Serum adiponectin levels and enzyme markers of liver dysfunction in diabetic and non-diabetic Caribbean subjects

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ABSTRACT

Low adiponectin levels are associated with elevated plasma alanine aminotransferase, a marker of reduced hepatic insulin sensitivity and a risk factor for type 2 diabetes. This study aims to determine the relationship between serum adiponectin level and alanine aminotransferase in diabetic and non-diabetic subjects. Fifty-six type 2 diabetic patients and 33 non-diabetic subjects participate in the study. Baseline plasma concentrations of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and glucose are measured on a chemistry analyser. Insulin and adiponectin are measured using enzyme-linked immunoassay techniques and insulin resistance is determined using the homeostatic model assessment method. Diabetic patients showed significantly lower levels of serum adiponectin than did the non-diabetic subjects, whereas levels of alanine aminotransferase and alkaline phosphatase were similar in both groups. While female non-diabetic subjects showed higher serum adiponectin levels than did female diabetic patients, alanine aminotransferase level did not differ ($P>0.05$). No significant relationship was seen between adiponectin and alanine aminotransferase in diabetic and non-diabetic subjects ($P>0.05$). Serum adiponectin levels were higher in non-diabetic subjects but there was no significant correlation between adiponectin and alanine aminotransferase in both groups of subjects. The data suggest that low serum adiponectin level may not be a suitable marker for impaired liver function in diabetic patients.

KEY WORDS: Adiponectin.
Diabetes mellitus.
Liver function.

Introduction

It is well established that the basic pathogenic mechanism leading to type 2 diabetes is insulin resistance.[1] In Trinidad, as well as in other developing countries, the common management protocol for type 2 diabetes involves a combination of sulphonylurea and metformin, or metformin alone;[2,3] metformin being used as an inducer of insulin sensitivity. However, the use of drug combination protocols notwithstanding, there are several reports of poor long-term glycaemic control in diabetic patients in developing countries.[2–6]

Previous reports suggest that the identification of molecules that would potentially sensitise the insulin signalling pathways in liver or muscle could be helpful in long-term glucose control in diabetic patients. Thus, the adipocyte-derived protein adiponectin, which has been shown to be an insulin sensitiser,[7,8] might play a role.

Adiponectin has been shown to down-regulate tumour necrosis factor-α· (TNFα) activity in humans,[9] and this is beneficial because TNFα is a major cytokine contributing to liver damage in non-alcoholic fatty liver disease.[10] Furthermore, recent reports indicate that adiponectin might also be important in the maintenance of liver integrity, as low adiponectin levels are associated with elevated plasma alanine aminotransferase (ALT) concentration.[8] Elevated plasma ALT concentration is associated with reduced hepatic insulin sensitivity and is a risk factor for type 2 diabetes.[11–13]

Other studies in animal models of alcoholic and non-alcoholic fatty liver disease show that the animals' condition improved when they were treated with recombinant doses of adiponectin.[14] As adiponectin sensitises hepatic insulin to decrease glucose production,[15] and several studies[16–20] have confirmed that adiponectin is lower in type 2 diabetic patients than in healthy non-diabetic subjects, it appears plausible that the low concentration of adiponectin in type 2 diabetic patients might be a contributing factor to poor glycaemic control in diabetic patients.

This study aims to determine whether or not serum adiponectin concentration has a relationship with enzyme markers of liver dysfunction in diabetic and non-diabetic Caribbean subjects.

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Materials and methods

The subjects in the study were a subgroup of participants of a recently concluded study, the recruitment strategies of which have been published.[20] Briefly, type 2 diabetic patients

were recruited from a computer database of diabetic patients using the patients' recorded telephone contact numbers. They were contacted randomly and the study protocol and objectives of the study were explained thoroughly to them. Patients who expressed interest in participating in the study were asked to visit the laboratory to register and to sign a consent form.

Non-diabetic subjects were recruited through posters and flyers. Interested persons were asked to contact the laboratory for a thorough explanation of the study protocol and objectives and to perform a standard oral glucose tolerance test (OGTT), which was used to exclude subjects who might have undiagnosed diabetes.

