposure to unhealthy diets, and diabetes predispose offspring to develop metabolic syndrome even in a healthy lifestyle [125] through epigenetic modifications (Figure 3D). These include S-adenosylmethionine-mediated methylation and Acetyl-CoA or NAD^+-dependent (de)acetylation, leading to transgenerational effects on offspring phenotypes [126–129]. Huypens et al. have conducted *in vitro* fertilization of embryos using sperm and oocytes from parental (F0) mice fed a HFD into healthy foster mothers to generate offspring (F1), followed by a HFD challenge [130]. This study demonstrated that female, but not male, F1 offspring from obese parents exhibit more pronounced increases in body weight and fat mass compared to those from lean parents. This indicates the presence of epigenetic germline inheritance of diet-induced obesity and insulin resistance. In addition, Wan et al. demonstrated that HFD-induced deregulation of lipid metabolism and lipid accumulation are transmitted to multigenerational progeny in *C. elegans* through nuclear receptors NHR-49 (a functional homolog of mammalian peroxisome proliferator-activated receptor α) and NHR-80 (a homolog of mammalian hepatocyte nuclear factor 4) and transcription factors SBP-1 and DAF-16, which are conferred by histone H3K4 trimethylation [131]. Importantly, DNA methylation of 4,875 Cytosine-phosphate-guanine (CpG) sites was differently affected between a 7-week excessive intake of saturated fatty acids and PUFAs in human adipose tissue. This underscores the distinct impacts of dietary fatty acid composition on epigenetic changes [132]. These findings provide evidence of transgenerational inheritance of obesity and diabetes even preceding pregnancy. Moreover, a growing body of evidence indicates that the nutritional status of parents may predispose their offspring to diabetic cardiomyopathy. In *Drosophila*, HFD-induced diabetic cardiomyopathy was transmitted to two subsequent generations, a phenomenon associated with increased systemic H3K27 trimethylation and the downregulation of ATGL and PGC-1 [133], a regulatory network modulating lipotoxicity in the heart [134]. These findings provide compelling evidence of intergenerational inheritance of cardiometabolic disease, including diabetic cardiomyopathy.

Epigenetic modifications can be reversible through lifestyle interventions. In a recent study, the genome-wide DNA methylation analysis was conducted using cord blood DNA collected from the Treatment of Obese Pregnant women (TOP)-study populations. This study has demonstrated that lifestyle intervention during pregnancy in women with obesity has an obvious impact on DNA methylation, 379 sites in 370 genes, in cord blood, which is notably linked to the body composition in the offspring [135]. The genes containing lifestyle intervention-related DNA methylation significantly associate with gene ontology biological processes for striated muscle cell proliferation, response to fatty acid, and adipose tissue development. In another study, Son et al. showed that maternal exercise enhances brown adipogenesis and thermogenesis through increased DNA demethylation in the *Prdm16* promoter via an exercise-induced apelin/α-KG-dependent axis in mice. This modification mitigated offspring obesity when challenged with a HFD [136]. These findings underscore the critical impact of obesity and lifestyle interventions on the development and prevention of cardiometabolic disease, not only for oneself but also future generations.

Epitranscriptomic effects of lipotoxicity

In addition to epigenetic modifications of histone and DNA, epitranscriptomic modifications of post-transcriptional mRNA play a pivotal role in gene expression by regulating RNA stability, subcellular localization (such as cytoplasmic export of RNAs containing methyladenosine), and alternative splicing (such as the efficient recognition of splice sites by methyladenosine) [137] (Figure 3E). Internal N^6-methyladenosine (m^6A) modifications and the 5′ cap are the most prevalent modifications in mRNA. Aberrant deposition of RNA modifications has been implicated in the pathogenesis of human diseases, including neurological deficits, cancer, obesity, and diabetes, by, for example, causing RNA degradation or structural changes (reviewed in Ref. [138]). Fat mass and obesity associated (FTO) is one of the well-studied epitranscriptomic regulators. A genome-wide association study initially identified single nucleotide polymorphisms in the first and second introns of the *FTO* gene as being associated with increased BMI and a predisposition to obesity that persists from childhood into old age, thereby increasing the susceptibility to diabetes [139, 140]. The FTO expression is elevated in mice fed a HFD [141]. Genetic deletion of *FTO* in mice leads to postnatal growth retardation and a significant reduction in adipose tissue by enhancing energy expenditure and systemic sympathetic activation [142], while ubiquitous overexpression of FTO results in obesity by increasing food intake [143]. FTO has been reported as a demethylase of N6, 2′-O-dimethyladenosine (m^6A_m) in the 5′ cap, which controls mRNA stability [144], and internal m^6A_m modifications. The m^6A-sequencing in human diabetic islets revealed several hypomethylated transcripts, including insulin secretion pathway [145]. Furthermore, reduced m^6A levels by Mettl14 β-cell specific deletion in mice recapitulated the islet phenotype in human diabetes. Additionally, endothelial cell-specific deletion of FTO attenuated retinal vascular endothelial dysfunction and inflammation against streptozotocin-induced diabetes in mice [146]. The methylated RNA immunoprecipitation sequencing (MeRIP-Seq) analysis combined with RNA-Seq has elucidated that FTO represses

the *TNFAIP3 interacting protein 1* (*Tnip1*) mRNA expression by erasing m^{6}A methylation in the 3′-UTR of *Tnip1* mRNA, thereby increasing NF-κB activity.

Aberrant RNA modifications have been detected in the cardiac tissues of patients with CVD, including atherosclerosis, heart failure, cardiac hypertrophy, and cardiomyopathy. The m^{6}A methyltransferase Mettl3 and m^{6}A RNA methylation levels are increased in endothelial cells in both *in vitro* and *in vivo* atherogenic environments, resulting in hypermethylation of m^{6}A sites predominantly at coding sequences near the 3' UTR of NLRP1 and KLR4 mRNA [147]. The short-hairpin (sh)RNA-mediated knockdown of Mettl3 effectively prevented atherosclerotic lesion formation in the ApoE$^{-/-}$ mouse model. The levels of m^{6}A RNA methylation are increased in both human failing hearts [148] and rat neonatal cardiomyocytes following serum stimulation [149]. The αMHC-promoter-driven overexpression of Mettl3 in cardiomyocytes was sufficient to induce physiological cardiac hypertrophy, while cardiac-specific deletion of Mettl3 exacerbated aging-related and pressure overload-induced cardiac remodeling in mice, concomitant with reduced cardiomyocyte hypertrophy [149]. A recent study demonstrated that cardiac-hypertrophy-associated piRNA (CHAPIR), a PIWI-interacting noncoding RNA (piRNA), directly interacts with Mettl3 to inhibit the m^{6}A RNA methylation of *Parp10* mRNA. This methylation enhances PARP10 expression in cardiomyocytes, facilitating pathological cardiac hypertrophy in response to pressure overload [150]. Conversely, while FTO is downregulated in both human and mouse failing hearts, adeno-associated virus (AAV)-mediated upregulation of FTO attenuated contractile dysfunction in response to myocardial infarction through FTO-mediated demethylation and stabilization of the *SERCA2a* mRNA transcript [148]. Mettl14 expression is decreased in both H9C2 cells treated with high glucose and streptozotocin-induced diabetic hearts in rats [151]. Lentivirus-mediated upregulation of Mettl14 attenuated diabetes-associated heart dysfunction by augmenting the m^{6}A RNA methylation of long noncoding RNA (lncRNA) TINCR and subsequently suppressing pyroptosis through inhibition of NLRP3 mRNA expression [151]. These results underscore the essential role of m^{6}A RNA methylation in maintaining cardiac homeostasis. Collectively, these findings implicate that translational changes related to obesity and diabetes across various cell types are, in part, modulated by mRNA modifications.

Lipotoxicity-mediated alternative splicing

Alternative splicing provides the complexity of transcriptomes, allowing species, tissue, and cell type-specific regulation of diverse processes. The 2 landmark papers were published in 2012 that address the fundamental question as to what generates differences in organs between species [152, 153]. Although tissue-specific gene expression is highly conserved among vertebrates, these papers demonstrated that alternative splicing patterns are dominated by species-specific differences. The article by Merkin et al. also demonstrated that differential splicing, rather than the abundance of protein kinase, primarily influences the regulation of protein kinase activity by including or excluding exons related to kinase reactions [153]. Global and gene-specific modulations of alternative splicing regulate a wide range of physiological and pathological processes, such as cell death, cell differentiation, and metabolism. These mechanisms contribute to the development of neurological and developmental disorders, CVD, and cancer [154]. The Genotype-Tissue Expression (GTEx) project has identified detailed gene expression regulation with genetic rare variants and alternative splicing for the majority of genes across human tissues by conducting DNA sequencing and multi-tissue RNA sequencing. The GTEx consortium identified functional rare genetic variation and cell type-specific genetic regulation of gene expression [155]. By generating a large number of human full-length well-known proteins and their novel spliced isoforms, Yang et al. demonstrated the protein-protein interaction profiling using yeast two-hybrid screens and a protein complementation assay for validation in human HEK293T cells [156]. This study clearly demonstrated that the majority of isoform pairs share less than 50% of their interactions, and the interaction partners are expressed in a highly tissue-specific manner. These findings indicate that alternatively spliced transcripts may function as distinct proteins rather than minor variants of each other. Nevertheless, the functional implications of genetic rare variations and alternatively spliced variants in the context of human diseases remain largely unknown.

In a recent rodent study, Keller et al. disrupted the 5' alternative splicing site in the *Bcl2l1* gene to inhibit alternative splicing of *Bcl-x short-isoform* (*Bcl-xS*) in mice *in vivo*, and demonstrated that the suppression of Bcl-xS induces systemic inflammation, splenomegaly, cardiac fibrosis, and cardiomyopathy. This study suggests that *Bcl-xS* alternative splicing is essential for maintaining organ functions in a tissue-specific manner [157]; however, whether this alternative splicing impacts on lipotoxicity and diet-induced cardiomyopathy and, if so, how it regulates lipid metabolism remain to be investigated. Importantly, RNA-sequencing analysis has identified altered expression of 17 RNA splicing factors (e.g., SRSF3) and alternative splicing of 3,525 transcripts corresponding to 2,858 genes in human islet cells in response to palmitate *in vitro* [158] (Figure 3F). The data presented suggests that β-cell dysfunction may be attributable to lipotoxicity-induced alternative splicing. Furthermore, Vernia et al. have identified an alternative splicing program in adipose tissue in response to a HFD challenge in mice *in vivo*. Mice fed a HFD displayed widespread changes in alternative splicing in adipose tissue, a phenomenon correlated with a

reduction in the expression of NOVA splicing factors following HFD consumption [159]. Adipocyte-specific NOVA deficient mice showed increased adipose tissue thermogenesis and less weight gain following a HFD, indicating that NOVA enhances an alternative splicing program that suppresses thermogenesis and promotes diet-induced obesity. A recent study using genomic and proteomic analyses has identified enhanced spliceosome proteins expression and alternative splicing machinery in the liver of mice fed a HFD [160]. A major isoform of splicing factor RBFOX2 expression and its activity were suppressed in the liver of diet-induced obese mice, which deregulates alternative splicing of lipid-regulatory genes, impairing cholesterol metabolism [160]. These findings suggest that targeting RNA splicing could be a potential therapeutic approach for mitigating lipotoxicity-induced tissue damage. However, further research is required to elucidate the mechanisms by which excessive lipid accumulation induces alternative splicing events and to determine whether and how alternatively spliced variants in the heart or other tissues contribute to diabetic cardiomyopathy in the context of obesity.

Clinical relevance of lipotoxicity in cardiovascular disease

Cardiovascular disease in individuals with metabolic syndrome, including obesity, insulin resistance, diabetes, and dyslipidemia, manifests as coronary artery disease (e.g., angina pectoris and myocardial infarction), cardiomyopathy, left ventricular hypertrophy, systolic and/or diastolic dysfunction, arrhythmia, and valvular heart disease [2]. The presence of metabolic syndrome, particularly with an increasing number of comorbidities, associate with a higher risk of age-dependent development of heart failure [161, 162]. A recent pooled analysis of community-based NHLBI cohorts has demonstrated that higher BMI (overall obesity), abdominal obesity, waist circumference, and fat mass are strongly associated with a greater risk of heart failure development among older adults, particularly among those with diabetes [163]. This indicates the pivotal role of diabetes in modifying the association between obesity and heart failure development. Alternatively, clinical studies have demonstrated that obesity can be a therapeutic target for the prevention of heart failure development. For example, the Look AHEAD trial showed that reductions in fat mass and waist circumference, but not lean mass, by an intensive lifestyle intervention, are each significantly associated with a lower risk of heart failure development but not myocardial infarction in adults with diabetes [164]. Although the etiologies of cardiometabolic diseases are multifactorial, obesity-mediated lipotoxicity plays a critical role in developing cardiometabolic disease. This section discusses the clinical relevance of lipotoxicity in cardiometabolic disease (Table 1).

Lipotoxicity in cardiometabolic disease

Circulating free fatty acids are elevated in obesity, a consequence of their release from enlarged adipose tissue and inadequate utilization in peripheral tissues. This, in turn, induces β-cell dysfunction and insulin resistance, accompanied by ectopic lipid accumulation in peripheral tissues, all contributing to the pathogenesis of type 2 diabetes [165]. Pancreatic islets consist of multiple cell types. To delineate the islet cell type-specific gene expression in healthy individuals and those with type 1 and type 2 diabetes, single-cell RNA sequencing has been employed through the Human Pancreas Analysis Program. Using these datasets, a recent study revealed that the downregulated genes in β-cells are enriched for processes related to mitochondrial function, with minimal reduction of β-cell numbers in type 2 diabetes. This reduction in genes for mitochondrial function contributes to oxidative stress, impaired insulin secretion, and β-cell dysfunction [166]. These findings align with previous reports [167]. In addition to pancreatic β-cell dysfunction, obesity and diabetes contribute to the accumulation of triglycerides in lipid droplets in adipocytes, which induce adipocyte hypertrophy and hyperplasia, resulting in the rapid expansion of adipose tissue. This, in turn, triggers the production of proinflammatory cytokines and further increases circulating free fatty acids [168]. Lipotoxicity in adipose tissue plays a critical role in establishing a state of chronic inflammation in obesity and diabetes. The complex interactions between metabolic and inflammatory pathways in immune cells and metabolic tissues have recently been extensively studied in the field of immunometabolism [169]. These findings indicate that lipotoxicity underlies the pathogenesis of insulin resistance and diabetes in obesity.

Diabetic cardiomyopathy

Diabetic cardiomyopathy can be clinically diagnosed by the presence of diabetes and cardiomyopathy in the absence of hypertension and coronary artery disease [2, 170]. It is important to exclude primary cardiomyopathy (e.g., hypertrophic cardiomyopathy, dilated cardiomyopathy, tachycardia-induced cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, left ventricular non-compaction cardiomyopathy, restrictive cardiomyopathy, ion channelopathies, takotsubo cardiomyopathy, and myocarditis) and cardiomyopathies secondarily developed by coronary artery disease, valvular heart disease, and hypertension. Individuals with overweight, obesity, dyslipidemia, or insulin resistance without diabetes, hypertension, and atherosclerotic cardiovascular disease (ASCVD) may also develop a similar cardiomyopathy. Considering the absence of diabetes, these patients may not be diagnosed with diabetic cardiomyopathy in the clinical setting. These similar metabolism-related cardiomyopathies may be referred to as obesity-related cardiomyopathy, insulin resistance-induced cardiomyopathy,

TABLE 1 Lipotoxicity-related cardiomyopathy based on the primary pathogenesis of lipid accumulation.

Obesogenic environment or obesity-related cardiomyopathy	
Disease	**Underlying diseases**
Diabetic cardiomyopathy	Diabetes and insulin resistance
Non-diabetic lipotoxic cardiomyopathy	Metabolic syndrome in the absence of diabetes and insulin resistance
Ischemic or hypertensive cardiomyopathy	Atherosclerotic coronary artery disease, renal disease, and hypertension
Inherited lipotoxic cardiomyopathy caused by mutations in genes for lipid droplets biology	
Disease	Responsible genes
Triglyceride deposit cardiomyovasculopathy (TGCV), Neutral lipid storage disease with myopathy (NLSDM)	*PNPLA2*
Lipodystrophy syndromes	*AGPAT2*
	Lpin1
	CCTα
	Seipin
	FIT2
Cholesteryl ester storage disease	*LIPA*

lipotoxic cardiomyopathy, or metabolic cardiomyopathy. Although there may be distinct underlying mechanisms, considering the key role of dysregulated metabolism and lipotoxicity in developing these cardiomyopathies and the frequent overlapping of obesity and diabetes, we collectively refer to these cardiomyopathies as diabetic cardiomyopathy in this review article.

The clinical features of diabetic cardiomyopathy are left ventricular hypertrophy and subclinical evidence of diastolic dysfunction in the early stage and systolic dysfunction in the late stage, which promotes heart failure, especially heart failure with preserved ejection fraction (HFpEF). Importantly, there may be a difference in cardiac morphology and function between obese and non-obese HFpEF patients. The echocardiographic and catheter-based functional analyses revealed that obese patients with HFpEF exhibit more pronounced biventricular remodeling and more severe right ventricular dysfunction compared to their non-obese counterparts with HFpEF [171]. A recent report based on the 7 community-based cohorts have demonstrated that approximately 6.2% of diabetic participants without ASCVD develop heart failure in 5 years, contrasting with a rate of 12.7% among diabetic participants with ASCVD [172]. Risk stratification using NT-proBNP and other cardiac biomarkers (e.g., echocardiography and high-sensitivity cardiac troponin T) has identified high-risk participants for heart failure, although a substantial number of participants initially classified as low risk developed heart failure [172]. This underscores the challenge of accurately predicting the development of heart failure in individuals with metabolic syndrome but without a history of ASCVD.

Clinical studies provide compelling evidence that the prominent accumulation of lipids is a hallmark of diabetic cardiomyopathy. Cardiac ^{1}H-magnetic resonance spectroscopy (MRS) analysis revealed that diabetic patients, regardless of obesity status, exhibit increased levels of myocardial triglyceride content and impaired energetics in comparison to normal-weight control subjects. Notably, there is no discernible disparity in the level of cardiac steatosis between diabetic patients with and without obesity [173]. The DMCM-AHEAD prospective study showed a significant early and progressive lipid accumulation in transplanted hearts among diabetic recipients when compared to their non-diabetic counterparts. This indicates that the early pathogenesis of human diabetic cardiomyopathy involves significant lipid accumulation in cardiomyocytes [174]. However, further investigation is warranted to elucidate the precise mechanisms by which excessive lipid accumulation contributes to diabetic cardiomyopathy in humans.

Triglyceride deposit cardiomyovasculopathy (TGCV)

TGCV is congenital heart disease associated with mutations in the *Patatin-like phospholipase domain containing protein 2* (*PNPLA2*) gene encoding adipose triglyceride lipase (ATGL)

[175]. This is also known as neutral lipid storage disease with myopathy (NLSDM). The ATGL-mediated lipolysis of cellular triglycerides plays a pivotal role in modulating the PPARs-PGC-1 complex activity. Deficiency of ATGL specifically in cardiac and skeletal muscle results in excessive lipid accumulation and subsequent cardiomyopathy in mice [176]. The clinical features of TGCV include skeletal myopathy and severe cardiomyopathy, characterized by excessive triglyceride accumulation in the myocardium and smooth muscle cells of coronary arteries. This differs from diabetic cardiomyopathy and ASCVD in two key aspects: first, coronary artery disease is evident in TGCV, and second, the predominant deposition in the arteries of TGCV is triglyceride, as opposed to cholesterol in the arteries of ASCVD [177]. Based on recently published diagnostic criteria, TGCV is classified into primary TGCV, which involves a mutation in the *PNPLA2* gene, and idiopathic TGCV, which lacks a mutation in the *PNPLA2* gene. The latter is likely to overlap with other congenital heart diseases that have mutations in genes related to the hydrolysis of triglycerides [178, 179]. Definite primary TGCV demonstrates the presence of the typical Jordan's anomaly (apparent vacuoles >1 μm in size) in over 90% of polymorphonuclear leucocytes in peripheral blood smears. The diagnostic criteria for TGCV include: 1) defective triglyceride lipolysis, as assessed by a reduced washout rate (<10%) of ^{123}I (iodine)-β-methyl-p-iodophenylpentadecanoic acid (BMIPP), a radiolabeled LCFA analogue, in myocardial scintigraphy: and 2) myocardial accumulation of triglycerides, as assessed by magnetic resonance spectroscopy/computed tomography scan or biopsy [179]. According to the Japan TGCV study group, the mean age of male and female patients at TGCV diagnosis was 63.6 years (range: 24–87) and 68.6 years (range: 33–93), respectively. The prevalence of coronary artery disease and heart failure were 74.9% and 71.0%, respectively. The 5-year overall and cardiovascular event-free survival rates after diagnosis were 71.8% (70.9% for males and 74.2% for females) and 54.0%, respectively [180]. Treatment with a medium-chain triglyceride, tricaprin, reduced triglyceride accumulation and improved contractile dysfunction in an *ATGL* knockout mouse model [181]. Additionally, in an investigator-initiated, multicenter, randomized, double-blind trial (Phase IIa) comprising 17 patients with idiopathic TGCV, 8-week oral administration of tricaprin (1.5 g/day) significantly increased the washout rate of BMIPP after baseline adjustments compared to placebo control, but there were no significant changes in the 6-min walk distance [182].

