Original Article

Evaluation of Basal Serum Adrenocorticotropic Hormone and Cortisol Levels and Their Relationship with Nonalcoholic Fatty Liver Disease in Male Patients with Idiopathic Hypogonadotropic Hypogonadism

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Abstract

Background: Prolonged gonadal hormone deficiency in patients with idiopathic hypogonadotropic hypogonadism (IHH) may produce adverse effects on the endocrine homeostasis and metabolism. This study aimed to compare basal serum adrenocorticotropic hormone (ACTH) and cortisol levels between male IHH patients and healthy controls. Moreover, this study compared the basal hypothalamic-pituitary-adrenal (HPA) axis in patients with and without nonalcoholic fatty liver disease (NAFLD), and also evaluated the relationship between basal HPA axis and NAFLD in male IHH patients.

Methods: This was a retrospective case-control study involving 75 Chinese male IHH patients (mean age 21.4 ± 3.8 years, range 17–30 years) and 135 healthy controls after matching for gender and age. All subjects underwent physical examination and blood testing for serum testosterone, luteinizing hormone, follicle-stimulating hormone, ACTH, and cortisol and biochemical tests.

Results: Higher basal serum ACTH levels (8.25 ± 3.78 pmol/L vs. 6.97 ± 2.81 pmol/L) and lower cortisol levels (366.70 ± 142.48 nmol/L vs. 452.82 ± 141.53 nmol/L) were observed in male IHH patients than healthy subjects (all $P < 0.05$). IHH patients also showed higher metabolism parameters and higher prevalence rate of NAFLD (34.9% vs. 4.4%) than the controls (all $P < 0.05$). Basal serum ACTH (9.91 ± 4.98 pmol/L vs. 7.60 ± 2.96 pmol/L) and dehydroepiandrosterone sulfate (2123.7 ± 925.8 μg/L vs. 1417.1 ± 498.4 μg/L) levels were significantly higher in IHH patients with NAFLD than those without NAFLD (all $P < 0.05$). We also found that basal serum ACTH levels were positively correlated with NAFLD ($r = 0.289$, $P < 0.05$) and triglyceride levels ($r = 0.268$, $P < 0.05$) in male IHH patients. Furthermore, NAFLD was independently associated with ACTH levels in male IHH patients by multiple linear regression analysis.

Conclusions: The male IHH patients showed higher basal serum ACTH levels and lower cortisol levels than matched healthy controls. NAFLD was an independent associated factor for ACTH levels in male IHH patients. These preliminary findings provided evidence of the relationship between basal serum ACTH and NAFLD in male IHH patients.

Key words: Adrenocorticotropic Hormone; Cortisol Level; Dehydroepiandrosterone Sulfate; Idiopathic Hypogonadotropic Hypogonadism; Nonalcoholic Fatty Liver Disease

Introduction

Idiopathic hypogonadotropic hypogonadism (IHH) is a rare congenital disorder due to an isolated deficiency or dysfunction of gonadotropin-releasing hormone (GnRH) neurons.[1] IHH is characterized by delayed or absent sexual maturation and infertility associated with inappropriately low gonadotropin and sex steroid levels.[2] Prolonged gonadal

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hormone deficiency in IHH patients may produce adverse effects on the endocrine homeostasis and metabolism.

The hypothalamic-pituitary-adrenal (HPA) axis is essential for the regulation and maintenance of homeostasis.[3] Gonadal hormone deficiency will inevitably lead to an imbalance of endocrine homeostasis, which may affect the HPA axis. Animal studies have shown that adrenocorticotropic hormone (ACTH) and corticosterone levels in male rats were increased by gonadectomy.[4,5] However, investigations on the influence of androgen deficiency on the basal HPA axis in humans are rare and still inconsistent. One of the main reasons for this is that previous studies have focused on healthy subjects by provisionally manipulating sex hormones to evaluate the effects on the HPA axis.[6] Therefore, the effects of prolonged androgen deficiency on the HPA axis in human males are unclear and limited to speculations based on the observations in lower animals and healthy men. To further characterize the basal HPA axis in human male with prolonged androgen deficiency, the present study compared basal serum ACTH and cortisol levels between male IHH patients and healthy controls.

