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Original Article

Sex hormones are altered in male type 2 diabetes complicated with non-alcoholic fatty liver disease

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Abstract: Non-alcoholic fatty liver disease (NAFLD) frequently occurs in type 2 diabetes patients and has differing effects in males and females. Therefore, this study investigated whether sex hormone levels were altered in male type 2 diabetes patients with NAFLD and evaluated metabolic indexes associated with these two diseases. A total of 112 patients were enrolled and divided into NAFLD (*n*=54) and non-NAFLD (*n*=58) groups. Waist circumference, body mass index (BMI), metabolic index, sex hormone levels, and transaminase were measured in the patients. Compared with the non-NAFLD group, the level of fasting blood glucose (8.75±2.58 vs 7.79±1.89 mmol/L), 2-hour postprandial blood glucose (18.12±3.95 vs 15.63±3.89 mmol/L), and glycosylated hemoglobin (11.96±4.85 vs 10.05±4.15%) were significantly elevated ($P<0.05$) in the NAFLD group. Additionally, total cholesterol (14.97±1.02 vs 4.15±0.92 mmol/L), triglyceride (2.74±2.25 vs 2.01±1.45 mmol/L), BMI (27.34±3.93 vs 22.38±3.39 kg/m^2), fasting insulin (6.18±4.21 vs 9.62±5.80 uIU/mL), and postprandial insulin (31.72±42.27 vs 72.71±109.70 uIU/mL) were significantly elevated ($P<0.05$) in the NAFLD group. In contrast, total testosterone (18.21±6.14 vs 14.18±6.39 nmol/L) and free testosterone (0.39±0.08 vs 0.32±0.03 ng/dL) were dramatically reduced ($P<0.05$) in the NAFLD group. Altogether, these results suggest that male patients with type 2 diabetes mellitus complicated with NAFLD are more prone to disruption in lipid metabolism, obesity, and lower levels of testosterone.

Keywords: Diabetes mellitus, type 2, non-alcoholic fatty liver disease, testosterone

Introduction

Type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) are both caused by the integration of genetic, environmental, and metabolic stress. Many factors contribute to these diseases, including glucose and lipid metabolism disorders, insulin resistance, and oxidative stress. In addition, gender differences have been observed in NAFLD and type 2 diabetes and are presumably related to differences in sex hormone levels, glucose and lipid metabolism, enzyme expression and activity, and other factors [1]. However, the connection between these factors and NAFLD in type 2 diabetes patients remains unknown. Therefore, the objective of this study was to investigate the level of sex hormones and the metabolism of glucose and lipids in male patients with type 2 diabetes complicated with NAFLD compared to male type 2 diabetes patients without NAFLD.

Materials and methods

Patients

One hundred and twelve male patients with type 2 diabetes were recruited from January 2013 to June 2015 in the Department of Endocrinology and Metabolism. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine. All patients were divided into either non-NAFLD (54 cases) or NAFLD (58 cases) groups. The following were exclusion criteria: (1) acute or chronic liver or kidney failure; (2) heart failure; (3) acute complications of diabetes; (5) a history of sex gland disease; and (6) infectious and autoimmune diseases.

Diagnostic criteria of NAFLD

According to the "Guideline for management of non-alcoholic fatty liver disease: an update and revised edition by the Chinese national workshop on fatty liver and alcoholic fatty liver disease for the Chinese liver disease association [2]" a diagnosis of NAFLD should consider the patient's symptoms, signs, laboratory tests, and imaging findings. A diagnosis of NAFLD can then be made based on the following three criteria: (1) no alcohol history or alcohol intake of less than 140 g per week for males and 70 g for females; (2) absence of viral hepatitis, drug-induced liver disease, total parenteral nutrition, hepatolenticular degeneration, autoimmune liver disease, and other diseases that cause fatty liver; and (3) histological changes in liver biopsy consistent with the pathological diagnostic criteria of fatty liver disease. Due to the difficulties of liver biopsy, current diagnoses primarily depend on noninvasive studies, such as ultrasonography and liver test result abnormalities.

