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ORIGINAL ARTICLE

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Pancreas transplantation modulates reverse cholesterol transport

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Abstract Hyperinsulinemia secondary to insulin resistance in type-II diabetes or in the metabolic syndrome is associated with the "atherogenetic lipoprotein phenotype": high triglycerides, small, dense low-density lipoprotein (LDL) cholesterol, and low high-density lipoprotein (HDL) cholesterol. In contrast, hyperinsulinemia in pancreas–kidney transplant recipients (PKT-R), secondary to systemic venous drainage of the heteropically implanted pancreas graft, leads to high lipoprotein lipase (LPL) activity and a presumably antiatherogenic lipoprotein profile with very attenuated postprandial lipemia, high HDL cholesterol, and a preponderance of large-sized HDL (HDL_2) and large buoyant LDL particles. We interpret these findings to suggest that in PKT-R, peripheral hyperinsulinemia upregulates LPL activity in peripheral tissues, which induces rapid clearance of chylomicron triglycerides from plasma and, thus, attenuates postprandial lipemia. Low postprandial lipemia allows little net cholesteryl ester transfer from HDL to triglyceride-rich lipoproteins, keeping the levels of the antiatherogenic lipoprotein HDL high and potentially increasing, thereby reverse cholesterol transport. The type of lipoprotein metabolism and pattern present in PKT-R is associated with a low cardiovascular risk in the general population; it cannot be excluded, however, that hyperinsulinemia as found in PKT-R may contribute to atherosclerosis by effects unrelated to lipoprotein metabolism.

Key words Pancreas transplantation · Hyperinsulinemia · Lipoprotein metabolism · Atherogenesis

Introduction

Combined pancreas–kidney transplantation is the treatment modality of first choice for type-I diabetic (IDDM) patients with chronic renal failure. The procedure eliminates the need for exogenous insulin treatment and affords near-normoglycemia in both the fasting and postprandial state. However, pancreas grafts, implanted heterotopically in the pelvic cavity, release insulin into the iliac rather than into the portal vein. This procedure decreases first-pass insulin clearance by the liver and results in peripheral hyperinsulinemia in recipients of pancreas grafts employing systemic venous endocrine drainage [3].

Based mainly on epidemiological studies, hyperinsulinemia has been suspected to cause atherosclerosis [16]. However, hyperinsulinemia usually occurs in states of insulin resistance, which per se bear the risk of atherosclerosis, at least in part, through an atherogenic lipoprotein distribution characterized by high triglycerides, small, dense low-density lipoprotein (LDL) cholesterol and low high-density lipoprotein (HDL) cholesterol. The hyperinsulinemia in pancreas–kidney transplant recipients (PKT-R), in contrast, is not the result of insu-

Table 1 Fasting lipids in pancreas-kidney transplant recipients (*PKT-R*), nondiabetic kidney transplant recipients (*NKT-R*), IDDM kidney transplant recipients (*DKT-R*), and healthy control subjects (*CO*)

Parameter (mg/dl)	PKT-R	NKT-R	DKT-R	CO
Cholesterol	208 ± 32	235 ± 42	216 ± 44	192 ± 29
Triglycerides	94 ± 30[a]	190 ± 126[b,c]	105 ± 28	101 ± 30
Non-HDL-chol	131 ± 19[d]	177 ± 50[e]	144 ± 33	135 ± 26
HDL cholesterol	76 ± 15[d,f]	59 ± 14	72 ± 24	58 ± 15
HDL_2 cholesterol	22 ± 11[d,g]	12 ± 6	24 ± 18[h]	11 ± 6
HDL_3 cholesterol	54 ± 8	46 ± 10	48 ± 10	47 ± 10

