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requests for materials
should be addressed to
X.Q.D.
(xiaoqun_dong@mail.
uri.edu)

Clinical implication of changes in serum adiponectin in patients with hepatogenic diabetes

Zhongying Bao¹, Xiaodong Yuan¹, Shuhong Duan¹ & Xiaoqun Dong²¹Department of Infectious Diseases, Beijing Shijitan Hospital, Capital Medical University (The 9th affiliated hospital of Peking University), Beijing, 100038, P.R. China, ²Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA.

Adiponectin is a protein hormone that modulates glucose metabolism and fatty acid oxidation. We explored the clinical implication of serum adiponectin in hepatogenic diabetes. Serum adiponectin levels were determined using enzyme-linked immunochemistry assay in 78 individuals including 19 hepatogenic diabetes, 20 type 2 diabetes (T2D), 20 chronic liver disease and 19 healthy controls. Cases and controls were matched by gender and body mass index (BMI). There is no difference in serum adiponectin levels among hepatogenic diabetic, T2D and healthy control groups. The levels of adiponectin are highest in chronic liver disease and lowest in T2D. Insulin levels are highest in hepatic diabetics and lowest in T2D. Hepatic diabetics have the lowest insulin sensitivity index (ISI). Serum adiponectin levels were negatively correlated with triglycerides and total cholesterol in T2D. Serum adiponectin is significantly increased in chronic liver disease, but lacks association with hepatogenic diabetes.

Adiponectin is an adipocyte-derived hormone encoded by *ADIPOQ* gene. Several genome-wide association studies have mapped *ADIPOQ* gene located on chromosome 3q27 as a susceptibility locus for type 2 diabetes (T2D) and metabolic syndrome¹. Decreased expression of adiponectin may confer insulin resistance in murine models of altered insulin sensitivity¹. Adiponectin is proposed to reverse insulin resistance by decreasing triglyceride content in muscle and liver in obese mice¹, which results from increased expression of molecules involved in fatty-acid combustion and energy dissipation in muscle¹⁻³. In addition, insulin resistance in lipotrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone¹. Thus the replenishment of adiponectin may provide a novel treatment modality for insulin resistance and T2D¹.

Accumulating evidence has shown that adiponectin is involved in multiple important biological functions such as regulating glucose transporter, enhancing insulin sensitivity, and attenuating inflammation⁴. Notably, chronic hepatitis has been shown to cause changes in adiponectin levels^{5,6}. However, there are very few studies on the change in adiponectin levels in patients with hepatogenic diabetes. Therefore, we compared the content of the plasma adiponectin of healthy controls to patients with hepatogenic diabetes, chronic liver disease and T2D. We tested whether the changes in adiponectin levels correlates with the above-mentioned diseases and evaluated the value of adiponectin as an early diagnostic marker for hepatogenic diabetes.

Results

The differences in age, waist, body mass index (BMI), serum adiponectin, fasting glucose, fasting insulin, total cholesterol, triglycerides, and insulin sensitivity index (ISI) were compared among the four groups (Table 1).

Plasma adiponectin levels. A significant difference in plasma adiponectin levels was observed among the four groups ($P < 0.01$). Patients with chronic liver disease had the highest, followed by the healthy control and hepatogenous diabetic group, T2D had the lowest level of adiponectin.

Fasting glucose. There was a significant difference in fasting glucose levels among the four groups ($P < 0.01$). Compared to healthy controls or patients with chronic liver disease, T2D and hepatogenous diabetics had increased fasting glucose levels ($P < 0.05$).

Table 1 | Clinical indicator (mean \pm SD) in study population

	Groups			
	Healthy control (n = 19)	Hepatogenic diabetes (n = 19)	Type II diabetes (n = 20)	Chronic liver disease (n = 20)
Age	48 \pm 4.5	53 \pm 8.6	57 \pm 6.2	51 \pm 4.1
Waist (WC)	82.9 \pm 5.12	84.69 \pm 6.48	86.1 \pm 7.07	83.28 \pm 4.34
BMI	21.7 \pm 3.3	22.7 \pm 2.6	23.3 \pm 1.8	22.0 \pm 1.9
Adiponectin (μ g/ml)	7.16 \pm 3.45	5.56 \pm 3.04	2.84 \pm 0.85	10.24 \pm 6.23*
Glucose (mg/dl)	3.3 \pm 2.5 ^a	8.8 \pm 2.4	9.6 \pm 4.0 ^a	4.2 \pm 2.0*
Insulin (μ U/ml)	9.8 \pm 3.1 ^a	15.5 \pm 4.1	8.2 \pm 3.1 ^a	10.6 \pm 4.4*
Triglycerides (mmol/L)	1.22 \pm 0.29	1.06 \pm 0.64	2.04 \pm 0.51 ^a	1.13 \pm 0.30
Total cholesterol (mmol/L)	3.61 \pm 0.46	3.32 \pm 0.85	5.53 \pm 1.03 ^a	3.28 \pm 0.88
Insulin sensitivity index	-1.66 \pm 0.14 ^a	-2.04 \pm 0.17	-1.83 \pm 0.24 ^a	-1.68 \pm 0.21*

*P < 0.05, comparison between hepatogenic diabetes and chronic liver disease.
^aP < 0.05, comparison between hepatogenic diabetes and healthy control.
^bP < 0.05, comparison between hepatogenic diabetes and type 2 diabetes.

