



# Factors Related with Non-Alcoholic Fatty Liver Disease in Normolipidemic Patients with 1-Hour Hyperglycemia

## *Birinci Saat Hiperglisemisi Olan Normolipidemik Hastalarda Alkole Bağlı Olmayan Yağlı Karaciğer Hastalığı İle İlişkili Faktörler*

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### Abstract

**Purpose:** Oral glucose tolerance tests (OGTT) use fasting and 120-minute values for the diagnosis of diabetes, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), however, in the medical literature, various studies suggest the probable importance of one-hour hyperglycaemia. Non-alcoholic fatty liver disease (NAFLD) is also shown to be related with insulin resistance and cardiovascular risks. We aimed to evaluate the factors related with NAFLD in patients with elevated one-hour plasma glucose levels in the absence of IGT or IFG, hyperlipidemia and hypertension.

**Material and Method:** Among 746 subjects who underwent physical examination, evaluation of drug and alcohol use, lipid profiles, transaminases, and hepatitis markers as well as OGTT, 69 patients (21 males, 48 females) had 1-hour glucose levels higher than 155 mg/dL. The hepatic steatosis ultrasound examination was conducted to grade (between 0 and 3) the presence of fat within the liver parenchyma. Ordinal regression analysis was performed to evaluate factors related with the degree of liver steatosis.

**Results:** Overall, hepatosteatosis grade was significantly related with glycohemoglobin (HbA1c) levels, gender and obesity (body mass index  $\geq 30$  kg/m<sup>2</sup>). Considering only non-obese patients, ultrasonographic grading was significantly associated with age and homeostasis model assessment (HOMA) of insulin resistance.

**Discussion:** Even if the diagnosis of diabetes, IGT and IFG is not established, insulin resistance can be related with hepatosteatosis and elevated HbA1c level and, homeostasis model assessment may be predictive for NAFLD grade on ultrasonography in patients with high 60-minute glycaemia on standard 75-g OGTT. *Türk Jem 2012; 16: 85-8*

**Key words:** Non-alcoholic fatty liver disease, hepatosteatosis, 1-hour hyperglycaemia, oral glucose tolerance test, metabolic syndrome

### Özet

**Amaç:** Diyabet, bozulmuş açlık glukozu ve bozulmuş glukoz toleransı tanılarında oral glukoz tolerans testi (OGTT) açlık ve 120.dakika değerleri kullanılmaktadır ancak literatürde farklı çalışmalar 1.saat hiperglisemisinin de önemi olduğunu göstermektedir. Alkole bağlı olmayan karaciğer hastalığının insulin direnci ve kardiyovasküler risk faktörleri ile beraberliği gösterilmiştir. Çalışmamızda diyabet, bozulmuş açlık glukozu ve bozulmuş glukoz toleransı, dislipidemi veya hipertansiyonu olmayan, sadece 1.saat hiperglisemisi bulunan hastalarda alkole bağlı olmayan yağlı karaciğer hastalığı ile ilişkili faktörleri araştırmayı amaçladık.

**Gereç ve Yöntem:** Çalışmaya katılan 746 kişinin 69'unda (21 erkek, 48 kadın) 1.saat OGTT (75g) glukoz düzeyi 155 mg/dL üzerindeydi. Fizik muayene, serolojik testleri ve OGTT değerlendirmeleri yapılan hastaların ultrasonografik olarak karaciğer parankimi derece 0 ile 3 arasında steatoz açısından değerlendirildi. Steatoz ile ilişkili faktörleri araştırmak için regresyon analizi yapıldı.

**Bulgular:** Tüm hastalarda hepatosteatoz derecesi glikohemoglobin (HbA1c), cinsiyet ve obezite ile ilişkili bulunurken, obez olmayan hastalarda ultrasonografik derecelendirme yaş ve HOMA (homeostasis model assessment) indeksi ile ilgili bulundu.

**Tartışma:** Standart 75 g OGTT ile 60. dakika hiperglisemisi olan hastalarda diyabet, bozulmuş açlık glukozu ve bozulmuş glukoz toleransı tanısı konmasa bile insulin rezistansı ile ilişkili olarak HbA1c ve HOMA değerleri ultrasonografik olarak derecelendirilen hepatosteatoz ile ilişkili görülmektedir. *Türk Jem 2012; 16: 85-8*

**Anahtar kelimeler:** Alkole bağlı olmayan karaciğer hastalığı, hepatosteatoz, 1.saat hiperglisemisi, oral glukoz tolerans testi, metabolik sendrom

## Introduction

Diabetes mellitus is a chronic disease with high mortality and morbidity and with increasing prevalence. Although the oral glucose tolerance test (OGTT) takes fasting and 2-hour plasma glucose levels into consideration, there are some studies that showed 1 hour glycaemia to be an important predictor of type 2 diabetes (1,2) and diabetes complications. For instance, in a study by Succoro et al. (3), increased carotid intima media thickness was observed in non-diabetic patients with serum glucose levels higher than 155 mg/dL showed. Higher prevalence of polyneuropathy (4) and subclinical inflammation (5) were also linked either to insulin resistance due to inflammatory cytokines and molecules or impairment of early insulin release (6).

