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# Ovaries are more vulnerable than hepatocytes for insulin resistance and hyperinsulinemia

**LIVER**

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## ABSTRACT

**Background/Aims:** Non-alcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS) are common metabolic disorders. We aimed to evaluate the underlying mechanisms in the development of NAFLD and PCOS.

**Materials and Methods:** Thirty female patients with NAFLD and without PCOS; 12 female patients with PCOS; and a control group with 17 healthy females were included. Pancreatic homeostatic model assessment-Beta cell function was measured by the homeostasis model assessment (HOMA)-B test.

**Results:** The body mass index (BMI) of the NAFLD patients was higher than that of the PCOS patients (29.4±3.8 kg/m$^2$ vs 25.6±5.2 kg/m$^2$, p<0.05). There was no significant difference between the PCOS patients and controls with respect to BMI. The fasting insulin levels of the NAFLD patients were higher than those of the PCOS patients (5.06 unit more than PCOS, p<0.05) and 12.8 unit more than controls (p<0.001). The HOMA scores of the NAFLD patients were more than those of the PCOS patients (1.41 unit more than PCOS, p<0.05) and 2.95 unit more than controls (p<0.001). The HOMA-B score was higher in the NAFLD patients than in the PCOS patients. There was no statistical difference among the groups for serum triglyceride (p>.05) and cholesterol (p>.05).

**Conclusion:** This study showed that rather than pancreatic beta-cell hyperfunction, insulin resistance plays a central role in the development of ovarian abnormalities.

**Keywords:** Non-alcoholic fatty liver disease, polycystic ovary syndrome, obesity, insulin resistance, hyperinsulinemia, pancreatic beta-cell function

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver enzymes (1). The estimated prevalence of NAFLD ranges between 10% and 30% in the general population (2). NAFLD is the liver component of metabolic syndrome and is found with increased frequency in people with certain medical conditions such as obesity, type II diabetes mellitus (DM), and dyslipidemia (3-5). A direct correlation between the degree of obesity with or without DM and the prevalence and severity of NAFLD and NAFLD-associated hepatocellular carcinoma (HCC) was reported (6). Insulin resistance plays a central role in NAFLD pathogenesis (7,8). Hyperinsulinemia leads to fatty liver (simple steatosis) and blocks mitochondrial fatty acid oxidation leading to necroinflammation, followed by fibrosis nonalcoholic steatohepatitis (NASH) (9). NAFLD occurs in all age groups with equal frequency in both females and males (1,3,4). The most commonly reported liver test abnormality is mildly to moderately elevated serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) (1). The ultrasound finding of NAFLD is a diffusely hyperechogenic or bright liver (1).

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders characterized with oligomenor-

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rhea-amenorrhea, infertility, enlarged cystic ovaries, hirsutism, and hyperandrogenemia (10,11). It affects 5%–26% of women. It occurs in reproductive-age women and generally starts in the peripubertal period. Most of the PCOS patients are obese or overweight and had peripheral insulin resistance and hyperinsulinemia (12).

Non-alcoholic fatty liver disease and PCOS are two common metabolic disorders in general clinical practice, and the therapy, which is metformin, is the same for both diseases (13-16). In this study, we aimed to evaluate the underlying molecular pathogenesis of NAFLD or PCOS by examining clinical cases.

## MATERIALS AND METHODS

Thirty women with well-defined biopsy-proven NAFLD (1), 12 subjects who fulfilled the criteria for PCOS (10,11) and a sex-matched community control group of 17 healthy subjects were included in this study. Ethics committee approval and informed consent were obtained.

**Diagnosis of NAFLD:** All NAFLD patients had unexplained elevations in serum aminotransferases (more than 6 months) and hyperechogenic liver on ultrasound. Because NAFLD is an exclusion diagnosis, patients using hepatotoxic drugs or alcohol (>20 g/day), those with hereditary liver disease or autoimmune hepatitis, or those with a history of blood transfusion or viral hepatitis such as chronic hepatitis B and C infection were excluded from this study. The diagnosis of NAFLD was then established by liver biopsy in each case.

