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# Relationship Between Serum Betatrophin Levels and Non-alcoholic Fatty Liver Disease in Hypogonadal Males

## ABSTRACT

**Objective:** Betatrophin is a hepatokine that modulates hepatic glucose and lipid metabolism and contributes to non-alcoholic fatty liver disease (NAFLD) pathogenesis. Therefore, this study aimed to investigate the relationship between NAFLD and betatrophin levels in hypogonadal males.

**Methods:** The study included 56 newly diagnosed hypogonadal males aged 18-60 and 60 eugonadal males of similar age and body mass index. All participants were assessed for anthropometric and metabolic parameters, liver function tests, and betatrophin levels. Transient elastography was used to evaluate liver steatosis [controlled attenuation parameter (CAP)] and fibrosis [liver stiffness measurement (LSM)]. Accordingly, hypogonadal and control groups were divided into NAFLD (n = 64) and non-NAFLD (n = 52).

**Results:** Controlled attenuation parameter, LSM, waist circumference (WC), triglycerides (TG), IR index homeostasis model assessment (HOMA-IR), and betatrophin were significantly higher in the hypogonadal group than controls. Hepatic steatosis and fibrosis (67.9%-43.3%) were higher in hypogonadal males. Triglycerides, HOMA-IR, and betatrophin were higher, and total testosterone was significantly lower in the NAFLD group. Serum betatrophin was also significantly higher in patients with fibrosis than without. There was a significant positive correlation between WC, TG, HOMA-IR, betatrophin, and LSM and CAP. The predictive factors were TG ($\beta = 0.329$, $P < .001$), betatrophin ($\beta = 0.221$, $P = .029$), HOMA-IR ($\beta = 0.213$, $P = .019$) for CAP, and betatrophin for LSM ($\beta = 0.466$, $P < .001$).

**Conclusion:** Non-alcoholic fatty liver disease is more common in hypogonadal males than in eugonadal males. Betatrophin is an independent risk factor for developing and progressing NAFLD. However, more research is needed to explain the causal relationship between betatrophin and NAFLD.

**Keywords:** Hypogonadism, non-alcoholic fatty liver disease, betatrophin

## Introduction

Non-alcoholic fatty liver disease (NAFLD) covers a wide clinical spectrum. It ranges from hepatic steatosis to fibrosis and cirrhosis.[1] It is an integral part of metabolic diseases that develop in the center of insulin resistance (IR), such as obesity and type 2 diabetes (T2DM). It is becoming an increasingly important problem worldwide.[2] Hypogonadism in males is a clinical situation that occurs as a result of insufficient testicular sperm production or testosterone (TT) or both.[3] It also causes an increased risk of cardio-metabolic diseases.[4,5] Low sex hormone levels have a negative effect on glucose and lipid metabolism. Several studies revealed that low TT levels are associated with abdominal obesity, metabolic syndrome (MS), IR, and NAFLD.[5-9]

Recently, betatrophin has been shown to contribute to the pathogenesis of MS, NAFLD, and T2DM.[10] It has even been suggested that it may be a possible biochemical marker of NAFLD progression.[11,12] Many mechanisms have been speculated to explain this relationship. Betatrophin is a hepatokine and has been shown to modulate liver glucose and lipid metabolism.[13] Insulin markedly increases betatrophin in adipose tissue and the liver.[14] It has been shown in animal studies that mice with IR have significantly increased betatrophin levels as a compensatory response.[15] While insulin directly activates lipoprotein lipase (LPL), it also indirectly regulates LPL by modulating betatrophin levels. It has been reported that betatrophin, which increases in the presence of IR, increases triglyceride (TG) synthesis by inhibiting LPL.[14,16]

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A liver biopsy is considered the best method to assess NAFLD severity. However, it is an invasive method with some issues regarding inter-observer variability, sampling errors, and cost.[17] Therefore, noninvasive procedures have been developed to assess inflammation and fibrosis. Liver transient elastography (FibroScan®) is currently used as a noninvasive imaging method to determine the risk of hepatic steatosis and fibrosis.[18] The controlled attenuation parameter (CAP) feature applied in the FibroScan® device is one of the most promising assessment methods for NAFLD. The risk of liver fibrosis can be evaluated by liver stiffness measurement (LSM).[19]

In this regard, we evaluated the frequency of NAFLD using FibroScan®, a noninvasive technique, in hypogonadal males at increased risk of metabolic disease. We also aimed to identify factors that could predict the severity of NAFLD using serum betatrophin levels and other metabolic risk factors.

## Materials and Methods

This study (retrospective, case–control) was carried out between September 2018 and 2019 in collaboration with the Departments of Endocrinology, Gastroenterology, and Medical Biochemistry, Faculty of Medicine, Kahramanmaraş Süçtü İmam University (KSU). Before commencing the study, approval was obtained from the Ethics Committee of the KSU Faculty of Medicine (approval no: 17, approval date: July 25, 2018). Furthermore, signed consent was obtained from the volunteers who participated in the study.

