

# Influence of Plasma Cortisol and Other Laboratory Parameters on Nonalcoholic Fatty Liver Disease

## Authors

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## Key words

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## Abstract

The objective of the present study was to analyse the association between the plasma cortisol concentration and nonalcoholic fatty liver disease (NAFLD). A total of 1326 subjects (age 18–65 years) were examined in the context of an epidemiological study of a population-based random sample. Medical history and anthropometric data of 662 women and 664 men were documented. In addition, laboratory examinations were performed and the fat concentration of the liver was esti-

mated by ultrasound examination. Mean cortisol concentration in plasma was  $260.4 \pm 156.8$  nmol/l for women and  $295.8 \pm 161.2$  nmol/l for men. NAFLD was identified in 17.7% in women and 35.1% in men. Plasma cortisol concentration showed no association with the existence of NAFLD. NAFLD correlated positive with age, body-mass index (BMI), waist-to-hip-ratio (WHR), alanine aminotransferase (ALT), and triglycerides. The present study failed to establish any association of plasma cortisol concentrations and NAFLD.

## Abbreviations

ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartat aminotransferase
BMI	Body-mass-index
EMIL	Echinococcus multilocularis in Leutkirch
GGT	$\gamma$ -glutamyl-transferase
HD	High-density lipoprotein
MRS	Magnetic resonance spectroscopy
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic Steatohepatitis
WHR	Waist-to-hip-ratio
11 $\beta$ -HSD1	11 $\beta$ -Hydroxysteroid dehydrogenase type 1

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterised by a liver triglyceride content >5% by weight (liver fat content of the liver >95<sup>th</sup> percentile for normal-weight, healthy subjects) or the presence of fat droplets the cytoplasm of >5% of hepatocytes [1]. With a prevalence of up to 30%

of adults in the general population, NAFLD represents the major chronic liver disease in Western industrial nations [2]. The prevalence may reach 70% and even exceed 90% in patients with diabetes and extreme obesity [3]. The degree of hepatic steatosis can be detected by imaging techniques. For example, magnetic resonance spectroscopy (MRS) allows for exact identification of the fat content in the liver [4]. Ultrasonography diagnoses NAFLD with a sensitivity of 85–94% and a specificity of 84–93% [5,6]. NAFLD includes both benign, nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), which is accompanied by histological evidence of inflammatory markers. Ultrasound cannot differentiate between NAFL and NASH. The latter may be associated with progressive fibrosis and may ultimately lead to cirrhosis of the liver and hepatocellular carcinoma [7]. Histological examination of the organ is required for identifying the exact state of the liver disease [8].

Possible causes for NAFLD include an increased supply of fat with the diet [9], an increased supply of free fatty acids from the adipose tissue that reaches the liver and increased de novo lipogenesis in the liver [1,10]. In addition, an increased supply of carbohydrates may promote development of NAFLD by increasing the de novo synthesis of fat in the liver [1,11–13]. In particular, an

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increased intake of fructose increases the fat content of the liver by stimulating hepatic de novo lipogenesis [14]. Whether this is due to insulin resistance, which is closely associated with an increased fat content of the liver [15], or as a result of a NAFLD remains unclear [1].

Genetic factors are also known to play a role in the pathogenesis of NAFLD. For example, a high percentage of children with NAFLD have siblings and parents who share this disorder [16]. The genetical basis, however, remains largely unknown [17].

Glucocorticoids and the regulation of glucocorticoid metabolism may also play an important role in the pathogenesis of NAFLD. Hepatic changes in glucocorticoid metabolism attributable to an increased cortisol production have been demonstrated in patients with NAFLD [18].

Objective of the present study, which was performed in the context of an epidemiological study of an urban population in Leutkirch (Southwestern Germany), was to analyse the association of plasma cortisol concentrations and NAFLD.