On the other hand, it is now established that low serum testosterone levels in men are associated with an increased risk of obesity, metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD).[7-9] Recently, a number of cross-sectional studies have reported that the clearance rate of cortisol was increased and the hepatic regeneration of cortisol was decreased in patients with NAFLD. Moreover, those studies have also shown the association of NAFLD with chronic, subclinical compensatory activation of the HPA axis in humans.[10,11] In addition, compensatory activation of the HPA axis may drive increased adrenal androgen secretion.[10,12] To date, the association between basal HPA axis and NAFLD in IHH is not clear. Hence, we compared basal serum ACTH, cortisol, and dehydroepiandrosterone sulfate (DHEAS) levels in IHH patients with and without NAFLD.

Methods

Study population

We performed a retrospective case-control study of 75 Chinese male IHH patients (aged from 17 years to 30 years), who were admitted to the Chinese PLA General Hospital from January 2010 to December 2013. A total of 135 healthy controls, whose data were obtained from the Health Examination Center from September 2013 to November 2013, were also included. All patients were diagnosed according to clinical findings of hypogonadism (such as low testis volumes, decreased body and facial hair, delayed puberty, and eunuchoid stature), subnormal testosterone (T) levels in the presence of low gonadotropins. Healthy controls were selected as study subjects with normal serum T, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels after matching for gender, age, and race. Medical history and lifestyle, including information on liver disease and alcohol consumption, were obtained from each subject using questionnaires.

All patients and healthy controls were excluded if they had liver disease (hepatitis B or C, autoimmune liver disease, drug- or alcohol-induced liver disease, cholestatic liver disease, or inherited liver disease).

This study was approved by the Ethics Committee of the Chinese PLA General Hospital, and written informed consents were obtained from all participants or their parents.

Laboratory assessments

Fasting blood samples of all participants were collected from the cubital vein between 7:00 a.m. and 9:00 a.m. Ethylenediaminetetraacetic acid plasma samples for the measurement of serum ACTH were stored at 2°C to 4°C before the test. Serum ACTH and DHEAS levels were measured by chemiluminescence on an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc., Munich, Germany). The normal reference values of ACTH and DHEAS at 8:00 a.m. were <10.12 pmol/L and 350.0–4300.0 μg/L, respectively. Serum cortisol, T, LH, and FSH levels were measured by chemiluminescence on an ADVIA Centaur XP Analyzer (Siemens Healthcare Diagnostics, Inc., Co Dublin, Ireland); and normal reference values were as follows: cortisol 198.7–797.5 nmol/L, T 8.4–28.7 nmol/L, LH 1.5–9.3 mU/ml, and FSH 1.4–18.1 U/L. Other laboratory examinations included the tests for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), fasting blood glucose (FBG), total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and creatinine (Cr) levels. All the processes of drawing blood were successful without any stress. All assays were performed according to the manufacturers' recommendations. All biochemical determinations were conducted in the same laboratory with standard methods.

Physical examination and abdominal ultrasonography

Height and weight were measured for the calculation of body mass index (BMI). The blood pressure of each participant was recorded. NAFLD was diagnosed based on abdominal ultrasonography performed by experienced ultrasonologist. Fatty infiltration of the liver was diagnosed if hepatorenal contrast and liver brightness were detected. The subjects, thus, identified as having a fatty liver in the absence of other potential causes of hepatitis such as excessive alcohol consumption (>140 g/week) were diagnosed with NAFLD.[13]

Statistical analysis

Data were expressed as mean ± standard deviation (SD) when normally distributed, and as median (interquartile range) when not normally distributed. Kolmogorov-Smirnov test was used to test the parameter distribution. Student's *t*-test was used for comparison of normally distributed parameters. In all other cases, Mann-Whitney *U*-test was used for comparisons between groups. In addition, Chi-square test

was used for comparing group ratios. Correlations were assessed using Pearson's or Spearman's method for normally or nonnormally distributed data, respectively. Multiple linear regression analysis was used to determine the association of ACTH levels with the metabolism parameters. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). A two-tailed $P < 0.05$ was considered statistically significant for all analyses.