It is well-known that diabetes and non-alcoholic fatty liver disease (NAFLD) has reciprocal relationship (7), but there is still not any study evaluating the effects of 1-hour hyperglycaemia without hyperlipidemia, high fasting or 2-hour plasma glucose levels, as a sole factor in NAFLD in medical literature.

In this context, we aimed to evaluate the factors related with ultrasonographically-evaluated hepatosteatosis in patients with 1-hour hyperglycemia.

## Materials and Methods

### Patients and procedure

We performed 75-g OGTT in 746 subjects who visited came for check-up to our endocrinology and metabolic diseases department outpatient clinic without known diabetes, thyroid, cardiovascular or pituitary disease. Among 96 patients with only 1-hour hyperglycemia (1-hour glucose levels of higher than 155 mg/dL), we enrolled 69 adult patients (21 males, 48 females) without any medication history (Figure 1) (1). Patients with any medication history, hypertension (systolic blood pressure  $\geq 130$  mmHg and/or diastolic blood pressure  $\geq 85$  mmHg), dyslipidemia (low-density lipoprotein cholesterol (LDL-C)  $> 160$  mg/dL, triglycerides (TG)  $> 150$  mg/dL, and/or high-density lipoprotein cholesterol (HDL-C)  $< 40$  mg/dL), cardiac diseases, malignancy, liver diseases, kidney diseases, thyroid disease, alcohol consumption, and smokers were excluded in order to limit confounding factors. Patients with alanine transaminase (ALT) or aspartate transaminase (AST)  $\geq 40$  U/L were excluded to avoid including higher degree of liver injury, non-alcoholic "steatohepatitis" (NASH) and other causes of hepatitides. Tests for hepatitis B and hepatitis C included screening for hepatitis B surface antigen (HBsAg), anti-HBc IgM, HBeAg and anti-HCV antibodies. Patients who have a HBsAg, anti-HBc IgM and HBeAg, were excluded. Finally, we enrolled 69 adult patients (21 males, 48 females) without any medication history, applying to our endocrinology and metabolic diseases department outpatient clinic. All patients were examined and their height, weight and waist circumferences were recorded during the physical examination. Body mass index (BMI) was calculated as  $\text{kg/m}^2$  and subjects with  $\text{BMI} \geq 30 \text{ kg/m}^2$  were grouped as obese.

### Biochemical analyses

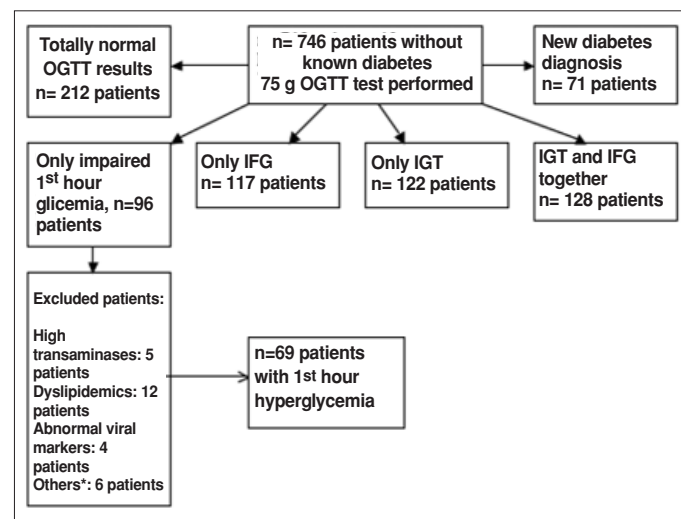
Venous blood samples were obtained early in the morning after a 12-hour fasting period, for measuring fasting blood glucose, HbA1c, total cholesterol, HDL-C, LDL-C, and TG levels. All laboratory measurements were carried out with standardized methods in laboratories of Ufuk University Dr. Ridvan Ege Hospital. All patients underwent 75-g OGTT early in the morning. Plasma glucose was measured with an automatic colorimetric method with Cobas Integra 400 Analyzer. Electrochemiluminescence immunoassay (ECLIA) was used for insulin measurements, with inter-assay coefficient of variation of 2.6%. Total cholesterol, LDL and HDL levels, triglycerides, ALT and AST were measured by automated colorimetric assay by Cobas Integra 400 Analyzer. HbA1c was measured by high-performance liquid chromatography method with inter-assay coefficient of variation of 2.3%.