## Participants and Methods

### Participants

The EMIL study (*Echinococcus multilocularis* in Leutkirch), a cross-sectional study of the prevalence of *Echinococcus multilocularis* infection in an urban population-based sample in Southwestern Germany, was conducted over a 4-week period in November and December, 2002. A secondary objective was to estimate the prevalence of NAFLD in the general population. Four-thousand inhabitants randomly selected out of an urban population of 22 093 inhabitants were contacted with the request to take part in the study. Of these, a total of 3893 persons aged 10–65 years were successfully contacted. The study was conducted in accordance with the principles of the Helsinki Declaration and the guidelines of Good Clinical Practice (GCP). The study was approved by the ethics committee the Landesärztekammer Baden-Württemberg. All subjects were informed about the study and its procedure in detail. After adequate time for consideration and before the beginning of the study, all participants provided written consent to participate in the study. In order to compare the study results with those of others, only the results of the adults were considered in this study. Participants with self-reported daily alcohol consumption exceeding 60 g/day in men and 20 g/day in women were not included in the study. Subjects with acute or chronic liver diseases and disorders with potential liver involvement, (autoimmune or metabolic) diseases of the heart, lung, gastrointestinal tract and kidney, as well as those with hormonal disorders and those treated with glucocorticoids were excluded from the study. However, women receiving estrogen therapy (n=845) were not excluded. Subjects with a fasting time less than 1 h were also not considered because of the food-dependent influence on the cortisol level. Also, subjects whose records were incomplete due to missing data were not included in the statistical analysis. The results of the remaining 1 326 subjects [662 (49.9%) women and 664 (50.0%) men, age 18–65 years] were included in the statistical analysis (► Fig. 1).

### Examination methods

Subjects' medical histories, laboratory test results, and anthropometric data {body-mass index (BMI) and waist-to-hip ratio (WHR) were calculated according to the WHO recommendations

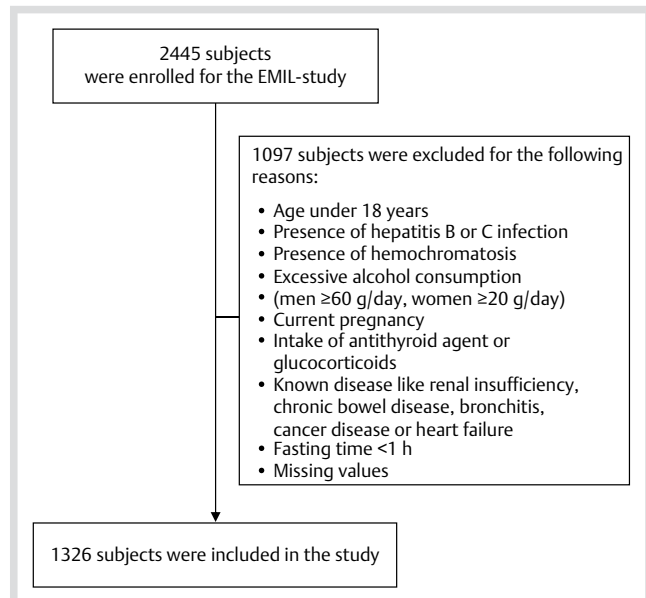


Fig. 1 Flow of the subjects across the study.

[5]} were recorded and ultrasound examination of the liver was performed.

### Ultrasonography examination

Diagnosis of NAFLD was made based on sonographic comparison of the hepatic and renal parenchyma, the liver's dorsal echo attenuation, the penetration of the diaphragm and the ability to assess the hepatic vessels [6, 7]. Based on these findings, the disease was graded as no NAFLD (normal, grade 0), and NAFLD grades I, II, and III.

Although liver biopsy remains the gold standard for confirming the diagnosis of NAFLD [9] and for measuring the fat content in this organ, in clinical practice, ultrasound has been proven valuable for assessing liver fat content. For the diagnosis of NAFLD, ultrasound has a reported specificity of 84–93% and a sensitivity of 85–94% [6, 7, 9]. The sonographic examination of fatty liver is a noninvasive and low-cost practical method appropriate for surveys under field conditions [19].