Atherosclerosis and coronary artery disease

The ASCVD is linked to lipotoxicity in arterial cells, including smooth muscle cells, endothelial cells, and immune cells. Atherosclerosis is developed by accumulation of cholesterol esters in the lipid droplets of macrophages (foam cells), giving rise to atherosclerosis. Atherosclerosis manifests as coronary artery disease, cerebrovascular disease, renal disease, and peripheral artery disease, which frequently leads to ischemic cardiomyopathy, stroke, and hypertensive heart disease. It is noteworthy that coronary artery disease is one of the exclusion criteria for diagnosis of diabetic cardiomyopathy. The thickening and stiffening of the arterial wall, known as arterial stiffness, as assessed by brachial-ankle pulse wave velocity, can be a risk stratification index for prognosis in patients with type 2 diabetes, irrespective of the presence or absence of coronary artery disease [183, 184]. Cholesterol released from oxidized LDL is esterified to cholesteryl esters by the acyl-CoA cholesterol acyltransferase (ACAT1) and stored in lipid droplets in macrophages, which become foamy (foam cells) in atherosclerotic plaques. Genetic inhibition of *ACAT1* exacerbates atherosclerosis in LDL receptor-deficient mice [185]. Consistent with this finding, the ACAT1 inhibitor, pactimibe, exhibited adverse effects on atheroma volume and may contribute to the progression of coronary atherogenesis in humans [186]. In addition, pactimibe treatment was associated with increases in mean carotid intima-media thickness and major cardiovascular events in patients with familial hypercholesterolemia [187]. These data indicate that the formation of cholesteryl esters in the lipid droplets in macrophage represents an adaptive response to hypercholesteremia. It is important to emphasize that vascular smooth muscle cells also accumulate cholesteryl esters, exhibiting features reminiscent of macrophage-derived foam cells in atherosclerotic plaques in the human coronary artery. Smooth muscle cell-derived foam cells account for 50% of the total foam cell population, underscoring the significant contribution to smooth muscle cells to the storage of excess cholesterol [188]. A recent study has demonstrated that the expression of transforming growth factor-β2 (TGF-β2) is more closely linked to the content of smooth muscle cells in human atherosclerotic tissue, showing an inverse association with plaque rupture and inflammation. The data presented indicates the role of smooth muscle cell TGF-β2 in stabilizing plaque and the protective role against atherosclerotic complications [189]. Presently, histological, fate mapping, and single-cell RNA sequencing studies suggest that smooth muscle cells play a major role in the pathogenesis of atherosclerotic plaques. Whether macrophage-like smooth muscle cells exert a protective or pro-atherosclerotic influence, and the regulatory mechanisms governing this phenomenon, are yet to be fully comprehended [190].

Lipodystrophy syndromes

Lipodystrophy syndromes represent a diverse group of fat storage disorders, characterized by the inability to maintain subcutaneous body fat, leading to an aberrant and excessive

distribution of body fat, accompanied by manifestations, such as diabetes, dyslipidemia, and CVD. The dysregulation of proteins or mutations in genes related to the formation or maintenance of lipid droplets are underlying mechanisms of lipodystrophy syndrome (reviewed in Refs. [191, 192]). Loss-of-function mutations in genes for triglyceride biosynthetic enzymes, encoded by *AGPAT2* (1-acylglycerol 3-phosphate o-acyltransferase 2) or *Lpin 1* (encoding lipin 1, phosphatidic acid phosphatase) result in lipodystrophy syndrome [192]. Lipin 1 expression is decreased in failing human hearts [193]. Cardiac-specific deletion of lipin1 in mice resulted in normal systolic function and mild cardiac hypertrophy with increased phosphatidic acid content as well as unexpected increases in diglycerides and triglycerides in the heart [193]. Frameshift mutations in the *PLIN1* gene, encoding perilipin 1 (a lipid droplet surface protein that regulates lipolysis), result in autosomal dominant partial lipodystrophy associated with severe dyslipidemia and diabetes [194]. In addition to enzymes that synthesize or regulate neutral lipids core, mutations in *CCTα* (CTP: phosphocholine cytidylyltransferase-α) that encodes an enzyme that regulates lipid droplets expansion by synthesizing phosphatidylcholine, cause lipodystrophy syndromes [195, 196]. Seipin is the ER membrane protein critical for initiating cytoplasmic lipid droplets formation and maintaining the contact site between ER and lipid droplets. Hereditary *seipin* deficiency causes the severe phenotype of lipodystrophy [197]. Furthermore, mutations in *FIT2* (Fat storage-inducing transmembrane protein 2, an ER membrane protein critical for lipid droplet biosynthesis) may be associated with lipodystrophy [198].

Therapeutic targets and interventional strategies for lipotoxicity

Lifestyle modifications with diet and physical activity interventions lose weight, which improves lipid metabolism, inflammation, and cardiometabolic health. However, the efficacy of medical and surgical weight loss interventions shows mixed results for ischemic heart disease and heart failure. Lifestyle interventions are recommended for individuals of all ages, including children, adolescents, and adults, throughout life for not only primary prevention of CVD but overall healthy life, preceding pharmacotherapy [199]. Here we discuss pharmacotherapy aimed at targeting lipotoxicity.

Drugs for diabetes and dyslipidemia

Metformin is the first-line medication for type 2 diabetes, followed by consideration of a sodium-glucose cotransporter 2 inhibitor (SGLT2i) or a glucagon-like peptide-1 receptor agonist. An increasing body of evidence indicates that metformin exerts its influence on multiple organs, including the liver, gut microbial communities, and tissue-resident immune cells, where mitochondria and lysosomes are the primary organelles targeted to achieve the glucose-lowering effect [200]. Metformin treatment reduces lipid accumulation in transplanted donor hearts of diabetic recipients when compared to those not receiving metformin [174]. A well-documented mechanism of metformin action involves the activation of AMP-activated protein kinase (AMPK), partly through transient inhibition of complex I, which stimulates fatty acid oxidation, thereby mitigating lipotoxicity in the liver, adipose tissue, and heart (reviewed in Ref. [201]). Metformin is accumulated in the gut, where it inhibits the intestinal absorption of dietary glucose. Importantly, metformin exerts an acute glucose-lowering effect in liver-specific [201] and intestine-specific [202] AMPK knockout mouse models, indicating the presence of AMPK-independent mechanisms (reviewed in Ref. [200]). These mechanisms involve fructose-1,6-bisphosphatase-1 (FBP1) [203], hepatic glucagon signaling [204], and changes in gut microbiota composition. However, metformin administration fails to ameliorate HFD-induced obesity and glucose intolerance in intestine-specific AMPKα1 knockout mice [205]. This paper demonstrated that metformin activates intestinal AMPKα1, which regulates brown adipose tissue thermogenesis by modulating gut microbiota composition and their metabolites, suggesting that AMPK activation in intestinal epithelial cells is required for the therapeutic effects of chronic metformin administration in mice fed a HFD. Recent independent two studies demonstrated that metformin exerts its effects in intestinal epithelial cells to increase the biosynthesis of the anorexigenic (appetite-suppressing) metabolite *N*-lactoyl-phenylalanine (Lac-Phe) through the inhibition of complex I, thereby leading to anti-obesity effects in cells *in vitro*, in mice *in vivo*, and in individuals regardless of the presence of diabetes [206, 207]. Lysosomes are another targeted organelle of metformin [208]. A recent study showed that metformin binds to presenilin enhancer 2 (PEN2) in the lysosomes of hepatocytes, which is recruited to ATPase H^+ transporting accessory protein 1 (ATP6AP1), leading to the inhibition of v-ATPase and the activation of AMPK at the surface of lysosomes [209]. Whether metformin acts through the same mechanism involving the lysosomal pathway to target lipotoxicity in cardiomyocytes in the heart remains to be elucidated.

Recent clinical trials evaluating the impact of SGLT2i on CVD mark significant milestones in the treatment of heart failure. SGLT2i demonstrates a notable reduction in the risk of hospitalization for heart failure or cardiovascular death in patients with heart failure and a reduced ejection fraction (HFrEF) [210, 211] as well as in patients with heart failure with mildly reduced or preserved ejection fraction [212, 213], regardless

of the presence or absence of diabetes. These findings suggest the presence of an anti-diabetes-independent cardioprotective mechanism associated with SGLT2i. Furthermore, a comprehensive meta-analysis of five randomized controlled trials (DELIVER, EMPEROR-Preserved, DAPA-HF, EMPEROR-Reduced, and SOLOIST-WHF) has confirmed that SGLT2i displays substantial benefits for cardiovascular death and hospitalization for heart failure irrespective of left ventricular ejection fraction [214]. However, the precise mechanisms governing the cardioprotective actions of SGLT2i in heart failure remain largely indeterminate. One of the proposed mechanisms suggests that SGLT2i induces a physiological level of ketosis [215]. Metabolomics analysis of myocardium samples obtained from end-stage human failing hearts [216], quantitative mitochondrial proteomics on myocardium samples from a hypertrophic heart failure mouse model [217], and metabolomics profiling of blood samples obtained from the artery and coronary sinus of patients with or without heart failure [218] collectively revealed increased utilization of ketone bodies and reduced utilization of fatty acids in the failing hearts. Furthermore, findings from a rodent experiment using a mouse model with a disrupted ketolysis indicate that the upregulation of ketone utilization serves as an adaptive mechanism in response to cardiac hypertrophy and heart failure [219]. Ketone bodies have been demonstrated to serve as a viable fuel source for failing heart in an isolated working mouse heart model [220, 221]. The infusion of exogenous 3-hydroxybutyrate (3-OHB) for 3 h demonstrated favorable hemodynamic effects, as assessed by Swan-Ganz catheterization and echocardiography, in patients with HFrEF [222]. A randomized, controlled, double-blind trial demonstrated that bolus administration of ketone ester improves cardiac output and left ventricular ejection fraction in patients with cardiogenic shock [223]. Furthermore, supplementing ketone bodies through a low-carbohydrate ketogenic diet exhibited improvements in cardiac hypertrophy and heart failure in mice subjected to acute pressure overload, in part through the suppression of the mTOR pathway by ketone-mediated activation of AMPK [224]. These findings suggest that SGLT2i exerts cardioprotective effects in both HFpEF and HFrEF, partially through the induction of a therapeutic range of ketosis. In addition to the modulation of ketone metabolism, a recent *in vitro* study employing metabolomics, lipidomics, and proteomics analyses revealed that empagliflozin, an SGLT2i, influences lipid accumulation, including the restoration of DHA levels, in the AC16 human cardiomyocyte cell line treated with high glucose [225], suggesting a cell-autonomous effect of SGLT2i on mitigating lipotoxicity.

Statins (HMG-CoA reductase inhibitors) are the first-line medication for primary and secondary prevention of cardiovascular death and hospitalization for heart failure in patients with ASCVD without heart failure [226]. The anti-inflammatory and lipid lowering effects of statins may also provide benefits for diabetic cardiomyopathy; however, randomized controlled clinical trials have yet to substantiate the cardiovascular benefits of statin usage in patients with heart failure [227, 228]. Conversely, it is noteworthy that a meta-analysis [229], a retrospective cohort study based on data from the Kaiser Permanente Southern California [230], and a recent retrospective database analysis of large single health care practice [231] have suggested a potential benefit of selectively using statins for clinical outcomes in patients with HFrEF and ASCVD or in patients with HFpEF [230] in real-world clinical settings. Future research is required to determine the benefits of statin usage in patients with heart failure, particularly those with non-atherosclerotic CVD origins, such as diabetic cardiomyopathy.

Recent four clinical trials focusing on triglyceride-lowering therapy have yielded mixed results for CVD outcomes. In the REDUCE-IT trial, patients with established CVD or diabetes who received statins and exhibited elevated triglyceride levels showed a favorable effect from daily consumption of 4 g of icosapent ethyl (an eicosapentaenoic acid ethyl ester) against cardiovascular events than the placebo group [232]. In contrast, in the VITAL trial, supplementation with marine n-3 (also known as omega-3) fatty acids at a dose of 840 mg per day did not significantly lower the incidence of cardiovascular events [233]. Furthermore, in the STRENGTH randomized clinical trial, Epanova, a carboxylic acid formulation of omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), failed to reduce the incidence of cardiovascular events in statin-treated participants with established ASCVD or those with hypertriglyceridemia and low HDL cholesterol levels [234].

Fibrates, small molecule ligands of PPARα, have been clinically used to reduce circulating triglyceride levels. The extended follow-up study of the ACCORD Lipid Trial (ACCORDION) has demonstrated that fenofibrate effectively reduces cardiovascular events in diabetic patients treated with statins [235], indicating that fibrates may be beneficial for CVD independently of statin therapy. However, in a recent multinational, double-blind, randomized, controlled trial (PROMINENT trial), Pemafibrate, a selective PPARα modulator that has greater triglyceride-lowering and high-density lipoprotein (HDL) cholesterol-raising properties than other fibrates [236], failed to reduce cardiovascular events (a composite of nonfatal myocardial infarction, ischemic stroke, coronary revascularization, or cardiovascular death) in patients with hypertriglyceridemia and diabetes undergoing statin treatment (where 96% of patients were receiving statins), compared to the placebo group, despite successfully improving hypertriglyceridemia and low HDL cholesterol levels [237]. Pemafibrate, in addition, did not contribute to a reduction in hospitalizations for heart failure. These findings suggest that triglyceride-lowering therapy may not provide additional benefits for ASCVD events beyond statin treatments. Further studies are required to confirm whether triglyceride lowering by fibrates or omega-3 may play a role in mitigating the risk of non-atherosclerotic CVD, including diabetic cardiomyopathy.

Targeting gut homeostasis

Obesity and obesity-related comorbidities correlate with richness of human gut microbiome [56]. Observational studies identified associative effects of drugs, drug combinations, and previous exposure to antibiotics on variations in the gut microbiome in patients with cardiometabolic disease [84]. One such drug is metformin. The *in vitro* gut-simulator experiments, coupled with transcriptome and gene ontology analyses, revealed that metformin directly modulates the composition of gut microbiota, partially by regulating the expression of genes encoding metalloprotein or metal transporters in individual bacterial species [238]. These findings suggest that metformin may not directly target cardiac steatosis but may achieve the effects through the modulation of gut microbiota populations. In addition to pharmaceutical interventions, the intake of dietary nitrate (NO_3^-) through beetroot juice has been shown to enhance exercise capacity in patients with HFpEF [239] and increase the maximum rate of oxygen consumption during exercise in patients with diabetes [240]. Rodent studies demonstrated the beneficial effect of dietary nitrate on HFD-induced liver steatosis through the modulation of microbiota [241] and cardiomyopathy [242] in mice. These findings suggest that dietary nitrate could be a therapeutic option for diabetic cardiomyopathy through the modulation of gut microbiota. Notably, the fecal metagenomic analysis of the cross-sectional MetaCardis Body Mass Index Spectrum cohort identified statin therapy as a key covariate of microbiome diversification and revealed a negative association between obesity-related microbiota dysbiosis and statin treatment [243]. Given that statin treatment may be ineffective for chronic heart failure in the absence of ASCVD, it is imperative to acknowledge that beyond the modulation of gut microbiota diversity through drugs or dietary interventions, certain specific bacterial species or their bioactive products may play a critical role in lipotoxicity-associated CVD. If this is the case, it is crucial to ascertain the identities of these entities.

Targeting alternative splicing

Antisense oligonucleotides (ASOs), synthetic single-stranded short RNA or DNA molecules that control alternative splicing, have obtained clinical approval from both the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for application in hereditary diseases, including spinal muscular atrophy (SMA). Nusinersen, a modified ASO drug, modulates pre-mRNA splicing of the survival motor neuron 2 (SMN2) gene, thereby promoting the expression of full-length SMN protein. A multicenter, double-blind, sham-controlled trial showed that nusinersen significantly improves motor function compared with sham control group in infants with SMA [244] and children with later-onset SMA [245]. Clinical trials that assess the efficacy of ASOs for improving lipid metabolism have provided positive outcomes on lipid parameters. For example, Vupanorsen, an ASO drug that inhibits the synthesis of Angiopoietin-like 3 (ANGPTL3) protein, demonstrated a notable reduction in non-HDL cholesterol levels in patients with dyslipidemia under statin treatment [246]. Future pre-clinical and clinical studies would be needed to determine whether ASO treatment confers beneficial effects on diabetic cardiomyopathy associated with alternative splicing.

Discussion

Although much progress has been made in elucidating the underlying mechanisms of diets or obesity-mediated lipotoxicity, many issues remain to be investigated. First, to date, the majority of studies on the biology of lipid droplets have been extensively conducted in adipose tissue and the liver. However, the roles and functional consequences of their dysregulation, including impaired lipid droplet formation and lipolysis, in cardiomyocytes remain largely unexplored. Considering the cell and tissue-specific regulation of lipid droplets, future research employing *in vitro* cardiomyocyte models is required. *In vitro* experiments using human cardiomyocytes derived from induced pluripotent stem cells may provide new insights into the mechanisms of lipotoxicity in a species- and cell type-specific manner. Additionally, it is crucial to ascertain the functional significance of the proposed mechanism in the development of diabetic cardiomyopathy *in vivo*.

Second, in certain cases, lipotoxicity tends to preferentially emerge in smooth muscle cells or macrophage, potentially manifesting as ASCVD (e.g., coronary artery disease). Conversely, in other cases, lipotoxicity arises in cardiomyocytes, giving rise to diabetic cardiomyopathy. Alternatively, it is conceivable that the progression of lipotoxicity may occur uniformly in both cell types, leading primarily to coronary artery disease rather than diabetic cardiomyopathy in humans. The susceptibility of each cell type to lipid overload, toxic lipid species, or inflammatory cytokines may be determined by RNA modifications, including alternative splicing, epigenetic modifications, and genetic factors. In this case, future research is warranted to understand the underlying mechanisms by which DNA, RNA, or histone modifications confer cell type-specific effects of lipotoxicity in the cardiovascular system. It may also be possible that a particular fatty acid or composition of fatty acids could induce lipotoxicity exclusively in cardiomyocytes. If so, there would be a necessity to identify a toxic lipid species that is cell type-specific and to elucidate the underlying molecular mechanism governing its release from lipid droplets or the gut microbiota, as well as how it damages cardiomyocytes.

Finally, while toxic lipid accumulation specifically in the heart is sufficient to induce diabetic cardiomyopathy, it

remains uncertain whether exclusively targeting cardiac steatosis is sufficient for sustained improvement in diabetic cardiomyopathy over the long term. Given the complexity of systemic lipid metabolism involving factors, such as diets, physical activity, gut microbiota, medications, circulating fatty acids, cellular signaling, and DNA/RNA/histone modifications, it is conceivable that merely inhibiting cardiac lipotoxicity may not yield sustained long-term benefits for diabetic cardiomyopathy. In this regard, it is imperative to ascertain the systemic effectiveness of pharmacological approaches that target the core lipid droplet machinery or lipid-lowering drugs for obesity or diabetic cardiomyopathy in real-world clinical settings.

Author contributions

MN researched data for the review, contributed to the discussion of the content, wrote the review, edited the manuscript before submission.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work is supported in part by National Heart, Lung, and Blood Institute, U.S. Public Health Service, grant HL155766.

Acknowledgments

The author thanks Mayumi Nakamura for assistance with editing the manuscript and illustration.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Mini Review
PUBLISHED 14 August 2024
DOI 10.3389/jpps.2024.13193

OPEN ACCESS

EDITED BY
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RECEIVED 26 April 2024
ACCEPTED 31 July 2024
PUBLISHED 14 August 2024

CITATION
Shafaati T and Gopal K (2024), Forkhead box O1 transcription factor; a therapeutic target for diabetic cardiomyopathy.
J. Pharm. Pharm. Sci 27:13193.
doi: 10.3389/jpps.2024.13193

Forkhead box O1 transcription factor; a therapeutic target for diabetic cardiomyopathy

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Cardiovascular disease including diabetic cardiomyopathy (DbCM) represents the leading cause of death in people with diabetes. DbCM is defined as ventricular dysfunction in the absence of underlying vascular diseases and/or hypertension. The known molecular mediators of DbCM are multifactorial, including but not limited to insulin resistance, altered energy metabolism, lipotoxicity, endothelial dysfunction, oxidative stress, apoptosis, and autophagy. FoxO1, a prominent member of forkhead box O transcription factors, is involved in regulating various cellular processes in different tissues. Altered FoxO1 expression and activity have been associated with cardiovascular diseases in diabetic subjects. Herein we provide an overview of the role of FoxO1 in various molecular mediators related to DbCM, such as altered energy metabolism, lipotoxicity, oxidative stress, and cell death. Furthermore, we provide valuable insights into its therapeutic potential by targeting these perturbations to alleviate cardiomyopathy in settings of type 1 and type 2 diabetes.

KEYWORDS

FoxO1, diabetic cardiomyopathy, energy metabolism, oxidative stress, cell death

Introduction

Diabetes has evolved exponentially, affects 463 million people worldwide, and prevalence is expected to increase to 700 million by 2045 [1]. Despite numerous advancements in the management of hyperglycemia, cardiovascular diseases including myocardial infarction or heart failure remain the number one cause of death in people with type 1 diabetes (T1D) or type 2 diabetes (T2D) [2]. Although macrovascular dysfunction, endothelial dysfunction, atherosclerosis, and hypertension are increased in diabetic individuals, the increased risk of heart failure is often independent of these comorbidities [3, 4]. Moreover, people with diabetes frequently develop an asymptomatic diastolic dysfunction, a hallmark of diabetic cardiomyopathy (DbCM) [5]. Although the definition of DbCM is still evolving, it is unequivocally considered as ventricular dysfunction with altered myocardial metabolism in the absence of underlying coronary artery diseases and/or hypertension in people with diabetes [5, 6]. Our understanding of pathological changes and molecular mediators of DbCM has greatly improved in last few decades [5], yet there is no approved therapy.