Methods

General patient characteristics were measured and included height, weight, blood pressure, and waist circumference (Wc). The body mass index (BMI) of each patient was calculated with the following formula: weight (kg)/height $(m)^2$.

Biochemical indicators were measured after an 8-10 h fast and were assessed between 6:00 and 7:00 AM. A range of biochemical indicators were measured, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), liver and kidney function, cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c). Blood glucose was determined using the glucose oxidase method, and blood lipid composition was determined by enzymatic analysis (Roche Diagnostics, Mannheim, Germany).

Measurement of insulin and sex hormones also occurred after an 8-10 hour fast, and the patients were tested in the morning between 6:00 and 7:00 AM. Fasting insulin (FINS) was measured by chemiluminescence. Sex hormones, including testosterone (TT), sex hormone binding globulin (SHBG), progesterone (P), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and dehydroepiandrosterone (DHEA), were also measured by chemiluminescence (Siemens Healthcare Diagnostics, New York, USA). The Homeostatic Model Assessment of insulin resistance (HOMA-IR) index was calculated according to the standard formula: fasting plasma glucose × fasting plasma insulin/22.5.

Statistical analysis

All values are expressed as the mean ± standard deviation (SD) for quantitative variables and as a percentage for categorical variables. The characteristics of the two groups were compared by *t*-test or nonparametric Mann-Whitney *U* test for quantitative variables and Fisher's exact test or χ^2 test for categorical variables. One-way analysis of variance (ANOVA) was used to analyze differences between multiple groups. Factors associated with hypogonadism were estimated using univariate analysis by logistic regression. All data analyses were performed using SPSS 16.0, and *P* values <0.05 were considered statistically significant.

Results

Comparison of general indicators

Compared with the non-NAFLD group, BMI and waist circumference in the NAFLD group were increased, as were the levels of alanine aminotransferase, aspartate aminotransferase, and glutamyl peptide transferase ($P<0.05$, **Table 1**).

Comparison of glucose and lipid metabolism indicators

FBG, 2-hour postprandial blood glucose (2hPBG), and HbA1C were significantly higher in the NAFLD group than in the non-NAFLD group ($P<0.05$). Similarly, FINS, postprandial insulin, total cholesterol, and triglyceride levels were also significantly increased in the NAFLD group ($P<0.05$). In contrast, high-density lipoprotein cholesterol was reduced in the NAFLD group compared to the non-NAFLD group (**Table 1**).

Comparison of sex hormones

Compared with the non-NAFLD group, serum levels of total testosterone, free testosterone, SHBG, and FSH were decreased in type 2 diabetic male patients with NAFLD, whereas estradiol levels were increased ($P<0.05$, **Table 1**).

Table 1. Comparison of general, glucose and lipid metabolism indicator ($\bar{x} \pm s$)

Group	N	Age (year)	BMI (kg/m^2)	Wc (cm)	SBP (mmHg)	DBP (mmHg)	ALT (u/L)	AST (u/L)	ALP (u/L)
Non-NAFLD	58	50.15±11.35	22.38±3.39	85.51±9.52	131±18	84±14	20.93±18.47	18.30±14.13	82.38±28.20
NAFLD	54	52.48±10.67	27.34±3.93[b]	92.29±11.11[b]	134±19	82±13	29.78±23.74[b]	23.29±17.37[a]	78.33±25.26
Group	N	GGT (u/L)	HbA_{1c} (%)	FBG (mmol/L)	2hPBG (mmol/L)	FINS (uIU/mL)	FCP (pmol/L)	PINS (uIU/mL)	
Non-NAFLD	58	49.32±42.97	10.05±4.15	7.79±1.89	15.63±3.89	6.18±4.21	554.10±229.95	31.72±42.27	
NAFLD	54	41.20±38.79[a]	11.96±4.85[a]	8.75±2.58[a]	18.12±3.95[a]	9.62±5.80[a]	712.14±343.23[a]	72.71±109.70[a]	
Group	N	PCP (pmol/L)	HOMA-IR	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TT (nmol/L)	FT
Non-NAFLD	58	1535.13±771.03	3.53±7.45	4.15±0.92	2.01±1.45	2.75±0.68	1.13±0.23	18.21±6.14	0.39±0.08
NAFLD	54	2232.73±1354.21[a]	3.38±2.28	4.97±1.02[b]	2.74±2.25[b]	2.81±0.93	0.86±0.24[a]	14.18±6.39[a]	0.32±0.03[a]
Group	N	BT	SHBG (nmol/L)	P (nmol/L)	PRL (uIU/mL)	LH (miu/ml)	FSH (miu/ml)	E2 (pmol/L)	DHEA (umol/L)
Non-NAFLD	58	8.22±2.24	34.42±19.14	1.32±0.31	234.02±119.51	7.87±3.78	9.83±5.72	97.53±39.94	5.43±2.72
NAFLD	54	7.92±2.83	25.92±13.63[b]	1.34±0.64	304.24±133.83	7.53±2.93	6.947±4.242[a]	108.70±46.67[a]	5.84±2.69

NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; Wc, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl peptide transferase; HbA_{1c}, glycosylated hemoglobin; FBG, fasting blood glucose; 2hPBG, 2 h postprandial blood glucose; FINS, fasting insulin; FCP, fasting serum C-peptide; PINS, postprandial insulin; PCP, postprandial C peptide ; HOMA-IR, Homeostatic Model Assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TT, total testosterone; FT, free testosterone; BT, bioactive testosterone; SHBG, sex hormone binding globulin; P, progesterone; PRL, prolactin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol; DHEA, dehydroepiandrosterone. [a]$P<0.05$, [b]$P<0.01$.

Table 2. Correlation analysis of testerone and SHBG with metabolic indexs

	HbA_{1C}	FINS	PINS	HOMA-IR	TC	TG	BMI
r	0.01	-0.21	-0.21	-0.30	-0.28	-0.27	-0.40
P	0.97	0.05	0.04	0.01	0.01	0.01	0.00
r	0.08	-0.16	-0.14	-0.23	-0.24	-0.08	-0.36
P	0.42	0.12	0.17	0.03	0.02	0.44	0.00
r	-0.04	-0.04	-0.10	-0.08	-0.21	0.00	-0.17
P	0.65	0.66	0.32	0.46	0.04	0.99	0.10
r	-0.00	-0.22	-0.20	-0.29	-0.24	-0.37	-0.33
P	0.95	0.03	0.05	0.01	0.02	0.00	0.00

Table 3. Logistic regression analysis of risk factors of NAFLD

Indicators	*β*	*SE*	*Wald*	*P*	*OR*	95% *CI*
TG	0.052	0.023	5.126	0.012	1.042	1.012-1.115
BMI	0.104	0.046	6.017	0.041	1.120	1.024-1.217
TT	0.007	0.008	8.130	0.046	1.006	1.002-1.014
HOMA-IR	0.101	0.042	5.971	0.038	1.132	1.031-1.218

Correlation analysis and logistic regression analysis

There was no significant correlation between total testosterone, SHBG, and HbA1C. However, there was a negative correlation between total testosterone and FINS, postprandial insulin, total cholesterol, triglyceride, BMI, and HOMA-IR. After adjusting for waist circumference and BMI, total testosterone was still negatively correlated with FINS, postprandial insulin, and HOMA-IR ($P<0.05$). Using NAFLD as the dependent variable and fasting blood glucose, 2hPBG, HbA1C, FINS, postprandial insulin, total cholesterol, triglyceride, waist circumference, BMI, HOMA-IR, and total testosterone as independent variables, binary logistic regression analysis showed that triglycerides, BMI, HOMA-IR, and total testosterone were risk factors for male diabetic patients with NAFLD (**Tables 2**, **3**).

Discussion

Currently, the incidence of type 2 diabetes complicated with NAFLD is high, and approximately 50% to 75% type 2 diabetes cases are associated with NAFLD [3]. Here is reported that the proportion of hypogonadism in patients with type 2 diabetes with NAFLD increased significantly compared to type 2 diabetes patients without NAFLD. Additionally, the level of sex hormones was significantly disrupted in type 2 diabetes patients with NAFLD. The main manifestations of this disruption included lower serum total testosterone levels, higher estradiol levels, imbalanced estradiol/testosterone, and lower SHBG and FSH.