HBsAg, anti-HBc IgM and HBeAg were measured by ECLIA using Elecsys 2010 analyzer and anti-HCV was analyzed with standardized microparticle enzyme immunoassay.

The OGTT results of the patients were evaluated according to the American Diabetes Association (ADA) criteria for type 2 diabetes, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (8). The reciprocal index of homeostasis model assessment (HOMA) was calculated as the product of fasting plasma glucose (mg/dL) by plasma insulin (mU/L), divided by a constant (405), as a measure of insulin resistance (9). The area under the curve (AUC) of OGTT glucose levels was calculated by trapezoid formula (10).

### Liver ultrasound evaluations

Ultrasonographic grading of hepatosteatosis was made by LOGIQ 10 ultrasonography device using 10 MHz probes, by a radiologist who was blind for the test results of the patients. Grade 0 hepatosteatosis represents normal liver echogenicity. Grade 1 hepatosteatosis - mild hepatosteatosis with a slight increase in liver echogenicity and slight exaggeration of liver-kidney difference in



**Figure 1.** Patient enrollment to study according to results of 75 g OGTT test.  
\* See the text for details of other exclusion criteriae.

echo amplitude with relative preservation of portal vein walls. Grade 2 hepatosteatosis-moderate hepatosteatosis with loss of echos from the walls of the portal veins, especially from the peripheral branches and less echo penetration into the deep portion of the liver. Grade 3-severe steatosis with higher reduction in beam penetration and loss of echos even from the main branches of the portal veins, and large echo difference between the kidney and liver (11,12).

### Statistical analyses

A full descriptive analysis of the evaluated variables has been carried out. Continuous and normally distributed variables have been compared by the t-test for unpaired samples. An ordinal regression analysis was performed to evaluate the association of the available parameters with the degree of liver steatosis. A p value of <0.05 was considered significant for all tests. All tests have been carried out by the SPSS version 14.0 for Windows.

## Results

The characteristics of patients and differences between the genders are shown in Table 1; women showed significantly higher HDL levels. Percentage of obesity was 47.6% among men and 43.8% for

women. Distribution of obese and non-obese among gender was homogeneous. There were 1 man and 6 women with Grade 0, five men and 25 women with Grade 1 and 15 men and 17 women with Grade 2 steatosis. None of the patients had Grade 3 hepatosteatosis on ultrasonography.

Ordinal regression analysis was performed to reveal the factors related with ultrasonographic grading. Hepatosteatosis severity was directly related to HbA1c level, (OR= 1.86; 95% CI: 0.330, 3.401; p=0.017), to male sex (OR=1.80; 95% CI: 0.239, 3.376; p= 0.024), and inversely to normoweight condition (OR=-1.80; 95% CI: -3.463, -0.145; p= 0.033). When we evaluated only non-obese people, the main predictors of the higher grading for NAFLD were age (OR=0.12; 95% CI: 0.017, 0.243; p=0.024), and HOMA (OR=19.55; 95% CI: 2.648-36.419; p=0.023).

## Discussion

In Western countries, approximately 20- 30% of adults have NAFLD, whose prevalence increases to 70% to 90% among those who are obese or diabetic and it is also related with increased cardiovascular risk.(13) Increased subclinical inflammation in patients with 1-hour hyperglycaemia (5) may contribute to the increased cardiovascular

Table 1. Characteristics of men and women enrolled into study.