Forkhead box O (FoxO) transcription factors, including FoxO1, FoxO3, FoxO4, and FoxO6, have important roles in several signaling pathways involved in human health and diseases [7]. Out of these subtypes, FoxO1 and FoxO3 are known to be essential for the maintenance of cardiac health by having pivotal roles in the regulations of cellular processes [8]. Lately, with the availability of various pre-clinical models of DbCM, mounting evidence has shown that FoxO1 activity is upregulated in diabetic myocardium [9, 10]. It is also widely accepted that FoxO1 could contribute to the pathogenesis of DbCM via direct or indirect regulations of molecular targets involved in metabolism, oxidative stress, endothelial dysfunction, and apoptosis [10].

In this review, we will provide an overview of the pathology of DbCM and discuss the FoxO1-driven regulations of its key mediators. While our focus will primarily be DbCM in the context of T2D, we will also consider these aspects in the setting of T1D. Furthermore, we will interrogate whether FoxO1 could be a potential therapeutic target for the treatment of DbCM.

Diabetic cardiomyopathy

The DbCM was first described by Rubler and colleagues in 1972 through findings from an autopsy of four diabetic individuals with no sign of myocardial infarction but with left ventricular (LV) hypertrophy, gross cardiomegaly, and congestive heart failure [11]. These observations led to the very first definition of DbCM, a ventricular dysfunction in the absence of underlying coronary artery disease and/or hypertension in people with diabetes. Although the clinical phenotype of DbCM is still under active investigation, our understanding of it has greatly advanced by utilizing modern non-invasive imaging technology [12, 13]. The growing recognition of diastolic dysfunction and alterations in myocardial metabolism mainly an elevation in fatty acid oxidation and a reduction in glucose oxidation in early-stage T2D is reshaping perspectives of DbCM, re-terming or redefining it as “diabetic heart disease” [5] or “diastolic dysfunction with altered myocardial metabolism without other known causes of cardiomyopathy and/or hypertension” [6]. However, re-terming it as “diabetic heart disease” could mistakenly encompass all cardiovascular conditions linked to diabetes, not just those affecting the myocardium, but also vascular diseases. Moreover, diastolic dysfunction often lacks symptoms and remains undiagnosed in diabetic individuals until a noticeable decline occurs, yet its prevalence in T2D has been reported to range from 20 to 80% based on diagnostic criteria and patient group [14–17]. Indeed, we concur with these perspectives, particularly as diastolic dysfunction and DbCM are significant risk factors for the advancement of heart failure with preserved ejection fraction (HFpEF), which is quite prevalent in individuals with diabetes [18]. Although the advancements in the understanding of DbCM have been greatly appreciated in the last few decades [5, 19], it is still unclear why some individuals with diabetes develop HFpEF whereas others develop heart failure with reduced ejection fraction (HFrEF).

Our understanding of the various mechanisms that contribute to the pathology of DbCM has greatly enhanced with improved knowledge of animal models of obesity and insulin resistance (extensively reviewed by Heather et al. [20]). As of now, we are fully aware of several attenuated cellular processes identified within the myocardium of DbCM subjects. These include lipotoxicity, glucotoxicity, mitochondrial dysfunction, abnormal substrate metabolism, oxidative stress, inflammation, and abnormal calcium handling, many of which can lead to the death of cardiac cells, we encourage the reader to refer to the excellent reviews on this topic [5, 19, 21, 22]. Assessing how these factors individually affect diastolic dysfunction in T2D individuals is challenging due to their cross-talk. For instance, insulin resistance can alter metabolism, leading to mitochondrial dysfunction, oxidative stress, and lipotoxicity [23, 24]. Identifying the most effective target for improving diastolic dysfunction remains uncertain, highlighting the need for future studies to explore these mechanisms in T2D subjects.

Forkhead box O1 transcription factor (FoxO1)

The “Forkhead” protein was first identified in 1989 in *Drosophila melanogaster* as a transcriptional regulator containing a winged-helix DNA binding domain [25, 26]. Later in the 1990s, FoxO was identified as abnormal dauer formation-16 (DAF-16) in *Caenorhabditis elegans* and as forkhead in rhabdomyosarcoma (FKHR) in tumor tissues from eight patients with alveolar rhabdomyosarcomas [27, 28]. In humans, there are four FoxO proteins including FoxO1, FoxO3, FoxO4, and FoxO6 are known to be present in various tissues [29, 30]. Although FoxO6 was initially thought to be mainly in the brain, it is now known for a ubiquitous expression as well [31]. FoxO1/3/4/6 proteins through their conserved forkhead domain specifically recognize DAF-16 binding element (DBE) 5′-GTAAACAA-3′ and insulin-responsive element (IRE) 5′-(C/A)(A/C)AAA (C/T)AA-3′ to transcriptionally regulate the expression of genes (extensively reviewed in [32, 33]). FoxO’s nuclear transit and transcriptional activity are also regulated by various post-translational modifications such as phosphorylation, acetylation, O-glycosylation, methylation, and ubiquitination. Although several kinases (e.g., mitogen-activated protein kinases, c-Jun N-terminal kinases, cyclin-dependent kinase 2, nuclear factor κB, etc.) are known to be involved in the phosphorylation of FoxO1, protein kinase B (also known as AKT), a downstream target of

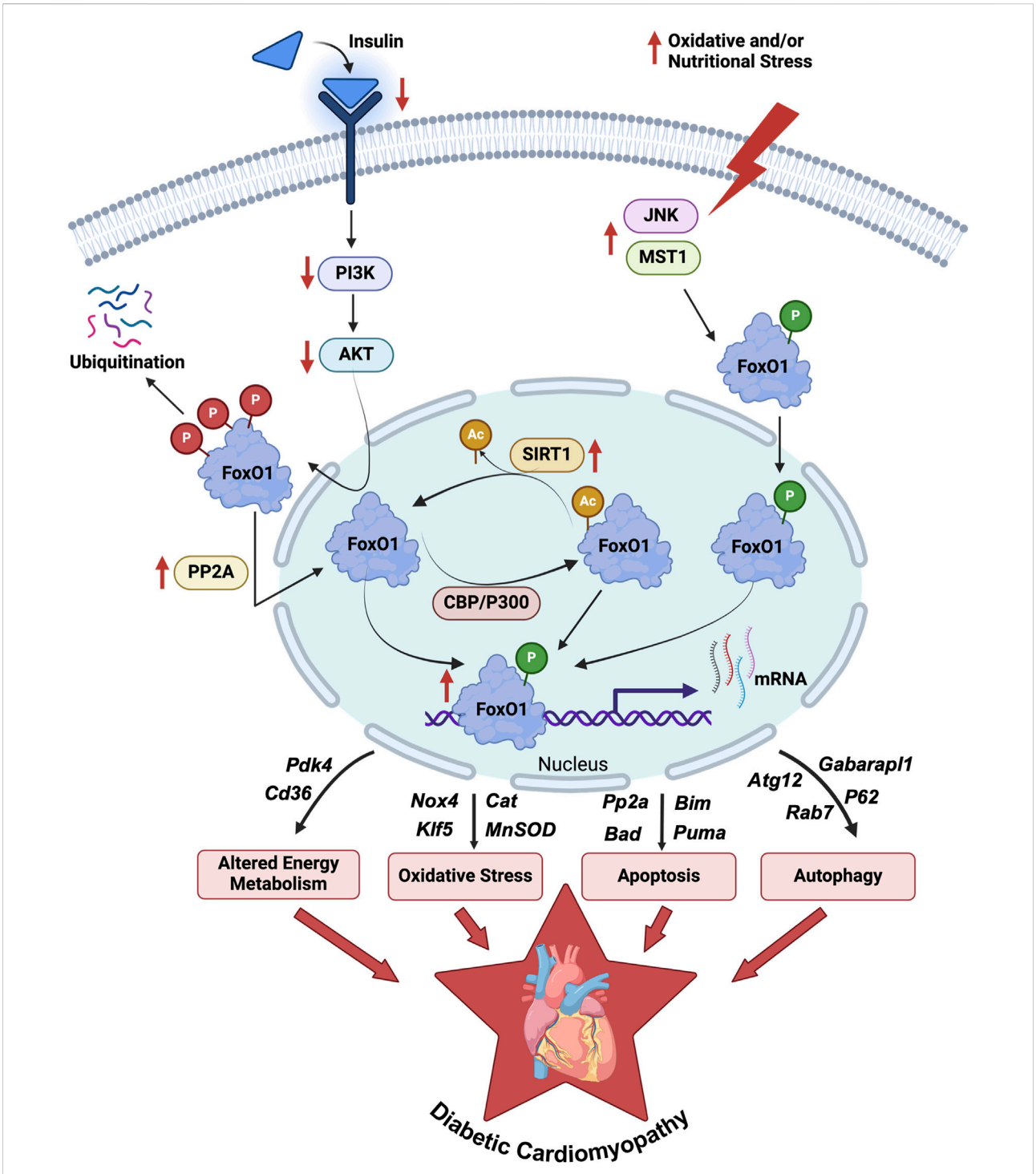

FIGURE 1
Regulations and role of FoxO1 in DbCM. An illustration depicts the regulation of FoxO1 activity via post-translational modifications such as phosphorylation, acetylation, and ubiquitination in the myocardium of diabetic individuals. The enhanced FoxO1 activity transcriptionally upregulates several genes (Table 1) involved in myocardial energy metabolism, oxidative stress regulations, apoptosis, and autophagy reported in preclinical and clinical studies of DbCM. DbCM, diabetic cardiomyopathy; FoxO1, Forkhead box O1 transcription factor; PI3K, Phosphoinositide 3-kinases; AKT, Protein kinase B; PP2A, Protein phosphatase 2A; CBP, cAMP-response element binding protein; P300, Histone acetyltransferase p300; SIRT1, Sirtuin-1; JNK, c-Jun N-terminal kinase; MST1, Macrophage stimulating 1; Pdk4, Pyruvate dehydrogenase kinase 4; Cd36, fatty acid translocase; Nox4, NADPH oxidase 4; Klf5, Kruppel-like factor 5; Cat, Catalase; MnSOD, Manganese superoxide dismutase; Bim, Bcl2 interacting mediator; Bad, Bcl2 antagonist of cell death; Puma, p53 upregulated modulator of apoptosis; Atg12, Autophagy related 12; Rab7, Rat sarcoma virus-related protein 7; Gabarapl1, Gamma-aminobutyric acid receptor-associated protein-like 1; P62, ubiquitin-binding protein p62; P, Phosphorylation (Red, AKT mediated; Green, JNK1/MST mediated); Ac, Acetylation.

insulin signaling have been considered a prime kinase which negatively regulates FoxO1 by phosphorylation and nuclear exclusion in the context of metabolism (Figure 1) [34, 35]. In the 21st century, a plethora of studies concluded the essential role of FoxO transcription factors in myocardial homeostasis through the regulation of cell proliferation, oxidative stress, energy metabolism, and cell death (extensively reviewed in [36, 37]). FoxO1, among other "O" subfamilies, has been considered the front-runner in controlling myocardial equilibrium in the settings of metabolic diseases (especially in DbCM) [10, 36, 38].

FoxO1 in diabetic cardiomyopathy

The plethora of evidence suggests that the pathophysiology of DbCM [9, 39, 40], and ischemic heart disease [41] are linked to upregulated FoxO1 activity. Although there is no direct clinical evidence of FoxO1 activation during DbCM, its DNA binding sites are overrepresented in the promoter sequences of heart failure genes in isolated RNA from the myocardium of heart failure patients with either ischemic or idiopathic dilated cardiomyopathy [42]. Additionally, RNA sequencing data of human hearts with dilated cardiomyopathy showed an enriched FoxO1-binding motif, suggestive of enhanced transcriptional activity [43]. However, the contribution of cellular mechanisms associated with FoxO1 signaling in the pathogenesis of DbCM is not yet fully understood. The connection between FoxO1 activation and the pathogenesis of DbCM mainly stemmed from *in vivo* animal models and *in vitro* studies [9, 44]. In the case of insulin resistance and diabetes, reduced growth signals and increased stress signals lead to weaker nuclear exportation mechanisms for FoxO1, resulting in increased FoxO1 transcriptional activity in cardiomyocytes [36]. The increased transcriptional activity of FoxO1 precipitates shifts in gene expression, consequently inducing modifications in myocardial energy metabolism, lipotoxicity, oxidative stress, and cellular damage in diabetic myocardium (Figure 1; Table 1).

FoxO1 in metabolic abnormalities during diabetic cardiomyopathy

Numerous studies have consistently affirmed the idea that disruptions in myocardial glucose and fatty acid metabolism serve as primary triggers for cardiac dysfunction in diabetic conditions, a topic thoroughly reviewed by Heather et al. [6]. FoxO1 is involved in various pathways related to myocardial energy metabolism. Battiprolu et al. have shown that 25 weeks of

TABLE 1 Potential effects of FoxO1 regulation in DbCM.

Animal models	Intervention/Targets	Effects	References
HFD-fed obese mice	Cardiac-specific FoxO1 knockout	↓ Myocardial TAG content ↓ LV Hypertrophy ↓ Cardiac systolic dysfunction Improves myocardial PDH activity	[9]
HFD and STZ-induced T2D mice	FoxO1-*Pdk4* axis targeted with AS1842856 and cardiac-specific FoxO1 knockout	↑ Myocardial glucose oxidation ↓ Diastolic dysfunction	[45, 46]
STZ-induced T1D Sprague-Dawley rats	FoxO1 inhibition by AS1842856	Improves cardiac function ↑ Glucose oxidation ↓ Apoptosis	[47]
Wistar rats with lipid overload	FoxO1-iNOS-CD36 pathway	Cardiomyocyte lipid accumulation	[48]
Db/db mice	FoxO1-CD36 axis targeted with Evogliptin	Protects against DbCM Prevents lipotoxicity	[49]
HFD-Fed mice	FoxO1-CD36 axis EP4-deficient mice	↓ Myocardial fatty acid uptake ↓ ATP production	[50]
STZ-induced T1D mice	FoxO1-KLF5 Cardiac-specific FoxO1 knockout	↓ Oxidative stress ↓ Cardiac dysfunction	[51]
Sprague-Dawley rats on high-glucose and HFD with STZ	Sirt1-FoxO1 and PI3K-Akt signaling pathways targeted with Curcumin	↓ Oxidative stress ↓ Apoptosis ↓ DbCM	[52]
Db/db mice	Akt-FoxO1 signaling by Diazoxide	↓ Apoptosis	[53]
STZ-induced T1D mice	Angiotensin IV AS1842856	↓ Autophagy	[54]
STZ-induced T1D mice	Resveratrol FoxO1- Rab7	Restores Autophagic flux	[55]

high-fat diet (HFD) (60% kcal from lard) feeding to male C57BL/6J mice induces myocardial nuclear enrichment of FoxO1, leading to enhancement in myocardial triacylglycerol (TAG) content, LV hypertrophy, and cardiac systolic dysfunction, which was not apparent in HFD-fed cardiac-specific FoxO1 deficient mice [9]. In addition, enhanced FoxO1 nuclear compartmentalization contributed to elevations in myocardial pyruvate dehydrogenase (PDH) kinase 4 (*Pdk4*) transcription and impairment in PDH activity in the myocardial tissues of HFD-fed mice. Concurrently, we have shown that FoxO1 binds to the DBE sequence in the promoter of the *Pdk4* gene to upregulate its expression in cardiomyocytes and reduce myocardial glucose oxidation rates [45]. As the glucose oxidation produces more ATP per mole of consumed oxygen than the fatty acid oxidation, reduced cardiac function correlated with higher oxygen consumption and lower cardiac efficiency in *ob/ob* mice with reduced glucose oxidation and increased fatty acid oxidation [56, 57]. Additionally, the increases in the myocardial delivery of fatty acids due to adaptive changes may lead to the uncoupling of the mitochondria, leading to a reduction in ATP production which aligns with the reduced cardiac performance. Moreover, recent studies from our lab targeting this FoxO1-*Pdk4* axis using either AS1842856 (FoxO1 inhibitor) or cardiac-specific FoxO1 elimination alleviated diastolic dysfunction via increasing myocardial glucose oxidation rates in male mice subjected to experimental T2D via HFD supplementation for 10 weeks with a single dose (75 mg/kg) of streptozotocin (STZ) at 4th week [45, 46]. Similarly, male Sprague Dawley rats induced with T1D using STZ (65 mg/kg) and treated with AS1842856 demonstrated improved cardiac function using pressure-volume conductance catheters [47]. The isolated cardiomyocytes from these rats demonstrated increased oxygen consumption rates in the presence of glucose or pyruvate (indicative of increased glucose oxidation). These findings strongly advocate the role of FoxO1 in the reduction of glucose oxidation in the myocardium of diabetic mice with cardiac dysfunction.

In diabetic myocardium, decreases in glucose oxidation with elevated PDK4 expression often result in increases in fatty acid uptake and oxidation by following Randle's cycle to meet constant energy demand, thereby promoting myocardial lipid accumulation [22]. Contrarily, mice with cardiac-specific overexpression of PDK4 were protected against HFD-induced myocardial lipid accumulation, likely due to adaptive metabolic re-programming for increased fatty acid oxidation [58]. However, Elevated myocardial TAG content-associated lipotoxicity has been verified in individuals with T2D and identified as an independent predictor of diastolic dysfunction [59]. A key early development in DbCM pathogenesis involves increased fatty acid transport across the sarcolemma, primarily controlled by fatty acid translocase (FAT/CD36) [60]. In conditions of lipid overload, the FoxO1/inducible NO-synthase (iNOS)/CD36 pathway was shown to mediate lipid accumulation in cardiomyocytes from adult male Wistar rats [48]. Palmitate exposure in isolated cardiomyocytes leads to a significant overload of intercellular TAG which triggers a chain reaction starting with the upregulation of FoxO1. The high expression of FoxO1 in the vascular endothelial cells leads to an overexpression of iNOS which activates the cell division control (Cdc) 42 protein through its nitration, resulting in cytoskeleton rearrangement. This process aids CD36 translocation and results in TAG accumulation in cardiomyocytes from adult male Wistar rats [48]. Moreover, Evogliptin (EVO), a dipeptidyl peptidase-4 (DPP-4) inhibitor known for its glucose-lowering effects in T2D, demonstrated the ability to prevent DbCM and associated lipotoxicity by suppressing CD36 protein expression and enhancing the phosphorylation of FoxO1 at Serine 256 position, indicative of its inactivation, in db/db mice [49]. Prostaglandin E receptor subtype 4 (EP4) is a G protein-coupled receptor (GPCR) highly expressed in cardiomyocytes. In a study involving mice supplemented with HFD for 8 weeks, EP4 was shown to protect against DbCM by modulating FoxO1/CD36-mediated fatty acid uptake [50]. The concentric hypertrophy and myocardial fibrosis in HFD-fed EP4-deficient mice converged with a reduction in myocardial fatty acid uptake and ATP production, which was corrected pharmacologically by activation of EP4. Thus, by targeting the FoxO1–CD36 axis, we could reduce the myocardial damage associated with lipotoxicity during diabetes.

FoxO1 in myocardial oxidative stress during diabetic cardiomyopathy

It is undebatable that hyperglycemia along with enhanced fatty acid oxidation and mitochondrial dysfunction contributes to oxidative stress by increasing reactive oxygen species (ROS) including superoxide and H_2O_2 levels in the diabetic myocardium [61]. FoxO1 has been known to play a dual role during oxidative stress regulation based on the cellular microenvironment and level of oxidative stress [37]. Recently, Krüppel-like factor (KLF) 5 directly transcriptionally regulated by FoxO1 was shown to cause oxidative stress via induction of NADPH oxidase (NOX) 4 expression, a major source of cytosolic ROS levels [62] in cardiomyocytes of STZ-induced T1D mice [51]. Cardiac-specific FoxO1 elimination remarkably reduced KLF5 expression and prevented oxidative stress and cardiac dysfunction, which was reverted by over-expression of FoxO1 or KLF5 in cardiomyocytes of T1D mice. Concurrently, Curcumin, a natural antioxidant, treatment in male Sprague-Dawley rats fed a high-glucose and HFD (40% fat, 41% carbohydrates, and 18% protein) and supplemented with STZ (60 mg/kg; 3 days) was shown to alleviate oxidative stress and DbCM by FoxO1 modulation via sirtuin 1 (Sirt1) and phosphoinositide 3-kinases (PI3K)-AKT signaling pathways

[52]. Moreover, high glucose upregulated thioredoxin (Trx) interacting protein (Txnip) expression by binding of FoxO1 to its promoter and subsequently inhibited Trx activity in human aortic endothelial cells [63]. These effects were Trx system-mediated reduction of oxidized cysteine groups on proteins through an interaction with the redox-active center of Trx and activated FoxO1 pathway.

