OBJECTIVE: This study was performed to evaluate the effects of metabolic parameters and thyroid dysfunction on the development of non-alcoholic fatty liver disease (NAFLD).

METHODS: The current study evaluated a total of 115 patients, 75 female and 40 male. Physical examination and anthropometric measurements were applied to all participants. Hypothyroidism was considered at a thyroid stimulating hormone level > 4.1 mIU/L. Patients with euthyroidism and patients with hypothyroidism were compared. Abdominal ultrasonography was used to diagnose non-alcoholic fatty liver disease. The participants were further compared with regard to the presence of non-alcoholic fatty liver disease and independent variables, such as metabolic parameters and insulin resistance.

RESULTS: Non-alcoholic fatty liver disease was identified in 69 patients. The mean waist circumference, body mass index, fasting plasma insulin, HOMA-IR (p<0.001) and FT3/FT4 ratio (p=0.01) values were significantly higher in the patients with NAFLD compared to those without it. Multivariate regression analysis revealed that FT3/FT4 ratio, waist circumference and insulin resistance were independent risk factors for non-alcoholic fatty liver disease.

CONCLUSION: Insulin resistance, enlarged waist circumference, elevated body mass index, higher FT3/FT4 ratio and hypertriglyceridemia are independent risk factors for NADLF, whereas hypothyroidism is not directly related to the condition.

KEYWORDS: FT3/FT4 ratio; Insulin resistance; Non-alcoholic fatty liver disease; Hypothyroidism; Euthyroidism.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a pathological spectrum of chronic liver diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with inflammation, has a high risk for progression to cirrhosis (1-3). NAFLD is a growing diagnosis and the most commonly encountered liver pathology in clinical practice (4,5). NAFLD is commonly asymptomatic and discovered incidentally. The diagnosis of NAFLD is based on exclusion criteria, such as alcohol consumption (more than 20 g/day), autoimmune liver disease, viral hepatitis infection, hemochromatosis, Wilson’s disease, and drug consumption. All of these must be excluded before considering NAFLD (6). The prevalence of NAFLD is associated with abdominal obesity, diabetes mellitus and other metabolic risk factors (7, 8). NAFLD is a strong determinant for the development of metabolic syndrome, which has potentially relevant clinical implications with regard to diagnosis, prevention and treatment (9,10). Moreover, metabolic syndrome, insulin resistance, diabetes, obesity and mixed hyperlipidemia are major metabolic risk factors for NAFLD (11). Because of the hyperinsulinism, pro-thrombotic potential, and subclinical inflammation associated with NAFLD, patients with this condition are at increased risk for cardiovascular mortality (12). In addition, the correction of insulin resistance may not be sufficient to successfully treat NASH in the majority of patients, conflicting with previous studies on NAFLD pathogenesis (13).

The thyroid gland is significantly involved in lipid and carbohydrate metabolism, regulation of body weight and adipogenesis (14). Recent studies have suggested that thyroid dysfunction may play a role in NAFLD. Subclinical hypothyroidism is associated with metabolic syndrome, cardiovascular mortality, and disturbance of lipid metabolism.
(15, 16). Thyroid dysfunctions in the form of overt or subclinical hypothyroidism are prevalent among patients with NAFLD/NASH (17).

NAFLD is a risk factor for the development of type 2 diabetes, which is, in turn, a major contributor to progressive liver disease (18). In contrast, chronic infections, such as that caused by hepatitis C virus, have an association with the development of NAFLD, insulin resistance and metabolic parameters (19). The identification of risk factors is essential for preventing NAFLD. Therefore, in the current study, we evaluated the effects of metabolic parameters and thyroid dysfunction on the development of NAFLD.

## METHODS

### Participants

The current study evaluated 115 individuals, 75 female and 40 male, who were admitted to the Haseki Training and Research Hospital’s outpatient clinic for routine care from July 2014 through January 2015. Anthropometric measurements were taken, and thyroid function tests were performed. Hypothyroidism was described according to Clinical Practice Guidelines for Hypothyroidism in Adults: Co-sponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association (20). Euthyroidism (ET) was described as a thyroid stimulating hormone (TSH) level of 0.5–4 mIU/L and no history of chronic disease. Hypothyroidism (HT) was described as a TSH level of 4.1 mIU/L. Patients meeting the following criteria were excluded: chronic liver and kidney disease, viral hepatitis, diabetes mellitus, undergoing corticosteroid treatment, malignancy, alcohol consumption greater than 20 g/d, and pregnancy. Informed consent was obtained from all participants. The study protocol was approved by the local ethics committee of Istanbul Haseki Training and Research Hospital.