### *Study Design and Inclusion Criteria*

The study included 56 hypogonadal males admitted to the Endocrinology outpatient clinic between 18 and 60, newly diagnosed, and 60 eugonadal males (control group) of similar age and body mass index (BMI).

Hypogonadism was diagnosed in males with clinical symptoms and a low total TT level (≤230 ng/dL or ≤8 nmol/L) measured at least twice in the morning.[20] Primary hypogonadism was defined as high FSH or LH, whereas secondary hypogonadism was defined as low or normal FSH or LH.[21] Eugonadal males were accepted as the control group. The control group had normal sexual development and erectile function and/or fertility with normal TT levels (>350 ng/dL).[20]

### MAIN POINTS

- Hypogonadism in males is associated with abdominal obesity, metabolic syndrome, and insuin resistance and is considered an independent cardiovascular risk factor. There are few studies investigating the relationship between non-alcoholic fatty liver disease (NAFLD) and low testosterone levels.
- Nowadays, noninvasive biochemical markers that can evaluate NAFLD progression are being investigated. Although controversial, betatrophin has recently been suggested as a possible noninvasive marker for NAFLD progression.
- Our study results indicate that the frequency of NAFLD is increased in hypogonadal males compared to eugonadal males. Additionally, serum betatrophin level is a positive predictive factor for liver steatosis and fibrosis in hypogonadal males. However, we believe that further studies are necessary to explain the causal relationship between betatrophin and NAFLD.

Body mass index, waist circumference (WC), fasting plasma glucose (FPG), lipid profile, liver function tests, fasting insulin (FI), TT, and serum betatrophin level were assessed in both the patient and control groups. Additionally, all participants were evaluated using the liver FibroScan® technique to establish liver steatosis and fibrosis.

### *Exclusion Criteria*

Individuals with additional drug use (statin, steroid, methotrexate, non-steroidal anti-inflammatory drugs, etc.) that may affect androgen levels and the liver, other pituitary hormone deficiency, history of malignancy, ethanol consumption >20 g/day, chronic liver disease, type 1 diabetes mellitus, acute or chronic pancreatitis, uncontrolled thyroid dysfunction, adrenal insufficiency, heart failure, recent parenteral nutrition, severe psychiatric disorders or mental retardation, and female patients were excluded from the study. Additionally, patients with morbid obesity (BMI ≥ 40 kg/m$^2$) and ascites, which could affect FibroScan® measurement, were not included in the study.

### *Anthropometric Measurements*

The formula BMI = weight (kg)/height$^2$ (m$^2$) has been used to calculate the body mass index. For consistency, WC was measured twice with a non-extendable tape at the midpoint between the iliac crest and the last rib.

### *Biochemical Measurements*

After an 8-10 hour fast, blood samples for biochemical parameters were collected between 8:00 AM and 9:00 AM. Biochemical parameters were measured using a spectrophotometric assay (Advia 1800 chemistry system, Siemens, Germany) and hormonal parameters using an electrochemiluminescence assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA).

### *Betatrophin Measurement*

All blood samples were collected from the groups. The serum was immediately separated in a centrifuge and laid at −20°C until analysis. Serum betatrophin levels were measured according to the manufacturer's instructions using a commercial kit (MBS761140, MyBioSource Company, Southern California, San Diego, USA) by quantitative sandwich enzyme-linked immunosorbent assay. The absorbance of each parameter was measured at 450 nm using a microplate reader (model 680; Bio-Rad). Calculations are made with GraphPad PRISM 5.0 (GraphPad Software Inc.). Logarithmic transformation was used for betatrophin analysis. All samples were tested twice. Coefficients of variation within and between assays are <8% and <10%, respectively.

Normal reference values were the following: FPG 70-100 mg/dL, total cholesterol 0-200 mg/dL, TG 0-150 mg/dL, high-density lipoprotein 26-86 mg/dL, low-density lipoprotein 0-130 mg/dL, aspartate aminotransferase (AST) 7-32 U/L, alanine aminotransferase (ALT) 7-45 U/L, FI 2.6-24.9 µU/mL, TT 300-1100 ng/dL, and betatrophin 12-400 pg/mL.

### *Insulin Resistance Index*

The IR index homeostasis model assessment (HOMA-IR) was calculated for all participants using the following formula based on FI and FPG levels: HOMA-IR = FPG (mg/dL) × fasting plasma insulin (µIU/mL)/405.[22]

### *Non-Alcoholic Fatty Liver Disease Diagnosis and Liver Elastography (FibroScan®)*

FibroScan® was used to measure the CAP and LSM. All patients were asked to fast for at least 3 hours before testing. Each patient

was placed supine with the right arm in full abduction for the procedure. Scanning the right liver lobe through an intercostal space was the measurement method. The FibroScan® procedure was carried out by a single experienced operator using a standard M probe and FibroScan® 530 compact (Echosens, Paris, France). Liver steatosis (CAP score) and fibrosis evaluation (LSM) were the median values for 10 successful measurements. The final results of the CAP and LSM were expressed in dB/m and kPa, respectively.