On the other hand, FoxO1 may also protect against oxidative stress in cardiomyocytes by promoting the expression of antioxidant enzymes such as catalase (CAT) and manganese superoxide dismutase (MnSOD) via Yes-associated protein (YAP) pathways to neutralize ROS [64]. STZ-induced diabetic rats with myocardial metabolism and functional abnormalities showed oxidative stress by reduced activity of SOD, and elevated malondialdehyde [MDA] levels [52]. Curcumin treatment in these rats rescued the activity of SOD by restoring Sirt1-FoxO1 signaling, resulting in reduced ROS and alleviation of DbCM. Moreover, Exenatide, a glucagon-like peptide-1 (GLP-1) receptor agonist, attenuated ROS production through increases in expression of MnSOD and catalase in cardiomyocytes of HFD-fed T2D mice and STZ-induced T1D mice [65]. These protective actions might be mediated through Sirt1-FoxO1 pathways, as the cardioprotective effects of Exendin-4 against ischemia/reperfusion (I/R) injury in male rats involves upregulated activity Sirt1-FoxO1 pathways and associated MnSOD production [66]. Thus, in varying microenvironments such as the level of stress in various cell types of diabetic myocardium or ROS-mediated signaling activation, FoxO1 may play a destructive rather than protective role during oxidative stress regulations [67]. In diabetic myocardium, conditions like hyperglycemia, insulin resistance, and metabolic disturbances such as elevated serum glucose or lipids can induce FoxO1 expression, shifting its function from antioxidant to prooxidant.

FoxO1 in diabetic cardiomyopathy-associated myocardial cell death

Apart from its roles in energy metabolism and oxidative stress, FoxO1 also has substantial roles in myocardial cell death via apoptosis and autophagy during diabetes [68]. In diabetic myocardium, upregulated FoxO1 activity stimulates the expression of various proapoptotic regulators such as B-cell lymphoma 2 (Bcl2)-associated agonist of cell death (BAD), Bcl-2 Interacting Mediator (BIM), Puma, and caspases [46, 48]. Puthanveetil et al. demonstrated that FoxO1 regulates BAD via up-regulation of protein phosphatase 2A (PP2A) in the diabetic myocardium [48]. However, in cardiomyocytes, overexpression of the wild-type or constitutively active form of FoxO1 has been associated with inhibition of the PP2A/B activity and attenuation of insulin signaling [69]. This divergence likely stems from FoxO1/CD36-mediated lipid buildup in diabetic cardiomyocytes, which may reactivate PP2A and trigger BAD activation, similar to how ceramides stimulate PP2A during arterial dysfunction in obese mice [70]. It is noteworthy that, myocardial apoptosis may not be regulated by FoxO1 in all available pre-clinical models of DbCM. Our recent study demonstrated the attenuation of diastolic dysfunction and altered myocardial metabolism but no effect on apoptosis by pharmacological or genetic inhibition of FoxO1 in T2D mice [46]. However, FoxO1 inhibition by AS1842856 in T1D male Sprague Dawley rats mitigated the apoptosis, evident by a reduction in cleaved caspase 3 expression and tunnel staining [47]. Similarly, curcumin was shown to alleviate apoptosis in cardiomyocytes which was associated with inhibition of FoxO1 acetylation and modulation of Sirt1-FoxO1 signaling in STZ-induced T2D rats [52]. Moreover, the opening of mitochondrial ATP-sensitive potassium (mitoKATP) channels by diazoxide was found to improve cardiac function and attenuate cardiomyocyte apoptosis in db/db mice [53]. The protective effect of diazoxide was associated with a reduction in AKT-FoxO1 signaling and the activity of caspase 3 in cardiomyocytes.

While the significance of autophagy in DbCM is still subject to debate, FoxO1 has been implicated in its regulation. In starvation, FoxO1 can activate the expression of autophagic genes such as autophagy-related protein 12 (Atg12) and γ-aminobutyric acid receptor-associated protein-like 1 (Gabarapl1) in cardiomyocytes [71]. Similarly, glucose-deprived cultured cardiomyocytes showed increased autophagic flux accompanied by Sirt1-associated FoxO1 deacetylation and a decreased expression of ubiquitin-binding protein p62 [72]. Contrarily, acetylated FoxO1 has been shown to upregulate autophagy in a transcription-independent manner by interacting with Atg7 in the cytosol of cancer cells [73]. FoxO1 also plays an essential role in the regression of cardiac hypertrophy via upregulating autophagy during mechanical unloading by reversal of transverse aortic constriction (TAC) in mice [74]. Similarly, FoxO1 contributes to exercise-induced physiological hypertrophy by regulating autophagy markers independent of the PI3K-AKT signaling [75]. Moreover, cardiac-specific overexpression of FoxO1 in transgenic mice exhibited a decrease in the size of hearts and upregulation of autophagy. Concurrently, it has been demonstrated that the cardioprotective effect of angiotensin (Ang) IV in T1D mice was through suppression of FoxO1-induced excessive autophagy [54]. The protective effects of Ang IV were completely blocked by over-expression of FoxO1, which was reversed by the additional administration of AS1842856. However, resveratrol has been shown to protect against DbCM by restoring autophagic flux [55]. The effect was achieved through the upregulation of FoxO1-mediated transcription of rat sarcoma virus-related protein (Rab) 7, a small GTP-binding protein that mediates late autophagosome-lysosome fusion. Thus, the enhancement of FoxO1 activity contributes to dysregulated apoptosis and autophagy in diabetes, and targeting these perturbations could alleviate the progression of DbCM.

Discussion

Taken together, FoxO1 dysregulations could exacerbate damages in myocardial cellular processes, accelerating the development of diastolic dysfunction during DbCM, a major complication in people with diabetes. Metabolic alterations, oxidative stress, and cell death are implicated in both the progression of DbCM and the regulatory processes involving FoxO1. Enhanced FoxO1 expression and activity appear to promote alteration in myocardial glucose and fatty acid metabolism, oxidative stress, and cell death in DbCM. Notably, the above-discussed findings are mainly based on animal models of T1D or T2D and clinical applications of FoxO1 signaling in cardiac injury in DbCM are still unknown. Moreover, the interplay between different molecular mediators of DbCM and their regulation by FoxO1 in pre-clinical models is largely unknown to predict a translational aspect of these findings. Thus, we currently lack enough information on whether FoxO1 or its pathways could be a therapeutic target during DbCM in people with diabetes. A promising approach could be the optimization of cardiac energy metabolism, though an improved understanding of how FoxO1-mediated modulations of myocardial energy metabolism, oxidative stress, cell death, and its interplay regulate diastole may direct us to better molecular targets for future drug development.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. The work is supported by the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta.

Acknowledgments

The figure in this review article was created with BioRender.com.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Review
PUBLISHED 23 July 2024
DOI 10.3389/jpps.2024.13080

OPEN ACCESS

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RECEIVED 02 April 2024
ACCEPTED 08 July 2024
PUBLISHED 23 July 2024

CITATION
Ketema EB and Lopaschuk GD (2024), The role of acetylation in obesity-induced cardiac metabolic alterations. *J. Pharm. Pharm. Sci* 27:13080. doi: 10.3389/jpps.2024.13080

The role of acetylation in obesity-induced cardiac metabolic alterations

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Obesity is a growing public health problem, with its prevalence rate having tripled in the last five decades. It has been shown that obesity is associated with alterations in cardiac energy metabolism, which in turn plays a significant role in heart failure development. During obesity, the heart becomes highly dependent on fatty acid oxidation as its primary source of energy (ATP), while the contribution from glucose oxidation significantly decreases. This metabolic inflexibility is associated with reduced cardiac efficiency and contractile dysfunction. Although it is well recognized that alterations in cardiac energy metabolism during obesity are associated with the risk of heart failure development, the molecular mechanisms controlling these metabolic changes are not fully understood. Recently, posttranslational protein modifications of metabolic enzymes have been shown to play a crucial role in cardiac energy metabolic changes seen in obesity. Understanding these novel mechanisms is important in developing new therapeutic options to treat or prevent cardiac metabolic alteration and dysfunction in obese individuals. This review discusses posttranslational acetylation changes during obesity and their roles in mediating cardiac energy metabolic perturbations during obesity as well as its therapeutic potentials.

KEYWORDS

obesity, cardiac energy metabolism, protein lysine acetylation, heart failure, fatty acid oxidation

Introduction

Obesity is a growing public health problem, with its worldwide prevalence rate having tripled in the last five decades [1]. Nearly 40% of the global adult population is overweight, while 13% of adults are clinically obese [1]. Similarly, the prevalence of both overweight and obesity has increased over fourfold in children [1, 2]. Overweight and obese accounts for over 4 million deaths each year worldwide [2, 3]. Obesity-related disability-adjusted life years (DALYs) has increased significantly in the last few decades, and the trend is projected to rise by 39.8% from 2020 to 2030 [3].

Obesity and its burden on heart failure (HF)

Obesity is a major risk factor for various diseases, including cardiovascular diseases [1]. Cardiovascular diseases contribute to more than two-thirds of mortalities in obese individuals [2, 4]. Obese individuals also have a two times higher risk of heart failure (HF) development compared with subjects with a normal body weight [5]. It is projected that the prevalence of obesity will increase significantly in the coming years, while other risk factors for HF, such as hypertension, are expected to decline [1, 6, 7]. Regarding the specific HF pathologies, the incidence of HF with preserved ejection fraction (HFpEF) is far more common in obese individuals compared to the incidence of HF with reduced ejection fraction (HFrEF) [8]. Moreover, approximately 80% of HF patients with preserved ejection fraction are either overweight or obese [9].

Obesity leads to cardiac dysfunction and risk of HF development through both direct and indirect mechanisms. The indirect mechanisms include increased levels of circulating free fatty acids, pro-inflammatory cytokines, and adipokines that can lead to the development of metabolic risk factors which includes, insulin resistance, dyslipidemia, and diabetes [10, 11]. On the other hand, direct mechanisms of obesity-induced HF include myocardial lipotoxicity, changes in neurohormonal and hemodynamic balances, and microvascular dysfunction [10, 12]. Both direct and indirect mechanisms listed here are strongly associated with cardiac energy metabolic alterations seen in obesity. Alterations in cardiac energy metabolism, in turn, play a significant role in obesity-related HF development [13].

Energy metabolism in the heart

The heart has the highest energy demand of any organ in the body on a per gram weight basis [14]. In the healthy heart this high energy demand is fulfilled by metabolizing various fuel substrates, predominantly fatty acids and glucose, but also ketones, lactate, and amino acids depending on the supply, demand and neurohormonal states [15]. This ability of the heart to use different types of fuel for energy production is often described as "metabolic flexibility" or being "metabolically omnivorous." Oxidation of fatty acids is the primary source of ATP production by the heart, accounting for approximately 60% of the ATP produced by the normal heart [14]. Myocardial fatty acid metabolism is regulated by both the supply of fatty acids to the heart, and by complex intracellular control mechanisms [14]. The intracellular control mechanisms involve allosteric, posttranslational, and transcriptional control of fatty acid oxidative enzymes [15]. The fatty acid supply and uptake into the myocardium is highly dependent on the circulating levels of fatty acids [16]. The subsequent uptake of fatty acids across the sarcolemma of cardiomyocytes is facilitated by at least three proteins: CD36, FA transport protein (FATP), and FA binding protein plasma membrane (FABPpm) (Figure 1) [17].

Within the cardiomyocytes, a key site involved in the regulation of fatty acid oxidation is at the point of mitochondrial fatty acid uptake by the enzyme carnitine palmitoyltransferase (CPT1) [14]. CPT1 facilitates the transport of long-chain fatty acids into the mitochondrial matrix by transferring the fatty acyl moiety from fatty acyl-CoA to carnitine to form acylcarnitines, which can be transported into the matrix for fatty acid β-oxidation. CPT1 activity is regulated by malonyl-CoA, a strong inhibitor of CPT1 [18]. Malonyl-CoA in the heart is synthesized from acetyl-CoA by acetyl-CoA carboxylase (ACC) and degraded by malonyl-CoA decarboxylase (MCD). Additionally, AMP-activated protein kinase (AMPK) regulates malonyl-CoA levels, and therefore fatty acid oxidation rates, by inhibiting ACC through phosphorylation (Figure 1) [19]. Once in the mitochondria, fatty acyl CoA undergoes β-oxidation, a repetitive and cyclic reaction sequentially catalyzed by long-chain acyl CoA dehydrogenase (LCAD), enoyl-CoA hydratase (ECHA), L-3-hydroxy acyl-CoA dehydrogenase (β-HAD), and 3-ketoacyl-CoA thiolase (3-KAT), until it is completely converted to acetyl CoA [14].

Cardiac glucose metabolism has two major steps. In the first step, glucose is taken up into cardiomyocytes through the insulin-dependent glucose transporter, GLUT4, or to a lesser extent, insulin -independent glucose transporter, GLUT1 [20]. In cardiomyocytes, glucose passes through glycolysis and converted to pyruvate. In the next step, pyruvate is transported into the mitochondria and converted to acetyl CoA by pyruvate dehydrogenase (PDH). In the end, acetyl CoA is oxidized to CO_2 in the TCA cycle. In addition to fatty acids and glucose, the heart can also metabolize ketones and amino acids as sources of ATP [21, 22].

Cardiac energy substrate metabolic alterations in obesity

Obesity occurs when an abnormal or excessive fatty acid accumulation occurs in adipose tissue due to imbalances in calorie intake and consumption [1]. It is associated with increased circulating levels of free fatty acids and triacylglycerols [23]. Increased levels of fatty acid transporter proteins on the sarcolemma of cardiomyocytes also occur in response to obesity [23–25]. Since myocardial fatty acid uptake is mainly influenced by the free fatty acid levels in the circulation [26], the increased levels of circulating fatty acids and fatty acid transporters on cardiomyocytes leads to an increased myocardial fatty acid uptake during obesity (Figure 1) [23, 25, 26]. Several pre-clinical and clinical studies have also shown that the increased myocardial fatty acid supply and uptake in obesity

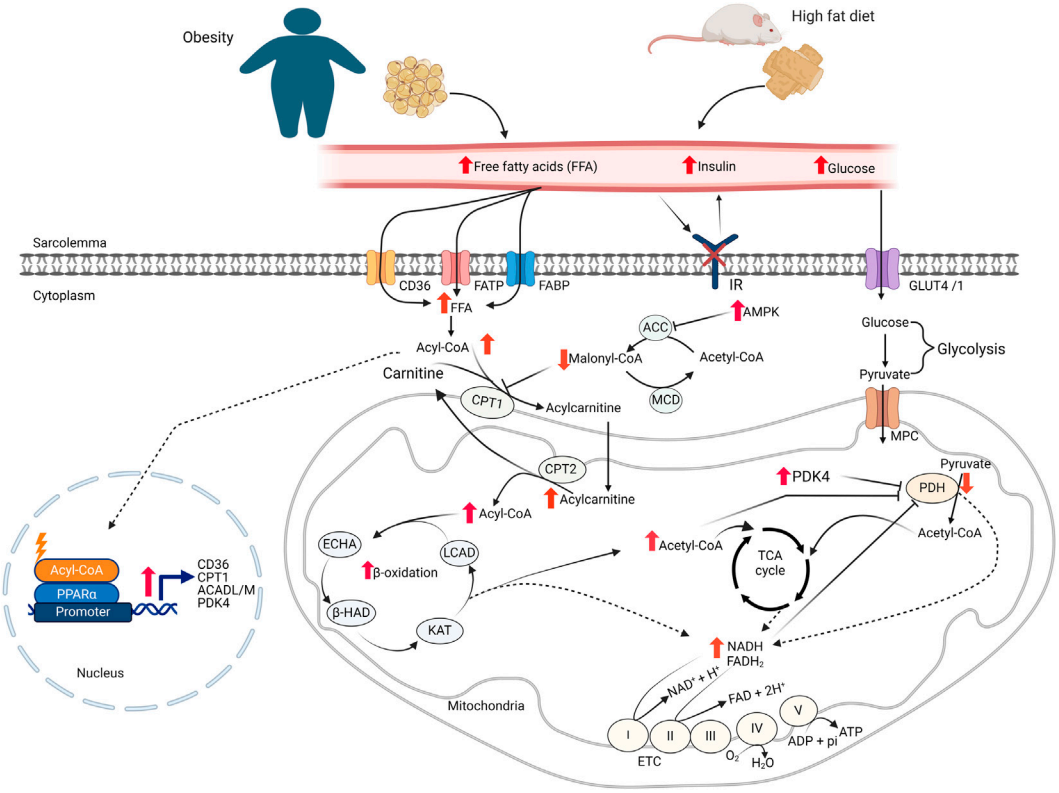

FIGURE 1
Cardiac energy metabolism changes in obesity FFA: free fatty acids; GLUT4, glucose transporter isoform 4; IR, insulin receptor; CD36, cluster of differentiation 36; FABP, fatty acid-binding protein; FATP, fatty acid transport protein; MCD, malonyl CoA decarboxylase; ACC, acetyl CoA carboxylase; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; LCAD, long-chain acyl CoA dehydrogenase; β–HAD, β-hydroxyacyl CoA dehydrogenase; KAT, 3-ketoacyl-coA thiolase; ECHA, enoyl-CoA hydratase; CPT, carnitine palmitoyltransferase; FAD/$FADH_2$, flavin adenine dinucleotide; NAD/$NADH_2$, nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate.

TABLE 1 Alterations in myocardial energy metabolism in obesity.

Experimental models/conditions	Changes in myocardial energy substrate metabolism		
	Fatty acid oxidation	Glucose oxidation	Other findings
HFD-induced obesity in rats	Increased [14] Unchanged [24]	Decreased [14]	• Increased PDK4 [14] • Increased UCP3 expression [14] • Increased FA uptake [24] • Decreased glucose uptake [24]
Obese Zucker rats	Increased [25, 27]	-	• Increased FA uptake and esterification [25] • Decreased glycolysis rate [27]
Ob/ob mice	Increased [28–30]	Decreased [28]	• Increased FA oxidation gene [30] • Decreased glycolysis rate [29] • Increased AMPK phosphorylation [29]
Db/db mice	Increased [31, 32]	Decreased [31]	• Decreased glycolysis rate [31] • Increased in UCP activity mice [32] • Increased FA oxidation genes [32]
HFD-induced obesity in mice	Increased [33–38]	Decreased [35–38] Unchanged [39]	• Decreased glucose uptake [39] Decreased glycolysis [36] • Increased PDK4 and UCPs expression [36]
Obese patients	Increased [40, 41]	Decreased [42]	• Increased fatty acid uptake [40] • Decreased glucose uptake [41]

HFD: high fat diet; PDK4: pyruvate dehydrogenase kinase 4; UCP: uncoupling protein; FA: fatty acid.

is accompanied by increased fatty acid oxidation rates in the heart (Table 1) [27, 28, 33, 34, 40, 41, 43, 44]. In addition to the increased fatty acid oxidation rates, excess fatty acid supply and uptake can lead to the accumulation of toxic lipid intermediates that have roles in the development of cardiac insulin resistance [45, 46]. The major lipid metabolite storage in the heart during obesity includes long-chain acyl CoAs, diacylglycerols (DAG), triacylglycerols (TAG), ceramides, and acylcarnitines [47]. The excess intra-myocardial storage of these lipid metabolites is associated with cardiomyocyte apoptosis, mitochondrial dysfunction, and lipotoxicity [13, 48].

The increased myocardial fatty acid supply and utilization in obesity suppresses cardiac glucose metabolism, a phenomenon known as the "Randle Cycle" [49]. In particular, myocardial glucose oxidation is markedly suppressed in obesity [28, 31, 35–37, 40, 42]. In addition to fatty acid oxidation-mediated inhibition of glucose oxidation, there are also several other mechanisms that can contribute to reduced glucose oxidation in the heart of obese subjects (Table 1). Firstly, the accumulation of lipid intermediates following excess supply or utilization is associated with the development of cardiac insulin resistance through different mechanisms [13, 29]. Secondly, the excess acyl CoAs in the heart of obese subjects is associated with decreased myocardial glucose uptake [29, 39, 41, 50]. Furthermore, increased acetyl CoA and nicotinamide adenine dinucleotide (NADH) production following high rates of fatty acid oxidation during obesity activates pyruvate dehydrogenase kinase 4 (PDK4), which inhibits PDH, the main enzyme of glucose oxidation, by phosphorylation [14, 36, 51]. As will be discussed, increased posttranslational acetylation of PDH may also be involved in this decrease in glucose oxidation.

Obesity can also affect myocardial fatty acid metabolism through transcriptional mechanisms. One of the important transcription factors controlling gene expression related to fatty acid metabolism is the peroxisome proliferator-activated receptors (PPARs). PPARs exist in three isoforms: PPARα (main isoform in the heart), PPARγ (predominantly in adipose tissue), and PPARδ. Increased levels of long-chain fatty acids in the myocardium are among the activators of PPARα. Activation PPARα has been shown to promote gene expression in myocardial fatty acid uptake, storage and oxidation [30, 52–54]. PPARα has also been shown to play a critical role in shifting energy substrate metabolism in the heart towards increased fatty acid utilization [55]. PPARα activation also suppresses glucose oxidation by increasing the expression of PDK4 [55, 56].

As discussed, obesity results in the heart becoming highly dependent on fatty acid oxidation as its ATP source, while the contribution from glucose oxidation significantly decreases [13]. This metabolic inflexibility is associated with reduced cardiac efficiency and contractile dysfunction [28, 57]. These adverse effects may be due, in part, to the fact that fatty acids are a less efficient energy substrate than glucose, leading to increased myocardial oxygen consumption per cardiac work [51]. The reduced cardiac efficiency due to high fatty acid oxidation rates is also associated with increased activity of uncoupling proteins (UCPs), which uncouples mitochondrial proton gradient from ATP synthesis by facilitating proton leak back into the mitochondrial matrix without generating ATP [32, 58]. Interestingly, increased activity and expression of cardiac UCPs have been shown in obese animals [14, 57–59]. An increased mitochondrial reactive oxygen species (ROS) production during lipid overload in obesity has also been shown to contribute to the increased activity of UCPs [32].