Values are means ± SD. Each group comprised 9–11 subjects. The Student's t test for independent samples was used to compare individual groups. [a] PKT-R vs NKT-R, $p < 0.005$; [b] NKT-R vs DKT-R, $p < 0.005$; [c] NKT-R vs CO, $p < 0.005$; [d] PKT-R vs NKT-R, $p < 0.05$; [e] NKT-R vs CO, $p < 0.05$; [f] PKT-R vs CO, $p < 0.05$; [g] PKT-R vs CO, $p < 0.005$; [h] DKT-R vs CO, $p < 0.05$. Adapted from (5)

lin resistance but purely iatrogenic because of the systemic insulin drainage of the pancreas graft [3]. To address the question of whether hyperinsulinemia in PKT-R also leads to a pro-atherogenic lipoprotein phenotype, we determined the levels and composition of plasma lipoproteins, triglyceride clearance capacity, and lipoprotein-modifying enzymes in PKT-R and compared them both with those of nondiabetic kidney transplant recipients (NKT-R) to control for immunosuppression and with those of healthy controls [5, 6].

Materials and methods

Simultaneous pancreas–kidney transplantation in IDDM patients with end-stage kidney disease has been performed at the Department of Surgery of the University of Innsbruck since 1979. PKT-R (segmental pancreas graft with endocrine drainage into the iliac vein), IDDM kidney transplant recipients without endogenous insulin secretion (DKT-R), NKT-R with end-stage kidney disease and healthy controls were matched for age, body mass index (BMI) and gender. Age and BMI averaged 41 ± 8 years and 23.3 ± 3.7 kg/m^2 in PKT-R ($n = 11$), 44 ± 13 years and 23.6 ± 2.3 kg/m^2 in DKT-R ($n = 9$), 43 ± 10 years and 22.3 ± 2 kg/m^2 in NKT-R ($n = 11$), and 44 ± 10 years and 23.6 ± 2.5 kg/m^2 in controls ($n = 11$), and the percentage of males in each study group ranged from 55% to 73%; none of these parameters was statistically different between the study groups. At least 6 months were required to have elapsed after the transplantation procedure and the average time after transplantation was 45 ± 35 months in PKT-R, 49 ± 40 months in DKT-R, and 53 ± 50 months in NKT-R ($P > 0.9$). Weight gain after transplantation failed to differ between the groups. The transplant groups were also matched for antihypertensive and immunosuppressive therapy. Transplant groups received triple-drug immunosuppressive therapy including prednisone, azathioprine, and cyclosporine; the dosage of cyclosporine was adjusted to maintain plasma levels of 150–200 ng/ml plasma. Two PKT-R, one DKT-R, and one NKT-R received only prednisone and cyclosporin at the time of the study. Dosages of prednisone, cyclosporin, and azathioprine (mg/day) were 7 ± 2, 243 ± 97, and 75 ± 42 in PKT-R; 4 ± 4, 236 ± 56, and 64 ± 28 in DKT-R, and 8 ± 3, 210 ± 112, and 77 ± 33 in NKT-R (all P values > 0.17). Five patients of each transplant group received antihypertensives, mainly calcium-channel blocking agents and prazosin. One patient per group was on metoprolol and/or enalapril; no diuretics were used. DKT-R received intensive insulin treatment employing two subcutaneous injections of NPH insulin at 0700 hours and 1900 hours, and three bolus doses of regular insulin at mealtime.

Cholesterol and triglycerides were measured by standard enzymatic methods. HDL, HDL_2, and HDL_3 cholesterol were determined using a stepwise precipitation procedure with dextran sulfate and magnesium chloride. Lipoprotein compositions were obtained by measuring protein, triglyceride, cholesteryl ester, cholesterol, and phospholipid concentrations in zonal rotor fractions [13]. Based on triglyceride levels measured before and at 2, 4, 6, 8, and 10 h after ingestion of a standardized liquid fatty meal, the magnitude of postprandial lipemia was quantified as the area under the postprandial triglyceride curve normalized to the fasting level [14]. As described elsewhere [12], lipoprotein lipase (LPL) and hepatic lipase in postheparin plasma were determined by immunochemical methods, and cholesteryl ester transfer protein (CETP) concentrations in plasma by an immunoradiometric assay; apoE phenotype distributions were comparable between the groups. The studies have been approved by the Ethics Committee of the University of Innsbruck and all participants gave their informed consent prior to inclusion in the study.