	All patients (mean±SD)	Men (mean±SD)	Women (mean±SD)	P (Independent T-test)
Age (years)	44.5±12.6	47.3±11.9	43.3±12.7	0.539
BMI (kg/m <sup>2</sup> )	29.9±5.6	29.5±3.9	30.2±6.2	0.054
Obesity (% in group)	44.9	47.6	43.8	0.639
Waist (cm)	94.8±11.2	100.4±9.6	92.3±11.3	0.487
ALT (U/L)	23.0±11.5	26.7±12.3	21.3±10.9	0.084
AST (U/L)	20.6±7.9	22.8±6.9	19.7±8.3	0.865
Fasting glucose**	95.1±4.4	94.6±5.5	95.4±3.9	0.093
30min. glucose	171.4±28.5	179.7±22.3	167.7±30.3	0.115
60min. glucose	187.3±27.8	190.8±31.0	185.8±26.6	0.619
90min. glucose	153.3±41.3	151.5±28.8	154.1±46.1	0.226
120min glucose	121.1±22.6	117.8±17.0	122.6±24.6	0.232
AUC	297.7±66.0	301.8±58.9	295.9±69.4	0.907
HbA1c (%)	5.41±0.55	5.35±0.42	5.44±0.60	0.055
Fasting insulin**	15.4±8.8	17.0±10.6	14.7±7.9	0.583
120min insulin	66.4±49.4	67.8±29.9	66.0±55.7	0.074
HOMA	3.6±2.1	3.9±2.6	3.5±1.9	0.442
Triglyceride	111.7±29.3	116.6±27.8	109.6±29.9	0.391
LDL-C	121.7±20.1	120.7±19.2	122.2±20.7	0.922
HDL-C	50.2±11.2	46.0±7.2	51.9±12.2	0.001*
USG grade	1.3±0.6	1.7±0.5	1.2±0.6	0.445

\*p<0.05 (statistically significant difference between two groups)

\*\* All plasma glucose, LDL, HDL, triglyceride measurements were in mg/dL, insulin measurements were mIU/L

risk, as well as to incident NAFLD and diabetes. Generally, at baseline, individuals with NAFLD have metabolic syndrome components more frequently (13). Thus, in pharmacologically untreated normolipidaemic and normotensive subjects with normal fasting and 2-hour glycaemia, high 1-hour plasma glucose levels could be a good model to evaluate the relationship of glycaemia with hepatosteatosis. This is of course complex, since, as above stated, it is very difficult to find patients with NAFLD and isolated metabolic abnormalities. Shiga et al. (14) tested the relationship between diabetes and hepatosteatosis and, hepatosteatosis was found to be independent from fasting plasma glucose levels, but 1- and 2-hour hyperglycemia were closely linked to steatosis. Although this study had different design, the results are in agreement with our findings, showing no relationship of plasma glucose levels with steatosis degree. In fact, in our study, none of the plasma glucose levels during OGTT revealed association with steatosis grade neither in the whole sample, nor in non-obese group, but the relationship of HbA1c (in all patients) and HOMA in non-obese group indicates probable effect of daily fluctuations of glucose levels and fasting insulin levels as predictors of hepatosteatosis.

In our study, evaluation of all patients revealed that women gender, people with lower HbA1c and non-obese people were strongly protected from NAFLD. Since the higher HDL-cholesterol level in women when compared to men was reflecting a physiologic difference between genders, these results mostly indicated the role of insulin resistance and glucose levels in the development of hepatosteatosis. Because the obesity per se was strong predictor for NAFLD, we repeated analysis for the non-obese subjects. In non-obese people, HOMA, fasting plasma glucose levels and age were related with NAFLD grades. Thus, in patients with only 1-hour hyperglycaemia on OGTT, insulin-resistance seem to play a role in NAFLD development and it should be checked in patients with suspected NAFLD or with other risk factors for diabetes or cardiovascular diseases.

Restrictions of our study were the relatively small patient sample and the absence of liver biopsy examinations for pathological degree of NAFLD, and PCR testing for any hepatitis B or C virus that could have been missed by classical antigen-antibody tests. Meanwhile, since most patients with high 1-hour glycemia also have high fasting glucose, high postprandial glucose or dyslipidemia, the study population is not large. On the other hand, it is one of the rare studies that have excluded metabolic syndrome components, except obesity, to evaluate only the effect of 1-hour glycemia on NAFLD. Another important point is the absence of grade 3 steatosis in our study. Since we had very strict inclusion criteria (such as normal

fasting and 2-hour glycemia, absence of dyslipidemia, and transaminases in normal range of laboratory values) we probably excluded higher (i.e. "steato-hepatitis") degree of liver damage and, therefore, our study evaluates the effects of 1-hour hyperglycemia independent of diabetes- or hyperlipidemia-related causes of hepatosteatosis. Meanwhile, even in the absence of abnormal transaminase levels, there was ultrasonographically diagnosed liver damage even in the absence of abnormal fasting and 2-hour glycemia or hyperlipidemia, which can be omitted or underestimated easily in routine clinical practice. Thus, our study emphasizes the importance of 1-hour hyperglycemia in organ damage.

In conclusion, in a sample of non-obese healthy patients with impaired 1-hour hyperglycaemia during OGTT, HbA1c levels and HOMA are associated with the degree of NAFLD.

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