Although alterations in cardiac energy metabolism during obesity are associated with the risk of HF development, the molecular mechanisms controlling these metabolic changes are not fully understood. Tremendous efforts have been made to characterize the allosteric and transcriptional mechanisms contributing to altered cardiac energy metabolism in obesity. However, transcriptome and metabolomics studies revealed these mechanisms alone are not sufficient to explain the significant alterations in cardiac energy metabolism during obesity or heart failure [60, 61]. Recently, several posttranslational protein modifications have been shown to play a crucial role in cardiac energy metabolic changes seen in obesity. There are numerous reversible posttranslational modifications of proteins, including phosphorylation, methylation, acetylation, O-GlcNAcylation, ubiquitylation, succinylation, nitrosylation, SUMOylation, glycation, and β-hydroxybutyrylation. This review focuses on post-translation acetylation changes and their roles in mediating the cardiac energy metabolic perturbations during obesity.

Posttranslational protein acetylation

Protein lysine acetylation is a reversible posttranslational modification that occurs by the addition of an acetyl group to the lysine residues of proteins. This acetylation modification alters the charge status on lysine residues, and adds an extra structural moiety, an acetyl group [62, 63]. This structural change impact proteins' native structure, interactions with other proteins, stability, and function [64].

Posttranslational protein acetylation was identified initially on histone proteins over half a century ago [65]. Since then, histone acetylation modification has been widely recognized as an important epigenetic mechanism that regulates the structure of chromatin and gene expression processes (Figure 2). Dysregulation of histone acetylation is linked to altered gene expression profiles and has been implicated in several diseases, including cancer and metabolic diseases [66]. More recently, non-histone protein acetylation was also identified as an important entity in regulating cellular function [67]. With the help of advances in mass spectrometry-based acetyl proteomics, research in non-histone protein acetylation has expanded

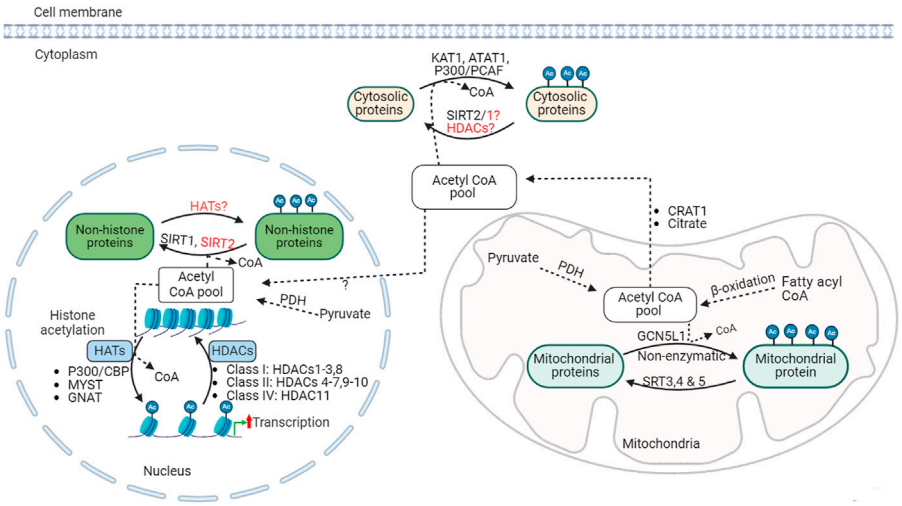

FIGURE 2
Acetylation regulation, acetyltransferases and deacetylases KAT1, lysine acetyltransferase; ATAT1, alpha-tubulin N-acetyltransferase; CBP, CREB-binding protein; PCAF, P300/CBP-associated factor; SIRT, sirtuin; GNAT, general control non-repressed N-acetyltransferases; MYST, MYST family acetyltransferase; GCN5L1, general control of amino acid synthesis 5 (GCN5) like-1; HAT, histone acetyltransferases; HDACs, histone deacetylases; PDH, pyruvate dehydrogenase; Ac, acetylated.

remarkably, leading to the discovery of thousands of lysine acetylation modifications in the cytosolic and mitochondrial proteins. Interestingly, many of these acetylated proteins are involved in energy substrate metabolism, including fatty acid and glucose oxidation [68, 69], (see [70] for review). However, even though it becomes apparent that non-histone protein acetylation is abundant, the exact contribution of these acetylation modifications to metabolic enzyme activity and metabolic flux regulation remains incompletely understood. In this review, we discuss recent progress made in understanding the role of posttranslational protein acetylation modification in relation to obesity-induced cardiac metabolic alterations.

Obesity and changes in protein acetylation

Numerous studies have demonstrated that obesity induces significant alterations in protein acetylation patterns, and suggesting that these changes may play an important role in the pathogenesis of obesity and obesity-related metabolic dysfunction (Figure 3) [71]. Accordingly, we demonstrated hyperacetylation of a number of myocardial metabolic proteins in obese mice induced by high-fat diet (HFD) feeding [35]. Similarly, hyperacetylation of mitochondrial protein has also been shown in obese subjects with HF in murine obesity models and human patient samples [72]. Dysregulation of acetylation proteins is also positively correlated with BMI values and mitochondrial dysfunction in obese-induced HF patients [72]. In another study, a large number of cardiac hyperacetylated proteins due to obesity were shown in a Zucker diabetic fatty/spontaneously hypertensive heart failure F1 (ZSF1) rat model of HFpEF [73]. The majority of these hyperacetylated proteins were related to fatty acid metabolism and other energy-generating pathways [73]. Similarly, several other studies have also shown that a HFD in mice leads to the hyperacetylation of several liver proteins involved in glucose and fatty acid metabolism [74, 75]. Pathway analysis of the hyperacetylated proteins in response to obesity also revealed the association of these acetylated proteins with metabolic dysfunction and cardiac remodeling [76].

Significantly increased histone acetylation levels have been shown in obese individuals with insulin resistance compared to lean individuals [77]. An association between HFD feeding and altered histone acetylation patterns has also been demonstrated in the liver [71, 78]. Interestingly, histone acetylation changes following HFD result in differential expression of genes associated with metabolic syndrome and NAFLD [71], highlighting the impact of histone acetylation changes in response to HFD on metabolic dysregulation. Some studies reported a dose-dependent increase in histone acetylation levels in response to acetyl CoA supplementation [79]. However, others reported different effects of acetyl CoA levels on histone acetylation across various tissues. For instance, acetyl CoA levels were correlated with histone acetylation changes in white adipose tissue and pancreas but not in the liver [80], indicating tissue-specific variations in histone acetylation patterns in response to dietary changes. In contrast, other

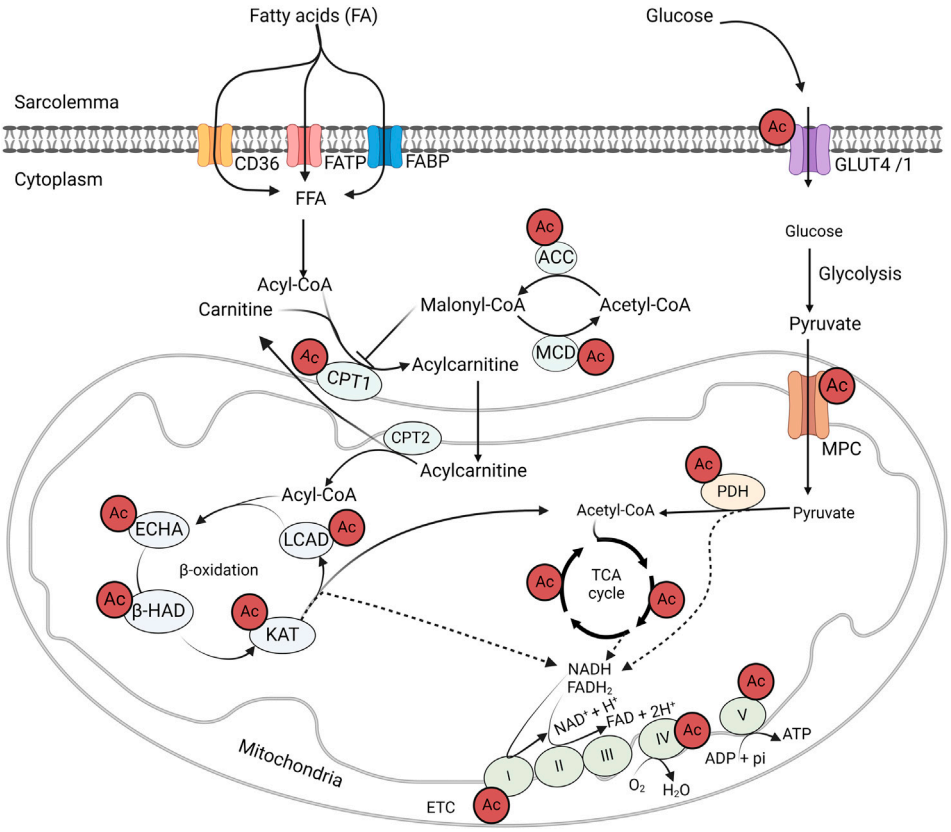

FIGURE 3
Acetylation control of cardiac metabolic enzymes and proteins GLUT4, glucose transporter isoform 4; CD36, cluster of differentiation 36; FABP, fatty acid-binding protein; FATP, fatty acid transport protein; MCD, malonyl CoA decarboxylase; ACC, acetyl CoA carboxylase; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; LCAD, long-chain acyl CoA dehydrogenase; β–HAD, β-hydroxyacyl CoA dehydrogenase; KAT, 3-ketoacyl-CoA thiolase; ECHA, enoyl-CoA hydratase; CPT, carnitine palmitoyltransferase; FAD/$FADH_2$, flavin adenine dinucleotide; NAD/NADH2, nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate.

studies in western-diet (WD) fed mice demonstrated decreased acetylation of hepatic histone proteins [81]. However, a recent study showed an enhanced histone hyperacetylation in the liver in response to chronic high carbohydrate HFD feeding, suggesting a different impact on dietary sources [82].

Links between acetyl CoA levels and protein acetylation during obesity

Acetyl CoA is a common intermediate in fuel metabolism pathways, and is also an acetyl group donor for acetylation modification. Acetyl CoA is produced in the mitochondria from catabolism of fatty acids, glucose, lactate, ketones and amino acids. As discussed earlier, HFD and obesity are associated with an increased rates of mitochondrial fatty acid ß-oxidation, leading to excess acetyl CoA generation [13]. This excess acetyl CoA has the potential to drive hyperacetylation of mitochondrial proteins. Using radioisotope tracing experiments, previous studies have demonstrated that acetyl CoA generated from fatty acid ß-oxidation is a key driver of mitochondrial hyperacetylation [83], indicating the association between fatty acid ß-oxidation and protein hyperacetylation. It has also been suggested that high acetyl CoA levels and alkaline mitochondrial pH promote non-enzymatic protein acetylation, independent of acetyltransferase enzymes [84].

Available evidence suggests a link between the metabolic state of the cell and histone acylation [85]. However, this relationship is highly affected by compartmentalization. It is not fully understood how the acetyl CoA is transported from mitochondria into the nucleus for histone acetylation modification as it cannot easily cross the mitochondrial membrane. Acetyl CoA export via citrate from the mitochondria and subsequent cleavage by ATP-citrate lyase in the cytosol is often suggested as the main source of acyl CoA in the nuclear-cytoplasmic compartment [79]. However, we have also recently shown an increased expression and activity of cytosolic carnitine acetyltransferase (CrAT) in hearts from

HFD-fed mice [86]. This increase in CrAT activity can play a significant role in facilitating the transport and availability of acetyl CoA to the cytosol for acetylation and other cellular processes in the cytosol. Some researchers suggest acetyl CoA can be transported to the nucleus through nuclear pores from the cytosol [87]. However, this concept has not been adequately explored. A recent study by the Sutendra group revealed the presence of mitochondrial subunits of PDH in the nucleus and suggested that this PDH in the nucleus generates acetyl CoA essential for histone acetylation [88]. Interestingly, deletion of PDH decreases acetyl CoA synthesis from pyruvate in the nucleus and lowers histone acetylation, indicating the role of nuclear PDH in acetyl CoA production for histone acetylation [88]. However, it is not yet clear how pyruvate is transported into the nucleus for such processes.

The acetyl CoA contribution of each fuel substrate for histone acetylation is still poorly defined. Recently, McDonnell et al., using a stable isotope tracing in AML12 cells, demonstrated that fatty acid-derived acetyl CoA leads to a significant increase in histone acetylation, while high glucose levels (25 mM) only modestly increases histone acetylation, suggesting a dominant role of fatty acids in regulating histone acetylation [89]. Interestingly, the authors also found that these changes in histone acetylation were associated with the upregulation of some of the genes related to lipid metabolism [89]. However, it is worth mentioning that the authors used high levels of octanoate (2 mM), which is not a common fatty acid, unlike palmitate and oleate in the heart. Furthermore, these studies primarily used cancer cell lines, and the generalizability of the results to the heart needs further investigation.

Regulation of protein lysine acetylation during obesity

Lysine acetyltransferases

The acetylation process is regulated by two opposing enzymes: lysine acetyltransferases (KATs) and lysine deacetylases (such as HDACs) (Figure 2). KATs catalyze the transfer of acetyl groups from acetyl CoA onto the ε-amino groups of lysine residues of histone or non-histone proteins. KATs can be described as histone acetyltransferase (HATs) in the case of histone acetylation. Several HATs have been identified in relation to histone and other nuclear protein acetylation, which can be broadly classified as type A or type B based on their subcellular origin and function [90, 91]. Type A HATs are localized in the nucleus and involved in acetylation of histone and nuclear proteins, and are linked with the regulation chromatin conformation and gene transcription process. Type A HATs are further divided into MYST (MOZ, YBF2/SAS3, SAS2, and TIP60), GCN-5-related N-acetyltransferases (GNAT), and CREB-binding protein and p300 (CBP/p300) families, which contains several HAT sub-families [90].

Type B HATs are cytosolic HAT enzymes responsible for the acetylation of newly synthesized histones before they are transported and incorporated into the newly replicated DNA in the nucleus [90]. These HAT subgroups have diverse substrate specificities in histone or non-histone proteins. While each HAT has specific and different lysine residue targets on histones, there is a huge overlap in the protein substrate [92]. However, the specificity is also determined by other factors, including the sequence, structure and interactions with other coactivators or transcription factors [92]. These complex substrate specificity and functional redundancy in several cellular processes among HATs pose a significant challenge in developing effective therapies targeting HATs.

There are limited evidence regarding the roles of HATs in obesity and other metabolic diseases. However, recent studies revealed the important roles of MYST member, HAT8 or MOF, in maintaining metabolic homeostasis in adipose tissue in response to HFD [93]. It has been shown that MOF-mediated histone acetylation (H4K16ac) is a crucial regulator of *Pparg* and *Ppargc1a* gene expression, which are responsible for glucose uptake and fat storage in adipocytes [93]. Interestingly, the authors demonstrated that deletion of MOF showed resistance to fat mass gain in adipocytes after HFD. The study also indicated that MOF deletion is associated with a decreased glycolysis rate in the heart [93]. Other studies demonstrated a positive correlation between oleic-palmitic acid-induced lipid accumulation and HAT activity in HepG2 cells [94]. An increased HAT activity following fatty acid-induced lipid accumulation was associated with increased acetylation of histones (H3K9, H4K8, and H4K16) and non-histone proteins as well as in upregulation of lipogenic genes such as PPARγ, ACLY, and FASN [94]. These effects are effectively reversed by the addition of a p300/CBP-specific inhibitor, C-646[94]. Similarly, upregulation of P300/CBP has been shown in the liver after HFD feeding and in *ob/ob* mice [95]. P300/CBP, in turn, hyperacetylates insulin receptors (IRS1/2), which impairs insulin signalling [95]. On the other hand, P300/CBP inhibition with C646 improves insulin sensitivity and decreases hyperglycemia in obese mice [95].

There is limited data regarding KATs involvement in non-nuclear proteins. While it is widely suggested that mitochondrial protein acetylation may occur through non-enzymatic acetylation, some studies indicated that the GNAT family, general control of amino acid synthesis 5-like 1 (GCN5L1) acetyltransferase, may contribute to the mitochondrial protein acetylation changes [96, 97]. GCN5L1 activity depends on acetyl CoA mitochondrial production [98]. Its expression is upregulated in response to HFD in the liver and heart [99]. Hyperacetylation of mitochondrial proteins has been shown in association with increased GCN5L1 expression in HFD [99]. Specifically, GCN5L1 targets several fatty acid oxidation (FAO) enzymes and PDH in the mitochondria [99–101]. While GCN5L1-induced hyperacetylation promotes the activity of fatty acid oxidation enzymes [97, 99], it impairs PDH [100].

On the contrary, GCN5L1 deletion in cardiomyocytes decreases mitochondrial acetylation levels [97, 99] and fatty acid oxidation while improving glucose oxidation in HFD [100]. We have also shown that GCN5L1 is vital in the maturation of mitochondrial fatty acid metabolism in newborn hearts [102]. Its expression increases during the newborn period, resulting in hyperacetylation of key fatty acid oxidation enzymes, LCAD and β-HAD. This leads to increased rates of fatty acid β-oxidation and the maturation of mitochondrial cardiac energy metabolism [102].

On the contrary, it is believed that the cytosolic protein acetylation requires the involvement of KATs. However, the specific KATs regulating cytosolic protein acetylation (acylation) modification remain unclear. Some studies suggest that HATs, such as p300/CBP families, may shuttle between the nucleus and cytoplasm and regulate the cytoplasmic acetylation processes [63, 103, 104]. Other studies indicated that type B HAT, KAT1, α-tubulin N-acetyltransferase 1 (ATAT1) and N-terminal acetyltransferases 10 and 60 (NAA10 and NAA60) are also cytoplasmic KAT enzymes [90, 105].

Histone deacetylases (HDACs)

Deacetylation is catalyzed by a group of HDACs. HDACs remove acetyl groups from ε-amino groups of lysine residues histone and non-histone protein substrates. Eighteen different HDACs have been characterized and grouped into 4 major classes based on sequence similarities: class I (HDACs 1-3, 8), class II (HDACs 4-7, 9, 10), class III (sirtuins or SIRTs 1-7), and class IV (HDAC11) (Figure 2) [90]. HDACs class I, II, and IV are described as classical HDACs and are dependent on zinc as a co-factor for their deacetylase activity. Classical HDACs regulate key aspects of cellular processes, including metabolism, inflammation, and vascular function, through altering chromatin structure and gene expression by deacetylation of histone proteins [106]. In addition, HDACs can also control the deacetylation of non-histone proteins in or outside of the nucleus. In support of this, HDAC1 and HDAC2 have been detected in the mitochondrial isolates from mouse hearts [107]. Class III HDACs are NAD^+-dependent deacetylases, also known as sirtuins or SIRTs, that can act as deacetylases outside the nucleus.

Emerging evidence on the relationship between obesity and HDACs highlights the complex and bidirectional mechanisms. Obesity influences HDAC expression levels and activities, leading to dysregulations in energy metabolic pathways, insulin sensitivity and adipogenesis. For instance, studies by Tian *et al.* [108] and Bricambert *et al.* [109] demonstrated that obesity, induced by both dietary and genetic interventions, upregulates HDAC8 activity in the liver during NAFLD, and HDAC5/6 in adipocytes, respectively. Increased activity of HDAC8 has also been associated with insulin resistance [108], while high HDAC5/6 activities led to adipocyte dysfunction [89].

Improvements in metabolic parameters have been shown in different obese models in response to HDAC inhibition. For instance, HDAC11 deletion in mice prevented obesity after HFD feeding, and significantly improves insulin sensitivity and glucose tolerance [110]. Some of these protective effects were attributed to increased expression and activity of UCP protein in adipose tissue [110]. Additionally, sodium butyrate (a pan HDAC inhibitor) treatment in obese mice led to an improved insulin sensitivity, adiposity reduction and increased energy expenditure [88]. Similar results were also observed with class I HDAC inhibition with MS-27 in HFD-fed obese mice [73]. Likewise, other studies have demonstrated improved glycemia and insulin secretion in obese diabetic rats in response to HDAC3 inhibition [72].

One of the hallmarks of obesity is an increase in leptin-resistant adiposity, which consequently leads to adipocyte dysfunction [111]. Interestingly, HDAC6 inhibition in *db/db* and obese mice increases leptin sensitivity and decreases obesity [112]. Furthermore, a negative association between HDAC1 activity and brown adipocyte thermogenesis has been shown, which was linked to histone acetylation changes and gene expression patterns [113]. Altogether, these findings offer insights into the therapeutic potential of targeting different HDACs to treat obesity. However, most of these studies were performed in non-cardiac tissues, mainly liver and adipose tissue. Although this has an indirect implication for the heart, the impact of obesity on cardiac HDAC expression/activity and the role of these alterations on obesity-induced cardiac metabolic perturbations and cardiac dysfunction remains to be elucidated.

Sirtuins (SIRTs)

Sirtuins are responsible for the deacetylation of cytoplasmic and mitochondrial proteins [114, 115]. Seven mammalian sirtuin proteins (SIRT1-SIRT7) have been identified [116]. SIRT2 predominantly functions in the cytoplasm [117], while SIRT1, 6, and 7 reside mainly in the nucleus [116, 118, 119]. SIRT 3, 4 and 5 are major mitochondrial deacetylases (Figure 2) [116, 120, 121]. However, this compartmentalization is not exclusive, and each sirtuin may shuttle across cellular compartments and regulate the acetylation state of diverse cellular proteins [118, 122–124]. While SIRT 1-3 possesses potent deacetylase activity [125–127], SIRT 4-7 have weak or no detectable deacetylase activity or have a high specificity for selective acetylation substrates [125, 128]. SIRT5 has potent lysine demalonylation and desuccinylation activity [124, 129, 130]. Altogether, sirtuins regulate diverse processes, including metabolism, gene expression, cell survival and several other processes in various tissues [131].

