Hepatic and Cardiovascular Consequences of Familial Hypobetalipoproteinemia

Raaj R. Sankatsing, Sigrid W. Fouchier, Stefan de Haan, Barbara A. Hutten, Eric de Groot, John J.P. Kastelein, Erik S.G. Stroes

Objective—Individuals with familial hypobetalipoproteinemia (FHB) have been reported to be prone to fatty liver disease (FLD). Conversely, the profound reduction of low-density lipoprotein (LDL) cholesterol in this disorder might decrease cardiovascular risk. In the present study, we assessed hepatic steatosis as well as noninvasive surrogate markers for cardiovascular disease (CVD) in subjects with FHB and in matched controls.

Methods and Results—Hepatic steatosis was assessed by abdominal ultrasonography. Carotid intima-media thickness (IMT) and distal common carotid arterial wall stiffness as surrogate markers for CVD risk were measured using high-resolution B-mode ultrasonography. Whereas transaminase levels were only modestly elevated, both prevalence (54% versus 29%; P=0.01) and severity of steatosis were significantly higher in FHB subjects compared with controls. Despite similar IMT measurements, arterial stiffness was significantly lower in FHB (P=0.04) compared with controls. Additionally, the increase in arterial stiffness as seen in the presence of traditional risk factors was attenuated, suggesting that very low levels of apoB-containing lipoproteins can negate the adverse effects of other risk factors on the vasculature.

Conclusions—FHB is characterized by an increased prevalence and severity of fatty liver disease. The observed decreased level of arterial wall stiffness, most pronounced in the presence of nonlipid risk factors, is indicative of cardiovascular protection in these subjects. (Arterioscler Thromb Vasc Biol. 2005;25:1979-1984.)

Key Words: apolipoprotein B ■ arterial stiffness ■ cardiovascular risk ■ familial hypobetalipoproteinemia ■ fatty liver disease

Familial hypobetalipoproteinemia (FHB) is a hereditary disorder of lipoprotein metabolism characterized by very low levels of apolipoprotein (apo) B-100. Plasma levels below the fifth percentile are distinctive for this condition that inherits as an autosomal dominant trait. The prevalence in the general population is estimated to vary from 0.1% to 1.9%. Genetic causes of hypobetalipoproteinemia include FHB, abetalipoproteinemia, and chylomicron remnant disease (OMIM numbers 601519, 246700, and 200100, respectively). A small percentage of FHB can be explained by mutations in the gene-encoding apolipoprotein B-100 (APOB). These include nonsense, frame-shift, and splicing mutations. Recently, it was reported that a missense mutation in the APOB gene can also lead to FHB. APOB gene mutations lead to truncated forms of apolipoprotein B (apoB) and are characterized by slower hepatic secretion, as well as more rapid plasma clearance compared with wild-type apoB-100 particles. Because apoB is the main constituent of such lipoproteins, including very low-density lipoprotein (VLDL), intermediate-density lipoprotein, and low-density lipoprotein (LDL), FHB subjects are characterized by exceptionally low levels of these proatherogenic particles from birth onwards. Whereas subjects with heterozygous FHB are generally asymptomatic, 2 potential implications have been attributed to this particular condition. First, the impairment of hepatic VLDL-triglycerides (TG) secretion in FHB may contribute to fat accumulation in the liver. Potential consequences of fat accumulation are highlighted by the occurrence of hepatic steatosis in subjects with nonalcoholic steatohepatitis in whom progression toward cirrhosis has been observed. Case reports and smaller studies have reported a relationship between fatty liver disease (FLD) and FHB. This is best illustrated by 2 recent studies by Schonfeld et al who showed that hepatic fat percentage, as assessed by magnetic resonance spectroscopy, was significantly increased in subjects with FHB as compared with healthy controls. Nevertheless, the natural course of this potential fat accumulation in FHB is as yet unknown. Second, FHB offers a unique opportunity to evaluate the impact of life-long exposure to unusually low levels of apoB-containing atherogenic lipoproteins. In this respect, FHB subjects might be regarded as a natural model for “intensive lipid-lowering therapy.” In fact, the potential success of intensive lipid-lowering therapy has recently been reinforced by data from the REVERSAL and PROVE-IT studies, showing a
more pronounced reduction of cardiovascular disease risk on intensive lowering of LDL cholesterol levels.

In the present study, we evaluated the impact of FHBL on hepatic steatosis as well as on surrogate markers for cardiovascular disease. Here we present the results of these investigations.