Results

The clinical and biochemical characteristics of 75 IHH patients and 135 matched controls are shown in Table 1. Serum T, LH, and FSH levels in all male IHH patients were significantly lower than those in healthy controls ($P < 0.001$). The male IHH patients showed significantly higher metabolism parameters (including BMI, FBG, TG, TC, and LDL-C) and lower HDL-C levels compared with healthy controls. The patients exhibited significantly higher ALT and AST levels than healthy controls (all $P < 0.05$). The Cr levels were significantly lower in the male IHH patients than that in healthy controls ($P < 0.001$). A diagnosis of NAFLD was made in 22 of 63 male IHH patients who underwent abdominal ultrasonography and in six of 135 healthy controls. For the HPA axis, basal serum ACTH level in the patients was significantly higher than that in the healthy controls ($P < 0.05$) whereas basal serum cortisol level in the patients was significantly lower than that in the healthy controls ($P < 0.001$). Although among 75 male IHH patients, 54 (72%) patients received hormone replacement therapy (testosterone replacement therapy, human chorionic gonadotropin therapy, GnRH pulse pump therapy, or random combination of them) before coming to our hospital, there were no statistical differences in clinical and biochemical characteristics between the male IHH patients with and without hormone replacement therapy [Supplement Material 1].

Among 75 male IHH patients, 63 patients underwent abdominal ultrasonography. The clinical and biochemical characteristics of these 63 patients grouped according to the presence of NAFLD are listed in Table 2. There was no statistically significant difference regarding serum T level between patients with and without NAFLD (0.95 nmol/L [0.61–1.57 nmol/L] vs. 1.11 nmol/L [0.49–3.09 nmol/L], $P > 0.05$). Patients with NAFLD had higher BMI than patients without NAFLD ($P < 0.001$). Both systolic and diastolic blood pressures were higher in patients with NAFLD than those in patients without NAFLD ($P < 0.05$). The serum ALT and GGT levels in the NAFLD group were higher than those in the non-NAFLD group ($P < 0.05$). Blood lipid levels also differed between the NAFLD group and the non-NAFLD group. Serum TC, TG, and LDL-C levels were significantly higher, and serum HDL-C levels were significantly lower in patients with NAFLD than those in patients without NAFLD (all $P < 0.05$). The FBG, serum AST, and Cr levels between two groups were different but without statistical significance (all $P > 0.05$). For the HPA axis, patients with NAFLD exhibited significantly higher basal serum ACTH and DHEAS levels than those

Table 1: The clinical and biochemical characteristics of the male IHH patients and matched healthy controls

Characteristics	Male IHH patients ($n = 75$)	Healthy controls ($n = 135$)	Statistical values	P
Age (years)	21.4 ± 3.7	21.1 ± 3.4	0.65*	0.487
BMI (kg/m²)	23.66 ± 5.49	21.72 ± 3.94	2.98*	0.008
SBP (mmHg)	118.79 ± 11.83	116.77 ± 12.76	1.19*	0.262
DBP (mmHg)	71.09 ± 8.86	74.21 ± 8.07	−2.62*	0.011
T (nmol/L)	0.95 (0.57–1.81)	18.32 (14.90–22.17)	20.00†	<0.001
LH (mU/ml)	0.21 (0.02–0.81)	3.51 (2.89–4.44)	759.00†	<0.001
FSH (U/L)	0.93 (0.47–1.60)	3.98 (2.91–5.14)	1077.50†	<0.001
FBG (mmol/L)	4.66 ± 0.45	4.37 ± 0.99	2.17*	0.031
TG (mmol/L)	1.32 ± 0.72	0.69 ± 0.31	7.87*	<0.001
TC (mmol/L)	3.98 ± 0.69	3.66 ± 0.64	2.94*	0.004
HDL-C (mmol/L)	1.17 ± 0.28	1.32 ± 0.33	−2.65*	0.003
LDL-C (mmol/L)	2.38 ± 0.65	2.03 ± 0.54	3.68*	0.001
ALT (U/L)	25.89 ± 20.60	17.14 ± 6.00	4.21*	0.003
AST (U/L)	20.35 ± 8.77	17.37 ± 3.11	2.20*	0.022
GGT (U/L)	19.87 ± 8.93	17.98 ± 8.73	1.30*	0.094
Cr (U/L)	61.85 ± 8.70	71.85 ± 8.65	−7.04*	<0.001
ACTH (pmol/L)	8.25 ± 3.78	6.97 ± 2.81	3.04*	0.017
Cortisol (nmol/L)	366.70 ± 142.48	452.82 ± 141.53	−0.88*	<0.001
With NAFLD	22 (34.9)‡	6 (4.4)	–	<0.001