### Measurements

All patients underwent basic physical examination. Blood pressure was measured using a mercury sphygmomanometer. Height (m), weight (kg), and waist circumference (WC) were also measured. WC was measured between the lowest rib and the crista iliaca superiori. Body mass index (BMI) was calculated as weight (kg)/height (m)$^2$. Plasma TSH, free T3 (FT3), free T4 (FT4), alanine aminotransferase (ALT), aspartate alanine aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), glucose, insulin, total cholesterol, triglycerides, HDL and LDL cholesterol, uric acid, fasting glucose, fasting insulin, HOMA-IR, NAFLD or ferritin in the subjects with ET (30.28 ± 5.19) versus those with HT. The mean FT3/FT4 ratio of the patients with HT was higher than that of the subjects with ET, at 4.61 ± 1.38 versus 3.63 ± 0.68, respectively ($p<0.001$). There was no difference in NAFLD status between the patients with ET and those with HT. NAFLD was identified in 69 of total 115 subjects: 33 patients with ET and 36 patients with HT.

The participants were compared according to the presence of NAFLD, and the parameters of the comparison are presented in Table 1. No significant differences were found in gender, age, mean BMI, systolic BP, diastolic BP, ALT, AST, ALP, GGT, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, uric acid, fasting glucose, fasting insulin, HOMA-IR, NAFLD or ferritin in the subjects with ET. The mean FT3/FT4 ratio of the patients with HT was lower than those in the patients with ET without NAFLD (30.28 ± 5.19) versus those with HT. The mean FT3/FT4 ratio of the patients with HT was higher than that of the subjects with ET, at 4.61 ± 1.38 versus 3.63 ± 0.68, respectively ($p<0.001$). There was no difference in NAFLD status between the patients with ET and those with HT. NAFLD was identified in 69 of total 115 subjects: 33 patients with ET and 36 patients with HT.

### Statistical Analysis

Numeric values were expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS 16.0 for Windows. The Kolmogorov-Smirnov Z test was used to determine the distributions of variables. Regular variances were assessed using a t test, and irregular variables were assessed using the Mann-Whitney U test. Logistic regression modeling was performed to assess independent risk factors of NAFLD. A $p$ value < 0.05 was considered statistically significant.

## RESULTS

In total, 115 participants were enrolled in this study: 54 presented with HT (F/M, 39/15) and 61 presented with ET (F/M, 36/25). The anthropometric and metabolic parameters of the patients with ET and HT were compared and are presented in Table 1. No significant differences were found in gender, age, mean BMI, free T3, free T4, total cholesterol, triglycerides, HDL cholesterol, HDL cholesterol, uric acid, fasting glucose, fasting insulin, HOMA-IR, NAFLD or ferritin in the subjects with ET (30.28 ± 5.19) versus those with HT. The mean FT3/FT4 ratio of the patients with HT was higher than that of the subjects with ET, at 4.61 ± 1.38 versus 3.63 ± 0.68, respectively ($p<0.001$). There was no difference in NAFLD status between the patients with ET and those with HT. NAFLD was identified in 69 of total 115 subjects: 33 patients with ET and 36 patients with HT.

The participants were compared according to the presence of NAFLD, and the parameters of the comparison are presented in Table 2. The mean WC, BMI, systolic and diastolic blood pressure values were statistically higher in the patients with NAFLD than those without the condition ($p<0.001$, < 0.001, 0.049 and 0.003, respectively). Additionally, the patients with NAFLD had significantly higher triglyceride levels (164.96 ± 77.27 mg/dl) than those without NAFLD (112.61 ± 89.80 mg/dl) ($p=0.001$). The patients with NAFLD also had significantly higher uric acid, fasting insulin, HOMA-IR and FT3/FT4 ratios.