Thus, after collecting a fasting blood sample, each non-diabetic subject consumed 75 g of anhydrous glucose (Cow & Gate Glucose, Nutricia, Rokkeveenseweg 49, Zoetermeer, Holland) dissolved in 250 mL water, and a blood sample was collected at 120 minutes. Subjects with fasting and two-hour post-prandial plasma glucose >7.0 and 11.1 mmol/L, respectively, were excluded from the study.[21] None of the diabetic and non-diabetic subjects had a previous history of liver disease.

Study protocol

Informed consent was obtained from each subject included in the study and the study protocol was approved by the Ethics Review Committee. All subjects were studied in the laboratory after an overnight (12–14 h) fast. During the visit, details of ethnic origin, previous medical history and age were recorded.

Waist measurement (in cm at the level of the umbilicus with the patient standing and breathing normally) and hip circumference (in cm at the level of the largest projection of the buttocks) were obtained by tape measure. Weight (in kg using a standard hospital balance) and height (in m using a metal rule) were measured (in light clothing, without shoes).

A fasting blood sample was collected from each subject. The samples were added to fluoride-oxalate and to plain tubes. Plasma and serum, respectively, were removed after centrifugation within 30 min of collection and stored at –20°C.

Biochemical analysis

Plasma ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and glucose were measured in a multichannel analyser using dry slide kits (Vitros 250, Ortho-Clinical Diagnostics). Glycated haemoglobin (HbA1c) was determined using a non-enzymatic reaction kit (DCA 2000, Bayer). Serum insulin (Mercodia AB, Uppsala, Sweden) and adiponectin (BioCat, Heidelberg, Germany) levels were determined by enzyme linked immunosorbent assay (ELISA). Intra- and inter-assay coefficients of variation (CV) for insulin and adiponectin were 3.7 and 6.4% and 2.3 and 3.4%, respectively.

Statistics and calculations

The results are expressed as mean±SE. The Statistical Package for the Social Sciences (SPSS, Chicago, USA) software was used for all analyses. Insulin resistance (IR), defined as the product of fasting serum insulin and plasma glucose divided by 22.5 was assessed using fasting serum insulin and plasma glucose concentrations in homeostasis model assessment (HOMA).[22]

For the purposes of statistical analysis only, all the biochemical parameters were log transformed to normalise data distribution. Comparisons of the mean differences in biochemical parameters between diabetic and non-diabetic subjects were performed using Student's t-test, while χ^2 analysis was used for categorical variables.

General linear modelling was employed to determine the effect of individual factors (gender, ethnicity and diabetes status) and the effect of their interactions on the levels of serum adiponectin, IR and liver enzymes. The relationships between adiponectin and liver enzymes were explored using Pearson correlation technique. $P<0.05$ was considered statistically significant on two-tailed testing for all analyses.

Results

None of the subjects (diabetic and non-diabetic) studied admitted a previous history of liver disease and Table 1 shows that the majority of the patients were treated with either a combination of metformin and sulphonylurea or metformin or sulphonlyurea. The diabetic patients (mean [SE] duration of 9.9±1.2 years) were older and had a larger waist circumference, but not body mass index [BMI], than did the non-diabetic subjects ($P<0.05$).

As expected, the diabetic patients had significantly higher levels of HbA1c, fasting glucose and HOMA-derived insulin resistance than did the non-diabetic subjects ($P<0.05$, Table 2). However, while the levels of AST and serum adiponectin were significantly higher ($P<0.05$) in the non-diabetic subjects, levels of ALT and ALP were similar in both groups.

Again, while female non-diabetic subjects had higher serum adiponectin levels than did female diabetic patients, the levels of ALT did not differ ($P>0.05$, Table 2). Furthermore, although the diabetic patients of either African or East Indian origin had significantly lower serum adiponectin levels than their ethnic non-diabetic

Table 1. Characteristics and self-reported medication profile of the diabetic patients.

Parameters	Diabetic subjects ($n=56$)	Non-diabetics ($n=33$)
M/F ratio	23/33	11/22
Age (years)	55.5±1.1*	50.1±1.9
Body mass index (kg/m^2)	29.5±0.8	27.4±0.8
Waist circumference (cm)	100.1±1.8*	93.8±1.8
Cigarette smokers (%)	6 (10.7)	2 (6.3)
Drinkers of alcoholic beverages (%)	30 (53.6)	15 (46.9)
African origin (%)	23 (41.1)	15 (45.5)
East Indian origin (%)	33 (58.9)	18 (54.5)
Treatment		
Diet and/or exercise (%)	2 (3.6)	NA
Metformin or sulfonlyurea (%)	13 (23.2)	NA
Metformin and sulfonlyurea (%)	27 (48.2)	NA
Insulin and/or tablets (%)	14 (25.0)	NA

NA: not applicable.