### Staging of Liver Steatosis

S0 (absent steatosis); CAP < 232.5 dB/m, S1 (mild steatosis); CAP 234-269 dB/m, S2 (moderate steatosis); CAP 270-300 dB/m and S3 (severe steatosis); CAP ≥ 301 dB/m were accepted.[23]

### Staging of Liver Fibrosis

F0-1 (absent or mild), LSM < 6 kPa; F2 (gray range), LSM = 6-8 kPa; F3 (severe fibrosis), LSM = 8-12.5 kPa; F4 (cirrhosis), LSM > 12.5 kPa were accepted.[24]

### Statistical Analysis

Mean ± standard deviation was used for normally distributed data and median (min, max) for non-normally distributed data. The data were analyzed using Statistical Package for the Social Sciences, version 25.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to determine both the sample distribution and Levene's test for homogeneity of variances. The independent 2 sample *t*-test compared 2 groups for normally distributed data. Mann–Whitney *U*, a nonparametric test, was used for data that did not show normal distribution. Chi-square and Fisher's exact tests were used to assess the frequency distribution of categorical variables. The Pearson and Spearman correlation tests assessed the direct relationship between the variables. A linear regression analysis was carried out to investigate the effect of age, WC, TG, TT, and betatrophin on the CAP and the LSM.

## Results

### Hypogonadal and Control Groups Compared

As shown in Table 1, CAP, LSM, WC, FPG, TG, FI, HOMA-IR, and betatrophin levels were significantly higher in the hypogonadal group compared to the eugonadal group ($P = .006$, $P < .001$, $P = .012$, $P = .031$, $P = .026$, $P = .002$, $P = .001$, and $P < .001$, respectively). The liver steatosis and fibrosis rate were higher in hypogonadal males than in controls (67.9% vs. 43.3%, $P = 0.012$, and 42.9% vs. 6.7%, $P < .001$).

### Comparing Individuals With and Without Non-alcoholic Fatty Liver Disease

The hypogonadal and control groups included in the study were divided into 2 groups: NAFLD (n = 64) and non-NAFLD (n = 52). In the NAFLD group, TG, HOMA-IR, and betatrophin levels were higher ($P = .048$, $P = .025$, and $P = .020$, respectively), and TT levels were significantly lower ($P = .045$) (Table 2).

Non-alcoholic fatty liver disease group was also divided into 2 groups: without fibrosis (n = 41) and with fibrosis (n = 23). Serum betatrophin levels were significantly higher in the fibrosis group than those without fibrosis ($P < .001$), as shown in Figure 1.

### Correlation Analysis

The correlation analysis of the demographic, biochemical, and FibroScan® data of the study participants (n = 116) with betatrophin levels is shown in Table 3. Betatrophin levels were significantly positively correlated with WC, AST, ALT, TG, FI, HOMA-IR, CAP, and LSM ($P = .012$, $P = .048$, $P = .022$, $P = .009$, $P < .001$, $P = .001$, $P = .005$, and $P < .001$, respectively) and negatively correlated with TT ($P < .001$).

The correlation analysis of CAP and LSM is also presented in Table 3. Controlled attenuation parameter and LSM were significantly negatively correlated with TT values ($P = .038$ vs. $P = .003$). Waist circumference, TG, FI, HOMA-IR, betatrophin, and LSM were significantly positively correlated with CAP ($P = .045$, $P = .044$, $P < .001$, $P < .001$, $P = .005$, and $P < .001$, respectively). Moreover, correlation analyses showed that WC, TG, FI, HOMA-IR, and betatrophin had significant positive relationships with LSM ($P = .002$, $P = .030$, $P < .001$, $P < .001$, and $P < .001$, respectively) (Figure 2).

### Multiple Linear Regression Analysis

Linear regression analysis was carried out to identify predictive variables for CAP score and LSM in hypogonadal males. The predictive factors were TG (beta = 0.329, $P < .001$), betatrophin (beta = 0.221, $P = .029$), and HOMA-IR (beta = 0.213, $P = .019$) for CAP and betatrophin (beta = 0.466, $P < .001$) for LSM when age, WC, TG, HOMA-IR, TT, and betatrophin levels were included in the model. The results are presented in Tables 4 and 5.

## Discussion

In the current study, we demonstrated a higher frequency of NAFLD in hypogonadal males compared to age and BMI-matched eugonadal males. We found significantly higher levels of liver steatosis (CAP) and fibrosis markers (LSM), WC, betatrophin, FPG, HOMA-IR, and TG levels in the hypogonadal group. We observed a negative association between betatrophin and TT levels and a positive association with CAP, LSM, WC, TG, and HOMA-IR. We also showed that betatrophin is an independent, effective factor in both NAFLD's steatosis and fibrosis stages. Our findings suggest that betatrophin can be a noninvasive biochemical marker that can predict NAFLD progression in male patients with hypogonadism.