### Methods

#### Subjects and Protocols
For a detailed description of methods please http://atvb.ahajournals.org.

Eighty-two subjects were enrolled in study, 41 with FHBL and 41 healthy controls, matched for sex and body mass index (Table 1). Autosomal codominant inheritance was a necessary characteristic for the clinical diagnosis of FHBL. FHBL subjects were identified from a group of individuals who were referred to our lipid clinic because of extreme low LDL levels. These subjects were characterized by direct sequencing of the entire apoB gene, as published previously.17 Secondary causes for low LDL cholesterol levels, ie, (strict) vegetarian diet or generalized diseases such as cancer, were excluded. The controls consisted of unaffected family members as well as unrelated volunteers. In the FHBL group, 4 subjects had diabetes mellitus (DM) compared with none in the control group. Because DM is strongly associated with both liver steatosis and carotid IMT/arterial stiffness,22–23 we repeated the analyses after excluding the 4 diabetic subjects in the FHBL group.

#### Liver Ultrasound
In all subjects ultrasound examination of the liver was performed by a single radiologist blinded to the disease state of the subjects. The extent of hepatic fatty infiltration was classified according to previously published criteria.26

#### Carotid Ultrasound

B-mode ultrasound intima-media thickness (IMT) measurements were performed in the far walls of the carotid arteries and M-mode arterial stiffness was measured bilaterally in the common carotid arteries.

### Statistical Analysis

Statistical analyses were performed using linear or logistic regression analyses with generalized estimating equations in the SAS procedure GENMOD to account for correlations within families. Analyses were performed using SAS software (release 8.02; SAS Institute Inc). $P<0.05$ was considered significant.

### Results

Clinical characteristics of FHBL subjects and controls are presented in Table 1. Forty-one subjects who met the clinical criteria of FHBL (apoB and LDL cholesterol less than fifth percentile adjusted by age, gender, and race) participated in the study. Thirty-three of these subjects had mutations in the apoB gene, characteristic of FHBL. These genetically affected subjects were recruited from 8 families with different apoB mutations. The following apoB mutations were identified in the FHBL group: 2534delA apoB-18, Q1309X apoB-29, R2507X apoB-55, and 11712delC apo-B8617 (Table 2). Subjects did not use lipid-lowering drugs. There was no significant difference in blood pressure, smoking, body mass

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>WT</th>
<th>MT</th>
<th>Bp Position</th>
<th>Predicted Size</th>
<th>No. of Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>2534delA</td>
<td>A</td>
<td>delA</td>
<td>2534</td>
<td>ApoB-18</td>
<td>14</td>
</tr>
<tr>
<td>25</td>
<td>Q1309X</td>
<td>CAA</td>
<td>TAA</td>
<td>4006</td>
<td>ApoB-29</td>
<td>4</td>
</tr>
<tr>
<td>26</td>
<td>R2507X</td>
<td>CGA</td>
<td>TGA</td>
<td>7600</td>
<td>ApoB-55</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>11712delC</td>
<td>C</td>
<td>delC</td>
<td>11712</td>
<td>ApoB-86</td>
<td>14</td>
</tr>
</tbody>
</table>

The reference sequence used was NM_000384, with the A of the ATG translation initiation codon numbered nucleotide +1 and the methionine numbered as amino acid –27. Adapted from Fouchier et al. J Med Genet. 2005;42:e23.

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**TABLE 1. Clinical Characteristics for FHBL Subjects and Controls**

<table>
<thead>
<tr>
<th></th>
<th>FHBL (n=41)</th>
<th>(minus DM) (n=37)</th>
<th>Controls (n=82)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>41.1±16.9</td>
<td>39.58±16.54</td>
<td>45.8±16.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>27 (66)</td>
<td>24 (65)</td>
<td>27 (66)</td>
<td>0.97</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.7±5.1</td>
<td>25.0±16.3</td>
<td>24.9±3.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.86±0.10</td>
<td>0.85±0.10</td>
<td>0.87±0.09</td>
<td>0.37</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67±12</td>
<td>67±13</td>
<td>70±12</td>
<td>0.37</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>57±13</td>
<td>56±12</td>
<td>56±19</td>
<td>0.88</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>138±19</td>
<td>137±18</td>
<td>136±25</td>
<td>0.72</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>81±12</td>
<td>80±12</td>
<td>80±11</td>
<td>0.70</td>
</tr>
<tr>
<td>Hypertension‡ (%)</td>
<td>3 (7)</td>
<td>1 (3)</td>
<td>3 (7)</td>
<td>0.48</td>
</tr>
<tr>
<td>DM (%)</td>
<td>4 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>§</td>
</tr>
<tr>
<td>Alcohol (U/d)</td>
<td>0.5 (0.0–1.3)</td>
<td>0.5 (0.0–1.8)</td>
<td>1.0 (0.0–2.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>21 (51)</td>
<td>19 (51)</td>
<td>13 (33)</td>
<td>0.09</td>
</tr>
<tr>
<td>Pack-years for smokers</td>
<td>21.0 (10.5–30.8)</td>
<td>18 (10–28.5)</td>
<td>10.0 (7.3–19.5)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or No. (percentage), except for alcohol consumption and pack-years, which are given as median (interquartile range).