**Diagnosis of PCOS:** The Rotterdam criteria for the diagnosis of PCOS included the following (10,11): Ultrasonographic evidence of polycystic ovaries, menstrual irregularity, and evidence of hyperandrogenism, either clinical (e.g., hirsutism, acne, male pattern balding) or biochemical (elevated androgen level), after the exclusion of other causes of hyperandrogenism. PCOS patients who deny alcohol abuse were selected for this study.

**Assessments of body mass index (BMI):** It is a mathematical formula in which a person's body weight (wt) in kilograms is divided by the square of their height (ht) in meters ($wt/ht^2$). According to the National Institute of Health criteria, a normal BMI is 18.5–24.9 $kg/m^2$ (17). Overweight was defined as a BMI of 25–29.9 $kg/m^2$. Obesity was defined as a BMI of ≥30 $kg/m^2$.

**Assessments of DM:** DM was defined on the basis of a fasting blood glucose level exceeding 140 mg/dl in two samples and/or a history of the use of insulin or oral hypoglycemic agents (18). All non-diabetic patients and healthy subjects underwent a 75 g oral glucose tolerance test with insulin measurement after a 3 day administration of 300 g carbohydrate diet and 12 h overnight fasting.

**Assessments of insulin resistance:** Insulin resistance and beta-cell function was calculated from plasma glucose (mmol/L) and insulin concentrations (μlU/mL) using homeostasis model assessment (HOMA) (18). Insulin resistance was calculated by the homeostasis model assessment method insulin resistance (HOMA-IR): (fasting glucose×fasting insulin)/22.5. HOMA <2.0 was accepted as no insulin resistance; HOMA >2.0 and ≤3.0 was considered mild; HOMA>3.0 and ≤4.0 was considered moderate; and HOMA >4.0 was considered severe insulin resistance.

**Assessments of beta-cell function (18):** Pancreatic beta-cell function was assessed by HOMA-beta-cell (β) in percent: 20×fasting insulin/ (fasting glucose–3.5).

**Assessments of dyslipidemia:** Hypertriglyceridemia and hypercholesterolemia were considered when the serum levels were over 170 mg/dL and 200 mg/dL, respectively.

**Assessments of serum aminotransferase levels:** Serum liver enzymes (ALT and AST) were measured in the PCOS patients for 3 months and in the NAFLD patients for more than 6 months.

**Assessments of transabdominal ultrasound findings:** All the PCOS patients beside the NAFLD patients underwent transabdominal ultrasound examination. If a liver was seen diffusely hyperechogenic or bright compared with the kidneys, then the patient was diagnosed with a fatty liver (19).

**Assessments of liver histology (20):** Liver biopsy was performed in all the NAFLD patients. Specific histologic findings of NAFLD included the following: (1) Steatosis, which generally is macrovesicular or mixed; (2) Inflammation that is mixed neutrophilic and mononuclear cell; and (3) Presence of mallory bodies, glycogen nuclei, and fibrosis.

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS) for Windows for 11.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. Means and standard deviations (SDs) were calculated for continuous variables. Mean values of markers were compared between groups by the one-way analysis of variance (ANOVA) test and univariate variance analysis. Then, if needed, proportions were compared across groups using Post Hoc Scheffe's test. A p value of less than 0.05 was considered to be statistically significant.

## RESULTS

The BMI, cholesterol, triglyceride, fasting glucose, insulin, insulin resistance, and beta-cell function in all groups are presented on Table 1 and 2 and Figure 1-7. The NAFLD patients (mean age

**Table 1.** Demographic characteristic of study participants

| Groups (no. of pts) | Age (years) | BMI ($kg/m^2$) | Cholesterol levels (mg/dL) | Triglyceride levels (mg/dL) |
|---|---|---|---|---|
| NAFLD (30) | 46.0±7.6 | 29.4±3.8 | 232±46 | 181±102 |
| PCOS (12) | 28.0±5.0 | 25.6±5.2 | 196±38 | 162±75 |
| Controls (17) | 43.4±10.2 | 24.2±2.8 | 199±40 | 138±62 |

NAFLD: non-alcoholic fatty liver disease; PCOS: polycystic ovary syndrome; BMI: body mass index