The gold standard method for diagnosing NAFLD is liver biopsy, but it is an invasive procedure. Thus, recently, it has been suggested that FibroScan®, a noninvasive technique, be used. In addition, elastosonography is the most sensitive, noninvasive method to assess liver fibrosis.[25] Our study assessed liver steatosis (CAP) and fibrosis (LSM) using FibroScan®.

Male hypogonadism is associated with abdominal obesity, MS, and IR and is considered an independent cardiovascular risk factor. There are a limited number of studies that have investigated the association between NAFLD and low TT levels. In a retrospective observational study by Kim et al,[26] which assessed steatosis using imaging techniques, they showed an independent association between low TT and NAFLD. Van de Velde et al[8] found an independent association between low TT levels and hepatosteatosis in their study, which included 80 obese males. Recently, Yang et al[27] demonstrated that a higher TT level is linked to a lower NAFLD prevalence in non-overweight/obese males with T2DM [(tertile 1: ≤2.66 ng/mL (63.3%), tertile 2: 2.67-3.58 ng/mL (53.7%), and tertile 3: >3.58 ng/mL (35.5%), respectively)]. In our study, we also found that the frequency of NAFLD was higher in hypogonadal males (TT (≤230 ng/dL)) than in similar age and BMI-matched eugonadal males (67.9% versus 43.3%).

The relationship between hypogonadism, obesity, and IR has been demonstrated in many studies. There has been evidence that there is an inverse relationship between IR and TT levels in men and that

**Table 1. Comparison of FibroScan®, Anthropometric, and Laboratory Parameters of Hypogonadal and Control Group**

| Parameters | Hypogonadal (n = 56) | Median (Minimum-Maximum) | Control (n = 60) | Median (Minimum-Maximum) | *P* |
|---|---|---|---|---|---|
| Age (years) | 33.37 ± 9.82 | 31 (19-56) | 30.71 ± 7.48 | 28.0 (19-51) | .103[a] |
| BMI (kg/m$^2$) | 27.11 ± 5.27 | 27.65 (15.4-39.2) | 25.66 ± 3.68 | 25.2 (17.2-36.5) | .091[a] |
| WC (cm) | 106.85 ± 17.65 | 109.5 (77-142) | 99.36 ± 13.46 | 97 (78-127) | **.012**[a] |
| Comorbidity, n (%) | 14 (25) | – | 12 (20) | – | .634[b] |
| FPG (mg/dL) | 96.44 ± 17.53 | 91 (72-158) | 88.65 ± 20.86 | 87.0 (65-228) | **.031**[c] |
| AST (U/L) | 22.94 ± 18.96 | 18 (6-135) | 20.81 ± 8.28 | 19.5 (12-65) | .430[c] |
| ALT (U/L) | 27.98 ± 23.64 | 22 (7-165) | 22.43 ± 10.04 | 21 (9-55) | .099[c] |
| Total-C (mg/dL) | 178.69 ± 44.15 | 176 (99-286) | 168.23 ± 31.51 | 169 (89-244) | .143[a] |
| LDL-C (mg/dL) | 115.05 ± 34.39 | 115 (43-205) | 117.33 ± 31.72 | 114.5 (55-212) | .711[a] |
| HDL-C (mg/dL) | 44.34 ± 7.58 | 44 (25.5-66) | 44.38 ± 9.47 | 44 (28-81) | .980[a] |
| TG (mg/dL) | 138.58 ± 55.35 | 184 (50-510) | 117.48 ± 89.83 | 98 (27-683) | **.026**[c] |
| TT (ng/dL) | 161.92 ± 97.60 | 148 (45-700) | 511.81 ± 18.21 | 495 (300-942) | **<.001**[c] |
| FI (µU/mL) | 13.29 ± 9.13 | 11.3 (1.30-40.8) | 9.00 ± 3.83 | 8.7 (2.60-18.30) | **.002**[c] |
| HOMA-IR | 3.20 ± 2.35 | 2.57 (12.09-11.83) | 2.01 ± 1.20 | 2.21 (0.55-11.84) | **.001**[c] |
| Betatrophin (pg/mL) | 143.85 ± 59.26 | 128.42 (34.7-298.7) | 97.99 ± 23.26 | 100 (52.5-177.6) | **<.001**[c] |
| TSH (mIU/L) | 1.49 ± 1.11 | 1.4 (0.54-3.75) | 1.88 ± 0.98 | 1.7 (0.45-2.9) | .093[c] |
| CAP (dB/m) | 275.83 ± 99.90 | 278.5 (107.5-510) | 232.70 ± 60.14 | 206.5 (97-396) | **.006**[a] |
| Liver steatosis, n (%) | 38 (67.9) | – | 26 (43.3) | – | .012[b] |
| S0 | 18 (32.1) | – | 34 (56.7) | – | |
| S1 | 6 (10.7) | – | 9 (15) | – | |
| S2 | 12 (21.4) | – | 9 (15) | – | |
| S3 | 20 (35.7) | – | 8 (13.3) | – | |
| LSM (kPa) | 5.49 ± 2.34 | 4.95 (2.00-13.80) | 4.19 ± 1.23 | 4.15 (1.90-7.90) | **<.001**[a] |
| Liver fibrosis, n (%) | 24 (42.9) | – | 4 (6.7) | – | <.001[d] |
| F0-1 | 32 (57.1) | – | 56 (93.3) | – | |
| F2 | 15 (26.8) | – | 4 (6.7) | – | |
| F3 | 9 (16.1) | – | 0 (0) | – | |
| F4 | 0 (0) | – | 0 (0) | – | |