*P indicates difference between FHBL group (n=41) and controls (n=41).

† P indicates difference between FHBL-DM group (n=37) and controls (n=41).

‡ Hypertension: SBP >140 mm Hg and/or DBP >90 mm Hg.

§ P could not be calculated.

DBP indicates diastolic blood pressure; DM, diabetes mellitus; FHBL, familial hypobetalipoproteinemia; SBP, systolic blood pressure.
Three of these 7 subjects had ALT levels more than twice the upper limit of normal (ULN) but still less than 3 times ULN. The highest value of ALT, observed in a diabetic patient, was 102. None of these 7 subjects had AST values > 2 times ULN. AST and ALT were both significantly associated with hepatic steatosis (P = 0.04 and P = 0.0002, respectively). Levels of high-sensitivity C-reactive protein (hs-CRP) and glucose were comparable between the two groups. HDL cholesterol levels were significantly higher in the FHBL minus DM group compared with the controls (1.76±0.59 mmol/L versus 1.55±0.33 mmol/L, P = 0.01).

Liver Ultrasound

We observed a significantly higher prevalence of liver steatosis in the FHBL group compared with the control group (54% versus 29%; P = 0.01). In addition, FHBL subjects were also characterized by a more severe degree of hepatic steatosis. Seven (17%) of the subjects in the FHBL group were classified as severe steatosis compared with none in the control group (Figure 1). The distribution of steatosis severity was significantly different between groups (P = 0.004). This was even more apparent when comparing the controls with the subgroup of FHBL with mutations (P = 0.001). The 4 diabetic subjects were equally distributed over the 4 steatosis categories. Separate analyses with exclusion of these 4 subjects lead to an equal statistical difference for steatosis severity between groups.

Hepatic steatosis was not more severe in those subjects carrying truncated apoB not secreted into the plasma (apoB-18 and ApoB-29) compared with the carriers of longer truncations (P = 0.68). Plasma LDL cholesterol and apoB levels were also not different between these subgroups (P = 0.77 and P = 0.81, respectively). Nine of the 14 carriers of Apo-86 had liver steatosis compared with 3 of the 8 FHBL subjects without mutations. FHBL was positively associated with liver steatosis on univariate analysis (P = 0.02). When adjusted for gender and smoking on multivariate analysis, FHBL, age, and body mass index were independent predictors for the development of liver steatosis (P = 0.0001, P < 0.0001, and P = 0.0014, respectively). Similar significant results were found when running the analyses with the

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**TABLE 3. Laboratory Characteristics for FHBL Subjects and Controls**

<table>
<thead>
<tr>
<th></th>
<th>FHBL</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=41</td>
<td>n=37 (minus DM)</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>2.95±0.88</td>
<td>2.96±0.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.73±0.59</td>
<td>1.76±0.60</td>
<td>0.08</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>1.04±0.50</td>
<td>1.02±0.50</td>
<td>0.01</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.40 (0.18–0.55)</td>
<td>0.39 (0.17–0.53)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.36±0.13</td>
<td>0.35±0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0 (4.8–5.1)</td>
<td>4.9 (4.8–5.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>1.7 (0.8–3.0)</td>
<td>1.8 (1.0–3.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>30 (23–35)</td>
<td>30 (23–35)</td>
<td>0.0002</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>32 (22–54)</td>
<td>32 (22–54)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>25 (18–43)</td>
<td>25 (18–43)</td>
<td>0.008</td>
</tr>
<tr>
<td>Alk phos, U/L</td>
<td>62 (51–73)</td>
<td>62 (53–73)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, except for glucose, TG, hs-CRP, ALT, AST, GGT and Alk phos, which are given as median (interquartile range).