### Laboratory testing

Phlebotomy was performed between 1 PM and 9 PM with fasting times of not less than one longer than 8 h after subjects' last meal. Samples containing 25 ml of whole blood were obtained from a cubital vein. Tubes were sent coded and cooled to the laboratory of the Department of Clinical Chemistry of the University Hospital of Ulm and processed on the same day. Aliquot samples were deep-frozen for replicate tests in case of need. Triglycerides, cholesterol, HDL cholesterol, AP, AST, ALT, and GGT were measured using the Dimension XL unit (Dade Behring Inc., Newark, DE, USA) on the same day. Following completion of the study, total cortisol concentrations of the until then deep-frozen serum samples were determined using the Cobas e411 testing unit (Roche) in samples that remained frozen until processing.

### Statistical analysis

The calculations were performed using the Statistics Analysis Software (SAS) statistical software package (version 9.2; SAS Institute, Cary, NC, USA). Data were first analysed descriptively. Qualitative features are given with absolute and relative fre-

quencies, quantitative variables are given with mean and standard deviation. In order to detect differences between female and male subjects, the Wilcoxon rank sum test (Mann-Whitney U-test) was used for continuous variables, while, for categorical variables, the  $\chi^2$ -test was used. In a further analysis, cortisol values were divided into 3 groups of equal size (tertiles) for subjects with and without hepatic steatosis. The ranges of the 3 respective tertiles for subjects without hepatic steatosis were as follows: first tertile: 11.8–184 nmol/l; second tertile: 185–319 nmol/l; third tertile: 320–827 nmol/l, respectively. For subjects with hepatic steatosis, the ranges of the 3 respective tertiles were as follows: first tertile: 16.4–178 nmol/l; second tertile:

180–321 nmol/l; third tertile: 324–816 nmol/l. In order to demonstrate differences between the 3 groups, the Kruskal-Wallis test for constant variables and the  $\chi^2$ -test for categorical variables were used. Multiple logistic regression was used to calculate the associations between the determining factors and NAFLD. All statistical calculations were performed 2-tailed; statistical significance was set at  $\alpha=0.05$ .

## Results

▼ The study collective consisted of 1326 subjects [662 (49.9%) women and 664 (50.0%) men; age 18–65 years, mean  $41.5 \pm 12.7$  years]. Broken down by gender, there were statistically significant differences in terms of the anthropometric variables BMI and WHR, in the prevalence of NAFLD, and in the laboratory parameters ALT, AST, GGT, AP, and triglycerides. By contrast, no significant differences were found between men and women in terms of age and total cholesterol (► **Table 1**). Sonographic evidence of NAFLD was returned in 353 subjects (26.4%); here, men showed a higher prevalence than women (35.1% vs. 17.1%;  $p < 0.0001$ ).

Total cortisol concentrations were further studied in subjects with and without hepatic steatosis. Cortisol values for subjects with and without hepatic steatosis were grouped in tertiles and analysed. There was a statistically significant difference for the parameters gender, age, ALT and total cholesterol among subjects without hepatic steatosis. In the group of subjects with evidence of hepatic steatosis there was no significant difference (► **Table 2**).

The association between NAFLD and possible risk factors are listed in ► **Table 3**. Increasing age was positively associated with NAFLD ( $p < 0.0001$ ). The risk of NAFLD increased with increasing BMI ( $p < 0.0001$ ) and with increasing WHR ( $p < 0.0001$ ). Elevated