Statistical analysis

The mean ± SD values of the parameters were calculated. Variables of the study groups were compared using Student's t-test for independent samples. Two-tailed tests and a significance level of P less than 0.05 were chosen. Relationships between variables were evaluated using Pearson product-moment correlation coefficients for all subjects combined.

Results

In PKT-R, insulin levels in fasting plasma were increased about twofold compared with NKT-R and threefold compared with controls; C-peptide levels, however, were similar in PKT-R and NKT-R [5, 6]. These findings suggest that decreased hepatic insulin clearance due to systemic venous drainage of the graft rather than increased insulin production is the major mechanism leading to peripheral hyperinsulinemia in PKT-R [3]. Non-HDL cholesterol (total cholesterol minus HDL cholesterol) and triglycerides in fasting plasma were increased in NKT-R when compared with healthy controls (Table 1) [5]. In contrast, the levels of these lipids in PKT-R were similar to controls. HDL choles-

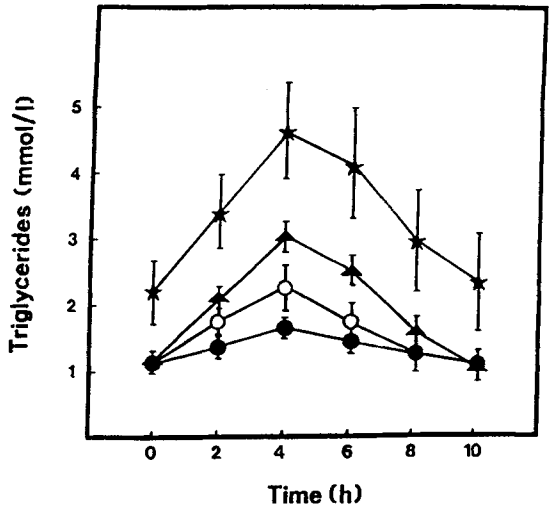

Fig. 1 Postprandial triglyceride values in pancreas–kidney transplant recipients (PKT-R) (*full circles*), nondiabetic kidney transplant recipients (NKT-R) (*stars*), type-I diabetic (IDDM) kidney transplant recipients (DKT-R) (*empty circles*), and healthy control subjects (*triangles*) after a standardized fatty meal [14]. Data are means (SEM). From Föger et al. [5]

terol was clearly increased in PKT-R compared with NKT-R and controls, mainly due to an elevation of the HDL_2 subfraction as determined by a precipitation procedure (Table 1). Notably, only HDL_2 cholesterol discriminated between the study groups and showed a much more pronounced relative increase in PKT-R and DKT-R than HDL_3 cholesterol (Table 1). To corroborate these findings, we isolated HDL_2 and HDL_3 by zonal ultracentrifugation, and, consistent with the precipitation data, observed a roughly twofold increase in HDL_2 cholesterol in PKT-R compared with NKT-R and healthy controls (both $P < 0.05$). HDL_3 cholesterol concentrations were not statistically different between the groups [6].

Analysis of very low density lipoprotein (VLDL) and LDL composition in PKT-R revealed no statistical differences compared with NKT-R and controls. The other lipoprotein classes of PKT-R, however, showed small but consistent differences with respect to surface components: LDL, HDL_2, and HDL_3 of PKT-R were enriched in unesterified cholesterol [6]. In PKT-R, HDL_2 was enriched in phospholipids while LDL was depleted of phospholipids. The only compositional change distinguishing NKT-R from both PKT-R and healthy controls was triglyceride enrichment of HDL_2 [6]. The LDL size mode, as determined by native polyacrylamide gel electrophoresis (PAGE), was increased in PKT-R relative to controls (28.5 ± 8 vs 27 ± 1 nm; $P < 0.005$); NKT-R showed intermediate values [6].