* Indicates difference between FHBL group (n=41) and controls (n=41).

† Indicates difference between FHBL-DM group (n=37) and controls (n=41).

ApoB indicates apolipoprotein B; HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
subgroup of FHBL subjects with mutations (n=33) compared with the healthy controls. Results were not significant when this was done for the subgroup of FHBL without mutations (n=8). However, the latter is likely to be caused by a lack of power attributable to the limited number of subjects in this group.

**Vascular Measurements**

Mean carotid IMT (±SD) was 0.63±0.14 mm in the FHBL group compared with 0.65±0.15 mm in the control group. The latter difference on univariate analysis (P=0.049) lost significance when adjusting for age, gender, smoking, and body mass index on multivariate analysis. Arterial stiffness was significantly lower in the FHBL minus DM group compared with the controls on univariate analysis (P=0.04), whereas a similar trend was seen for the whole FHBL group (P=0.06). When comparing the 2 subgroups of FHBL, (with and without mutations), FHBL was still significantly associated with arterial stiffness in the first group (P=0.04), but this was not significant for the subgroup without mutations. Again, this could reflect a lack of power, because the subgroup without mutations contained only 8 subjects. To evaluate a potential interaction between risk factors and apoB-containing lipoproteins, we attributed a cumulative risk score to each subject. The cumulative risk score comprised age, systolic blood pressure, and smoking. These variables were chosen because their individual relationship with cardiovascular risk has been proven beyond any doubt as attested to by their incorporation in both the PROCAM\(^27\) and Framingham Risk Score,\(^28\) the 2 most widely used risk calculators for predicting cardiovascular disease. Moreover, these risk factors have an independent strong association with arterial stiffness.\(^29-35\) In our study these parameters were also strongly correlated with arterial stiffness with the exception of smoking (r=0.732, P<0.001, r=0.550, P<0.001, and r=0.267, P=0.018, respectively). Both FHBL and control subjects showed a gradual increase in arterial stiffness with increasing cumulative risk scores. However, using linear regression analysis, the slope for the FHBL group was markedly decreased compared with the controls, indicating decreased stiffening in the presence of nonlipid risk factors in the FHBL group (P=0.03) (Figure 2). This difference remained significant (P=0.04) when comparing the FHBL minus DM group with the control subjects.

**Discussion**

Subjects with FHBL are characterized by an increased prevalence of fat accumulation in the liver as well as by a more severe degree of such hepatic steatosis. Whereas these findings are not novel, we show that FHBL subjects exhibit decreased arterial stiffness. Notably, the increase in arterial stiffness under the influence of “traditional” nonlipid risk factors was markedly attenuated in FHBL subjects.

**Biochemical Analyses**

Subjects with FHBL are characterized by significantly decreased levels of apoB-containing lipoproteins, including low LDL cholesterol as well as low triglyceride-rich particles. Also, slightly higher levels of HDL cholesterol were found in these FHBL subjects. Presumably, the latter is the consequence of low levels of triglycerides, thus minimizing exchange of cholesterol esters from HDL cholesterol to apoB-containing particles through the action of cholesterol ester transfer protein. Another explanation could be that the truncated apoB are only present in the density range of HDL. However, we excluded the latter option by performing agarose gel electrophoresis on HDL fractions, from patients with apoB-55 and apoB-86, which were isolated after ultracentrifugation. No LDL bands were present in the HDL density range. Additionally, apoB could also not be detected in the HDL fraction using immunonephelometry (data not shown).