Data are presented as n (%) and mean ± standard deviation. Values in bold indicate statistical significance. Between group comparisons were made by independent sample *t*-test, chi-square test, Mann–Whitney *U*, and Fisher's exact test. Comorbidity: hypertension, diabetes mellitus, hyperlipidemia, atherosclerotic cardiovascular disease. Liver steatosis grade; S0 <248 dB/m; S1 (mild), 248-268 dB/m; S2 (moderate), 268-280 dB/m; S3 (severe), ≥280 dB/m. Liver fibrosis stage; F0-1, ≤7 kPA (absent or mild), F2,7-10 kPA (moderate), F3, 10-14 kPA (severe), F4, ≥14 (cirrhosis).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, FibroScan's controlled attenuation parameter; FI, fasting insulin; FPG, fasting plasma glucose; HDL-C, high density lipoprotein; HOMA-IR, Homeostatic Model Assessment—Insulin Resistance; LDL-C, low density lipoprotein; LSM, liver stiffness measurement; Total-C, total cholesterol; TG, triglyceride; TT, total testosterone; TSH, thyroid-stimulating hormone; WC, waist circumference.
[a]Independent sample *t*-test.
[b]Chi-square test.
[c]Mann–Whitney *U*-test.
[d]Fisher's exact test.

TT may have an effect on insulin signaling in peripheral tissues.[6,7] In the pathogenesis of NAFLD, obesity and IR play a central role. Body fat distribution is more important in the pathogenesis of NAFLD, although obesity is associated with NAFLD. Cnop et al[28] have shown that excessive accumulation of abdominal fat has a strong relationship with IR and that it plays an important role in the pathogenesis of NAFLD as a source of free fatty acids. In another study, intra-abdominal fat accumulation was positively associated with hepatosteatosis in both men and women.[29] Kim et al[26] reported that the rate of visceral steatosis evaluated by computed tomography was significantly higher in hypogonadal patients. We found that WC and HOMA-IR were significantly higher in patients with both hypogonadism and NAFLD. Our findings, in line with the literature, suggest that low TT levels may contribute to the development of NAFLD in association with abdominal obesity and IR. Clinical noninvasive markers are needed to identify patients with NAFLD, predict their progression, and allow early intervention. Betatrophin is a newly identified adipokine secreted by adipose tissue and the liver. It has been shown to regulate glucose homeostasis and lipid metabolism. Zhang and Abou-Samra[13] showed that betatrophin significantly stimulated the growth of pancreatic beta cells, increased the mass of beta cells, and improved glucose tolerance in mouse models of IR. Recent studies have suggested that betatrophin is a circulating protein secreted by the liver in IR. It plays a role in the compensatory response to IR.[16] Betatrophin also plays a significant role in lipid metabolism.[30] Insulin regulates the expression and function of LPL directly and affects its activity indirectly by expressing LPL modifiers, like betatrophin.[12,13] Overexpression of betatrophin results in elevated serum TG, while knockout reduces fatty acid uptake by adipose tissue and increases LPL activity.[11] In line with the literature, we found that betatrophin

**Table 2. Comparison of FibroScan®, Anthropometric, and Biochemical Parameters of Individuals With and Without Non-Alcoholic Fatty Liver Disease**

