

# Prevalence of and Risk Factors for Hepatic Steatosis in Northern Italy

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**Background:** Although hepatic steatosis is seen with increasing frequency in clinical practice, its prevalence and risk factors are unknown.

**Objective:** To investigate the prevalence of and risk factors for hepatic steatosis, such as alcohol consumption and obesity.

**Design:** Cross-sectional, observational study.

**Setting:** Participants in the Dionysos Study.

**Patients:** 257 participants assigned to one of four categories (67 controls, 66 obese persons, 69 heavy drinkers, and 55 obese heavy drinkers).

**Measurements:** Ethanol intake, assessed by a validated questionnaire and expressed as daily (g/d) and lifetime (kg) consumption, and body mass, expressed as body mass index. Biochemical tests of liver and metabolic function and hepatic ultrasonography were done.

**Results:** The prevalence of steatosis was increased in heavy drinkers (46.4% [95% CI, 34% to 59%]) and obese persons (75.8% [CI, 63% to 85%]) compared with controls (16.4% [CI, 8% to 25%]). Steatosis was found in 94.5% (CI, 85% to 99%) of obese heavy drinkers. Compared with controls, the risk for steatosis was higher by 2.8-fold (CI, 1.4-fold to 7.1-fold) in heavy drinkers, 4.6-fold (CI, 2.5-fold to 11.0-fold) in obese persons, and 5.8-fold (CI, 3.2-fold to 12.3-fold) in persons who were obese and drank heavily. In heavy drinkers, obesity increased the risk for steatosis by twofold (CI, 1.5-fold to 3.0-fold) ( $P < 0.001$ ), but heavy drinking was associated with only a 1.3-fold (CI, 1.02-fold to 1.6-fold) increase in risk in obese persons ( $P = 0.0053$ ). Elevated alanine aminotransferase and triglyceride levels are the most reliable markers of steatosis.

**Conclusions:** Steatosis is frequently encountered in healthy persons and is almost always present in obese persons who drink more than 60 g of alcohol per day. Steatosis is more strongly associated with obesity than with heavy drinking, suggesting a greater role of overweight than alcohol consumption in accumulation of fat in the liver.

Unexplained abnormalities on liver function tests are common and often perplexing. Fatty liver, or hepatic steatosis, is a common clinical and histologic finding. When hepatic steatosis does not coexist with alcoholic hepatitis or steatohepatitis, it is a benign condition (1, 2). Fatty liver is usually attributed to alcohol abuse. Although the hepatotoxicity of ethanol has been well established, only 8% to 20% of persons with chronic alcoholism develop cirrhosis (3). In the Dionysos Study, a survey of the prevalence of chronic liver disease in the general population that included 6917 participants (3, 4), the risk threshold for ethanol-induced liver disease was the ingestion of more than 30 g of alcohol per day. However, only 74 of 1349 participants at risk (5.5%) showed persistent signs of alcoholic liver damage (4).

Steatosis can also occur in association with other conditions, such as obesity, hypernutritional support, drug- or toxin-induced hepatitis, type 2 diabetes mellitus, and cachexia, and it can occur after jejunoleal bypass surgery for refractory obesity (1, 5–12). The role of obesity compared with that of alcohol in inducing fatty liver or abnormal results on liver function tests is still controversial. Although the presence of excess weight for at least 10 years has been claimed to be a potential additional risk factor for acute alcoholic hepatitis and cirrhosis (13), most data have been collected from selected series or retrospectively.

To explore the relative roles of obesity and alcohol abuse, alone and in combination, in inducing steatosis, we performed an intracohort study in the general population of two towns of northeastern Italy (Campogalliano, in the province of Modena, and Cormons, in the province of Gorizia) that participated in the Dionysos Study (3). Data indicate that obesity plays a greater role than excessive alcohol intake in inducing fatty liver.