*$P<0.05$.

Table 2. Levels of serum adiponectin and liver enzymes in all subjects.

Parameters	Diabetic subjects			Non-diabetics		
	All (n=56)	Male (n=23)	Female (n=33)	All (n=33)	Male (n=11)	Female (n=22)
Body mass index (kg/m^2)	29.5±0.8	26.4±0.7	31.6±1.1†	27.4±0.8	26.1±0.9	28.1±1.1
Waist circumference (cm)	100.1±1.8*	96.2±1.7	102.8±2.7†	93.8±1.8	94.8±2.4	93.2±2.5
Glycated haemoglobin (%)	8.7±0.3**	8.1±0.3‡‡	9.1±0.4††	5.6±0.1	5.5±0.2	5.6±0.1
Fasting plasma glucose (mmol/L)	8.7±0.5**	7.9±0.7‡	9.3±0.6††	5.5±0.1	5.7±0.2	5.5±0.1
Fasting serum insulin (mu/L)	13.3±2.4	16.3±5.3	11.1±1.8	8.5±1.3	7.1±1.5	9.2±1.8
Insulin resistance (pmol/mol/L)	37.8±6.9**	41.3±15.4‡	35.4±5.0††	15.5±2.6	13.3±3.3	16.7±3.6
Alanine aminotransferase (U/L)	18.0±0.6	18.5±0.6	17.7±0.9	17.1±0.9	18.8±1.9	16.2±1.0
Aspartate aminotransferase (U/L)	19.1±0.6**	21.1±1.1‡	17.7±0.5††	22.8±1.0	26.2±2.1	21.1±0.8
Alkaline phosphatase (U/L)	85.0±2.9	75.2±3.4	91.6±4.0	80.8±2.9	82.4±5.4	80.0±3.4
Fasting adiponectin (μg/mL)	5.2±0.5**	4.9±0.9	5.3±0.6††	10.4±1.4	4.5±0.7	12.8±1.7

Values expressed as mean±SE. For statistical analysis only, insulin, insulin resistance, AST, ALT, ALP and adiponectin were log-transformed.

$^{*}P< 0.05$, $^{**}P< 0.01$ between all diabetic and all non-diabetic subjects.

$^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$ between diabetic and non-diabetic female subjects.

$^{\ddagger}P< 0.05$, $^{\ddagger\ddagger}P< 0.01$ between diabetic and non-diabetic male subjects.

counterparts ($P<0.05$, Table 3), the levels of ALT were similar in diabetic and non-diabetic subjects. Similarly, levels of ALT did not differ between the diabetic patients of East Indian and of African origin (Table 3).

General linear modelling confirmed that gender and diabetes status are the two major factors that determine the level of serum adiponectin, IR and AST ($P<0.05$, Table 4). Further investigations on the effect of interactions between these factors showed that interaction between ethnicity and diabetes status significantly affect the levels of IR ($P<0.05$, Table 4).

Tables 5 and 6 show that the relationship between serum adiponectin level and the markers of liver dysfunction was not significant in either diabetic or non-diabetic subjects on partial correlation analysis controlling for the contributions of age, BMI and insulin resistance ($P>0.05$).

Discussion

The present study showed that although the type 2 diabetic patients had significantly lower serum adiponectin levels than did the non-diabetic subjects, ALT levels were similar in both groups, and the correlations between serum adiponectin levels and liver enzymes were not statistically significant in diabetic and non-diabetic subjects.

Table 3. Levels of serum adiponectin and liver enzymes in two major ethnic groups.