Obesity is associated with the reduction in expression or activity of some sirtuins. For instance, we have previously shown

a decreased cardiac SIRT3 expression in HFD-fed mice, which was associated with cardiac protein hyperacetylation [35]. Similarly, significantly reduced SIRT3 expression has also been found in patients with obesity and HF [72]. The authors demonstrated a negative correlation between protein hyperacetylation and reduced SIRT3 expression [72]. In the hearts of SIRT3 KO mice fed HFD, we have shown a significant increase in cardiac protein acetylation, including the hyperacetylation of LCAD and β-HAD [35]. This acetylation change is accompanied by a shift in cardiac energy metabolism toward high rates of fatty acid oxidation [35]. Similarly, HFD-feeding in SIRT3 KO mice led to hyperacetylation of the PDH enzyme and suppression of glucose oxidation in skeletal muscle [84].

A reduction in SIRT1 expression and activity associated with HFD-induced obesity has also been reported in various studies [100, 108]. Intriguingly, some studies suggest that weight loss through calorie restriction correlates with increased SIRT1 expression [100]. However, there are also conflicting results regarding the effects of SIRT1 in obesity-induced metabolic dysfunction. For instance, Xu et al. [101] found brown adipose tissue (BAT) degeneration and exacerbated dysfunction in response to SIRT1 deficiency in mice, while studies by White et al. [132] reported no beneficial effects of SIRT1 overexpression on HFD-induced glucose intolerance, weight gain, or insulin resistance. Other studies have shown that SIRT6 expression is reduced in adipose tissue both in HFD-fed and *ob/ob* mice as well as in aged mice [133]. On the contrary, adipose tissue-specific deletion of SIRT6 sensitized mice to HFD obesity and led to a decreased adipose triglyceride lipase (ATGL) levels due to acetylation changes on its transcriptional regulator, FOXO1 in the SIRT6 KO mice [133].

NAD^+ is a critical co-substrate for the sirtuins. Some studies have demonstrated an association between HFD or obesity and decreased NAD^+ levels in the heart [113]. On the contrary, other studies indicated that increasing NAD^+ levels through NR supplementation enhances SIRT1 and SIRT3 activity and protects against HFD-induced metabolic abnormalities [134].

Contribution of protein acetylation to cardiac metabolic alterations in obesity

One of the most notable findings in the mass discovery of non-histone protein acetylation is the abundance of acetylation modifications on energy metabolic enzymes (Figure 3). Since the first landmark study by Kim et al. in 2006 [135], numerous acetylated proteins have been identified in the cytosol and mitochondria [118, 119]. Of these acetylated proteins, fatty acid and glucose metabolic enzymes are abundantly represented [127]. For instance, all of the enzymes involved in fatty acid β-oxidation, including long-chain acyl CoA dehydrogenase (LCAD), enoyl-CoA hydratase, L-3-hydroxy acyl-CoA dehydrogenase (β-HAD), and 3-ketoacyl-CoA thiolase (3-KAT), are subjected to acetylation modification (Figure 3) [35, 75]. In addition, other proteins involved in fatty acid transport and metabolism have been identified with acylation modification [128]. Similarly, acetylation of key enzymes of glucose oxidation, such as PDH, has been reported [84, 100]. Moreover, at every step of glycolysis, glycolytic enzymes are subjected to acetylation modifications [136]. Although acetylation modifications are widespread among metabolic enzymes, its real impact on enzyme activity and metabolic flux in glucose and fatty acid metabolism are still incompletely understood. In the following section, we will discuss recent findings on the impact of protein acetylation on cardiac energy metabolism in association with obesity.

Impact of acetylation on myocardial fatty acid oxidation rates

Increased acetylation of myocardial fatty acid oxidation enzymes, including LCAD and β-HAD, in response to a HFD, obesity and diabetes have been reported [35, 101, 137, 138]. Similarly, increased acetylation of fatty acid oxidation enzymes has been observed in HFpEF hearts [139] and in the liver of obese animals [74, 140, 141]. However, there are conflicting views regarding the actual impact of acetylation on the fatty acid oxidation enzyme activities and fatty acid oxidation rates in the heart. In HFD-fed and SIRT3 KO mice, we have shown that chronic HFD led to an overall myocardial protein hyperacetylation as well as increased acetylation of myocardial LCAD and β-HAD [35]. Importantly, increased acetylation of these enzymes was positively correlated with their activity and increased myocardial fatty acid oxidation rates (Table 2) [35]. In a separate study, we have also replicated the same results in obese mice subjected to transverse aortic constriction (TAC) induced HF [38]. Interestingly, weight loss in these obese mice decreased the acetylation of these enzymes and fatty acid oxidation rates [38].

Other studies have also shown a correlation between hyperacetylation and increased activities of cardiac fatty acid oxidation enzymes (Table 2) [101]. In mice fed a HFD for 24 weeks, Thapa et al. found an increased expression of GCN5L1 along with hyperacetylation of fatty acid oxidation enzymes [101]. The authors further demonstrated that deletion of GCN5L1 in H9c2 decreased the acetylation status and activity of fatty acid oxidation enzymes [101]. We have also shown a decrease in fatty acid oxidation and acetylation of fatty acid oxidation enzymes in newborn hearts that have undergone hypertrophic remodelling [145]. Furthermore, deletion of the GCN5L1 in H9c2 cells or hypertrophic remodelling of newborn hearts leads to a decreased myocardial acetylation and impaired maturation of myocardial fatty acid oxidation [145].

TABLE 2 Effects of lysine acetylation on cardiac fatty acid and glucose metabolic enzymes.

Metabolic pathways	Target enzymes	Effects on enzyme activity	References
Fatty acid oxidation	LCAD	Increased, Decreased	[35, 97, 139, 142, 143]
	β-HAD	Increased, Decreased	[35, 97, 139, 143]
	MCAD	Increased	[144]
	MCD	Increased	[128]
Glucose oxidation	PDH	Decreased	[35, 38, 97, 100, 143]
	MPC	Decreased	[83]

GLUT4, glucose transporter isoform 4; MCT, monocarboxylate transporter 1; PDH, pyruvate dehydrogenase; LCAD, long-chain acyl CoA dehydrogenase; β–HAD, β-hydroxyacyl CoA dehydrogenase; MCAD, medium-chain acyl CoA-dehydrogenase; MCD, malonyl CoA decarboxylase; MPC, mitochondrial pyruvate carrier; PGM, phosphoglucomutase; HK, hexokinase.

A positive association between acetylation and fatty metabolism has also been shown in the hearts of diabetic animals [83, 144, 146–148]. In both type 1 and type 2 diabetes, increased acetylation of myocardial fatty acid metabolic enzymes promotes their enzyme activity and is associated with cardiac metabolic inflexibility during diabetes [147, 149]. Further evidence supporting the link between hyperacetylation and enhanced activity of fatty acid metabolic enzymes has been found in the liver and skeletal muscles. For instance, the association between excessive acetylation and increased palmitate oxidation rates has been observed in skeletal muscle in mice lacking SIRT3 [84]. Similar results have also been reported in liver cells exposed to high fat and deacetylase inhibitor [68]. Altogether, these data suggest that hyperacetylation of cardiac fatty acid oxidation enzymes in obesity and diabetes has a stimulatory effect on fatty acid oxidation. Thus, acetylation may contribute to cardiac metabolic inflexibility characterized by increased heart's reliance on fatty acid oxidation during obesity.

On the contrary, an inhibitory effect of hyperacetylation on fatty acid metabolism has also been suggested in various studies [69, 139, 142, 143, 150]. In mice with HFpEF, induced by a two-hit model of a chronic HFD and hypertensive stress (L-NAME treatment), Tong et al. showed an increased acetylation of cardiac fatty acid oxidation enzymes [139]. By measuring the activities of some of the fatty acid oxidation enzymes in isolated mitochondria, the authors suggested that hyperacetylation is associated with reduced activity of fatty acid oxidation enzymes and impaired mitochondrial fatty acid metabolism [139]. However, this contrasts with recent evidence showing an actual increase in myocardial fatty acid oxidation rates in HFpEF hearts [15, 151]. Similarly, reduced activity of LCAD due to hyperacetylation has been shown in the liver of SIRT3 deficient mice [69]. In the same study, the authors also showed that increasing SIRT3 expression through fasting decreased LCAD acetylation and increased its enzyme activity [69].

Various factors may contribute to the conflicting results regarding the effects of acetylation on fatty acid oxidation enzymes. Firstly, the effect of acetylation and its regulation is complex and context-dependent. Thus, the impact of acetylation can vary depending on the specific disease conditions, target cells/organs, and substrate availability or metabolic state. Secondly, the methodological variation used to measure fatty acid oxidation or enzyme activity may also contribute to the discrepancies in these studies. For instance, some studies used isolated muscle fibers/mitochondria to measure the effect of acetylation on enzyme activity. However, these cells are in a quiescence state, and the important role of workload on fatty acid oxidation is missing. The necessary signalling pathways are also lacking, which could affect the overall outcome of the study. Additionally, the composition and substrate concentration of buffers used to determine metabolic rates could affect the accuracy of such measurements. Some studies used non-physiological concentrations involving single substrates or imbalanced free fatty acid to albumin ratio, which can influence the rate of fatty acid metabolism.

Impact of acetylation on cardiac glucose oxidation rates

Unlike fatty acid oxidation, most researchers agree that acetylation has an inhibitory effect on glucose oxidation (Table 2). Increased acetylation of the cardiac PDH enzyme occurs in obesity [38, 100, 101]. A recent study showed that a long-term HFD in aged mice led to a diastolic dysfunction, a pre-HFpEF state, and an increased acetylation of cardiac PDH [100]. This hyperacetylation of PDH was inversely correlated with its enzyme activity [100]. Furthermore, the acetylase GCN5L1 was also shown as a regulator of PDH acetylation and activity, as its deletion reversed the hyperacetylation of PDH and increased PDH's enzyme activity [100]. Similarly, previous studies from our lab have also shown obesity-induced hyperacetylation of PDH in the heart, which is associated with a marked decrease in cardiac glucose oxidation rates [38]. In contrast, switching to a

low-fat diet or caloric restriction significantly reduces PDH acetylation status and enhances glucose oxidation rates in the heart [38]. In addition to the heart, other studies have also revealed a negative impact of acetylation on PDH in skeletal muscles in mice lacking SIRT3 [84]. SIRT3 deletion impairs glucose oxidation in skeletal muscle and leads to lactate accumulation and a shift towards excessive fatty acid oxidation [84]. Importantly, it was also shown that increased acetylation of PDH promotes its phosphorylation, which further suppresses its enzyme activity.

In addition to PDH, increased acetylation of the mitochondrial pyruvate carrier protein (MPC) has also been shown in diabetic mice hearts. It was shown that hyperacetylation of MPC leads to a significant decrease in its function, as indicated by impaired pyruvate uptake and suppressed pyruvate-based mitochondrial respiration [83]. Overall, these studies suggest the potential contribution of acetylation to impaired cardiac glucose oxidation in HFD and obesity. Together with the altered acetylation of fatty acid oxidation enzymes, which has a stimulatory effect on fatty acid oxidation, the acetylation dysregulation in HFD and obesity may potentially contribute to cardiac energy metabolic perturbations seen in obese subjects. Hyperacetylation of cardiac metabolic enzymes during obesity may lead to a metabolic rewiring characterized by increased fatty acid oxidation and decreased glucose oxidation.

Acetylation as a therapeutic approach in obesity-induced cardiac metabolic alterations

HDACs as a therapeutic target in obesity

In recent years, HDAC inhibitors have gained significant research attraction as a potential therapeutic option for various diseases. Numerous HDAC inhibitors have been developed and several others are being investigated for various therapeutic applications, including for HF and metabolic diseases [152]. HDAC inhibitors have also been proven to be effective and promising for certain cancer treatments. Vorinostat (class I and II HDAC inhibitor) was the first HDAC inhibitor to gain FDA approval in 2006 for the treatment of cutaneous T-cell lymphoma (CTCL). Since then, three other HDAC inhibitors, panobinostat (pan-HDAC inhibitor), belinostat (pan-HDAC inhibitor) and romidepsin (class I HDAC inhibitor), have also been approved by FDA for the treatment of hematological malignancies [137].

Emerging evidence suggests that HDAC inhibitors have therapeutic potential for a wide variety of diseases, including HF and obesity. Several studies indicated the promising therapeutic effects of HDAC inhibitors against cardiac hypertrophy [138, 153] and myocardial ischemia and reperfusion injury [140, 141, 154]. As discussed, changes in KAT and HDAC may contribute to the pathogenesis of obesity-induced metabolic alterations during HF. Furthermore, these studies have also shown that HDAC can affect the expression of genes involved in adipogenesis, lipolysis and energy metabolism. In fact, several animal models of obesity and HFD have also demonstrated that certain HDAC inhibitors can effectively reduce adiposity, improve leptin sensitivity, increase energy expenditure, and improve insulin sensitivity and glucose homeostasis in obese animals. This suggests that HDAC inhibitors are a potentially promising avenue for the treatment of obesity and related metabolic alterations. However, similar studies are lacking in obesity-related HF.

There are still several challenges in improving the specificity of HDAC inhibitors. HDACs have complex substrate specificity, functional redundancy and multidimensional interaction with other proteins that can affect their specific action. Often, multiple transcriptional and non-transcriptional mechanisms are involved in HDAC inhibitors' mechanism of action. For instance, several pathways, including cell proliferation, differentiation, inflammation and apoptosis across different tissues and organs, may be affected by HDAC inhibitors, which may lead to potential off-target effects. Additionally, several HDAC isoforms are shown to affect the pathogenesis of obesity. Thus, for better outcomes, it is crucial to elucidate the most significant underlying pathogenesis mechanisms modified by HDAC inhibitors in relation to obesity-induced cardiac metabolic alterations. Further research is needed to identify a subset of HDACs that are more relevant for treating obesity and metabolic alterations in HF.

Sirtuins as a therapeutic target in obesity

The discovery that protein acetylation is a widespread PTM modification of metabolic proteins and is dysregulated in various diseases has attracted interest in the development of acetylation and/or sirtuin modulators as therapeutic tool. Several plant extracts, such as honokiol, resveratrol, quercetin, curcumin, and berberine have been developed [146]. Similarly, numerous synthetic small molecules such as SIRT1-activating compounds (STACs), SRT1720, and SRT2104 have been developed to modulate sirtuin activities [145, 146]. Despite the availability of many of these sirtuin activators and inhibitors, most of them suffer from low specificity, lack of a unique target, weak potency, and unclear mechanism of action. There is still a lack of comprehensive data and consensus on the effective pharmacological activator of sirtuins. This poses a significant challenge in studying the translational potential of targeting sirtuins/acetylation modulation as a therapeutic option for the treatment of cardiac metabolic alterations.

Acetylase inhibition as a therapeutic target in obesity

Comparatively, there are few available KAT inhibitors. As KATs often form large complexes with other proteins, targeting them selectively is challenging [155]. In addition, the structural and functional diversity of KATs complicates the development of specific drugs targeting each KAT[90]. There are naturally occurring pan-HAT inhibitors derived from plants, such as anacardic acid, garcinol, and curcumin [155]. While anacardic acid and garcinol inhibit P300/CBP and PCAF HAT enzyme activities [156], and curcumin selectively inhibits P300/CBP [155]. However, there is a lack of well-designed pre-clinical animal studies on the effects of these HAT inhibitors in disease settings such as obesity. Besides, these HAT inhibitors are poorly soluble and have poor cell permeability, and their pharmacokinetics are not fully characterized [155]. Recently, new small molecule or synthetic HAT inhibitors, including C646 and WM-1119, have been developed [157]. While these new promising drugs offer more specificity and potency, further research is needed to evaluate the effectiveness of these new generations of HAT inhibitors in specific disease models.

Discussion

Obesity is associated with hyperacetylation of several cardiac energy metabolic enzymes, including those involved in fatty acid oxidation and glucose oxidation. Hyperacetylation during obesity may contribute to cardiac metabolic inflexibility by stimulating fatty acid oxidation and suppressing glucose oxidation. Although acetylation modulation holds a potential therapeutic value, there is still a lack of well-designed studies with rigorous experimental approaches and adequately validated acetylation-modulating drugs in relevant disease models.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by a Canadian Institutes for Health Research Foundation grant to GL.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Mini Review
PUBLISHED 06 June 2024
DOI 10.3389/jpps.2024.13065

OPEN ACCESS

EDITED BY
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RECEIVED 30 March 2024
ACCEPTED 22 May 2024
PUBLISHED 06 June 2024

CITATION
Morissette A and Mulvihill EE (2024), Obesity management for the treatment of type 2 diabetes: emerging evidence and therapeutic approaches.
J. Pharm. Pharm. Sci 27:13065.
doi: 10.3389/jpps.2024.13065

Obesity management for the treatment of type 2 diabetes: emerging evidence and therapeutic approaches

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Excess adiposity can contribute to metabolic complications, such as type 2 diabetes mellitus (T2DM), which poses a significant global health burden. Traditionally viewed as a chronic and irreversible condition, T2DM management has evolved and new approaches emphasizing reversal and remission are emerging. Bariatric surgery demonstrates significant improvements in body weight and glucose homeostasis. However, its complexity limits widespread implementation as a population-wide intervention. The identification of glucagon-like peptide 1 (GLP-1) and the development of GLP-1 receptor agonists (GLP-1RAs) have improved T2DM management and offer promising outcomes in terms of weight loss. Innovative treatment approaches combining GLP-1RA with other gut and pancreatic-derived hormone receptor agonists, such as glucose-dependant insulinotropic peptide (GIP) and glucagon (GCG) receptor agonists, or coadministered with amylin analogues, are demonstrating enhanced efficacy in both weight loss and glycemic control. This review aims to explore the benefits of bariatric surgery and emerging pharmacological therapies such as GLP-1RAs, and dual and triple agonists in managing obesity and T2DM while highlighting the caveats and evolving landscape of treatment options.

KEYWORDS

type 2 diabetes mellitus, gut hormones, glucagon like peptide 1, metabolic surgery, obesity

Introduction

Obesity represents a multifaceted, chronic condition characterized by an accumulation of excessive body fat, known as adiposity, which can impair health and decrease lifespan [1]. Epidemiologic studies define obesity using the body mass index (BMI), which can stratify obesity-related health risks at the population level. Obesity is clinically defined as a BMI exceeding 30 kg/m^2 and is subdivided further into class 1 (30–34.9 kg/m^2), class 2 (35–39.9 kg/m^2) and class 3 (≥40 kg/m^2). At the population level, complications from obesity rise as BMI increases [2]. At the individual level, the relationship between health complications and BMI is influenced by diverse factors such as the extent of adiposity, its distribution throughout the body, and an array of

environmental, genetic, biological, and socioeconomic influences [3]. Excessive adiposity can predispose to metabolic complications, such as type 2 diabetes mellitus (T2DM) [4]. T2DM is defined by hyperglycemia resulting from tissue insulin resistance and relative insulin deficiency [4]. Estimates indicate that approximately 537 million individuals worldwide had T2DM in 2021, a figure that is expected to increase by 46%–783 million by 2045 [5]. Individuals with T2DM are at high risk for microvascular complications, including retinopathy, nephropathy and neuropathy, and macrovascular complications such as cardiovascular comorbidities [6].

For years, T2DM has been viewed as a chronic, progressive condition necessitating continual adjustment of pharmacotherapy, with estimates that 50% of patients will require insulin dependence within 9–10 years [7]. However, a growing body of research challenges this timeline by introducing surgical and pharmacotherapy approaches to managing the disease, emphasizing reversal and remission [8]. Indeed, sustained weight loss of at least 15% of body weight has a positive effect on the progression of T2DM, inducing remission in a large proportion of patients and markedly improving metabolic status in many others [9, 10]. The World Health Organization now openly acknowledges that a window of time exists in which T2DM is metabolically reversible - which is defined as a normal HbA1c without glucose-lowering medications for at least 3 months [11]. Pioneering work by Pories et al. [12] laid the foundation for the notion that bariatric surgery could effectively address T2DM owing to its substantial impact on weight reduction and significant improvements in blood glucose levels, fasting insulin, and HbA1c. Subsequent studies have consistently reaffirmed the efficacy of bariatric surgery in enhancing glucose homeostasis, diminishing the requirement for glucose-lowering medications, and mitigating both microvascular and macrovascular complications associated with T2DM [13]. Notably, some patients have experienced complete remission of T2DM following surgery [13]. Furthermore, evidence suggests that individuals undergoing bariatric surgery are significantly less likely to receive a diagnosis of T2DM even 15 years post-surgery compared to those who do not undergo the procedure [14]. Despite its considerable benefits, a complex surgical procedure is not feasible or scalable as the mainstay for a population-wide intervention.

The discovery that glucagon-like peptide-1 (GLP-1) enhances insulin secretion in a glucose-dependent manner and suppresses glucagon release while minimizing the risk of hypoglycemia has led to the development of various structurally distinct GLP-1 receptor (GLP-1R) agonists (GLP-1RAs) with longer circulation times for the management of T2DM [15–17]. Beyond their now well-defined role in managing glucose levels, GLP-1RAs have emerged as important tools in weight management strategies for individuals living with obesity and T2DM. This effect on body weight primarily stems from their ability to reduce food intake and slow gastric emptying [18]. Innovative treatment approaches combining GLP-1RAs with other gut hormone-derived agonists, such as glucose-dependent insulinotropic polypeptide (GIP), and pancreatic hormone-derived agonists, such as glucagon (GCG) and amylin, are demonstrating promising outcomes, further enhancing both weight loss and glycemic control [19, 20]. This new line of pharmaceuticals to reduce body weight and decrease glucose levels could therefore be a more accessible treatment alternative for individuals living with obesity and T2DM (Figure 1).