Data are shown as mean ± SD, median (interquartile range), or *n* (%).*t values; †U values. ‡Among 75 male IHH patients, 63 patients were undergoing abdominal ultrasonography. IHH: Idiopathic hypogonadotropic hypogonadism; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; T: Serum testosterone; LH: Serum luteinizing hormone; FSH: Serum follicle-stimulating hormone; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; Cr: Serum creatinine; ACTH: Adrenocorticotropic hormone; NAFLD: Nonalcoholic fatty liver disease; SD: Standard deviation.

in patients without NAFLD (all $P < 0.05$). The basal serum cortisol levels in the NAFLD group were lower than that in the non-NAFLD group but without statistical significance ($P > 0.05$).

Furthermore, basal serum ACTH levels were positively correlated with NAFLD and TG levels in the male IHH patients. However, there was no significant correlation between serum cortisol levels and NAFLD, BMI, TC, TG, and HDL- and LDL-C levels [Table 3]. In our study, only 43 patients had records for DHEAS levels. Moreover, no significant correlation was found between DHEAS and NAFLD ($r = 0.276$, $P = 0.089$).

A multivariate linear regression model was used to study which kinds of clinical and biochemical factors were independently associated with ACTH levels. In our model, NAFLD was independently associated with ACTH levels in male IHH patients [Table 4].

Discussion

In the present study, although ACTH and cortisol levels were within the normal ranges, we observed higher ACTH and lower cortisol levels in male IHH patients. In particular, ACTH and DHEAS levels were higher in IHH patients with NAFLD than those in IHH patients without NAFLD; however, serum cortisol levels were similar between two groups. Basal serum ACTH levels were positively correlated with NAFLD and triglyceride. NAFLD was an independent associated factor for ACTH levels in male IHH patients. This study evaluated the basal HPA axis in male IHH patients, but the underlying precise molecular mechanism needs further study.

Extensive experimental evidence has showed that androgens exert an inhibitory influence on HPA axis.[4,14] To date, the mechanism(s) by which androgens may act to influence HPA function have not been completely resolved.[15] Animal studies have revealed that basal ACTH release was regulated by testosterone-dependent effects on arginine vasopressin synthesis.[4] Furthermore, the increase in adrenal size in response to gonadectomy may also be explained by the ability of testosterone to repress expression of the corticotropin-releasing hormone (CRH).[16] Moreover, prior studies also showed that castration can also disturb basal and stimulated concentrations of circulating glucocorticoids in rats.[17] A variety of central mechanisms, such as decreased CRH, decreased arginine vasopressin, and increased glucocorticoid receptor concentrations, have been postulated to underlie the suppressive effects of testosterone on corticosterone in rodents.[18] In our study, male IHH patients exhibited higher basal serum ACTH levels than healthy controls. However, the serum cortisol

Table 2: The clinical and biochemical characteristics of the 63 IHH patients with and without NAFLD