**Table 1** Characteristics of the subjects.

	Female n=662 (49.9%)	Male n=664 (50.0%)	p-Value
<b>Demographics</b>			
Age (years)	41.8±12.6	41.2±12.7	0.3925
BMI (kg/m <sup>2</sup> )	24.9±5.1	26.2±4.2	<0.0001
WHR	0.8±0.1	0.9±0.1	<0.0001
<b>Laboratory parameters</b>			
ALT (U/l)	12.4±4.6	18.3±9.4	<0.0001
AST (U/l)	8.6±2.4	10.6±4.4	<0.0001
GGT (U/l)	9.8±11.0	17.1±16.5	<0.0001
AP (U/l)	78.8±25.7	85.1±19.5	<0.0001
Total cholesterol (mmol/l)	5.5±1.0	5.5±1.1	0.3089
Triglycerides (mmol/l)	1.3±0.8	1.9±1.4	<0.0001
Total cortisol (nmol/l)	260.4±156.8	295.8±161.2	<0.0001
<b>Disorders</b>			
Hepatic steatosis n (%)	117 (17.7%)	233 (35.1%)	<0.0001

Values are mean ± SD

ALT: Alanine aminotransferase; AP: Alkaline phosphatase; AST: Aspartat aminotransferase; GGT: Gamma glutamyltransferase; HDL: High-density lipoprotein; WHR: Waist-to-hip-ratio

**Table 2** Demographics and other characteristics of subjects with and without hepatic steatosis according to cortisol tertiles.

	Subjects without hepatic steatosis			Subjects with hepatic steatosis		
	Tertile 1 (n=327)	Tertile 2 (n=323)	Tertile 3 (n=326)	Tertile 1 (n=116)	Tertile 2 (n=117)	Tertile 3 (n=117)
<b>Demographics</b>						
Gender n (%)						
Female	208 (63.6)	186 (57.6)	151 (46.3) <sup>a</sup>	44 (37.9)	41 (35.0)	32 (27.4)
Male	119 (36.4)	137 (42.4)	175 (53.7)	72 (62.1)	76 (65.0)	85 (72.7)
Age (years)	40.9 (11.2)	40.3 (12.3)	36.0 (12.6) <sup>a</sup>	47.9 (10.1)	50.4 (10.7)	47.0 (12.5)
BMI (kg/m <sup>2</sup> )	24.1 (3.8)	24.5 (4.2)	23.7 (3.5)	29.9 (4.0)	29.6 (4.8)	29.4 (4.8)
WHR	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
<b>Laboratory parameters</b>						
ALT (U/l)	12.9 (4.9)	13.3 (5.0)	14.1 (6.3) <sup>b</sup>	20.6 (10.3)	19.6 (10.0)	21.9 (12.1)
AST (U/l)	8.8 (2.3)	9.0 (2.1)	9.2 (2.9)	10.9 (4.4)	10.6 (4.3)	12.2 (7.1)
GGT (U/l)	10.9 (12.9)	10.9 (8.5)	12.1 (13.9)	17.8 (14.0)	18.9 (18.3)	22.0 (22.6)
AP (U/l)	78.9 (22.1)	79.9 (22.7)	82.6 (24.5)	84.7 (21.3)	87.5 (22.2)	86.1 (23.0)
Triglycerides (mmol/l)	1.3 (0.8)	1.4 (1.0)	1.3 (0.8)	2.4 (1.7)	2.2 (1.4)	2.2 (1.4)
Cholesterin (mmol/l)	5.3 (1.0)	5.5 (1.1)	5.2 (1.0) <sup>c</sup>	5.9 (1.1)	5.8 (1.1)	5.8 (1.0)
<b>Sonographic findings</b>						
Hepatic steatosis n (%)						
No	327 (100)	323 (100)	326 (100)	–	–	–
Grade I	–	–	–	46 (39.7)	54 (46.2)	48 (41.0)
Grade II/III	–	–	–	70 (60.3)	63 (53.8)	69 (59.0)

Values are mean ± SD

ALT: Alanine aminotransferase; AP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT:  $\gamma$ -Glutamyl transferase; HDL: High-density lipoprotein; LDL: low-density lipoprotein; WHR: waist-to-hip ratio

<sup>a</sup> $p < 0.001$ ; <sup>b</sup> $p < 0.05$ ; <sup>c</sup> $p < 0.01$

levels of ALT ( $p < 0.0001$ ) and triglycerides ( $p = 0.0001$ ) were associated positively with NAFLD. AST, GGT, AP, and cholesterol levels were not associated with NAFLD. Also, total cortisol concentrations showed no significant association with NAFLD ( $p = 0.1518$ ).