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**Table 2.** Laboratory assessments of non-diabetic study participants

| Non-diabetic patients (no. of pts) | Fasting Glucose (mmol/L) | Fasting Insulin (μIU/mL) | HOMA-A | HOMA-β (%) |
|---|---|---|---|---|
| NAFLD (19) | 5.1±1.0 | 17.8±6.5 | 4.1±2.0 | 509±185 |
| PCOS (10) | 4.8±1.1 | 12.8±2.7 | 2.7±0.8 | 98±20 |
| Controls (17) | 5.0±0.7 | 5.0±2.3 | 1.1±0.6 | 42±19 |

NAFLD: non-alcoholic fatty liver disease; PCOS: polycystic ovary syndrome; HOMA: homeostatic model assessment

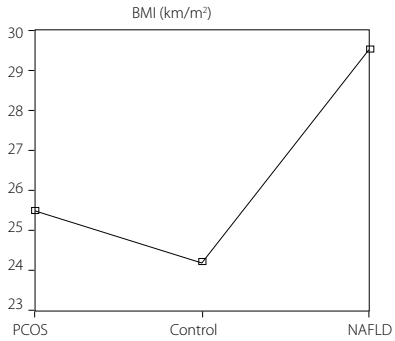


**Figure 1.** BMI of the NAFLD patients compared with the PCOS patients ($p<0.05$) and controls ($p<0.001$).
BMI: body mass index; PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

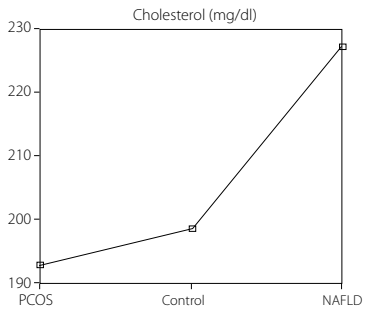


**Figure 2.** The fasting cholesterol insulin of the NAFLD patients compared with the PCOS patients.
PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

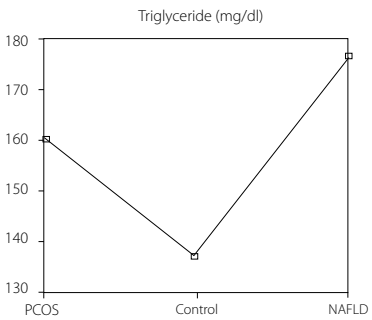


**Figure 3.** The fasting triglyceride of NAFLD patients compared with PCOS patients.
PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

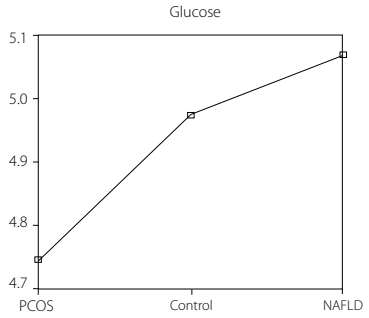


**Figure 4.** The fasting glucose level of NAFLD patients compared with PCOS patients.
PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

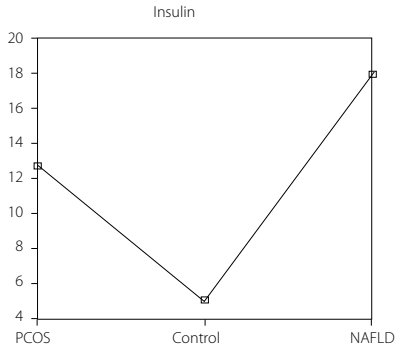


**Figure 5.** The fasting insulin of NAFLD patients compared with PCOS patients.
PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

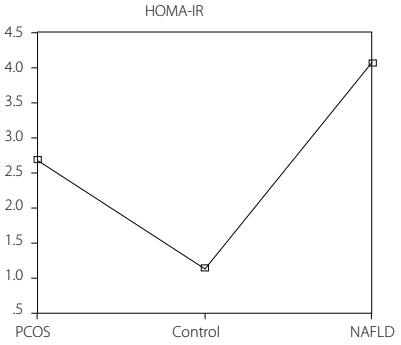


**Figure 6.** Insulin resistance which was measured by HOMA-A in study groups.
HOMA-A: homeostatic model assessment A; PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease

46.0±7.6 years, range 28–61 years) were significantly older than the PCOS patients (28.0±5.0 years, range 21–38 years) ($p<0.05$). There was no difference between the NAFLD patients and the controls (mean age 43.4±10.2 years, range 24–55 years) ($p>0.05$). On the other hand, the PCOS patients were significantly younger than the controls ($p<0.05$).