Characteristics	NAFLD group ($n = 22$)	Non-NAFLD group ($n = 41$)	t	P
Age (years)	22.91 ± 4.73	20.66 ± 2.89	−2.34	0.051
BMI (kg/m²)	28.73 ± 5.42	20.57 ± 3.58	−7.18	<0.001
SBP (mmHg)	123.41 ± 10.70	116.10 ± 10.74	−2.58	0.012
DBP (mmHg)	74.50 ± 9.53	68.24 ± 7.42	−2.88	0.005
FBG (mmol/L)	4.68 ± 0.53	4.62 ± 0.43	−0.76	0.625
TG (mmol/L)	1.59 ± 0.68	1.06 ± 0.64	−2.58	0.013
TC (mmol/L)	4.33 ± 0.74	3.76 ± 0.60	−2.80	0.007
HDL-C (mmol/L)	1.07 ± 0.28	1.22 ± 0.20	2.20	0.033
LDL-C (mmol/L)	2.64 ± 0.62	2.16 ± 0.61	−2.57	0.013
ALT (U/L)	29.15 ± 14.40	22.85 ± 23.59	−1.00	0.031
AST (U/L)	21.33 ± 10.51	19.52 ± 8.22	−2.23	0.069
GGT (U/L)	24.49 ± 11.41	16.08 ± 6.82	−7.98	<0.001
Cr (U/L)	62.01 ± 7.31	61.05 ± 8.59	−0.39	0.696
ACTH (pmol/L)	9.91 ± 4.98	7.60 ± 2.96	−2.21	0.026
Cortisol (nmol/L)	349.43 ± 166.08	376.06 ± 146.47	3.58	0.553
DHEAS (μg/L)*	2123.7 ± 925.8	1417.1 ± 498.4	−2.40	0.020

Data are shown as mean ± SD. *Among 63 patients who underwent abdominal ultrasonography, 39 subjects had records for DHEAS levels (11 in NAFLD group and 28 in non-NAFLD group). IHH: Idiopathic hypogonadotropic hypogonadism; NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; Cr: Serum creatinine; ACTH: Adrenocorticotropic hormone; DHEAS: Dehydroepiandrosterone sulfate; SD: Standard deviation.

Table 3: Correlation between ACTH, cortisol and metabolism parameters in male IHH patients

Parameters	NAFLD	BMI	TC	TG	HDL	LDL	ALT	GGT	ACTH
ACTH	0.289*	0.143	0.239	0.268*	−0.143	0.135	−0.066	0.208	–
Cortisol	−0.001	−0.052	0.098	−0.086	−0.138	0.079	−0.173	−0.047	0.484*

*P<0.05. NAFLD: Nonalcoholic fatty liver disease; TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase; ACTH: Adrenocorticotropic.

Table 4: Multiple linear regression analysis for ACTH levels

Variables	*B*	*P*	95% *CI* for *B*
Constant	8.303	0.000	6.738–9.868
BMI	–0.087	0.554	−0.380–0.206
NAFLD	3.232	0.013	0.704–5.761
TC	0.114	0.915	−2.028–2.257
TG	1.324	0.215	−0.797–3.446
FBG	2.150	0.120	−0.588–4.888

CI: Confidence interval; BMI: Body mass index; NAFLD: Nonalcoholic fatty liver disease; TC: Total cholesterol; TG: Triglycerides; FBG: Fasting blood glucose.

levels were lower in IHH patients. We speculated that it might result from dysregulation of cortisol metabolism caused by prolonged testosterone deficiency in male IHH patients. Cortisol metabolism is regulated by the activities of 11β-hydroxysteroid dehydrogenase 1 (11βHSD1), interconverting hormonally inactive cortisone to active cortisol, and the A-ring reductases (5α- and 5β-reductases) which inactivate cortisol.[10] In rats, hepatic 11βHSD1 is expressed in lower amounts in females than males, and castration of male rats does suppress hepatic 11βHSD1 activity toward female levels.[19] In humans, both hepatic 5α- and 5β-reductases are suppressed by higher circulating androgens. Previous studies have shown that 5α-reduced metabolites of glucocorticoids were more abundant in women compared to men.[19] Therefore, prolonged testosterone deficiency may result in decreased activation and increased clearance of cortisol, and it is plausible that the serum cortisol levels in male IHH patients were lower than those in matched healthy controls.