These results are consistent with a previous study in China, which reported that male patients with fatty liver exhibited decreased serum testosterone levels, increased estradiol levels, and estradiol/testosterone imbalance compared with healthy people [4]. Of note, sex hormones, including estradiol and testosterone, are primarily metabolized by the liver. Thus, liver involvement in sex hormone metabolism offers a potential explanation for the differences observed in sex hormone levels between type 2 diabetes patients with NAFLD and those without NAFLD [5]. Moreover, liver diseases can interfere with the metabolism of sex hormones in the body. The main manifestations are reduced ability to inactivate estrogen, conversion of androgens to estrogens, and decreased androgen levels. The current study also confirmed the presence of imbalance between estradiol/testosterone in male patients with diabetes complicated with NAFLD. Additionally, patients with diabetes are prone to hypogonadism. Therefore, patients with diabetes and NAFLD are particularly susceptible to reduced testosterone levels, leading to disordered sex hormone levels.

In addition, HOMA-IR in patients with diabetes and NAFLD was found to be negatively correlated with total testosterone, free testosterone, and SHBG. Previous studies have shown that there is a close relationship between insulin sensitivity and sex hormone levels, however the relationship between low testosterone levels and insulin resistance remains unclear [6]. On one hand, low testosterone levels are considered to be a cause of insulin resistance and diabetes [7], and, on the other hand, insulin resistance and hyperglycemia are known to lead to hypogonadism [8, 9]. Thus, the causality of the relationship between low testosterone levels and insulin resistance remains unknown.

The present study found that diabetic patients with NAFLD had a higher BMI and demonstrat-

ed stronger relationships to sex hormone levels. In fact, BMI was negatively correlated with testosterone levels in type 2 diabetes patients with NAFLD. Consistent with these results, a survey showed that low testosterone levels in a middle-aged male population were closely related to metabolic syndrome. In particular, after age-adjusted regression analysis, testosterone showed a significant negative correlation with obesity-related BMI and insulin resistance, suggesting that androgens may be an important factor in obesity-related fatty liver disease. This conclusion has also been confirmed in animal experiments. Insulin resistance in male rats deficient in androgen receptors is clear, and these rats are prone to hepatic steatosis [10, 11].

Furthermore, lipid metabolism disorder was found to be more apparent in diabetic patients with NAFLD, and testosterone levels were also negatively correlated with total cholesterol and triglycerides. These findings suggest that hyperlipidemia may be involved in the pathogenesis of fatty liver and hypogonadism. Moreover, these effects may be attributed to conversion of androgen to estrogen by the action of aromatase in obese patients or those with hyperlipidemia, leading to a further decrease in testosterone and an increase in estrogen levels [12].

There are some limitations to our study. First, this was a retrospective study, and, as a result, sexual dysfunction assessment scale could not be employed, such as the International Index of Erectile Function or the Sexual Health Inventory for Men. Second, because free testosterone levels can reflect the extent of the biological activities of testosterone, the free testosterone concentration should be used to assess hypogonadism. However, the determination of free testosterone is difficult, and free testosterone is frequently calculated with a formula in practical work. For this reason, the formula method was employed to calculate free testosterone in the present study.

Despite these limitations, the results suggest that diabetic patients complicated with NAFLD and hyperlipidemia require additional monitoring of serum testosterone levels. This additional monitoring combined with clinical evaluation of gonadal function could promote the timely detection of late onset hypogonadism. Therefore, it is of great importance to control hyperglycemia, alleviate insulin resistance, reduce weight, and lower lipid profiles to prevent and reduce the incidence of type 2 diabetes complicated with NAFLD as well as delay the decline of testosterone levels. In the future, the functional status of patients with type 2 diabetes complicated with NAFLD and the role of sex hormone replacement therapy will require further study.

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Disclosure of conflict of interest

None.

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