## Methods

### Patients

The details of the overall design of the Dionysos Study have been described elsewhere (3, 4). Briefly, 6917 of the 10 150 inhabitants (age range, 12 to 65 years) of two northern Italian communities were

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screened. For each participant, an extensive medical history was obtained that included history of chronic liver disease in first-degree relatives; a detailed history of acute hepatitis, gallstone disease, surgical operations, blood transfusions, drug abuse, dental procedures, dog or other domestic animal bites, homosexual sex, or tattooing; previous diagnosis of chronic hepatitis, cirrhosis, hemochromatosis, Wilson disease, primary biliary cirrhosis, other congenital liver diseases, diabetes, hyperlipoproteinemia, pericarditis, or heart diseases; careful questioning for any current symptoms of liver or biliary diseases, including biliary colic, anorexia, pruritus, jaundice, dark urine, and ascites; and personal habits, including tobacco and coffee consumption and type and duration of past and present professions and duties. Each participant also underwent a detailed physical examination aimed at detecting liver diseases or physical signs related to chronic liver disease, including jaundice, excoriation of skin, ascites, pretibial edema, flapping tremor, spider nevi, palmar erythema, liver enlargement, and palpable spleen; measurement of height, weight, body mass index (calculated as weight in kg/height in square meters), and left wrist circumference; and assay for serum levels of alanine aminotransferase, aspartate aminotransferase,  $\gamma$ -glutamyltransferase, glucose, mean corpuscular volume of erythrocytes, platelet count, and presence of hepatitis B surface antigen and antibody to hepatitis C virus. The current study involves 257 persons 19 to 70 years of age from the Dionysos cohort who tested negative for infection with hepatitis B virus or hepatitis C virus and had no evidence of chronic liver disease.

### Design

The intracohort study began in March 1997. The study was approved by the ethical committee of Fondo Studio Malattie del Fegato, Trieste, Italy. Of 6917 participants in the initial Dionysos Study cohort (3), 3618 were excluded: Three hundred twelve tested positive for hepatitis B surface antigen ( $n = 86$ ) or antibody to hepatitis C virus ( $n = 226$ ), 2 had histologically proven hemochromatosis or primary biliary cirrhosis, 19 had histologically proven cryptogenetic cirrhosis, and 3285 of them were overweight but not obese (body mass, 25 kg/m<sup>2</sup> to 30 kg/m<sup>2</sup>) or were obese but drank less than 60 g of alcohol per day. The remaining 3299 participants were divided into four groups according to body mass index and daily (g/d) and lifetime (kg) alcohol intake. The control group consisted of 2753 persons of normal weight (body mass index < 25 kg/m<sup>2</sup>) who drank less than 100 kg of alcohol over a lifetime and did not drink more than 30 g of alcohol per day. The obese group included 283 obese persons (body mass index > 30 kg/m<sup>2</sup>) who drank less than

100 kg of alcohol over a lifetime and did not drink more than 30 g of alcohol per day. The heavy drinker group comprised 168 persons of normal weight who drank more than 100 kg of alcohol over a lifetime and drank more than 60 g of alcohol per day. Finally, the obese and heavy drinker group consisted of 95 obese persons who drank more than 100 kg of alcohol over a lifetime and drank more than 60 g of alcohol per day.