The subjects with ET or HT in this study were also compared according to the presence or absence of NAFLD, as shown in Table 3. The patients with ET and NAFLD had higher WC ($p=0.001$), total cholesterol ($p=0.042$), triglycerides ($p<0.001$), fasting insulin ($p<0.001$) and HOMA-IR ($p=0.001$) levels compared to the subjects with ET without NAFLD. While the FT4 levels in the patients with ET and NAFLD were lower than those in the patients with ET without NAFLD, the patients with ET and NAFLD had increased FT3/FT4 ratios, as well as uric acid, fasting insulin and HOMA-IR levels, compared to the patients with ET without NAFLD ($p=0.01$).

The patients with HT and NAFLD had lower FT4 levels compared to the patients with HT without NAFLD (Table 3). Additionally, the patients with HT and NAFLD had higher WC, total cholesterol, triglycerides, fasting insulin and HOMA-IR levels than the patients with HT without NAFLD.

Logistic regression analysis was performed to delineate the nature of the relationships that exist between NAFLD, metabolic parameters and insulin resistance as independent variables (Table 4). WC (OR: 1.087, $p=0.01$), HOMA-IR (OR: 2.978, $p=0.005$), and FT3/FT4 ratio (OR: 1.834, $p=0.02$) were independent risk factors for NAFLD in all study participants. Additional regression analysis was performed to evaluate HT patients with NAFLD with respect to metabolic parameters (Table 5). WC (OR: 1.189, $p=0.02$), triglycerides (OR: 1.031, $p=0.04$), uric acid (OR: 0.318, $p=0.03$), HOMA-IR
Euthyroidism, hypothyroidism n: number of patients. WC: waist circumference. BMI: body mass index. BP: blood pressure. ALT: alanine aminotransferase. AST: aspartate aminotransferase. ALP: alkaline phosphatase. GGT: gamma glutamyl transferase. HOMA-IR: homeostasis model assessment for insulin resistance. NAFLD: non-alcoholic fatty liver disease.

OR: 8.042, p=0.02) and FT3/FT4 ratio (OR: 3.540, p=0.01) were independent risk factors for NAFLD in patients with HT.

### DISCUSSION

NAFLD is a burgeoning health problem and is currently recognized as the most common metabolic liver disease. Insulin resistance and obesity contribute to the development of NAFLD, which has become the most prevalent liver disease worldwide, affecting one-third of the global adult population (22,23). NAFLD can lead to NASH and/or hepatocellular cancer (24).

It has been suggested that a relationship exists between NAFLD and thyroid dysfunction (25). Despite the precise physiological mechanism underlying the development of NAFLD, the relationship between NAFLD, hypothyroidism and metabolic syndrome remains unclear. Because of the importance of thyroid hormones in lipid metabolism (26), HT may result in hyperlipidemia, thereby initiating the development of NAFLD. Several studies have indicated that hypothyroidism is a risk factor for NAFLD and can result in metabolic syndrome (16,27,28). FT3/FT4 ratio can be considered an indicator of peripheral deiodinase activity. Bilgin and Pirgon (29) suggested that augmented conversion from FT4 to FT3 due to increased deiodinase activity is a compensatory mechanism for fat accumulation to improve energy expenditure. FT3/FT4 ratio positively correlates with HOMA-IR in patients with NAFLD (18). Moreover, positive associations have been reported between FT3/FT4 ratio and both waist circumference and BMI in patients with obesity (30). Ittermann and Haring (31) reported that low FT4 levels, but not low TSH and FT3 levels, are associated with hepatic steatosis. In the present study, the mean BMI values in patients with ET and patients with HT were similar; however, the patients with HT had significantly higher FT3/FT4 ratios (p<0.001). The patients with NAFLD had significantly elevated BMI, WC, HOMA-IR values and FT3/FT4 ratios; their FT4 levels were low, leading to increased FT3/FT4 ratios, but their TSH levels were unaffected. The results of this study suggest that elevated FT3/FT4 ratio is an independent risk factor for NAFLD.

Patients with HT have elevated triglyceride and LDL cholesterol levels due to decreased plasma lipoprotein lipase activity. Hyperlipidemia associated with fatty accumulation in the liver and cellular oxidative stress is one potential mechanism underlying the development of NAFLD (32,33). The results of the present study support this relationship, as TC (p=0.002), LDL cholesterol (p=0.001), triglyceride (p=0.008), and uric acid (p=0.006) levels were significantly higher and HDL cholesterol levels lower (p=0.022) in the patients with NAFLD.