**Hepatosteatosis**

The prevalence of fatty liver disease in healthy controls (29%) is in the same order of magnitude as reported by others.\(^19,36\) The increased prevalence of hepatosteatosis in subjects with FHBL is in agreement with previous results from Schonfeld and Tanoli who showed that these subjects had a 3-fold increase in mean liver fat content, as assessed by magnetic resonance spectroscopy.\(^13,14\) The most likely cause for this increase in hepatic steatosis is the impaired secretion of VLDL-TG from the liver, leading to accumulation of VLDL-TG in the liver. There is a large body of evidence suggesting that accumulation of liver triglycerides may give rise to increased oxidative stress in the hepatocytes.\(^37-39\) However, distinct proof, in the human setting, that this process invariably translates into progression of liver steatosis to nonalcoholic steatohepatitis is lacking.\(^40\) Recent work by Yousef et al revealed that up to 25% of patients with FLD may progresses to nonalcoholic steatohepatitis,\(^21\) of whom 20% may eventually even progress into cirrhosis.\(^19,20\) Notably, in our FHBL group, transaminase levels were only modestly elevated with none of the subjects exceeding a 3-fold increase in upper limit of normal. Most studies...
reporting long-term outcome of fatty liver disease, use the AST/ALT ratio as a marker for the risk of disease progression. A ratio of <1 indicates a “low risk” for steatosis and in our FHBL subjects, AST/ALT ratios were all <1. It should be kept in mind, however, that the absence of liver enzyme elevations does not completely preclude advanced fibrosis or cirrhosis in these subjects. To date, long-term follow-up data with regard to liver outcome in FHBL are lacking. Nevertheless, risk factors for FLD such as hypertriglyceridemia, obesity, alcohol, diabetes mellitus, and certain drugs are likely to aggravate hepatic steatosis in FHBL. Hence, it is prudent to avoid these risk factors and recommend a diet with low to moderate amounts of fat and energy, limited use of alcohol, as well as avoiding obesity in these individuals.

Cardiovascular Risk
Numerous studies have established a strong correlation between levels of LDL cholesterol and progression of IMT. In the present study, however, we could not show an independent statistical difference in terms of carotid IMT values between FHBL subjects and controls. Nevertheless, data have accumulated recently that show the predictive value of the assessment of vascular function, such as arterial stiffness, for future cardiovascular events. Arterial stiffness is closely correlated with increasing age, smoking, and hypertension. The impact of these risk factors is augmented in the presence of hypercholesterolemia and can be reverted by statin therapy. In our FHBL group, we observed a significant decrease in arterial stiffness. Of note, this difference was observed despite the fact that traditional risk factors such as smoking and diabetes occurred more frequently in the FHBL group compared with controls. In earlier studies, apoB-containing lipoproteins have been put forward as a pivotal “permissive” factor for the development of atherogenic changes of the vessel wall. To evaluate a potential interaction between apoB-containing lipoproteins and other traditional risk factors, we constructed a cumulative risk index including age, smoking, and systolic blood pressure in FHBL subjects as well as controls. In both groups, there was a linear relationship between increased risk score and arterial stiffness. Interestingly, the increase in arterial stiffness, also in presence of these risk factors was decreased significantly in the FHBL group compared with controls. These data suggest that apoB-containing lipoproteins indeed have the ability to potentiate the impact of traditional risk factors on vascular function. Tentatively, these observations might suggest that lowering of apoB-containing lipoproteins should have a beneficial impact also in subjects with “non-cholesterol” risk factors. Recent studies have validated the beneficial effects of statin therapy in normocholesterolemic subjects with nonlipid risk factors, such as hypertension.

This study has some limitations. We used the less sensitive ultrasonography method to evaluate fatty liver disease rather than magnetic resonance spectroscopy. However, in view of the carefully standardized methodology and the fact that both patients and controls were evaluated using the same methodology, it is unlikely that the latter has affected our outcomes. With regard to the IMT measurement, we could not find a clear relationship between LDL cholesterol levels and carotid IMT. Several reasons may have contributed to the absence of a relation. First, we studied a relatively young cohort with an inherently low risk for cardiovascular disease and hence low IMT values. Second, we studied IMT in a case control design to show thinner IMTs compared with healthy controls. A priori, it is very difficult to demonstrate decreased IMT thickness in “low-risk” groups compared with healthy controls. We have estimated that inclusion of more than 1000 subjects per group would have been necessary to be able to detect significantly thinner IMTs compared with healthy controls with a “normal” risk factor distribution, as seen in western populations.

In summary, our study shows that subjects with FHBL are at increased risk of developing FLD. Whereas long-term sequelae of FLD in FHBL subjects remain to be established, it is prudent to give lifestyle advice in affected individuals. As is illustrated by decreased vascular wall stiffness, our findings suggest that the vessel wall in FHBL subjects is relatively protected by the (life-long) reduced levels of exposure to apoB-containing lipoproteins. The attenuated gradual increase in vascular stiffness in the presence of classical, nonlipid cardiovascular risk factors in FHBL subjects is of interest and suggests that apoB-containing particles constitute a central factor in atherogenesis, amplifying any risk mediated by nonlipid risk factors. Further confirmation of this finding is needed in larger cohorts to ascertain its impact on cardiovascular risk.