The definition of *heavy drinker* as consumption of 30 g of alcohol per day or 100 kg of alcohol over a lifetime was based on previous data indicating these amounts of alcohol as the risk threshold for alcoholic liver disease in both sexes (4). Alcohol intake was calculated by using data from a semi-quantitative, color food questionnaire (14, 15) that included detailed questions on the use of alcoholic beverages (16, 17). Each participant was asked, in multiple-choice form, whether he or she drank beer, red wine, white wine, alcoholic aperitifs, or hard liquors daily, weekly, monthly, or hardly ever or never. For each type of alcoholic beverage, a color picture of a glass containing a standard unit of the beverage (200 mL for beer, 100 mL for wine, 70 mL for aperitif, and 40 mL for hard liquor) was printed in the questionnaire. Each participant was asked if he or she usually drank a glass like the one reported in the picture, a smaller one, or a larger one. If the participant answered that he or she usually drank a glass that was smaller or larger than the one pictured, the amount of the beverage consumed per day was reduced or increased by 25%. To calculate accurately the amount of alcohol in grams for each unit (glass), the brand of beverage was also recorded. Daily alcohol intake was computed by multiplying the frequency of consumption of each unit of beverage by the alcohol content of the specified portions, according to standard procedures (14–17). Total lifetime intake of alcohol was computed by multiplying the daily alcohol intake by years of alcohol intake. As described in detail elsewhere (4), alcohol consumption was validated by monitoring wine intake (liters per year per person) from purchase records of wine sellers in the two participating towns and by cross-checking the alcohol consumption declared by the participant with family members.

Among the participants who fit the inclusion criteria, 300 (150 residents of Campogalliano and 150 residents of Cormons, pair-matched for age) were randomly selected from the four groups by using the random procedure of the SPSS software package (SPSS, Inc., Chicago, Illinois). Informed consent to participate was obtained from 285 participants. Twenty-eight persons were excluded: Nineteen stated that they had reduced their daily alcohol intake to less than 30 g/d for at least 2 years or that

**Table 1. Features of the Study Groups\***

Group	Men	Women	Age	Body Mass Index	Ethanol Intake	Total Lifetime Intake of Ethanol
	<i>n</i>		<i>y</i>	<i>kg/m<sup>2</sup></i>	<i>g/d</i>	<i>kg</i>
Control ( <i>n</i> = 67)	34	33	46.8 ± 11.7 (49)	22.3 ± 1.8 (22.7)	5.8 ± 0.9 (0)	33.5 ± 6.0 (0)
Heavy drinker ( <i>n</i> = 69)	62	7	49.7 ± 10.7 (46)	23.3 ± 1.3 (23.5)	71.1 ± 31.9 (64)†	619 ± 477 (449.7)†
Obese ( <i>n</i> = 66)	27	39	47.8 ± 10.2 (44.5)	32.3 ± 2.8 (31.1)‡	6.5 ± 8.2 (0)†	53.3 ± 83.4 (0)
Heavy drinker and obese ( <i>n</i> = 55)	48	7	51.5 ± 9.2 (50)	32.5 ± 2.5 (33.2)‡	77.5 ± 44.3 (68)†	734 ± 612 (473)†

\* Data are reported as the mean ± SD (median).

† *P* < 0.001 for comparison between controls and obese participants.

‡ *P* < 0.001 for comparison between controls and heavy drinkers.

their body mass index was less than 30 kg/m<sup>2</sup> from 1993 to 1997; 6 who had type 2 diabetes mellitus (3 women and 1 man) or hyperlipoproteinemia type 2b (2 men) took oral antidiabetic drugs or cholestyramine, and 3 regularly took drugs. The remaining 257 participants, who were 19 to 70 years of age and had taken no drugs in the past 6 months, were enrolled in the study. Sixty-seven were assigned to the control group, 66 to the obese group, 69 to the heavy drinker group, and 55 to the obese and heavy drinker group.

Two consecutive blood samples were obtained 15 days apart from all of the enrolled participants, and the following were measured: serum levels of alanine aminotransferase, aspartate aminotransferase, and  $\gamma$ -glutamyltransferase; mean corpuscular volume of erythrocytes; platelet count; and levels of glucose, cholesterol, and triglycerides. All participants also underwent hepatobiliarysplenic ultrasonography, which was performed by two operators (one in each center). The ultrasonograph operators were unaware of the participants' clinical and biochemical profiles and the aim of the study.