The prevalence of NAFLD did not significantly differ between the ET (n=36, 59%) and HT (n=33, 64%) groups. Mazo and Lima (34) previously reported that no association exists between HT, hepatosteatosis and NASH. In addition, Eshraghian and Dabbaghmanesh (35) reported that no association exists between autoimmune thyroid disorder and elevated anti-thyroid peroxidase antibodies, anti-thyroglobulin levels and NAFLD. Furthermore, NAFLD was not correlated with thyroid dysfunction in the current study, as the included patients with ET and HT did not show significant differences in NAFLD prevalence, insulin resistance, abdominal obesity or BMI. The mean BMI of the patients with ET and HT was above 30, and abdominal obesity was considered to be more important to the development of NAFLD than HT. WC, FT3/FT4 ratio, triglyceride level and serum uric acid level were independent risk factors for NAFLD.
NAFLD in the patients with HT in our study. Abdominal obesity is a substantial component of metabolic syndrome and increases the risk for cardiovascular events. Visceral fat can be considered an important predictive factor for both the prevalence of NAFLD and ALT levels were higher in patients with HT. Serum ALT level is a surrogate marker for NAFLD in the absence of other causes of liver disease (17). In the current study, fasting insulin and HOMA-IR values were elevated in patients with NAFLD. Furthermore, insulin resistance and fasting insulin level formed a strong relationship with NAFLD, independent of HT. Additionally, it has been reported that hyperinsulinemia and HT can separately result in the development of NAFLD (39,40).

In the current study, abdominal USG was applied to diagnose NAFLD via the qualitative detection of steatosis. Abdominal USG detects changes in fatty accumulation in the liver of as low as $\geq 20\%$ and closely mirrors coronary and carotid atherosclerosis burden. In contrast, semi-quantitative USG indices (to exclude NASH) and sonoelastography (to exclude NAFLD) were used in patients with NAFLD. Chung and Kim (25) reported that

Table 4 - Logistic regression analysis of the association between non-alcoholic fatty liver disease and metabolic variables in all participants.

| Variables          | $p$ value | OR    | 95% CI         |
|--------------------|-----------|-------|----------------|
| WC                 | 0.01      | 1.087 | 1.018 - 1.061  |
| Triglycerides      | 0.12      | 1.010 | 0.997 - 1.031  |
| Total cholesterol  | 0.21      | 1.009 | 0.995 - 0.921  |
| Uric acid          | 0.79      | 1.056 | 0.706 - 1.528  |
| HOMA-IR            | 0.005     | 2.978 | 1.397 - 5.757  |
| FT3/FT4 ratio      | 0.02      | 1.834 | 1.089 - 3.569  |

WC: waist circumference. HOMA-IR: homeostasis model assessment for insulin resistance. OR: odds ratio. CI: confidence interval.

Table 5 - Logistic regression analysis of the association between non-alcoholic fatty liver disease and metabolic variables in patients with HT.

| Variables          | $p$ value | OR    | 95% CI         |
|--------------------|-----------|-------|----------------|
| WC                 | 0.02      | 1.189 | 1.024 - 1.381  |
| Triglycerides      | 0.04      | 1.031 | 1.001 - 1.061  |
| Total cholesterol  | 0.03      | 1.004 | 0.977 - 1.031  |
| Uric acid          | 0.03      | 0.318 | 0.011 - 0.921  |
| HOMA-IR            | 0.02      | 8.042 | 1.261 - 51.283 |
| FT3/FT4 ratio      | 0.01      | 3.540 | 1.309 - 9.575  |

WC: waist circumference. HOMA-IR: homeostasis model assessment for insulin resistance. OR: odds ratio. CI: confidence interval.
quantify fibrosis) help predict liver histology and can be used to help select patients to submit to liver biopsy (41). According to the above, semi-quantitative steatosis indices must be further investigated.

In conclusion, FT3/FT4 ratio, HOMA-IR and WC are risk factors for the development of NAFLD. FT3/FT4 ratio is a predictor of NAFLD independent of insulin resistance both in patients with ET and in patients with HT. Elevated serum triglyceride and uric acid levels are independent risk factors for NAFLD in patients with HT.

**AUTHOR CONTRIBUTIONS**

Gökmen FY and Alhab S participated in the study design, study coordination and drafting of the manuscript. Ataöğlu H, participated in statistic analysis and helped in drafting the manuscript. Türker BC, Çetin F, Türkler F and ManaçRY participated in data collection. Yenigün M participated in study design and coordination.

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