Recently published studies in general Chinese population have reported that the prevalence of NAFLD in children and adolescents is between 2.1% and 15%.[19] According to our study, the prevalence rate of NAFLD in male IHH patients was 34.9%. The high prevalence of NAFLD in IHH patients observed in the current study was concordant with anecdotal reports and findings of other observational studies in hypogonadism patients.[20-22] Furthermore, a recent large observational study in 1912 German men and a Korean retrospective cross-sectional study in 495 men showed an inverse association between serum testosterone levels and NAFLD.[7,12] There are several mechanisms that might explain this inverse association, such as increased adipose tissue lipolysis, increased hepatic lipogenesis, decreased hepatic fatty acid β-oxidation, and decreased export of lipids from the liver.[23,24] Low-grade inflammation could also be a link between testosterone and NAFLD. Low serum testosterone is associated with markers of inflammation.[25] The chronic inflammatory state is fundamental to the progression of NAFLD.[26] In addition, there are two studies that demonstrated an inverse association between low serum testosterone levels and the metabolic syndrome.[8,27] Therefore, our results suggested that the high metabolism parameters and the high prevalence of NAFLD in male IHH patients might be caused by low serum testosterone levels, which resulted from low levels of gonadotropin.

Some cross-sectional studies have reported the association of NAFLD with chronic, subclinical general activation of the HPA axis in humans.[28,29] Human study has showed that patients with hepatic steatosis gave rise to decreased 11βHSD1 activity and increased 5α- and 5β-reductase activities, which resulted in increased the metabolic clearance rate of cortisol. To maintain normal circulating cortisol concentrations, the HPA axis was activated by a negative feedback pathway, which enhanced ACTH and ACTH-dependent DHEAS production.[11] Therefore, it was reasonable that in our study, basal serum cortisol levels were similar between patients with and without NAFLD, and basal serum ACTH and DHEAS levels were increased in IHH patients with NAFLD. Furthermore, we also found that basal serum ACTH level was positively correlated with NAFLD. NAFLD was an independent associated factor for ACTH level in male IHH patients. The characteristics of this association in our study suggested that higher ACTH levels could better reflect the interplay between NAFLD and the HPA axis. The exact mechanisms leading to high serum ACTH levels in male IHH patients with NAFLD need to be established by further studies. We hypothesized that this association might result from an increased glucocorticoid metabolism. Due to limited sample size of our study, no significant correlations were found between DHEAS and NAFLD. However, the above mentioned study in 1912 German men has showed that NAFLD was associated with high serum DHEAS levels in men.[12] Moreover, there was also no significant correlation between NAFLD and serum cortisol levels in male IHH patients. Similar to our results, a recent study in 1326 German subjects also found no significant correlation between plasma cortisol concentration and the existence of NAFLD.[30] Therefore, we conjectured that the HPA axis may be influenced by low testosterone levels on the one hand and may also be affected by liver fat deposition in male IHH patients on the other hand.

In our study, we also found that basal serum ACTH level was positively correlated with triglyceride levels. One explanation could be that ACTH has been shown to increase apolipoprotein E levels in humans, which is a key protein in determining triglyceride metabolism.[31] However, previous study demonstrated that the association between ACTH and triglycerides may be secondary to the association between ACTH and insulin resistance,[32] and the IHH patients always suffered from insulin resistance[33] although the data of insulin resistance in IHH patients were not shown in our study. It was reasonable that triglyceride levels were not independently associated with ACTH level in our study. The exact mechanism underlying the association between ACTH and triglyceride deserves further investigation.