Steatosis was defined as the presence of an ultrasonographic pattern consistent with "bright liver," with evident ultrasonographic contrast between hepatic and renal parenchyma, vessel blurring, focal sparing, and narrowing of the lumen of the hepatic veins, according to international guidelines (18, 19). Previous studies indicated that ultrasonography can detect and quantitate hepatic fat accumulation with an accuracy similar to that of computed tomography and liver biopsy (20). The biochemical and ultrasonographic findings (presence or absence of steatosis and gallstones) were reported to the physician of the study participant.

### Statistical Analysis

Statistical analysis was performed by using the SPSS statistical package, version 7.1 (SPSS, Inc.). Data are expressed as the mean ± SD or the median with 95% CIs for continuous variables. Statistical comparison was done by using the F-test (analysis of variance) (21). Bivariate risk ratios (22, 23)

with 95% CIs (StatXact 3 for Windows, CYTEL Software Co., Cambridge, Massachusetts) and positive predictive values were calculated.

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The funding source did not have any role in the analysis or interpretation of the data or in the decision to submit the paper for publication.

## Results

**Table 1** shows the main demographic characteristics and drinking behavior of the 257 participants. Mean participant age was similar across groups. No statistically significant differences in daily alcohol intake or total lifetime alcohol consumption were found between nonobese and obese heavy drinkers or between controls and obese persons. As observed in the whole cohort (3, 4), the ratio of men to women was similar between heavy drinkers and obese heavy drinkers; more women than men were classified as obese.

As shown in **Table 2**, the prevalence of steatosis increased progressively from 16.4% (95% CI, 8% to 27%) in controls to 46.4% (CI, 34% to 59%) in heavy drinkers, 75.8% (CI, 63% to 85%) in obese persons, and 94.5% (CI, 85% to 99%) in obese heavy drinkers. Compared with controls, steatosis was more common by 2.8-fold in heavy drinkers, 4.6-fold in obese persons, and 5.8-fold in obese heavy drinkers. Obesity was associated with a two-fold increase in risk among heavy drinkers (*P* < 0.001), but heavy drinking in obese persons was associated with only a 30% increase in risk (*P* = 0.0053). The risk for steatosis was 1.6-fold higher for an obese nondrinking participant than for a nonobese heavy drinker (*P* < 0.001).

Two consecutive blood samples were obtained from each participant at a 15-day interval, and the mean of the two determinations was used. Biochemical values varied by less than 3% in the two consecutive blood samples. The upper limit of normal was set according to the international cut-points

(24). When both obesity and heavy drinking were present, the mean values of all biochemical variables, except for cholesterol level, were elevated compared with values in controls ( $P = 0.012$  for aspartate aminotransferase levels;  $P < 0.001$  for alanine aminotransferase,  $\gamma$ -glutamyltransferase, and triglyceride levels). Mean levels of aspartate aminotransferase ( $P < 0.012$ ) and  $\gamma$ -glutamyltransferase ( $P < 0.002$ ) and mean corpuscular volume ( $P < 0.0016$ ) were higher in heavy drinkers than in controls. Compared with controls, obese heavy drinkers had elevated alanine aminotransferase ( $P < 0.001$ ), triglyceride ( $P < 0.001$ ), and glucose ( $P < 0.005$ ) levels.

The prevalence of elevation of alanine aminotransferase ( $>633$  nkat/L),  $\gamma$ -glutamyltransferase ( $>0.88$   $\mu$ kat/L), glucose ( $>6.11$  mmol/L [ $>110$  mg/dL]), and triglyceride ( $>1.92$  mmol/L) levels was 20% to 22% in controls, confirming our previous observation that abnormal results on these tests are common in the general population (3). The mean  $\gamma$ -glutamyltransferase level was elevated in heavy drinkers with or without obesity but not in obese persons who were not heavy drinkers. In heavy drinkers, mean corpuscular volume and  $\gamma$ -glutamyltransferase levels were 1.8-fold (CI, 1.4-fold to 2.4-fold) and 1.6-fold (CI, 1.2-fold to 2.9-fold) higher than in controls ( $P < 0.0016$  and  $P < 0.009$ , respectively) and were 2-fold (CI, 1.5-fold to 2.6-fold) and 1.9-fold (CI, 1.5-fold to 2.6-fold) higher than in obese persons ( $P < 0.001$ ). Triglyceride levels were 1.7 (CI, 1.3 to 2.3) times more likely to be elevated in obese persons than in controls ( $P = 0.0028$ ).