Fasting insulin. We found a significant difference in fasting insulin levels among four groups were ($P < 0.01$). Higher levels of fasting insulin were detected in T2D and hepatogenous diabetics than in healthy controls or chronic liver disease ($P < 0.01$). T2D patients had even higher insulin levels than hepatogenous diabetics ($P < 0.01$).

Triglycerides. T2D had higher triglycerides than other subjects ($P < 0.05$).

Total cholesterol. T2D showed increased total cholesterol levels than other subjects ($P < 0.01$).

Insulin sensitivity Index (ISI). Patients with hepatogenous diabetes presented significantly decreased ISI compared with other subjects ($P < 0.01$).

Correlation of serum adiponectin with other clinical indicators. Serum adiponectin levels negatively correlated with triglycerides and total cholesterol levels in all subjects as demonstrated in Table 2. Compared with healthy control, serum adiponectin levels in T2D negatively correlated with triglycerides and total cholesterol levels, but positively correlated with ISI ($P < 0.01$) as illustrated in Table 3.

Discussion

Recent studies suggest that adiponectin can significantly improve insulin resistance and enhance peripheral tissue sensitivity to insulin, which may serve as a target of T2D treatment. Hyperinsulinemia and insulin resistance are the main features of hepatogenous diabetes. The role of adiponectin in hepatic diabetes is unclear. We are interested in whether adiponectin is a diagnostic indicator and a potential therapeutic target of hepatogenous diabetes. Insulin resistance has been proposed as one of the most important causes of liver-induced diabetes⁷. A precise method to determine insulin resistance was the insulin clamp technique; however, the high expense has limited its clinical application. Thus amendments have been proposed to improve the minimal model⁸. Insulin sensitivity index (ISI) may replace the insulin clamp technique, as an alternative marker to reflect insulin resistance of hepatogenous diabetes⁹. Consistently, we found ISI in hepatogenous diabetics was decreased significantly comparing with other groups, suggesting the presence of insulin resistance⁸. A recent study on rhesus monkeys observed that circulating concentrations of the adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to T2D¹⁰.

Adiponectin levels were gradually reduced with the development of insulin resistance. In our study, patients with T2D had relatively low levels of baseline insulin compared to healthy controls. We suggest that some patients had low levels of insulin due to dysfunction in secretion or delayed peak at diagnosis.

Hepatogenous diabetes develops in chronic liver disease; especially liver cirrhosis and sex hormone metabolism disorders, where estrogen levels are elevated but androgen are relatively low, resulting in elevated levels of adiponectin. Interestingly, serum adiponectin levels in mice presented gender dimorphism, where the females had higher adiponectin than the males¹¹. Adiponectin receptor 1 (AdipoR1) and 2 (AdipoR2) have been implicated in adiponectin hoist caused by hepatic diabetes¹². Human AdipoR1 was expressed in skeletal muscle and AdipoR2 mainly in the liver. Hepatogenous diabetics may have reduced expression of AdipoRs and thus elevated serum adiponectin due to the destruction and dysfunction of liver cells^{5,13}.

Adiponectin can be produced by human hepatoma HepG2 cells after treatment with IL-6 or damaged hepatocytes in response to CCl₄-induced injury in mice¹⁴. Adiponectin levels were elevated during the progression from chronic hepatitis B to liver fibrosis, which declined in response to antiviral therapy¹⁵. Serum adiponectin levels were significantly increased in liver cirrhosis, associated with the reaction of active inflammation in chronic liver disease¹⁶.

Adiponectin negatively regulates fatty acid oxidation. We found that the level of triglyceride and total cholesterol were lower in chronic liver disease and hepatogenous diabetes, but higher in T2D. We observed increased insulin levels but decreased insulin sensitivity index in hepatogenous diabetes, which are consistent with a previous study¹⁰. Another study found no association between adiponectin levels and insulin resistance of varying degrees of liver cirrhosis¹⁶. In our study, serum adiponectin in healthy controls and T2D negatively correlated with BMI, triglycerides and cholesterol, but positively correlated with insulin sensitivity index.

Hepatogenous diabetics often accompany with varying degrees of liver inflammation and metabolic disorders, which led to the changes in serum adiponectin levels. Although many studies suggest that decreased serum adiponectin levels closely related with T2D, it may not serve as a diagnostic marker for hepatogenous diabetes. Accumulating evidence indicates that dynamic measurements of adiponectin levels may predict hepatogenous diabetes. Mice feed

Table 2 | Correlation of serum adiponectin with clinical indicators

	Triglycerides	Total cholesterol	Insulin sensitivity index
R values	-2.79	-1.6	0.032
P values	0.013	0.043	0.78

Table 3 | Correlation of serum adiponectin with other indicators in healthy controls and type II diabetics

	Triglycerides	Total cholesterol	Insulin sensitivity index
R values	-0.485	-7.11	0.391
P values	0.001	<0.001	0.01



by high-fat and high-sugar diet can lose weight and present reduced glucose, fatty acids, triglycerides and insulin resistance when treated with the globular domain of adiponectin¹⁷. Additionally, adiponectin could reduce glucose levels of murine type I diabetes by reducing hepatic glucose output¹⁸. In our study, adiponectin levels were lower in hepatic diabetics than healthy controls and chronic liver disease. Hepatogenous diabetes may benefit from exogenous adiponectin. The limitations of this study include a small sample size and the absence of other clinical indicators such as tumor necrosis factor. Our findings need to be expanded in a large patient population. The metabolic characteristics and mechanisms of serum adiponectin in hepatic diabetes need further investigation.