## Discussion

In the present random population sample, the prevalence of NAFLD was 26.2%. This corresponds with the prevalences of 23% to 31% reported in the literature for developed countries [2,20–22]. Differences in prevalence rates result from the characteristics of the studied population. In Asia, the reported prevalences of 11.5–20.8% are significantly lower [23,24] than in European countries. Recently, however, the variable increases in obesity and diabetes in different Asian populations is reflected in the prevalence of NAFLD ranging from only 5% to as high as 32% [25], showing that not only obesity and diabetes, but also ethnicity have an influence on the prevalence of NAFLD. In the USA, the prevalence rates of 45%, 33%, and 24% for Hispanics, Caucasians, and African-Americans, respectively, would appear to bear this out [22].

Divergent prevalence data may also arise by reason of different diagnostic methods to identify NAFL [26]. Compared to MRI, the sensitivity of sonography for determining the fat content of the liver, especially when there is a low fat content, is weak [27]. Thus, when the fat content of the liver is  $< 20\%$ , the prevalence of NAFLD could be underestimated [28]. Liver biopsy remains the gold standard for confirming the diagnosis of NAFLD and for measuring the fat content in this organ [9]; nevertheless, sonographic diagnosis of fatty liver is easy to perform and has an established role in hospitals and outpatient settings [29]. It has proven to be valuable in our study although NASH and fibrosis

cannot be adequately recognised and distinguished from benign NAFL [8].

In the present study, the NAFLD risk in the general population increased significantly with age. This result corresponds with reports in the literature [26]. The influence of age on the incidence of NAFLD is particularly evident in women [30,31]. In our population with a 50.1% proportion of women, the prevalence of NAFLD was 17.3% for women and 35.1% men. The difference between the sexes is already well known. Compared to rates observed in women, NAFLD is found about twice as frequently in men in studies in the USA (42% in men and 24% in women [22]), Israel (38% in men and 21% in women [20]), and China (15.8% in men and 7.5% in women [30]), but not in Hispanics and African-Americans [22].

In our study of healthy subjects, risk factors for NAFLD such as increasing age, male sex, increased levels of BMI, WHR, ALT, and triglycerides were associated with NAFLD corresponding to reports in the literature [21,25,31]. No association was observed between the presence of NAFLD and serum cortisol concentrations. The lack of correlation between NAFLD and serum cortisol does not rule out that glucocorticoids nevertheless play a pathogenetic importance in the development of NAFLD. Supporting that hypothesis is the finding that development of severe fatty liver was observed under treatment with the synthetic glucocorticoid, dexamethasone [32]. In line with these clinical observations, animal studies demonstrate that dexamethasone increases hepatic triglyceride synthesis and intrahepatic storage of triglycerides [33]. To accompany this pathogenetic significance, it is also suggested that the presence of NAFLD is correlated with increased activity of the pituitary-adrenocortical axis (HPA) resulting in subclinical hypercortisolism [34,35]. Compared to controls, patients with NAFLD show an increased cortisol turnover [19,35]. The increased excretion of cortisol metabolites in urine could lead to compensatory increases of cortisol secretion and furthermore to a subclinical hypercortisolism. A corresponding increased cortisol secretion could not be detected in our study by simply determining the concentration of cortisol in serum.