The mean BMI was 29.4±3.8 kg/m$^2$ in the NAFLD group and 24.2±2.8 kg/m$^2$ in controls ($p<0.001$) as shown in Table 1

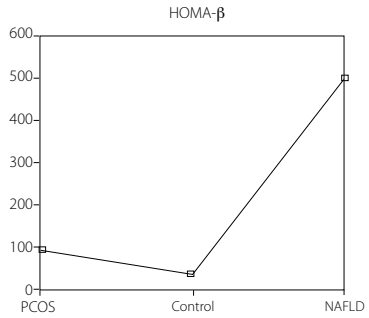


**Figure 7.** Pancreatic homeostatic model assessment-Beta cell function which was measured by HOMA-B test in study groups.
HOMA-B: homeostatic model assessment B; PCOS: polycystic ovary syndrome; NAFLD: Non-alcoholic fatty liver diseaseq

and Figure 1. There was no difference between controls and 25.6±5.2 kg/m$^2$ in the PCOS patients (p>0.05). The BMI of the NAFLD patients was significantly higher than that of the PCOS patients (p<0.05).

In the NAFLD group, 50% of the patients were obese (12 patients were class 1 and 3 patients were class 2), 40% were overweight, and 10% had normal BMI; 36% of the patients had DM, 83% had hyperlipidemia; and 89% of non-diabetic NAFLD patients (17/19) were insulin resistant (of these, 5 were mildly, 4 were moderately, and 8 were severely insulin resistant). In the PCOS group, 33% of the patients were obese (class 1), 67% had a normal BMI, 17% had an impaired glucose tolerance, 58% had hyperlipidemia, and 80% of the non-diabetic (8/10 patients) were insulin resistant (5 were mildly, 3 moderately; and none were severely insulin resistant).

In the NAFLD group; the mean serum levels of fasting cholesterol, triglyceride glucose, and insulin were higher than those in the PCOS group [232±46 mg/dL vs. 196±38 mg/dL, p>0.05 in Figure 2; 181±102 mg/dL vs. 162±75 mg/dL, p>0.05 in Figure 3; 5.1±1.0 mmol/L vs 4.8±1.1 mmol/L, p>0.05 in Figure 4; and 17.8±6.5 µIU/mL vs 12.8±2.7 µIU/mL, p<0.05 in Figure 5; respectively].

Then, we evaluated the insulin resistance and pancreatic beta-cell function in 10 non-diabetic PCOS patients and 19 non-diabetic NAFLD patients as shown in Table 2 and Figure 6 and 7, respectively. Insulin resistance shown by HOMA-IR was 4.1±2.0 in NAFLD-nondiabetic patients and 2.7±0.8 in PCOS-nondiabetics; p<0.05, as shown in Figure 6. Pancreas beta-cell function by HOMA-B was 509±185% in NAFLD and 98±20% in PCOS; p<0.0001, respectively, as shown in Figure 7.

In the control group; there was no obese subject. Nine subjects were overweight, eight had a normal BMI, and 53% had hyperlipidemia. One patient showed mild insulin resistance. The mean serum levels of fasting glucose, fasting insulin, triglyceride, and cholesterol of the control group were 5.0±0.7 mmol/L, 5.0±2.3 µIU/mL, 138±62 mg/dL, and 199±40 mg/dL, respectively.

A difference was only observed between controls and NAFLD for fasting insulin (p<0.0001), but not for glucose, triglyceride, and cholesterol (p>0.05). We compared controls with PCOS for fasting insulin (p=0.001). There was no difference for glucose, triglyceride, and cholesterol (p>0.05).

Compared with the control group, 19 non-diabetic NAFLD patients had increased insulin resistance by HOMA-IR of 4.1±2.0 (in control 1.1±0.6; p<0.0001) and pancreatic beta-cell function of 509±185% (in control 42±19%; p<0.0001).

Compared with the control group, 10 non-diabetic PCOS subjects showed an increased insulin resistance with HOMA-IR of 2.7±0.8 (in control 1.1±0.6; p=0.001) and an increased beta-cell function of 98±20% (in control 42±19%; p>0.05).