Parameters	Diabetic subjects		Non-diabetics	
	African (n=23)	East Indian (n=32)	African (n=15)	East Indian (n=18)
Body mass index (kg/m^2)	31.6±1.5*	28.0±0.8	27.0±1.5§	27.8±0.7
Waist circumference (cm)	103.9±3.4	97.4±1.8	92.2±3.4§	95.0±1.8
Glycated haemoglobin (%)	9.1±0.5	8.4±0.3‡	5.7±0.2§§	5.5±0.2
Fasting plasma glucose (mmol/L)	8.6±0.8	8.8±0.6‡‡	5.2±0.2§§	5.8±0.1††
Fasting serum insulin (mu/L)	12.1±2.7	14.1±3.7	5.8±0.6	10.7±2.2†
Insulin resistance (pmol/mol/L)	34.8±7.3	39.9±10.7‡	9.6±1.0§§	20.5±4.4††
Alanine aminotransferase (U/L)	17.1±0.7	18.6±0.9	16.3±1.2	17.7±1.4
Aspartate aminotransferase (U/L)	19.6±0.7	18.7±0.8‡‡	22.0±1.1	23.4±1.5
Alkaline phosphatase (U/L)	93.1±4.8*	79.3±3.4	81.1±4.2	80.1±3.9
Fasting adiponectin (μg/mL)	5.5±0.8	4.9±0.6?	12.7±2.3§§	8.4±1.4

Values expressed as mean±SE. For statistical analysis only, insulin, insulin resistance, AST, ALT, ALP and adiponectin were log-transformed.

$^{*}P<0.05$ between diabetic patients of African and East Indian origin.

$^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$ between non-diabetic subjects of African and East Indian origin.

$^{\ddagger}P< 0.05$, $^{\ddagger\ddagger}P< 0.01$ between diabetic and non-diabetic subjects of East Indian origin.

$^{\S}P< 0.05$, $^{\S\S}P< 0.01$ between diabetic and non-diabetic subjects of African origin.

Table 4. Multivariate analysis showing the effect of individual factors (gender, ethnicity and diabetes status) and their interactions to the levels of serum adiponectin, insulin resistance and liver enzymes.

	Dependent variables (all log-transformed), F-statistic				
	Adiponectin	IR	ALT	AST	ALP
Individual factors					
Sex	5.050*	3.263	1.230	11.510†	1.075
Ethnicity	0.010	2.3A36	0.664	0.420	1.573
Diabetes status	5.119*	16.787†	1.424	6.139*	0.470
Interactions between factors					
Sex and ethnicity	0.001	0.812	0.314	1.513	0.001
Sex and diabetes status	0.916	0.475	0.000	0.682	2.391
Ethnicity and diabetes status	0.024	4.262*	0.089	1.942	1.009
Sex, ethnicity and diabetes status	0.036	0.122	1.181	2.215	0.009

*$P<0.05$, †$P<0.01$ for F-statistic values.

The finding that diabetic patients had lower serum adiponectin concentration than did the non-diabetic subjects is consistent with previous reports in other population groups,[16–19] which has now been confirmed in the groups investigated in the present study. The difference between the diabetic and non-diabetic subjects with respect to adiponectin level appears more apparent in the female diabetic patients, who showed lower serum concentrations than their non-diabetic counterparts.[20]

Indeed, the sexual dimorphism of this protein has been described in other population groups,[23–25] and has been found consistently to be inversely associated with biochemical risk factors for diabetes and cardiovascular disease.[17–19] Nonetheless, there are some reports about the relationships between serum adiponectin levels and the various markers of liver dysfunction, particularly the aminotransferases ALT and AST that are widely considered to be indicators of hepatocellular function.

Although both AST and ALT are found in the liver, the former is also found in other tissues such as cardiac muscle and is thus a less-specific marker for liver dysfunction. Therefore, ALT is the primary liver enzyme known to be involved in gluconeogenesis, and it has been suggested as an indicator of impaired insulin signalling as well as a predictor of type 2 diabetes.[11–13]

The liver is known to play an important role in maintaining normal glucose homeostasis during fasting and post-prandial states. Thus, the loss of a direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver results in an increase in hepatic glucose production.[15] Interestingly, recent reports have shown that administration of physiological doses of recombinant adiponectin *in vitro* and *in vivo* in animal models caused a dramatic decrease in glucose production.[7,26] Indeed, adiponectin has been described as a potent hepatic insulin sensitiser, exhibiting an inverse relationship to plasma ALT.[8]

Although previous studies have associated high ALT levels with decreased hepatic insulin sensitivity and the risk of developing type 2 diabetes,[11–13,27] the results of the present study showed that the levels of ALT were similar in diabetic

Table 5. Correlations between adiponectin and liver enzymes before and after partial correlation controlling for age, BMI and insulin resistance in diabetic patients.