Methods

Patient population. A total of 78 male subjects were recruited at Beijing Shijitan Hospital including 19 cases of hepatogenous diabetes, 20 cases of T2D, 20 cases of chronic liver disease and 19 healthy controls. Among hepatic diabetics (mean age 53 ± 8.6 years, range 42–71 years), 17 cases were hepatitis B virus-related and 2 cases were hepatitis C virus-related. T2D patients were newly diagnosed (mean age 57 ± 6.2 years, range 37–76 years). Among the 20 patients with chronic liver disease (mean age 51 ± 4.1 years, range 42–67 years), 17 cases were hepatitis B virus-related and 3 cases were hepatitis C virus-related. The healthy control group consisted of 19 individuals (mean age 48 ± 4.5 years, range 37–52 years) with normal blood pressure, blood lipids, blood glucose levels and no previous history of chronic hepatitis. Each subject had a body mass index (BMI) less than 25 according to the Obesity Guidelines for Asia-Pacific region. Hypertensive patients were excluded from the study. Informed consent was obtained from all subjects. This study was approved by the institutional review board of Beijing Shijitan Hospital Affiliated to Capital Medical University (The 9th affiliated hospital of Peking University) and was carried out in accordance with established national and institutional ethical guidelines regarding the involvement of human subjects and the use of human tissues for research.

Diagnostic, inclusion and exclusion criteria. The diagnosis of T2D is based on the Diagnosis and Classification of Diabetes Mellitus of American Diabetes Association¹⁹. Diagnosis of chronic viral hepatitis and liver cirrhosis was based on American Association for the Study of Liver Diseases (AASLD) Practice Guideline in 2004²⁰. The diagnostic criteria for hepatogenous diabetes include⁸: 1) Chronic liver disease occurred before the diabetes; 2) The patient's liver function was abnormal; 3) Elevated glucose was consistent with the deterioration of liver function; 4) Symptoms of diabetes were mild or absent and without the complications of diabetes; 5) Fasting plasma glucose ≥ 7.0 mmol/L or normal, but the 2-hour postprandial blood glucose ≥ 11.1 mmol/L; oral glucose tolerance test (OGTT) or fasting blood glucose was normal or mildly elevated but postprandial glucose ≥ 11.1 mmol/L; 6) Insulin release test showed high levels of fasting plasma insulin and postprandial insulin response delay; C-peptide/insulin ratio was decreased; 7) Patients had no family history of diabetes. Patients with secondary hyperglycemia caused by the pituitary, adrenal, pancreas, and thyroid disease were excluded. Patients with glucose metabolism disorder caused by drugs such as diuretics, corticosteroids, antihypertensive drugs, and contraceptives were excluded.

Acquisition and processing of specimens. All subjects were fasted for 12–14 hours, and fasting venous blood 5 ml were collected in the next morning. Specimens were centrifuged at 3500 r/min for 10 minutes; serum was separated and kept at -80°C .

Determination of adiponectin. Adiponectin was determined using Enzyme-linked immunochemistry assay (ELISA) according to the manufacturer's instruction. The ELISA kit was purchased from Jingmei Biotech Co Ltd. The data was analyzed using the MRX-automatic production fluorescence microplate reader (DyNex Technologies, USA).

Measurement of Glucose, triglycerides, and total cholesterol. Concentrations of blood glucose, triglycerides, and total cholesterol applied Hitachi 7170 automatic biochemical analyzer.

Determination of fasting insulin. Fasting insulin was measured using chemiluminescence detection.

ISI (insulin sensitive index). ISI was calculated as the natural logarithm of the reciprocal of product fasting blood glucose and fasting insulin.

Statistical analysis. Statistical analysis was performed using SPSS software (version 11.5). The measurement data were expressed as mean \pm standard deviation (SD). For the data with normal distribution, the differences between groups were examined by pairwise comparisons; otherwise, data with skewed distribution were first transformed to log-normal distribution. Association between different indicators was evaluated by Pearson correlation. A $P < 0.05$ was considered as statistically significant.

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Author contributions

Z.Y.B. was responsible for experimental design and supervised the study. Z.Y.B., X.D.Y. and S.H.D. developed methodology. X.D.Y. and S.H.D. carried out the experiments. Z.Y.B. performed data analysis and prepared the figures and tables. Z.Y.B., X.D.Y., S.H.D. and X.Q.D. wrote, reviewed, and revised the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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