This review aims to explore bariatric surgery, currently considered the most effective intervention for addressing obesity and T2DM, and the potential pharmacological emerging therapies such as GLP-1 receptor agonists (GLP-1RAs), dual agonists, and tri-agonists in body weight and T2DM management. Additionally, we will discuss the caveats and potential future directions in treating patients living with obesity and T2DM.

Bariatric surgery

Bariatric surgery, also known as metabolic surgery, is an effective therapy that helps people with severe obesity achieve significant weight loss while decreasing related cardiometabolic comorbidities [21, 22]. The term metabolic surgery acknowledges the physiological changes caused by the procedure, which leads to a more favourable metabolic profile beyond the traditional belief that it is only provided through weight-dependant mechanisms [23–25]. The most widely performed bariatric surgeries are vertical sleeve gastrectomy (VSG), which consists of removing ~80% of the stomach along the greater curvature, and Roux-en-Y gastric bypass (RYGB), which involves gastric size restriction with the creation of a small gastric pouch and re-routing of the intestinal tract, such that ingested nutrients empty directly into the jejunum and bypass 95% of the stomach, duodenum and proximal jejunum [26]. Biliopancreatic diversion with duodenal switch (BPD-DS) is a less-common procedure consisting of a sleeve gastrectomy followed by re-routing of the small intestine so that the ileum now connects to the pylorus of the stomach, bypassing both the jejunum and the duodenum [27].

Studies have shown that patients living with T2DM undergoing BDP-DS tend to lose between 36% and 55% of their initial body weight after 10 and 3 years, respectively [28, 29], compared to 28% with RYBG [30] and 22% with sleeve gastrectomy after 10 years [30]. Similarly, BPD-DS is the procedure conferring the highest rate of long-term (2–5 years) diabetes remission, ranging from 90 to 100% [27, 31] compared to 50–84% [29, 32] for RYGB and 14–86% for sleeve gastrectomy [33–35]. The longer duration of diabetes and the type of antidiabetic therapy used before surgery could influence postsurgical glycemic outcomes, thus explaining the heterogeneity in diabetes remission following bariatric surgery

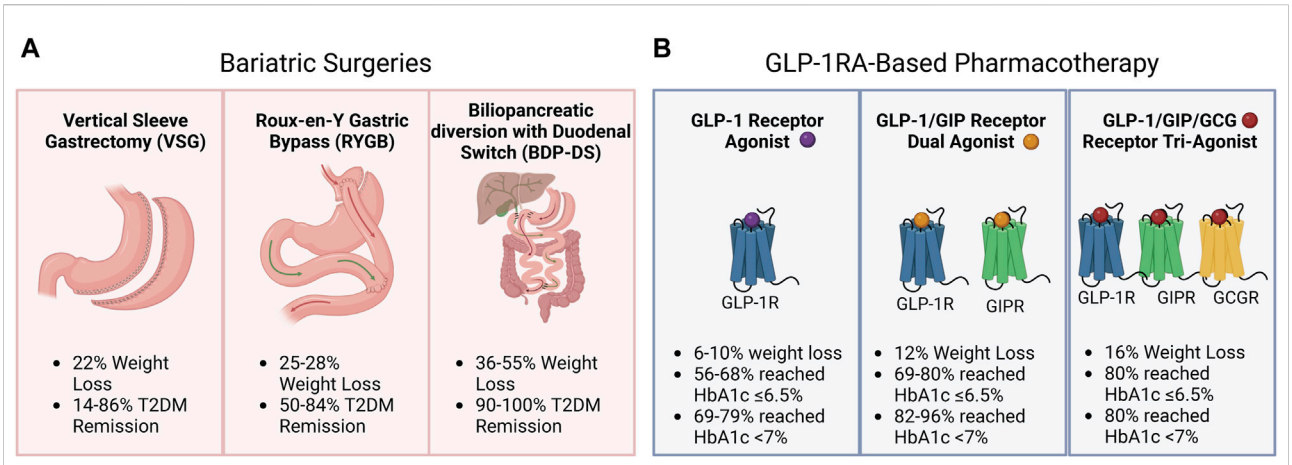

FIGURE 1
Weight loss and type 2 diabetes mellitus (T2DM) management with bariatric surgery and Glucagon-like peptide 1 receptor agonist (GLP-1RA)-based pharmacotherapy. **(A)** Weight loss (%) and type 2 diabetes mellitus (T2DM) remission (%) were observed following vertical sleeve gastrectomy, Roux-en-Y Gastric Bypass, or biliopancreatic diversion with duodenal switch. **(B)** Weight loss (%) and HbA1c targets of 6.5% and 7% reached by patients following GLP-1RA-based pharmacotherapy.

[36–38]. Despite being recognized for its durability in terms of weight loss and diabetes remission [29, 39, 40], DPB-DS constitutes only 2.2% of bariatric surgeries performed worldwide [41]. The technical complexity and demanding post-operative monitoring needed to avoid malnutrition due to the malabsorptive nature of this surgery may explain the reduced surgeries employing BPD-DS. As it is a more straightforward procedure that requires a shorter operative time, VSG is now the most widely performed bariatric surgery worldwide [21].

The precise mechanisms resulting in improved glucose control following bariatric surgery remain unclear. The degree of weight loss achieved is generally associated with the degree of resolution of T2DM [9, 42, 43], suggesting that those with greater weight loss after surgery have a greater propensity for improved management of T2DM and remission than those with less weight loss [44]. Indeed, weight loss yields a reduction in total, visceral and pancreatic adipose tissue, reductions in intrahepatic levels of lipids, and improved insulin sensitivity, all of which are expected to improve systemic glucose homeostasis [45]. One study demonstrated that in patients living with obesity and T2DM, 18% weight loss achieved either by RYGB or caloric restriction resulted in similar improvements in insulin sensitivity and β-cell function, suggesting that metabolic improvements are weight-related [44]. Metabolic surgery has also been found to have well-documented effects on improving blood glucose levels [13] and even achieving T2DM remission on a faster timeline that is disassociated from weight loss [21]. These weight-loss-independent improvements are thought to be in part related to changes in bile acid dynamics [46] and microbiota composition [47], a shift in gut physiology, including nutrient intake, gastric emptying, gastric acid production [48], and increases in postprandial gut hormone secretion [49]. Other factors to consider in T2DM remission following bariatric surgery include disease duration, age, and the level of glycemic control [9, 50]. These factors, linked to β-cell functional capacity, suggest that T2DM remission might be more achievable in patients with shorter disease duration, younger age, and better glycemic control. Nevertheless, it was reported in patients with T2DM using insulin before BPD-DS, 97% of patients had cessed insulin therapy after 10 years postoperatively [51].

Overall, the magnitude of change in body weight and glycemic control depends on the type of bariatric surgery performed and the improvements in T2DM management are related to both weight-loss-dependent and independent mechanisms.

GLP-1RA-based therapies

GLP-1 and GIP are incretin hormones released from gut enteroendocrine cells following a meal and potentiate glucose-dependent insulin secretion from the pancreas [52]. They exert their incretin actions through two distinct yet structurally related class B G protein-coupled receptors, the GIPR and the GLP-1R. These receptors are expressed in several organs tightly controlling energy homeostasis and metabolism, including the pancreas, cardiovascular system, and central and peripheral nervous system [52]. The essential role of incretin receptors in glucose homeostasis was demonstrated in single and double incretin receptor knockout mice. *Glp1r*$^{-/-}$ mice, and, to a greater extent, *Glp1r*$^{-/-}$ and *Gipr*$^{-/-}$ mice, exhibit impaired glucose tolerance and defective insulin secretion when fed a

high-fat diet [53]. GLP-1 also exerts anorectic effects by activating GLP-1R + neurons in the hypothalamus and brainstem, which reduces food intake and promotes weight loss [54]. The action of GLP-1 to reduce glycemia by stimulating insulin secretion in a glucose-dependent manner provided the rationale for exploring incretin-based therapies and led to the approval of the first GLP-1R agonist in 2005 for treating T2DM [15]. The use of two GLP-1RAs, liraglutide and semaglutide, for weight loss was later approved in 2014 [55, 56].

The observed reduction in body weight with the use of the GLP-1RA liraglutide (1.2 and 1.8 mg once daily) in individuals living with T2DM prompted the exploration of higher doses of liraglutide in the treatment of overweight and obesity in the Satiety and Clinical Adiposity—Liraglutide Evidence (SCALE) program [55, 57–60]. In the Scale Diabetes trial, an average of 6% weight loss was achieved over 52 weeks in 623 individuals living with T2DM treated with 3 mg liraglutide once daily, with 25,2% of the participants experiencing >10% weight loss. Furthermore, 56.5% of participants receiving 3 mg liraglutide daily achieved a HbA1c ≤ 6.5%, which is considered prediabetic, compared to 15% in the placebo group, and 69.2% reached the target HbA1c <7% set by the American Diabetes Association (ADA) compared to 27.2% in the placebo group [58, 61].

The GLP-1RA semaglutide was also evaluated for the treatment of obesity in the Semaglutide Treatment Effect in People with Obesity (STEP) program at a dose of 2.4 mg once weekly [56, 62–64]. STEP 2 evaluated weight loss in 1,210 individuals living with T2DM and overweight/obesity not treated with insulin (HbA1c 7–10%). Participants were randomized to placebo, semaglutide 1 mg or semaglutide 2.4 mg weekly, together with lifestyle interventions over 68 weeks. Those receiving the highest dose lost an average of 9.6% of their body weight, compared to 3.4% with the placebo. At the highest dose, more than a quarter of the participants lost over 15% of their weight, almost half lost 10%, while two-thirds lost a minimum of 5%. After 68 weeks, participants receiving 2.4 mg had an average HbA1c of 6.4%, in the prediabetic range, and therefore below the threshold to diagnose T2DM, compared to 7.8% in the placebo group. After 68 weeks, 78.5% and 67.5% of those receiving 2.4 mg semaglutide weekly reached the <7% HbA1c target and ≤6.5% prediabetic range, respectively, compared to 26.5% in the placebo group [62].

The efficacy of GLP-1RA in managing body weight and T2DM has spurred significant efforts toward developing next-generation therapies that surpass the effectiveness of GLP-1RA alone. Tirzepatide, a novel dual GLP-1 and GIP analogue, was investigated at weekly subcutaneous doses of 5mg, 10mg and 15 mg compared to 1 mg semaglutide for 40 weeks in patients living with T2DM in the SURPASS phase 3 clinical trial program. The highest tirzepatide dose led to an 11.2 kg (11.9%) weight loss and decreased HbA1c by 2.3%. A total of 82–96% of the patients who received tirzepatide and 79% of those who received semaglutide reached the HbA1c target of <7.0%. Furthermore, HbA1c ≤ 6.5%%, which is considered prediabetic, was met in 69–80% of patients receiving tirzepatide compared to 64% of patients receiving semaglutide [65]. These findings are encouraging, highlighting the promising potential of tirzepatide in the management of T2DM.

Recently, tri-agonists (GLP-1/GIP/GCG) were shown to provide even greater improvements in glycemic control and robust reduction in body weight in individuals living with T2DM. In a phase 2 clinical trial including 281 participants with T2DM and a mean HbA1c of 8.3%, weekly administration of 12 mg retatrutide (starting dose 2 mg) for 36 weeks decreased HbA1c by 2.16% and participants lost ≥15% of body weight compared to baseline. Approximately 80% of those receiving the highest dose of retatrutide reached the <7.0% HbA1c target established by the ADA and roughly the same percentage attained the ≤6.5% HbA1c prediabetic level [66]. These outcomes align with the potential reversal of T2DM [10, 67]. Another study investigating the combination of semaglutide with the long-acting amylin analogue cagrilintide in patients living with T2DM also resulted in significant improvements in body weight and HbA1c in a phase 2 trial. Compared to baseline, once-weekly 2.4 mg of CagriSema for 32 weeks resulted in a 2.2% decrease in HbA1c (mean HbA1c of 6.3%) and a 15.6% body weight loss. Eighty-nine percent of patients achieved the <7% HbA1c target, and 75% had a HbA1c ≤ 6.5% considered in the prediabetic range [68].

While additional studies are required to validate the safety and effectiveness of these newer medications in larger cohorts, GLP-1RA-based pharmacotherapy represents a very promising avenue for managing body weight and T2DM.

Discussion

Bariatric surgery induces significant weight loss and T2DM remission (Figure 1A). However, there are several contraindications to bariatric surgeries, and not all patients may be eligible. As with any other medical intervention, bariatric surgery poses a health risk, such as postoperative surgical complications, and dumping syndrome, and patients need to be closely monitored for micronutrient deficiencies after the intervention [69]. Furthermore, surgical interventions are difficult to scale to reach everyone who could potentially benefit. It is therefore worth investigating if GLP-1RA-based pharmacotherapy could be a more accessible alternative to bariatric surgery for managing body weight and T2DM (Figure 1B).

Despite their safety and efficacy, individuals may experience adverse side effects using GLP-1R agonists, dual and tri-agonists, such as nausea, vomiting, constipation and diarrhea [70]. Furthermore, GLP-1RAs generally require once-weekly subcutaneous injections [55–60, 62–64]. Orally administered

GLP-1RA, such as semaglutide and orforglipron represent another effective therapeutic strategy for managing body weight, blood glucose and other cardiometabolic risk factors [71, 72]. Additionally, an oral formulation for a GLP-1R/GIPR agonist is currently being tested in a phase 2 trial (ClinicalTrials.gov ID NCT06068946). One major limitation to the use of GLP-1RA-based therapies remains its high cost. A recent study even suggested that sleeve gastrectomy was cost-saving compared to semaglutide in the treatment of class II obesity and estimated that a 3-fold decrease in the price of semaglutide was needed to achieve nondominance [73]. Furthermore, long-term obesity and T2DM pharmacotherapy may also be required, as cessation of pharmacological treatment is frequently followed by weight regain, even with continued lifestyle intervention [64, 74]. Nevertheless, GLP-1RA-based pharmacotherapy remains a more accessible alternative than bariatric surgery for managing body weight and T2DM.

While both bariatric surgery and GLP-1RA-based pharmacotherapy represent promising options, surprisingly, few studies have directly compared surgery to pharmacotherapy for glycemic control and glycemic control in patients living with obesity and T2DM. Three studies have reported that RYGB and VSG surpass medical therapy in terms of weight loss, glycemic control and reduction in medical use among patients with T2DM [13, 38, 75]. However, it is important to mention that medical therapy, including various oral anti-hyperglycemic agents, insulin, GLP-1RAs and SGLT2 inhibitors, was heterogeneous across participants. Recent studies using GLP-1/GIP/GCG receptor agonists have demonstrated very promising results. It would therefore be interesting to explore whether these findings could be compared to the outcomes of surgery in regards to both weight loss and glycemic control.

In conclusion, bariatric surgery stands as a highly effective option for managing body weight and T2DM, yielding significant benefits. Yet, its widespread implementation faces scalability challenges, limiting access for many who could potentially benefit. In contrast, GLP-1RAs, and more particularly dual and triple agonists, offer a promising alternative, potentially extending to a larger patient population. Future research is imperative to ensure safety, and efficacy, and optimize treatment options, including decreasing side effects commonly reported by patients. Nevertheless, this novel pharmacotherapy could play a pivotal role in managing body weight, and T2DM, and preventing related micro- and macro-vascular complications.

Author contributions

AM and EM drafted and edited the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Diabetes Canada grant and a Heart and Stroke New from a Investigator Award to EM.

Acknowledgments

The figure was produced using Biorender (Publication license: DB26MXS3PJ).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CSPS | jpps Journal of Pharmacy and Pharmaceutical Sciences

TYPE Mini Review
PUBLISHED 28 May 2024
DOI 10.3389/jpps.2024.12861

OPEN ACCESS

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RECEIVED 18 February 2024
ACCEPTED 16 May 2024
PUBLISHED 28 May 2024

CITATION
Son JE (2024), Genetics, pharmacotherapy, and dietary interventions in childhood obesity. *J. Pharm. Pharm. Sci* 27:12861. doi: 10.3389/jpps.2024.12861

Genetics, pharmacotherapy, and dietary interventions in childhood obesity

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Childhood obesity has emerged as a major global health issue, contributing to the increased prevalence of chronic conditions and adversely affecting the quality of life and future prospects of affected individuals, thereby presenting a substantial societal challenge. This complex condition, influenced by the interplay of genetic predispositions and environmental factors, is characterized by excessive energy intake due to uncontrolled appetite regulation and a Westernized diet. Managing obesity in childhood requires specific considerations compared with adulthood, given the vulnerability of the critical juvenile–adolescent period to toxicity and developmental defects. Consequently, common treatment options for adult obesity may not directly apply to younger populations. Therefore, research on childhood obesity has focused on genetic defects in regulating energy intake, alongside pharmacotherapy and dietary interventions as management approaches, with an emphasis on safety concerns. This review aims to summarize canonical knowledge and recent findings on genetic factors contributing to childhood obesity. Additionally, it assesses the efficacy and safety of existing pharmacotherapies and dietary interventions and suggests future research directions. By providing a comprehensive understanding of the complex dynamics of childhood obesity, this review aims to offer insights into more targeted and effective strategies for addressing this condition, including personalized healthcare solutions.

KEYWORDS

childhood obesity, genetics, pharmacotherapy, dietary intervention, personalized therapy

Abbreviations: BMI, body mass index; GWAS, genome-wide association studies; FTO, fat mass and obesity-associated protein; IRX3/5, Iroquois homeobox 3 and 5; SIM1, single-minded family BHLH transcription factor 1; POMC, pro-opiomelanocortin; PCSK1, proprotein convertase subtilisin/Kexin type 1; LEPR, leptin receptor; AGRP, agouti-related protein; MC4R, melanocortin 4 receptor; SH2B1, SH2B adaptor protein 1; MRAP2, melanocortin 2 receptor accessory protein 2; PHIP, Pleckstrin homology domain-interacting protein; FDA, U.S. Food and Drug Administration; GLP1RAs, glucagon-like peptide-1 receptor agonists; GABA, γ-aminobutyric acid; GIPR, glucose-dependent insulinotropic polypeptide receptor; IF, intermittent fasting; TRF, time-restricted feeding; FMD, fasting-mimicking diet; MBS, metabolic and bariatric surgery.

Introduction

Childhood obesity has emerged as a critical global health concern, notably in developed countries where obesity rates among children and adolescents have nearly tripled in the last 30 years. Projections by the World Obesity Federation anticipate that by 2030, approximately 250 million children worldwide will be obese [1–3]. This condition markedly increases the risk of chronic diseases, including fatty liver and type 2 diabetes [1, 4–8]. The persistent transition from obesity in childhood to adulthood is especially concerning, with >80% of affected adolescents expected to remain obese as adults [9]. Beyond impacting physical health, this trend affects self-esteem, social relationships, and future economic prospects, underscoring the urgent need for action [1, 10, 11].

The etiology of childhood obesity is multifaceted, involving a complex interplay of genetic and environmental factors [1]. Advances in genetic research have illuminated the role of specific genetic factors influencing energy homeostasis, particularly appetite regulation [12, 13]. Studies on the genetics of obesity have identified key genes as pivotal contributors to the condition, enhancing our understanding of its biological mechanisms and opening new avenues for preventive and therapeutic strategies.

In addition to deepening our understanding of the mechanisms involved in obesity, genetic insights also drive the development of pharmacotherapies targeting specific metabolic pathways. Such treatments have shown promise in adults, signaling a potential shift toward more effective obesity management. However, their use in children and adolescents remains limited, being primarily reserved for cases of severe

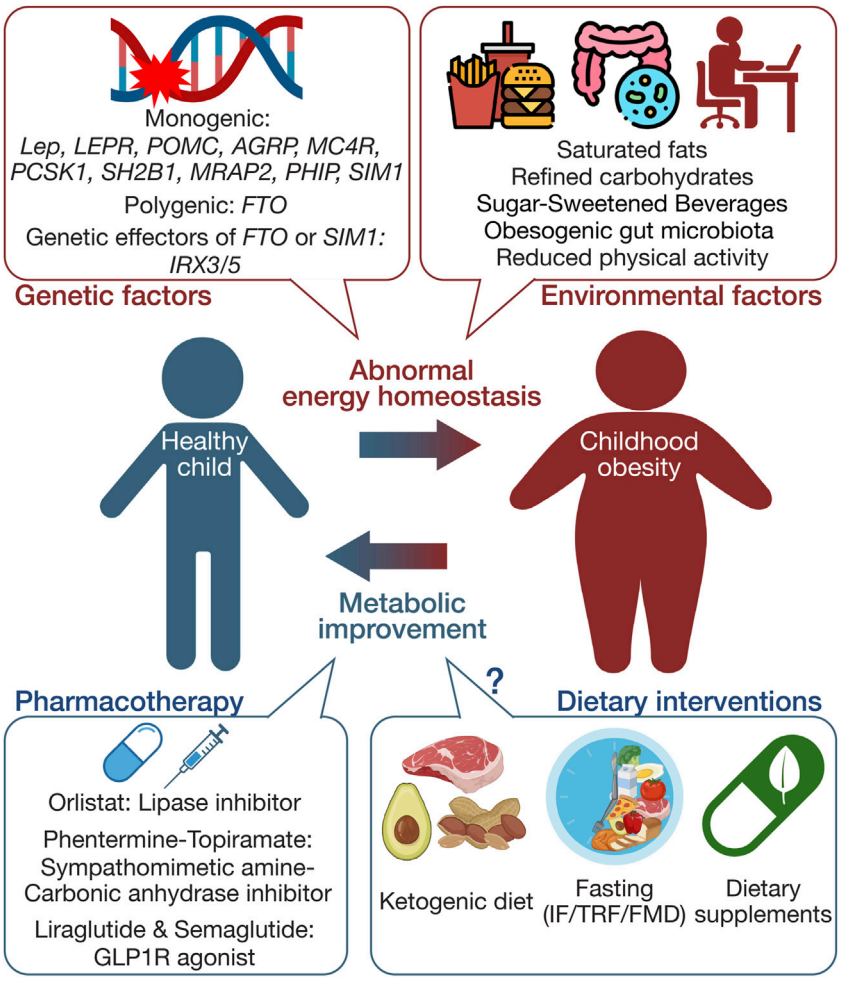

FIGURE 1
Schematic summary of the multifaceted etiology and management approaches in childhood obesity. This figure illustrates the complex interplay between genetic and environmental factors influencing the development of childhood obesity. It also delineates primary management strategies, including pharmacotherapy and dietary interventions. The schematic was created using illustrations from https://biorender.com.

obesity and diabetes in adolescents, where treatment benefits are considered to outweigh the risks [14]. Alongside this cautious approach, new medications are under development, emphasizing improved efficacy and safety, accompanied by more rigorous clinical validation.