In heavy drinkers, obesity increased the risk for elevated levels of glucose,  $\gamma$ -glutamyltransferase, alanine aminotransferase, and triglycerides by 2.3-fold (CI, 1.4-fold to 3.9-fold) ( $P < 0.001$ ), 2.1-fold (CI, 1.3-fold to 3.3-fold) ( $P < 0.001$ ), 2.4-fold (CI, 1.6-fold to 3.6-fold) ( $P < 0.001$ ), and 2.9-fold (CI, 1.6-fold to 5.4-fold) ( $P < 0.001$ ), respectively. In obese persons, heavy drinking increased the risk for abnormal glucose,  $\gamma$ -glutamyltransferase, and alanine aminotransferase levels by 2.3-fold (CI, 1.4-fold to 3.8-fold) ( $P < 0.001$ ), 4.0-fold (CI, 2.6-fold to

6.2-fold) ( $P < 0.001$ ), and 2.1-fold (CI, 1.3-fold to 3.2-fold) ( $P < 0.001$ ), respectively.

The prevalence of gallstones was 7% in controls, 6% in heavy drinkers, 13% in obese persons, and 11% in obese heavy drinkers). However, the presence of gallstones was not significantly associated with steatosis ( $P = 0.049$ ).

In the 257 participants, the overall prevalence of steatosis on ultrasonography was 58.3%; this value is similar to that in participants in the general Dionysos Study, who had persistent abnormal values on liver function tests (61%) (3). Using this prevalence, we calculated the positive predictive value of each biochemical variable for the presence of steatosis. Elevation of alanine aminotransferase levels above 633 nkat/L and triglyceride levels above 1.92 mmol/L had the highest positive predictive value (78% and 79%), whereas a  $\gamma$ -glutamyltransferase level greater than 0.88  $\mu$ kat/L had a positive predictive value of 71%.

## Discussion

Although the role of alcohol abuse and obesity in inducing fatty liver has been reported, the relative role of these factors is still undefined. This is the first intracohort study to compare four patient groups in which factors that may cause steatosis (alcohol consumption and body weight) were investigated simultaneously in a well-defined population and in the absence of confounding factors. The groups, which were selected from participants in the Dionysos Study, were fully comparable (3, 4). After exclusion of persons who were positive for hepatitis B virus or hepatitis C virus, those with type 2 diabetes mellitus or hyperlipoproteinemia, and those who regularly used any drug, the remaining participants were pair-matched for age, body mass index, and alcohol intake. The prevalence of steatosis on ultrasonography in nonobese, nondrinking participants in our study (16%) was similar to that in apparently healthy Japanese persons (25) and in middle-aged Japanese workers (26). The presence

**Table 2. Relative Risk for Steatosis in the Study Groups**

Study Group	Participants with Steatosis/All Participants, n/n (%)	Relative Risk (95% CI)		
		Controls	Heavy Drinkers	Obese Persons
Control	11/67 (16.4)	–	–	–
Heavy drinker	32/69 (46.4)	2.8 (1.4–7.1)	–	–
<i>P</i> value		<0.001		
Obese	50/66 (75.8)	4.6 (2.5–11.0)	1.6 (1.2–2.5)	–
<i>P</i> value		<0.001	<0.001	
Heavy drinker and obese	52/55 (94.5)	5.8 (3.2–12.3)	2.0 (1.5–3.0)	1.3 (1.02–1.6)
<i>P</i> value		<0.001	<0.001	0.0053

of steatosis progressively increased according to heavy drinking and obesity, reaching its maximum (94.5%) when both conditions were present. The relative risk for steatosis was significantly higher in an obese persons than in heavy drinkers (Table 2). The relative risk for steatosis was greater in obese persons and obese persons who drank more than 60 g of alcohol per day than in nonobese heavy drinkers, indicating that obesity may have a greater role than heavy drinking in causing fatty liver.