Our study still had some limitations. First, it is well known that the liver biopsy is the gold standard for detecting fatty liver.[34] However, the liver biopsy was also not

feasible in this retrospective case-control study. In this study, diagnosis of NAFLD has used the ultrasonography which is noninvasive, safe, sensitive (up to 84.8%), and specific (up to 93.6%) in terms of identifying fatty infiltration.[35] However, the ultrasonography could not identify fatty infiltration below 30% as the mode of diagnosis. Furthermore, 12 IHH patients had no records of abdominal ultrasound. Therefore, the prevalence of NAFLD in IHH may have been underestimated in this study. The second limitation was the evaluation of the HPA axis without the evaluation of urinary free cortisol. It was difficult to collect daily urine samples of all the participants. However, a single morning fasting cortisol measurement had been shown to be associated with chronic stress and metabolic disturbances.[36] Third, the gonadal hormone levels and metabolism in male IHH patients were significantly different from those in healthy controls.[37] We only assessed the correlation between the HPA axis and NAFLD in male IHH patients. On the other hand, the number of healthy controls with NAFLD is too limited to examine the association between the HPA axis and NAFLD. Thus, our findings may do not necessarily apply for healthy population, female, or other forms of hypopituitary patients. Fourth, our study was a cross-sectional design, in which we could not determine the effect of hormone replacement therapy on the HPA axis and metabolism parameters in IHH patients. Prospective studies might clarify this aspect. Although these findings were from a single cohort, these preliminary findings provided evidence for basal serum ACTH and cortisol levels and their relationship with NAFLD in male IHH patients. Longitudinal studies might clarify this aspect.

In summary, the male IHH patients exhibited higher basal serum ACTH level, lower cortisol level, and higher prevalence rate of NAFLD than those in healthy controls. Basal serum ACTH level was positively correlated with NAFLD and triglyceride; however, no significant correlation between serum cortisol level and NAFLD was found in male IHH patients. Furthermore, NAFLD was an independent associated factor for ACTH level in male IHH patients. These specific associations suggested complex mechanisms among the HPA axis and testosterone deficiency and metabolic impairments in male IHH patients.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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Supplement Material 1: The clinical and biochemical characteristics of the 75 male IHH patients with and without HRT

Characteristics	Patients with HRT (n = 54)	Patients without HRT (n = 21)	P
Age (years)	21.7 ± 3.7	20.8 ± 3.8	0.355
BMI (kg/m^2)	24.01 ± 5.15	22.76 ± 6.31	0.425
SBP (mmHg)	120.22 ± 12.49	115.10 ± 9.19	0.157
DBP (mmHg)	72.02 ± 8.63	68.71 ± 9.19	0.164
T (nmol/L)	1.00 (0.62–1.94)	0.76 (0.35–1.24)	0.078
LH (mU/ml)	0.27 (0.03–1.03)	0.15 (0.01–0.77)	0.560
FSH (U/L)	1.01 (0.49–1.89)	0.89 (0.42–1.56)	0.715
FBG (mmol/L)	4.66 ± 0.50	4.65 ± 0.30	0.931
TG (mmol/L)	1.34 ± 0.75	1.26 ± 0.67	0.718
TC (mmol/L)	4.07 ± 0.74	3.80 ± 0.56	0.139
HDL-C (mmol/L)	1.14 ± 0.28	1.21 ± 0.26	0.383
LDL-C (mmol/L)	2.47 ± 0.69	2.17 ± 0.54	0.097
ALT (U/L)	26.74 ± 23.20	22.28 ± 8.55	0.205
AST (U/L)	21.24 ± 10.17	18.35 ± 3.79	0.133
GGT (U/L)	20.33 ± 10.30	16.45 ± 4.17	0.094
Cr (U/L)	63.43 ± 9.25	58.33 ± 6.21	0.121
ACTH (pmol/L)	8.02 ± 3.85	8.84 ± 3.92	0.311
Cortisol (nmol/L)	358.24 ± 153.24	371.97 ± 126.18	0.439
With NAFLD	15 (27.8)	5 (23.8)	0.543

Data are shown as mean ± SD, median (interquartile range), or n (%). HRT: Hormone replacement therapy; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; T: Serum testosterone; LH: Serum luteinizing hormone; FSH: Serum follicle-stimulating hormone; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; Cr: Serum creatinine; ACTH: Adrenocorticotropic hormone; NAFLD: Nonalcoholic fatty liver disease; SD: Standard deviation.