Environmental factors, particularly diet and lifestyle with reduced physical activity, also play a crucial role in childhood obesity development [15]. Modern dietary patterns, often labeled the Western diet, are mainly characterized by a high caloric intake of saturated fats and refined carbohydrates and frequent consumption of sugar-sweetened beverages, which closely correlate with rising childhood obesity rates [16–20]. Consequently, management strategies for childhood obesity are increasingly focused on dietary interventions, such as ketogenic diets, fasting-based interventions, and dietary supplements. Ongoing research explores the effectiveness and safety of these dietary interventions in preventing and treating childhood obesity.

This review aims to summarize current knowledge on genetic factors contributing to childhood obesity, evaluate the efficacy and safety of existing pharmacotherapies and dietary interventions (outlined in Figure 1), and suggest directions for future research. By presenting a comprehensive understanding of the complex dynamics involved in childhood obesity, this review highlights potential approaches for more effective and safe treatment strategies, ultimately providing foundations for tailored interventions addressing genetic predispositions and environmental influences.

Genetic factors in childhood obesity

Twin studies have highlighted the heritability of obesity, with estimates for body mass index (BMI) heritability reaching up to 70% [21, 22]. The genetic landscape of childhood obesity has been extensively explored, revealing multiple genetic factors contributing to its development (Figure 1). Childhood obesity is predominantly polygenic, involving multiple genes, each contributing modestly but collectively exerting a substantial impact [23, 24]. In contrast to monogenic forms of obesity, resulting from single genetic defects with pivotal effects [25], the genetic predisposition to childhood obesity in the broader population is shaped by numerous common genetic variants, collectively exerting a substantial impact on the obesity phenotype.

The advent of genome-wide association studies (GWAS) has markedly advanced our understanding of the genetic basis of obesity. A landmark discovery involved identifying variants in the fat mass and obesity-associated (*FTO*) gene as a major risk factor for obesity in the general population and severe childhood obesity. The strongest association was noted for single-nucleotide polymorphisms in the first intron of *FTO*. The influence of *FTO* gene variants on energy homeostasis is mediated through their impact on appetite regulation, with certain variants linked to increased energy intake and high-calorie food preference [26–29].

Monogenic obesity, although rare, is predominantly identified in patient cohorts with severe and early-onset obesity, highlighting its strong correlation with severe childhood obesity. Monogenic obesity is mainly attributed to genetic mutations associated with the central regulation of energy homeostasis, particularly appetite control driven by the leptin–melanocortin signaling pathway [21]. Genes implicated in monogenic obesity include *Lep* (*leptin*), *LEPR* (*leptin receptor*), *POMC* (*pro-opiomelanocortin*), *AGRP* (*agouti-related protein*), *MC4R* (*melanocortin 4 receptor*), *PCSK1* (*proprotein convertase subtilisin/kexin type 1*), *SH2B1* (*SH2B adaptor protein 1*), *PHIP* (*proline-rich protein 5*), *MRAP2* (*melanocortin 2 receptor accessory protein 2*), and *SIM1* (*single-minded 1*) [30–42]. In most monogenic obesity cases, genetic mutations drive abnormal feeding behavior, resulting in early-onset, severe hyperphagic obesity.

Recently, the *Iroquois* homeodomain transcription factor genes *IRX3* and *IRX5* have emerged as novel genetic determinants in human obesity, revealing the complex genetic interactions underlying this condition. Known for their similar expression patterns and cooperative roles during mammalian development, the *IRX3* and *IRX5* genes have been implicated in obesity through interactions with intronic *FTO* locus variants. Chromatin conformation capture techniques revealed that these variants physically interact with *IRX3* and *IRX5* promoter regions, serving as enhancers that increase *IRX3*/*IRX5* expression levels in the hypothalamus and adipose tissue [43–45]. This upregulation influences crucial physiological processes, including feeding control, thermogenesis, and adipogenesis, positioning *IRX3*/*IRX5* as central mediators of *FTO* variant–associated obesity effects [46–48]. Notably, *Sim1* interacts with *IRX3*/*IRX5*. Specifically, loss-of-function mutations in *SIM1* are linked to hyperphagic childhood obesity, and *Sim1* haploinsufficiency leads to ectopic expression of *IRX3*/*IRX5* in the hypothalamus in mice, causing neurodevelopmental defects and contributing to appetite dysregulation and hyperphagic obesity [49]. Further research is warranted to explore the mechanistic evidence for the tissue- or cell-type-specific roles of *IRX3*/*IRX5*, particularly their involvement in regulating energy homeostasis. This evolving genetic narrative emphasizes the need to elucidate these pathways for further advancements in childhood obesity prevention and treatment.

Pharmacotherapy in childhood obesity

Managing childhood obesity often involves pharmacological intervention, especially in cases where a child presents with a

severe obesity phenotype and critical health issues. The cautious application of pharmacotherapy in young patients with obesity stems from concerns regarding potential long-term impacts on growth and overall development. Current pharmacotherapy options are predominantly limited to adolescents, particularly in cases of severe obesity with accompanying comorbid conditions. Pharmacotherapies currently used in childhood obesity cases are summarized in Figure 1.

Classical pharmacotherapy: orlistat and phentermine

Among the drugs approved for adults, only a few have received approval for childhood–adolescent obesity treatment. Until the early 2020s, orlistat and phentermine were the sole U.S. Food and Drug Administration (FDA)-approved medications for this purpose [50, 51]. Orlistat, a lipase inhibitor, reduces the hydrolysis of ingested triglycerides, decreasing gastrointestinal fat absorption. Clinical trials have demonstrated its efficacy in BMI reduction compared with placebo groups [52], leading to its approval for use in adolescents aged ≥12; however, orlistat has potential side effects, including diarrhea and hepatic injury, resulting in dropout rates of around 35%–75% within 3 months [52–54]. Long-term use of orlistat may disrupt the absorption of fat-soluble vitamins and minerals, negatively impacting growth or pubertal development [55, 56].

Phentermine, a sympathomimetic amine anorectic, is FDA-approved for monotherapy in adolescents aged ≥16 with severe obesity and additional related health complications. A recent clinical advancement involved the FDA approving the phentermine–topiramate combination for weight loss in obese individuals aged ≥12. Topiramate, originally an antiepileptic agent, contributes to weight loss by inhibiting carbonic anhydrase and increasing γ-aminobutyric acid (GABA) activity, suppressing appetite [57–59]. This combination, leveraging distinct mechanisms, offers a more effective weight loss solution than either drug alone, allowing for lower doses of each medication and enhancing overall treatment efficacy and safety profile. Phentermine/topiramate may pose safety concerns such as mood disorders, cognitive impairment, nephrolithiasis, cardiac risks, and teratogenic effects [60].

Setmelanotide, a melanocortin-4 receptor (MC4R) agonist approved by the FDA in 2020, offers a targeted pharmacological approach for managing monogenic obesity linked specifically to *POMC*, *PCSK1*, or *LEPR* genetic deficiencies [61, 62]. These genetic variants can disrupt signaling through the MC4R pathway, leading to hyperphagia and severe early-onset obesity [21, 63]. MC4R agonist serves as an alternative activator of the MC4R pathway in patients who have POMC deficiencies due to mutations in either *POMC* or *PCSK1* and in those with *LEPR* deficiencies caused by mutations in *LEPR*, which is crucial for POMC function. Hence, the MC4R agonist effectively reduces hyperphagia and promotes weight loss for treating severe obesity linked to these specific genetic disorders [62, 64, 65]. While its effectiveness in clinical trials is notable, its application is limited to these particular genetic disorders and not applicable to general childhood obesity. Setmelanotide therapy is associated with potential side effects, including skin hyperpigmentation, sexual dysfunction, depression, and suicidal ideation [66].

Innovative pharmacotherapy: GLP-1 receptor agonists

Glucagon-like peptide-1 receptor agonists (GLP1RAs), such as liraglutide and semaglutide, have become pivotal pharmacological agents for managing obesity. Originally developed to treat type 2 diabetes, GLP1RAs unexpectedly induce weight loss. Studies have indicated that GLP1RAs primarily act on the central nervous system to reduce appetite, delay gastric emptying to prolong satiety and alter brain pathways that decrease reward-driven eating behaviors. Ultimately, these actions lead to decreased energy intake and promote weight loss in general obesity and syndromic monogenic forms of obesity, including Prader-Willi syndrome and MC4R mutations [67–74]. Having successfully promoted weight loss in adults, GLP1RAs have received FDA approval, which was extended to adolescents. Specifically, liraglutide treatment has resulted in notable BMI reductions without negative impacts on pubertal development or growth, making it an appropriate option for adolescents aged ≥12 [75, 76]. Recent preliminary investigations into the safety and effectiveness of liraglutide in the 6–12 age group resulted in the initiation of the SCALE KIDS clinical trial, a study assessing its viability as a childhood anti-obesity treatment [77]. Additionally, semaglutide also received FDA approval for weight management in adolescents aged ≥12 with severe obesity in 2022 [78, 79]. This represents a major advancement in expanding therapeutic options for childhood obesity management.

Tirzepatide, recently approved for chronic weight management in adults, is a dual agonist targeting GLP1R and glucose-dependent insulinotropic polypeptide receptor (GIPR), offering a novel approach to obesity treatment by simultaneously enhancing glucose regulation and reducing appetite [80, 81]. It has been shown that GLP1R–GIPR dual agonist is superior in weight reduction to GLP1RAs and offers additional benefits, including improved insulin sensitivity, lipid profiles, and blood pressure [82, 83]. This demonstrates groundbreaking potential in the pharmacology of obesity and related metabolic diseases. Building on its success in adults, tirzepatide is currently in phase 1 clinical trials for children and adolescents aged 6–11 and 12–17 to assess its safety and efficacy. This expansion into pediatric studies reflects a proactive step towards addressing childhood obesity, providing hope for a

new, effective treatment option that could mitigate the long-term health consequences associated with early-onset obesity. In the future, the development of novel and effective drugs with favorable safety profiles is expected to revolutionize the approach to treating childhood obesity treatment, even within younger populations. Potential side effects of GLP1RAs or GLP1R–GIPR dual agonists include gastrointestinal symptoms, such as nausea, vomiting, diarrhea, cardiovascular conduction abnormalities, and sinus tachycardia [84, 85].

Off-label medications

Off-label medication refers to the use of pharmaceutical drugs for an unapproved age group, dosage, or condition. In the context of childhood obesity, metformin is a common example of an off-label medication. Metformin is a well-established, approved option for managing type 2 diabetes in adults and adolescents [86, 87]. Some research suggests that metformin may be effective for weight loss [88–91]; however, due to its modest and inconsistent weight-loss effects, the FDA has yet to approve metformin as a weight-loss agent. Consequently, its use in treating obesity in children has also not received official approval. Nonetheless, multiple lines of evidence demonstrate metformin's favorable effects on weight management in children and adolescents with obesity, along with a safe profile. This makes metformin a viable and accessible option for off-label use in combating childhood obesity [92, 93]. Although metformin's efficacy and safety profile are established for children, its off-label use still introduces potential risks. The lack of comprehensive clinical data specifically for treating childhood obesity means that the potential benefits must be cautiously weighed against risks that are not fully understood or might be underestimated. Consequently, the use of off-label medications such as metformin in treating childhood obesity requires careful consideration and underscores the necessity for more rigorous research to confirm their safety and effectiveness in these younger patients.

Dietary interventions in childhood obesity

Dietary interventions play a pivotal role as an alternative strategy for addressing childhood obesity, particularly as pharmacotherapy is often reserved for severe cases accompanied by additional metabolic complications [94]. These interventions, focusing on altering dietary habits and behaviors, aim to cultivate healthy eating practices conducive to long-term weight management and overall health enhancement. Emerging dietary strategies, including ketogenic diets, fasting-based interventions, and dietary supplements, are gaining attention for their potential in combating childhood obesity (Figure 1).

Ketogenic diet

The ketogenic diet, characterized by high fat and low carbohydrate intake, prompts the body to convert fats and ketone bodies for energy, entering ketosis [95]. This metabolic shift makes the diet a popular non-pharmacological option for obesity management, given its potential to promote weight loss through enhanced lipolysis and reduced insulin levels [96–98]. Although the ketogenic diet is considered beneficial for obesity-related metabolic and cardiovascular risk factors in adults [99, 100], its role in childhood weight management is still being explored. Clinical trials and animal studies have shown the diet's effectiveness in promoting weight loss and addressing metabolic issues caused by obesity [101]. However, the long-term safety and efficacy of ketogenic diets in the pediatric population require further investigation. Specifically, maintaining a ketogenic diet for extended periods may lead to elevated levels of circulating triglycerides, lipoproteins, and increased lipolysis, potentially increasing the risk of cardiovascular disease [102–104]. Challenges, such as limited food variety and maintaining long-term adherence, present additional considerations for young patients with obesity. Although ketogenic diets offer potential benefits, the associated risks, such as nutrient deficiencies, growth and developmental impacts, and metabolic complications, necessitate careful monitoring to ensure these diets are applied safely in children [105, 106].

Fasting-based interventions

Eating pattern-based dietary interventions, including intermittent fasting, time-restricted feeding, and the fasting-mimicking diet, are gaining attention for their potential metabolic benefits. Intermittent fasting (IF) involves alternating cycles of fasting and eating; time-restricted feeding (TRF) restricts daily food intake to a specific time window, typically 6–8 h, promoting a consistent daily fasting period; the fasting-mimicking diet (FMD) entails consuming an extremely low-calorie diet mimicking the physiological effects of fasting, achieving the advantages of fasting without complete food abstention. These approaches are being explored for adaptability and potential health benefits in animal experiments and clinical settings to trigger beneficial metabolic changes that aid weight management and improve overall health by leveraging the body's natural responses to fasting periods, including improved lipolysis and thermogenesis and glucose management [107–112]. Despite their simplicity and departure from traditional calorie counting, implementing fasting-based strategies in pediatric

populations warrants careful evaluation owing to the critical nutritional needs of growing children and adolescents and the potential impact on their physical and cognitive development [113, 114]. Although these fasting methods offer a fresh perspective on dietary management with demonstrated feasibility and positive outcomes [115–118], the evidence supporting their utility as acceptable therapeutic approaches, particularly for younger demographics, is still emerging. Comprehensive research is needed to establish their efficacy and safety and develop age-appropriate guidelines for children and adolescents.

Dietary supplements

Dietary supplements, including vitamins, nutrients, probiotics, plant extracts, and polyphenols, are increasingly recognized for their potential role in managing childhood obesity [119]. Omega-3 polyunsaturated fatty acids and vitamin D have been extensively researched in pediatric populations. Despite growing interest, their use in children is marked by controversy, largely due to inconsistent clinical outcomes that raise questions regarding treatment efficacy and reliability. For example, some studies have associated omega-3 supplementation with improvements in insulin resistance and fatty liver disease, as well as weight reduction in patients with obesity. In contrast, other studies have suggested no significant effect on body weight, indicating unclear impacts on anthropometric indices [120–122]. This emphasizes the need for larger pediatric studies to ascertain the effectiveness of omega-3.

The focus on probiotics, driven by insights into the role of the human microbiome in health, signals a shift in our understanding of the causes of obesity [123]. Probiotics, specifically *Lactobacillus* and *Bifidobacterium* species, show promise in reducing BMI and improving metabolic parameters, indicating their potential as an intervention for children with metabolic issues. However, cautious use of dietary supplements is recommended owing to limited evidence regarding their safety and effectiveness in children, potential interactions with medications, and unknown long-term health consequences [124–126]. This situation highlights the urgent need for comprehensive clinical trials to verify the safety and benefits of dietary supplement use in childhood obesity treatment.

Other approaches

Lifestyle interventions are foundational in managing childhood obesity, particularly through increased physical activity and exercise. These approaches are the first line of defense, especially in a preventive and managing manner. Encouraging a healthy diet and regular physical activity are essential, as these modifications can significantly impact overall health and prevent the progression of obesity [127]. Hence, lifestyle interventions are usually combined with pharmacological or dietary interventions to enhance the efficacy of these treatments [1]. This integrative approach is especially crucial when obesity reaches severe levels, as lifestyle changes alone often become insufficient [128–130]. Thus, most clinical treatment approaches for childhood obesity include combined treatment with lifestyle interventions as an effective integrative approach.

Metabolic and bariatric surgery (MBS), including procedures such as sleeve gastrectomy, gastric bypass, and gastric banding, is recognized as the most effective treatment for adolescents with severe obesity, notably reducing appetite and facilitating substantial weight loss alongside improvements in comorbidities and overall quality of life [131, 132]. These surgeries are considered for adolescents under stringent criteria, typically for those with a BMI ≥35 who also have severe comorbidities or a BMI ≥40. Despite the significant benefits, MBS carries potential risks, including nutritional deficiencies, the need for reoperations, and other surgical complications [131]. However, a recent large study indicated that MBS is effective across younger pediatric age groups without affecting vertical growth [133], affirming its utility as a crucial intervention in severe cases of childhood obesity and associated comorbidities. Consequently, this method is increasingly regarded as a viable final option for managing severe childhood obesity, prompting discussions about lowering the stringent criteria for surgery eligibility in younger patients [134].

Discussion

Managing childhood obesity necessitates a comprehensive approach, incorporating tailored pharmacological and dietary interventions to meet each child's unique requirements. While appropriate for severe cases, pharmacotherapy must be applied judiciously to prevent adverse impacts on childhood growth and development. Although dietary interventions aiming to alter immediate eating habits and establish long-term nutritional practices for prevention and treatment are perceived as safe, their safety warrants further investigation. The potential synergy between pharmacotherapy and dietary intervention is gaining recognition, showing promise in effectively managing childhood obesity while balancing metabolic control with overall health, as demonstrated in other diseases [129, 135, 136].

The undeniable role of genetics in obesity influences predisposition to the condition and impacts responses to various treatment options. As treatment options progress, obesity management is increasingly likely to prioritize personalized medicine and nutrition, advocating for interventions and dietary plans tailored to each individual's

genetic makeup. The polygenic risk score (PRS) is a tool that estimates an individual's genetic liability to a trait or disease based on their genotype profile and data from relevant GWAS. Regarding childhood obesity, PRS can be crucial in predicting obesity susceptibility and informing personalized intervention strategies [137]. Several studies have already constructed PRSs specifically for childhood obesity [23, 24, 138, 139], illustrating the potential of genetic insights to guide more effective prevention and treatment approaches.

This personalized approach requires a comprehensive understanding of the genetic factors contributing to obesity and how these interact with various treatment and dietary strategies. It is also essential to refine diagnostic measures to better and, more specifically, diagnose childhood obesity, given the limitations of using BMI as the sole parameter for assessing childhood obesity [140, 141]. Identifying genetic predispositions and tailoring treatments aims to enhance efficacy and minimize adverse effects. Ultimately, this approach would lead to safer and more effective management of childhood obesity, ensuring that interventions are as individualized as the genetic profiles they aim to accommodate.

Future research should explore the molecular mechanisms underlying the interactions among pharmacotherapies, dietary interventions, and genetic factors. In addition to genetic predispositions, understanding the role of gene–environmental interactions is becoming increasingly crucial. Epigenetics—modifications that change gene expression without altering the DNA sequence—mediates the effects of environmental variables on the expression of genes. These modifications include DNA methylation, histone alterations, and microRNA (miRNA) regulation. By affecting how genes are expressed in response to environmental cues, epigenetic mechanisms can contribute to the complexity of obesity pathogenesis and its related metabolic disorders [142–144]. Recognizing these interactions provides valuable insights into how personalized interventions can be tailored to individuals based on genetic makeup, environmental exposures, and lifestyle choices. This integrated approach emphasizes the necessity of advancing our understanding of epigenetics to develop more precise and effective strategies for preventing and managing childhood obesity. Advancements in computational technologies, such as artificial intelligence and high-throughput genomic analysis, promise increased accessibility to personalized treatments in the near future, marking a major step toward more effectively addressing childhood obesity.

Author contributions

JS conceived, designed, and wrote the manuscript.

Funding

The author declares that financial support was received for the research, authorship, and/or publication of this article. This work was supported by Kyungpook National University Research Fund, 2023.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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