Acknowledgments
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References


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Hepatic and Cardiovascular Consequences of Familial Hypobetalipoproteinemia (R1)
Raaj R. Sankatsing, Sigrid W. Fouchier, Stefan de Haan, Barbara A. Hutten, Eric de Groot,
John J.P. Kastelein and Erik S.G. Stroes

DETAILED METHODS (ON-LINE ONLY)

Subjects and protocols
All subjects completed a questionnaire, which comprised (family) history of cardiovascular
disease, alcohol use, cardiovascular risk factor inventory and current use of medication.
Physical examination consisted of measurement of blood pressure, pulse, weight, length,
waist- and hip circumference. The study protocol was approved by the Institutional Review
Board of the Academic Medical Center, University Hospital of Amsterdam. All subjects
provided written informed consent.

Biochemical analyses
All participants were asked to refrain from food and fluid intake at least 8 hours prior to the
examination. Blood samples were drawn in a fasted state.
Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were
determined with commercially available enzymatic methods (Roche Diagnostics GmbH,
Mannheim, Germany). LDL-C was calculated by the Friedewald formula. Liver function tests
were measured with standard laboratory methods. Glucose was measured using the
HK/Glucose-6-P dehydrogenase UV test (Roche Diagnostics GmbH, Mannheim, Germany).
High-sensitivity CRP (hs-CRP) levels were measured with a commercially available immune
turbidimetric test (Roche Diagnostics GmbH, Mannheim, Germany). Serum levels of apoB
were measured by rate immunonephelometry (Dade Behring Nephelometer BNII, Marburg,
Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards.

Liver ultrasound

Abdominal ultrasound examinations were performed with a Sonoline Elegra, 3.5C40H curved array transducer (Siemens Medical Solutions, Mountainview, CA, USA) and a ATL HDI 5000 SonoCT, curved array C5-2 40R transducer (Philips/ATL, Bothell, WA, USA).

The liver ultrasounds were classified into the following categories:

- **none**: normal liver echo structure – no steatosic infiltration
- **mild**: slight, diffuse increase of echogenicity in hepatic parenchyma; normal visualisation of diaphragm and intrahepatic vessel borders
- **moderate**: moderate, diffuse increase of echogenicity with slightly impaired visualisation of diaphragm and intrahepatic vessels
- **severe**: marked increase in fine echoes with poor or non visualisation of diaphragm and intrahepatic vessel borders, and posterior portion of the right lobe.

Images were stored electronically and analyzed by a separate radiologist, who was blinded to the disease-state of the subjects.

Carotid ultrasound

Carotid intima-media thickness measurements were performed as has been published previously. In summary, the common carotid, the carotid bulb and the internal carotid arterial walls were measured bilaterally. M-mode ultrasound lumen measurements were performed in the right and the left distal common carotid artery. The lumen and change in lumen diameter with the cardiac cycle were combined with oscillometric blood pressure
measurements. The arterial wall stiffness index (\(\beta\)) was calculated according to the following formula: \(\frac{\ln[SBP/DBP]}{\Delta D/D}\); where SBP represents systolic blood pressure; DBP, diastolic blood pressure; \(\Delta D\), difference between systolic and diastolic carotid arterial diameter; D, arterial diastolic diameter. The coefficient of variation is 15.2%. The intra-sonographer, the intra-analyst and the inter-analyst variability were less than 1%. The inter-sonographer variability accounts for most variability; therefore our study was performed with two well-trained sonographers who were randomly assigned to cases and controls. We used an Acuson 128XP/10v (Acuson Corporation, Mountainview, CA, USA) equipped with a L7 5-10 MHz linear array broadband transducer and Extended Frequency software. Two experienced sonographers scanned all subjects. B- and M-mode images were stored on a digital still recorder (SONY DKR-700 P). An analyst, blinded to the disease-state of the subject, performed image analyses off-line with semi-automated quantitative and qualitative video image analysis software.

Statistical analysis

Variables with a skewed distribution were log-transformed before statistical analysis. Differences in terms of clinical, lifestyle and laboratory characteristics between FHBL subjects and controls were evaluated using linear or logistic regression analyses with generalized estimating equations (GEE) in the SAS procedure GENMOD to account for correlations within families. We used the same SAS procedure -allowing for clustering within families- to explore univariately the relation between the outcome variables (mean IMT, steatosis) and baseline variables, with linear regression analysis. Using multivariate models, we identified independent predictors after stepwise backward selection. Differences in the distribution of steatosis severity between the FHBL and control group were evaluated using a GEE regression model for ordinal outcomes.
Reference List


Ref Type: Abstract