| Parameters | NAFLD (+) (n = 64) (55.2%) | Median (Minimum-Maximum) | NAFLD (–) (n = 52) (44.8%) | Median (Minimum-Maximum) | *P* |
|---|---|---|---|---|---|
| Age (years) | 32.84 ± 9.32 | 31 (19-56) | 30.96 ± 7.98 | 28 (19-53) | .252[a] |
| BMI (kg/m$^2$) | 26.67 ± 5.09 | 26.7 (15.4-39.2) | 25.98 ± 3.81 | 25.4 (19.8-37.7) | .416[a] |
| WC (cm) | 105.32 ± 17.14 | 105 (77-142) | 100.12 ± 14.48 | 99.5 (78-140) | **.027**[a] |
| FPG (mg/dL) | 94.15 ± 21.52 | 89.5 (72-228) | 90.26 ± 16.99 | 88 (65-158) | .279[b] |
| AST (U/L) | 19.73 ± 8.49 | 18.5 (9-59) | 24.44 ± 19.19 | 19.5 (6-135) | .105[b] |
| ALT (U/L) | 24.96 ± 12.58 | 22.5 (8-70) | 25.28 ± 23.26 | 20 (7-165) | .929[b] |
| Total-C (mg/dL) | 176.57 ± 42.03 | 172 (89-286) | 169.23 ± 33.18 | 172 (104-262) | .295[a] |
| LDL-C (mg/dL) | 119.37 ± 35.97 | 116.5 (43-212) | 112.36 ± 28.58 | 112 (59-163) | .245[a] |
| HDL-C (mg/dL) | 44.51 ± 8.29 | 44 (25-66) | 44.17 ± 8.98 | 43.5 (28-81) | .830[a] |
| TG (mg/dL) | 185.89 ± 122.86 | 145 (27-510) | 148.55 ± 95.66 | 134.5 (50-683) | **.048**[b] |
| TT (ng/dL) | 308.48 ± 216.20 | 217.5 (45-802) | 385.26 ± 210.12 | 393.5 (55-942) | **.045**[b] |
| FI (µU/mL) | 11.98 ± 7.29 | 10.5 (1.3-40.8) | 9.1 ± 4.25 | 8.5 (2.6-21) | **.011**[b] |
| HOMA-IR | 3.52 ± 2.20 | 2.46 (0.26-12.09) | 2.84 ± 2.19 | 2.2 (0.55-11.84) | **.025**[b] |
| Betatrophin (pg/mL) | 129.42 ± 56.99 | 109.9 (53.5-298.7) | 108.70 ± 36.80 | 106.2 (34.7-263) | **.020**[b] |
| CAP (dB/m) | 298.75 ± 77.34 | 284.5 (97-510) | 180.17 ± 34.36 | 187.5 (100-226) | **<.001**[a] |
| Liver steatosis grade, n (%) | – | – | – | – | – |
| S0 | 0 (0) | – | – | – | – |
| S1 | 15 (23.4) | – | – | – | – |
| S2 | 21 (32.8) | – | – | – | – |
| S3 | 28 (43.8) | – | – | – | – |
| LSM (kPa) | 5.62 ± 2.16 | 5.1 (2.0-13.8) | 3.83 ± 1.03 | 3.9 (1.9-6.7) | **<.001**[a] |
| Liver fibrosis stage, n (%) | – | – | – | – | – |
| F0-1 | 37 (76.7) | – | – | – | – |
| F2 | 18 (28.1) | – | – | – | – |
| F3 | 9 (14.1) | – | – | – | – |
| F4 | 0 (0) | – | – | – | – |

Data are presented as n (%) and mean ± standard deviation. Values in bold indicate statistical significance. Between group comparisons were made by independent sample *t*-test and Mann–Whitney *U*-test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, FibroScan's controlled attenuation parameter; FI, fasting insulin; FPG, fasting plasma glucose; HDL-C, high density lipoprotein; HOMA-IR, Homeostatic Model Assessment—Insulin Resistance; LDL-C, low density lipoprotein; LSM, liver stiffness measurement; NAFLD (+), steatosis is present on ultrasonography; NAYKH (–), no steatosis on ultrasonography; NAFLD, non-alcoholic fatty liver disease; Total-C, total cholesterol; TG, triglyceride; TT, total testosterone; TSH, thyroid-stimulating hormone; WC, waist circumference.
[a]Independent sample *t*-test.
[b]Mann–Whitney *U*-test.

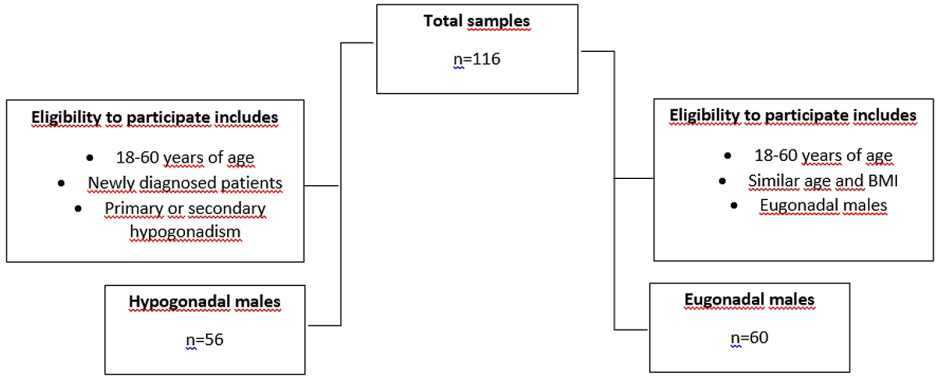


**Figure 1. Selection of the participants.**

and TG were significantly higher in those with NAFLD and hypogonadal males. We also showed a significant positive relationship between betatrophin and TG and HOMA-IR. These results suggest that IR and betatrophin play a role in the development of NAFLD by acting together and contributing to the increase in TG in hypogonadal patients.