Recently it was proven that the activity of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) in visceral adipose tissue leads to portal hypercortisolism and is associated with NAFLD [36]. Visceral adipose tissue is of considerable importance in terms of cardiovascular risk. Visceral fat is positively associated with waist circumference, blood pressure, triglycerides and negatively with HDL-cholesterol [37], and it also is significant in terms of the glucocorticoid metabolism [38]. At the same subcutaneous fat mass, male adolescents with NAFLD have more visceral fat than female adolescents with NAFLD [39]. Furthermore, the visceral fat mass correlates closely with a fatty liver [40]. Compared to women, increased visceral fat in men and in persons with increased BMI and/or an increased WHR might explain the different prevalences of NAFLD in men and women and the high prevalence in individuals with abdominal obesity, both in our own and in other studies [20,22,30].

The present study has weaknesses and strengths. One strength of the study is the large number of healthy persons examined ( $n = 1348$ ). For the diagnosis of fatty liver, sonography was selected as a noninvasive, fast, and low-cost method. Medical ultrasonics has proven to be sufficiently suitable in both in- and outpatient settings for establishing the diagnosis. Because of the lower sensitivity compared to the MRI, the high prevalence of NAFLD may have been underestimated with sonography. How-

**Table 3** Multiple logistic regression analysis to assess the independent predictors of hepatic steatosis.

Variable	OR (95% CI)	p-Value
Gender	Ref.	
Female	1.578 (1.083–2.297)	0.0174
Male		
Age		
18–30	Ref.	$< 0.0001$
31–40	3.063 (1.528–6.138)	
41–50	3.776 (1.823–7.819)	
51–65	7.271 (3.548–14.901)	
BMI		
$< 25$	Ref.	$< 0.0001$
25–30	4.092 (2.662–6.290)	
$\geq 30$	13.502 (8.161–22.340)	
WHR		
normal	Ref.	$< 0.0001$
elevated	2.536 (1.760–3.653)	
ALT (U/l)	1.092 (1.053–1.132)	$< 0.0001$
AST (U/l)	1.014 (0.938–1.097)	0.7241
GGT (U/l)	0.994 (0.981–1.006)	0.3081
AP (U/l)	0.993 (0.985–1.001)	0.0987
Cholesterol (mmol/l)	1.131 (0.956–1.339)	0.1920
Triglycerides (mmol/l)	1.360 (1.162–1.592)	0.0001
Total cortisol (nmol/l)	1.001 (1.000–1.0002)	0.1518

ALT: Alanine aminotransferase; AP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT:  $\gamma$ -glutamyl-transferase; OR: Odds ratio; 95% CI: 95% confidence interval

ever, for screening under field conditions ultrasonography is the only practical method for estimating the fat content of the liver. A weakness of the study is that not all investigations took place in the morning. Instead, examinations were performed between 1 PM and 9 PM and the serum cortisol reference range for the second half of the day was used. Although the schedule of each activity was known and every procedure has been previously explained and performed under quiet conditions, it cannot be excluded that individual stress influenced cortisol levels in some cases. Another weakness of the study is that only the current cortisol levels were quantified. The excretion of cortisol metabolites in 24-h urine could not be measured in the context of the study and no functional studies were performed. Furthermore, women taking oral contraceptives have not been excluded from the study, even if there is an influence on the cortisol value. This was done in favour of better comparability of men and women with approximately equal numbers of participation in this study. In addition, after excluding women taking estrogens, the informative values would have been too low. In 350 of the 1 326 study participants, ultrasound returned findings consistent with fatty liver. The small number of subjects limits the informative value. Nonetheless, the well-known associations between fatty liver and anthropometric measures, and with ALT and triglycerides were confirmed.

## Conclusion

The present population-based cross-sectional study found no association between serum cortisol concentrations and the presence of NAFLD. The determination of total cortisol in serum would appear to be of no independent value in the diagnosis of NAFLD in routine clinical practice. Nevertheless, glucocorticoid metabolism is an important factor in the pathogenesis of NAFLD. Ultrasonography of the liver is a practical, reproducible, and cost-effective method for detecting NAFLD.

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Members of the EMIL-Study-Group (in alphabetical order)

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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