In Italy and in other western countries, one of the most common reasons for referring patients to a hepatologist is the discovery of persistently abnormal aminotransferase or  $\gamma$ -glutamyltransferase levels without any symptoms and in the absence of known viral or toxic agents (2, 13, 25–32). In these patients, ultrasonography usually shows a “bright liver,” suggesting steatosis.

In our study, elevated biochemical values, with the exception of cholesterol, were observed more frequently in patients with steatosis than in controls. Bivariate analysis of the data indicates that elevated mean corpuscular volume and  $\gamma$ -glutamyltransferase level seem to be specifically related to heavy drinking and that an increased triglyceride level is associated with obesity. We and others (33) found that the risk for an elevated serum  $\gamma$ -glutamyltransferase level was fourfold higher in obese persons who drank more than 60 g of alcohol per day than in nondrinking obese persons, indicating that alcohol abuse is an important determinant of increases in the serum  $\gamma$ -glutamyltransferase level in obese persons. An elevated serum alanine aminotransferase level is found both in alcohol abuse and obesity, although this value cannot discriminate between the two conditions. Analysis of our data showed that increased levels of alanine aminotransferase and triglycerides, in addition to body mass index and alcohol consumption, were the most sensitive markers of steatosis. Because patients with steatosis in this study did not undergo liver biopsy and because this is a cross-sectional study of a well-defined population, caution should be used in extrapolating these results to other populations. In addition, the positive predictive value may be lower in groups with lower prevalence of steatosis.

Another important conclusion that can be derived from the data is that elevated levels of alanine aminotransferase, triglycerides, and, to a lesser extent,  $\gamma$ -glutamyltransferase are the best indicators of steatosis. They also indicate that when abnormal values on these tests are found in an apparently healthy person, the chance that he or she has fatty liver is high.

Although steatosis is generally a benign disease, the role of dietary intake of fat and development of fatty infiltration of the liver in the pathogenesis of

alcoholic hepatitis and cirrhosis has been recognized (33). From the present data, we cannot rule out whether obese heavy drinkers with steatosis will develop cirrhosis more rapidly than nonobese heavy drinkers or normal persons. This is related to the difficulty in using ultrasonography to assess the presence of inflammation or fibrosis in addition to fatty infiltration. However, we are regularly following all participants in the Dionysos cohort, and it will be interesting to see whether the natural history of steatosis differs among the various groups considered in this study.

From these data, we draw the following conclusions. In the general population of northern Italy, independent of confounding factors, 1) elevation of alanine aminotransferase and triglyceride levels is the most predictive condition for hepatic steatosis; 2) steatosis is associated more with obesity than with alcohol abuse; 3) an elevation of mean corpuscular volume and levels of aspartate aminotransferase and  $\gamma$ -glutamyltransferase indicates heavy drinking; 4) an elevated glucose level suggests the presence of both obesity and heavy drinking; and 5) in persons who are both obese and drink heavily (alcohol consumption > 30 g/d), steatosis is almost always present.

On the basis of these findings, we propose that persons with unexplained abnormalities on liver function tests (alanine aminotransferase and  $\gamma$ -glutamyltransferase levels in particular) and without a declared increased alcohol intake (keeping in mind the limitations of self-reporting) should be screened with hepatic ultrasonography. This is particularly true if the abnormalities are associated with an increased triglyceride level. The demonstration of hepatic steatosis should prompt a reduction in caloric or alcohol intake (or both) and follow-up with both ultrasonography and biochemical tests. If it is clinically indicated, a liver biopsy to assess the degree of inflammation and fibrosis could be performed during follow-up.

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