Diabetic patients					
	All ($n=56$)	Male ($n=23$)	Female ($n=33$)	African ($n=23$)	East Indian ($n=32$)
Before partial correlation					
log adiponectin versus:					
log aspartate aminotransferase (U/L)	0.049	0.183	0.109	–0.202	0.167
log alanine aminotransferase (U/L)	0.010	0.198	–0.044	–0.085	0.084
log alkaline phosphatase (U/L)	0.256	0.308	0.162	0.167	0.310
After partial correlation					
log adiponectin versus:					
log aspartate aminotransferase (U/L)	0.017	0.337	0.112	–0.312	0.164
log alanine aminotransferase (U/L)	0.021	0.363	0.085	–0.055	0.092
log alkaline phosphatase (U/L)	0.309*	0.292	0.271	0.231	0.379

*$P<0.05$ for significant correlation.

and non-diabetic subjects. It would appear that no previous study has compared the levels of ALT in diabetic and non-diabetic subjects; however, ALT values obtained in the diabetic and non-diabetic subjects in the present study were close to those reported in healthy Caucasian subjects of similar age and BMI.[8]

The role of adiponectin in intermediary metabolism is still contentious, however. For instance, Tacke and co-workers recently reported that high levels of adiponectin in patients with chronic liver disease correlated positively with markers of inflammation and liver cell injury (aminotransferase activity).[28] The latter observation would appear to be consistent with some findings in the present study (Tables 5 and 6).

The present data could not establish a statistically significant association between plasma ALT concentration and serum adiponectin levels in diabetic and non-diabetic subjects. It is possible that small sample size might have limited the statistical power of the tests, considering the correlation coefficients obtained (Tables 5 and 6). In this respect, the results reported here are in contrast to those obtained by Lopez-Bermejo and co-workers in a healthy Spanish population, which showed a significant inverse relationship between adiponectin and ALT.[8]

It should be noted, however, that the correlation coefficient between adiponectin and ALT in the Spanish study of 257 apparently healthy subjects was rather weak after adjusting for the effects of gender, age, insulin resistance and BMI ($r = -0.13$, $P = 0.033$). It is possible, therefore, that the statistical significance obtained in that study might be due to the larger sample size.

Although recent larger studies of non-diabetic populations show that high ALT concentrations are involved in the pathogenesis of type 2 diabetes,[11–13] they did not measure serum adiponectin levels to establish a link between ALT and adiponectin in the aetiology of type 2 diabetes. However, the present study could not establish that link in the subjects studied.

Although disruption of insulin signalling targeted to the liver can cause diabetes in animal models,[15] the aetiological role of the liver in the development of diabetes in humans remains controversial. Further studies are in progress to explore the link between serum adiponectin and ALT in the pathogenesis of type 2 diabetes.

In conclusion, the data presented here show that adiponectin levels are higher in non-diabetic subjects than diabetic patients, but there was no significant association between adiponectin and ALT concentration in diabetic and non-diabetic subjects. Future work is needed to explore the predictive value of low serum adiponectin as a marker for impaired liver function in type 2 diabetes. □

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Table 6. Correlations between adiponectin and liver enzymes before and after partial correlation controlling for age, BMI and insulin resistance in non-diabetic subjects.

Non-diabetic subjects					
	All ($n=33$)	Male ($n=11$)	Female ($n=22$)	African ($n=15$)	East Indian ($n=18$)
Before correlation					
log adiponectin versus:					
log aspartate aminotransferase (U/L)	–0.045	–0.221	0.128	0.064	–0.161
log alanine aminotransferase (U/L)	–0.235	–0.134	–0.183	–0.267	–0.195
log alkaline phosphatase (U/L)	–0.038	0.258	–0.077	0.008	–0.113
After partial correlation					
log adiponectin versus:					
log aspartate aminotransferase (U/L)	–0.091	–0.127	0.084	–0.002	0.193
log alanine aminotransferase (U/L)	–0.236	–0.220	0.223	–0.421	–0.033
log alkaline phosphatase (U/L)	–0.044	–0.265	–0.088	0.115	0.024

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