Because postprandial triglycerides have been shown on one hand to exhibit a strong inverse relationship with HDL cholesterol [14] and on the other hand to constitute an independent risk factor for coronary artery disease [15], we quantified the magnitude of postprandial lipemia after a standardized fatty meal [14] in PKT-R. Postprandial lipemia was clearly attenuated in PKT-R relative to healthy controls and even more so to NKT-R (Fig. 1) [5]. To explain the increased triglyceride tolerance of PKT-R, we examined the activity of LPL, the rate-limiting enzyme in the catabolism of triglyceride-rich lipoproteins (TGRLP) (Table 2) [5, 6]. Indeed, PKT-R had 50% higher LPL activities in postheparin plasma when compared with NKT-R and controls ($P < 0.05$).

Hepatic lipase activities tended to be lower in both patient groups with ectopic insulin release into the peripheral venous system, i.e. PKT-R and NKT-R compared with healthy controls (Table 2). Supporting the postulated sequence of events, we found a direct relationship between insulin levels and LPL activity, an indirect relationship between LPL activity and the postprandial triglyceride increase, and an indirect relationship between the postprandial triglyceride increase and HDL_2 cholesterol, respectively (all P values < 0.05).

Discussion

In NKT-R, the high cardiovascular risk can be explained best by the dyslipidemia, due mostly to steroid- and cyclosporin-based immunosuppression. When the pancreas is transplanted in addition to the kidney, dyslipidemia is not only avoided but even reversed to a particularly desirable phenotype distinguished in the fasting state mostly by the high levels of the antiatherogenic lipopro-

Table 2 Lipoprotein modifying-enzymes in pancreas-kidney transplant recipients (*PKT-R*), nondiabetic kidney transplant recipients (*NKT-R*), IDDM kidney transplant recipients (*DKT-R*), and healthy control subjects (*CO*)

Parameter	PKT-R	NKT-R	DKT-R	CO
Lipoprotein lipase	$471 \pm 164^{a,b}$	321 ± 111	369 ± 139	306 ± 37
Hepatic lipase	262 ± 140	397 ± 160	261 ± 105	364 ± 173
CETP	1.40 ± 0.51^{c}	1.26 ± 28^{d}	n.d.	1.02 ± 0.18

Values are means ± SD. Lipoprotein lipase and hepatic lipase activities are in mU/ml; CETP levels are in µg/ml. Each group comprised 7–14 subjects. The Student's t test for independent samples was used to compare individual groups. [a] PKT-R vs NKT-R, $p < 0.05$; [b] PKT-R vs CO, $p < 0.01$; [c] PKT-R vs CO, $p < 0.05$; [d] NKT-R vs CO, $p < 0.05$. Adapted from (5, 6)

tein, HDL [5, 6]. Our cross-sectional observations are corroborated by the study of Katz et al. [10] who found increased fasting triglycerides in NKT-R when compared with healthy controls, but normal fasting triglycerides in PKT-R. Furthermore, a longitudinal study by Larsen et al. [11] showed an increase in HDL cholesterol after successful pancreas–kidney transplantation. Interestingly, in our studies, fasting triglyceride levels were similar in PKT-R when compared with healthy controls. Because previous studies from our laboratory have shown that (1) the magnitude of postprandial lipemia varies widely among subjects with normal fasting triglycerides and that (2) postprandial triglyceride levels show a tighter inverse relationship to HDL than fasting triglycerides [14], we quantified the magnitude of postprandial lipemia in our PKT-R. As suspected from the high HDL levels, PKT-R showed much lower postprandial triglycerides when compared with the healthy control subjects. Thus, the increased triglyceride tolerance of PKT-R was not evident in the postabsorptive state but was uncovered in a state of challenge only. In contrast, the absence of a transplanted pancreas in NKT-R was associated with high triglycerides, not only in the postprandial but also in the postabsorptive state. We explain the excellent triglyceride tolerance of PKT-R by the high activity of LPL found in this setting, which leads to an increased capacity to clear chylomicron triglycerides. LPL, at least in adipose tissue, is upregulated by insulin. We, thus, propose that the type of peripheral hyperinsulinemia in PKT-R – due solely to systemic venous drainage of the pancreas graft – increases LPL activity in adipose tissue, which attenuates postprandial lipemia, which in turn allows for HDL cholesterol and HDL_2 levels to be high.