Although all of the NAFLD patients had fatty liver diagnosis based on ultrasound and liver function test abnormalities in this study, only 50% of the PCOS patients had fatty liver images on transabdominal ultrasound, but none had serum aminotransferase abnormalities.

Only one subject in the NAFLD group had simple fatty liver; the rest of the group had different degrees of inflammation, with or without fibrosis on liver biopsies. This subject was 52 years old and had a BMI of 28 kg/m$^2$, a fasting glucose level of 5.6 mmol/L, a fasting insulin level of 10 µIU/mL, cholesterol level of 211 mg/dL, triglyceride level of 240 mg/dL, HOMA-IR of 2.5, and beta-cell function of 286%. There was no cirrhosis case in this study.

There was no history or any clinical or laboratory findings of complete PCOS in the NAFLD group.

## DISCUSSION

Non-alcoholic fatty liver disease is becoming increasingly more prevalent worldwide. Currently, NAFLD is the most common cause of previous cryptogenic cirrhosis at hepatology clinics. On the other hand, PCOS is one of the most common endocrine disorders. The metabolic syndrome is an integral part of both NAFLD and PCOS. Insulin resistance and hyperinsulinemia play important roles in the pathogenesis of both NAFLD and PCOS. It is possible that hyperinsulinemia develops long before the appearance of NAFLD and PCOS at the clinical stage. Therefore, the early identification and treatment of insulin-resistant NAFLD patients may be important for prevention.

This study showed that metabolic abnormalities are common findings in patients with NAFLD and PCOS, as reported previously (21). However, our study also showed that metabolic abnormalities were more severely and more frequently expressed in the NAFLD patients than in the PCOS patients. The NAFLD

patients were more severely obese (p<0.05), more diabetic DM (p<0.05), had higher fasting insulin levels (p<0.05), and were more and severely insulin resistant (p<0.05) than the PCOS patients in this study. More importantly, the NAFLD patients had impaired beta-cell function, but the PCOS patients did not.

We only found statistically significant differences for fasting insulin levels and insulin resistance between PCOS group and controls, but not for BMI, fasting glucose, triglyceride, cholesterol, and pancreatic beta-cell function. On the other hand, there were statistically significant differences for BMI, fasting insulin, insulin resistance, and pancreatic beta-cell function between NAFLD and controls.

Several studies reported age as one of the most important factors related to the severity of clinical and metabolic changes in NAFLD, and the prevalence of NAFLD increases by age (1,2,4). In this study, the NAFLD patients were significantly older than the PCOS patients (p<0.05). These observations suggest that age is one of the main factors associated with the metabolic differences between NAFLD and PCOS, which we found in this study.

It is well known that insulin resistance is more severe in NASH than in fatty liver (4,22-24). However, although some of the PCOS patients had fatty liver images on ultrasound examinations without any serum aminotransferase abnormalities, only one case in the NAFLD group showed fatty liver, while others had NASH. Thus, we failed to compare fatty liver subjects in the NAFLD group with those in the PCOS group who had or not fatty liver image by transabdominal ultrasound.

In conclusion, fatty liver may have the best prognosis within the spectrum of NAFLD, but NASH also has a potential to progress to end-stage liver disease, even NASH-associated HCC. No effective medical therapy is currently available for the NAFLD patients. Therefore, food and drug administration (FDA) recently defined NAFLD as an orphan disease status. Thus, the early identification of the NAFLD patients is crucial for their prognosis. It is possible that PCOS subjects can progress to NAFLD over time. We have to provide clinical attention on that issue. We recommend the follow-up of the PCOS patients by liver function tests regularly, and if a patient shows persistent aminotransferase elevations, they should be directed to hepatology units for the evaluation of NAFLD.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of FNG (2002).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

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

**Author Contributions**: Concept - M.B.; Design - M.B.; Supervision - M.B.; Materials - M.B., Y.S.; Data Collection and/or Processing - M.B.; Analysis and/or Interpretation - M.B.; Literature Review - M.B., G.B., F.B., İ.G.; Writer - M.B.; Critical Review - M.B.; Other - İ.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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