**Table 3. Relationship Between Serum Betatrophin Levels, Controlled Attenuation Parameter, and Liver Stiffness Measurement Score with Age, Anthropometric, and Metabolic Parameters**

| | Betatrophin (pg/mL) | | CAP (dB/m) | | LSM (kPa) | |
|---|---|---|---|---|---|---|
| **Parameters** | ***r*** | ***P*** | ***r*** | ***P*** | ***r*** | ***P*** |
| Age (years)[a] | 0.041 | .658 | 0.097 | .302 | 0.181 | .052 |
| BMI (kg/m²)[a] | 0.162 | .083 | 0.193 | .038 | 0.278 | .003 |
| WC (cm)[a] | 0.234 | **.012** | 0.186 | **.045** | 0.288 | **.002** |
| FPG (mg/dL)[b] | 0.036 | .697 | 0.051 | .589 | 0.171 | .067 |
| AST (U/L)[b] | 0.184 | **.048** | 0.124 | .185 | -0.118 | .209 |
| ALT (U/L)[b] | 0.212 | **.022** | 0.047 | .613 | 0.010 | .919 |
| Total-C (mg/dL)[a] | 0.178 | .056 | 0.122 | .191 | 0.171 | .066 |
| LDL-C (mg/dL)[a] | 0.091 | .333 | 0.073 | .438 | 0.109 | .242 |
| HDL-C (mg/dL)[a] | -0.034 | .716 | 0.050 | .595 | 0.057 | .546 |
| TG (mg/dL)[b] | 0.243 | **.009** | 0.188 | **.044** | 0.245 | **.030** |
| Fasting insulin (μU/mL)[b] | 0.365 | **.000** | 0.442 | **.000** | 0.581 | **<.001** |
| HOMA-IR[b] | 0.313 | **.001** | 0.416 | **.000** | 0.560 | **<.001** |
| Total testosterone (ng/dL)[b] | -0.453 | **.000** | -0.193 | **.038** | -0.277 | **.003** |
| Betatrophin (pg/mL)[b] | – | – | 0.262 | **.005** | 0.348 | **<.001** |
| LSM (kPa)[a] | 0.348 | **.000** | 0.630 | **.000** | – | – |
| CAP (Db/M)[a] | 0.262 | **.005** | – | – | 0.630 | **<.001** |

Values in bold indicate statistical significance.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, FibroScan's controlled attenuation parameter; FI, fasting insulin; FPG, fasting plasma glucose; HDL-C, high density lipoprotein; HOMA-IR, Homeostatic Model Assessment—Insulin Resistance; LDL-C, low-density lipoprotein; LSM, liver stiffness measurement; Total-C, total cholesterol; TG, triglyceride; TT, total testosterone; TSH, thyroid-stimulating hormone; WC, waist circumference.
[a]Pearson correlation test.
[b]Spearman correlation test.

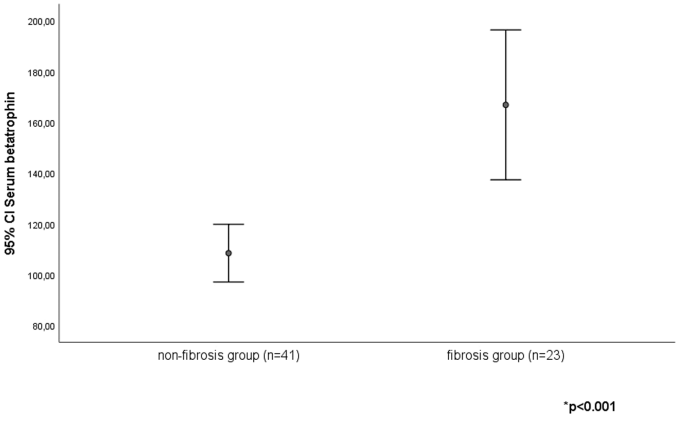


**Figure 2. Comparison of betatrophin levels of groups with and without fibrosis.**

Recent studies have suggested that betatrophin is a potential non-invasive marker for assessing NAFLD progression. Therefore, in this study, we also evaluated the relationship between betatrophin and NAFLD progression. We showed that there is a relationship between NAFLD progression and betatrophin levels in hypogonadal males for the first time in the literature. We showed that serum betatrophin levels were significantly higher in patients with fibrosis than those without. In addition, betatrophin, TG, and HOMA-IR were predictive of CAP. Only betatrophin was predictive of LSM. When we reviewed the literature, studies showed that betatrophin levels increased in patients with fibrosis, as in our research, and articles reported that they decreased. Hu et al[12] showed that serum betatrophin is an independent risk factor for NAFLD and a possible noninvasive marker of NAFLD progression in the Chinese population. In this study, participants in the highest tertile of serum betatrophin had greater chances of having NAFLD.