High HDL levels are thought to protect from atherosclerosis through facilitating the transport of cholesterol from the vessel wall back to the liver, a process termed reverse cholesterol transport [4]. According to this concept, small HDL precursor particles take up from peripheral cells unesterified cholesterol, which is subsequently esterified by lecithin:cholesterol acyltransferase to form the hydrophobic core of spherical HDL. These mature HDL then return cholesteryl esters (CEs) to the liver for biliary excretion, the only efficient mechanism available for clearing cholesterol from the body. Instead of going to the liver, HDL CE may be diverted to TGRLP in exchange for triglycerides, a process catalyzed by CETP. The magnitude of this diversion of CE from HDL to TGRLP is determined primarily by the plasma levels of TGRLP. Therefore, when TGRLP is not cleared efficiently from the circulation, CE transferred from HDL to TGRLP may instead become trapped in the vessel wall, promoting formation of atheroma. A desirable low rate of net CE diversion to TGRLP and resulting high HDL levels are found mainly under two circumstances: low levels of postabsorptive and/or postprandial TGRLP or low CETP levels. In PKT-R, postprandial TGRLP is very low, but the levels of CETP are high [2, 6]. The lipoprotein profile suggests that the rate of net CE transfer in vivo is low in PKT-R because the great majority of CE is contained in the larger sized HDL_2 and LDL, both of whose levels are high. Neither HDL nor LDL are enriched in triglycerides. This constellation strongly supports our concept [7] that increased CETP appreciably accelerates net CE mass transfer to TGRLP only in the simultaneous presence of fasting and/or postprandial hypertriglyceridemia, but not when fasting and/or postprandial triglycerides are low because they are handled very efficiently, as is the case in PKT-R.

Analysis of HDL composition in PKT-R revealed an alteration in surface components: HDL_2 and HDL_3 were enriched in unesterified cholesterol, and HDL_2 was enriched also in phospholipids. In previous, carefully conducted studies, Hughes et al. [8, 9] reported lower VLDL and LDL apoB and, similar to our findings, higher HDL free cholesterol in PKT-R. Altered lipoprotein composition was observed previously both in PKT-R and in IDDM patients who also display peripheral hyperinsulinemia due to subcutaneous insulin treatment [1, 8]. Based on direct analysis of PKT-R with systemic versus portal venous insulin drainage, Bagdade et al. demonstrated that peripheral hyperinsulinemia accounts for the changes in lipoprotein composition by modulating CE transfer [2]. The clinical relevance of these changes, however, remains unclear at present.

In summary, PKT-R do not show the atherogenic lipoprotein distribution typical for triglyceride intolerance [12] which is associated with the secondary hyperinsulinemia found in NIDDM or the metabolic syndrome. On the contrary, PKT-Rs show an antiatherogenic lipoprotein profile with high HDL levels and a preponderance of large-sized HDL (high HDL_2 levels) and LDL particles, all due to very good triglyceride clearance capacity. Whether the altered surface composition of HDL in PKT-R will affect any functional properties of these particles, i.e., with respect to reverse cholesterol transport, is currently unknown. Based on our current knowledge in lipoprotein metabolism and atherogenesis, the reported findings suggest that hyperinsulinemia in PKT-R, i.e., hyperinsulinemia in the absence of insulin resistance, would certainly not be expected to increase cardiovascular risk through altered lipoprotein metabolism. It cannot be excluded, however, that this type of hyperinsulinemia, by effects on systems other than lipoproteins, may promote atherosclerosis.

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