**Table 4. Linear Regression Analysis for the Controlled Attenuation Parameter**

| | CAP | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Unadjusted Analysis** | | | | | | | **Adjusted Analysis** | | | | | |
| Variables | *B* | SD | *Beta* | *t* | 95% CI | *P* | *B* | SD | *Beta* | *t* | 95% CI | *P* |
| Age (years) | 1.187 | 1.374 | 0.117 | 0.864 | −1.567-3.941 | .391 | – | – | – | – | – | – |
| WC (cm) | 1.238 | 0.486 | 0.232 | 2.544 | 0.274-2.201 | **.012** | – | – | – | – | – | – |
| TG (mg/dL) | 0.229 | 0.068 | 0.302 | 3.384 | 0.095-0.364 | **.001** | 0.356 | 0.097 | 0.329 | 3.669 | −0.548-0.164 | **<.001** |
| HOMA-IR | 10.780 | 3.513 | 0.276 | 3.068 | 3.820-17.739 | **.003** | 8.190 | 3.439 | 0.213 | 2.381 | 1.373-15.006 | **.019** |
| TT (ng/dL) | −0.104 | 0.036 | −0.264 | −2.919 | 1.038-1.416 | **.004** | – | – | – | – | – | – |
| Betatrophin (pg/mL) | 0.542 | 0.152 | 0.316 | 3.555 | 0.240-0.843 | **.001** | 0.374 | 0.169 | 0.221 | 2.214 | 0.039-0.708 | **.029** |

Values in bold indicate statistical significance. *P* < .05 was considered statistically significant.
BMI, body mass index; CI, confidence interval; HOMA-IR, Homeostatic Model Assessment—Insulin Resistance; TG, triglyceride; TT, total testosterone; WC, waist circumference.

**Table 5. Linear Regression Analysis for the Liver Stiffness Measurement**

**LSM**

| | Unadjusted Analysis | | | | | |
|---|---|---|---|---|---|---|
| Variables | B | SD | Beta | *t* | 95% CI | *P* |
| Age (years) | 0.040 | 0.021 | 0.181 | 1.960 | 0.000-0.081 | .052 |
| WC (cm) | 0.035 | 0.011 | 0.288 | 3.217 | 0.014-0.057 | **.002** |
| TG (mg/dL) | 0.005 | 0.002 | 0.295 | 3.295 | 0.002-0.008 | **.001** |
| HOMA-IR | 0.258 | 0.080 | 0.289 | 3.220 | 0.099-0.417 | **.002** |
| TT (ng/dL) | −0.003 | 0.001 | −0.277 | −3.074 | −0.004-0.001 | **.003** |
| Betatrophin (pg/mL) | 0.014 | 0.003 | 0.348 | 3.967 | 0.007-0.021 | **<.001** |

Values in bold indicate statistical significance. $P < .05$ was considered statistically significant.
BMI, body mass index; CI, confidence interval; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; LSM, Liver Stiffness Measurement; TG, triglyceride; TT, total testosterone; WC, waist circumference.

Hong et al[31] showed that individuals with moderate-to-severe NAFLD on magnetic resonance imaging had elevated serum betatrophin, in contrast to age- and gender-matched healthy individuals and those with mild NAFLD. Arias-Loste et al[32] proposed that plasma betatrophin levels increased with IR in cirrhotic patients, which was linked to cirrhosis severity. Contrarily, Sonmez et al[11] suggested in their study that betatrophin levels are higher in the early stages of NAFLD and tend to decrease as the disease progresses. Cengiz et al[33] also supported that the mild fibrosis group had higher serum betatrophin levels than the severe fibrosis group. The agreed view of both our study and the studies we have mentioned here is that betatrophin may be a guiding marker in the progression of NAFLD. However, conflicting results in studies may be due to differences in study design, population, and steatosis and fibrosis evaluation methods. When we evaluate our data, we think IR plays a central role in developing NAFLD in hypogonadal men. Still, that betatrophin is an important clinical marker, especially in predicting progression.

Although our study had various limitations, the most important strength was that it was the first study to show the relationship between NAFLD progression and betatrophin in hypogonadal males. The limitations of our study were as follows: First, the cross-sectional case–control design makes it difficult to determine the role of serum betatrophin in the development of NAFLD. Long-term cohort studies with larger populations are needed in the future. Second, our study did not confirm fibrosis by liver biopsy, which is the gold standard method. We are planning this in our future work.

The frequency of NAFLD is increased in hypogonadal males compared to eugonadal males. Serum TG, HOMA-IR, and betatrophin level are positive predictive factors for liver steatosis in hypogonadal males. Furthermore, serum betatrophin level is an independent risk factor for fibrosis. These results support the role of IR and secondarily increased betatrophin and TG levels in the development of NAFLD in hypogonadal males and that betatrophin may predict progression. However, more studies are needed to explain the causal relationship between betatrophin and NAFLD.

**Ethics Committee Approval:** This study was approved by the Ethics Committee of Kahramanmaras Sütçü İmam University (approval number: 17; date: July 25, 2018).

**Informed Consent:** Written informed consent was obtained from the patients who agreed to take part in the study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – F.S., A.O.; Design – F.S., K.G.; Supervision – A.O., K.G.; Resources – U.N.O., O.N.B.; Materials – F.S., A.O.; Data Collection and/or Processing – F.S., M.I.; Analysis and/or Interpretation – E.B.K., O.N.B.; Literature Search – F.S., M.I., A.O.; Writing – F.S., A.O., K.G.; Critical Review – K.G., A.O., E.B.K.

**Declaration of Interests:** The authors have no conflicts of interest to declare.

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