



Fatty liver predicts the risk for cardiovascular events

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4 **1 Fatty liver predicts the risk for cardiovascular events**

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3 20 **Disclosure summary:** Authors report no conflict of interests.
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7 22 **ABSTRACT**

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10 23 **Objective:** We investigated if the differences in liver fat accumulation would predict the
11
12 24 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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14 25 stroke).

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16 26 **Design, setting and participants:** Our study group is a population-based, randomly recruited
17
18 27 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.

19
20 28 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
21
22 29 registry of the National Institute for Health and Welfare and the National death registry.

23
24 30 **Main outcome measure:** The severity of liver adiposity was measured by ultrasound and
25
26 31 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.

27
28 32 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
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30 33 24.2% of subjects having moderate liver fat accumulation and 29.2% of the subjects having
31
32 34 severe fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$).
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34 35 Severe liver fat accumulation predicted the risk for future risk of cardiovascular event even
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36 36 when adjusted for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When
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38 37 further adjustments for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic
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40 38 blood pressure were conducted, the risk still remained statistically significant (HR 1.74, CI
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42 39 1.16-2.63, $p < 0.01$). Statistical significance disappeared with further adjustment for QUICKI.

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44 40 **Conclusions:** Liver fat accumulation increases the risk of future cardiovascular disease event
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46 41 in long-term follow-up but it seems to be dependent on insulin sensitivity.
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3 45 **Article focus**

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5 46 1 To investigate if the differences in liver fat accumulation predict the risk for development of
6 47 fatal or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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10 49 **Key messages**

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12 50 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
13 51 compared to the subjects without fat in the liver

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15 52 2 Severe liver fat accumulation increases the risk of a future cardiovascular event and
16 53 mortality to cardiovascular disease over the long-term follow-up but it does seem to be
17 54 dependent on insulin sensitivity

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19 55 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
20 56 traditional metabolic risk factors

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22 57 **Strengths and limitations of the study**

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24 58 1 Study seems to be the first follow-up study with a large population-based study group and a
25 59 very long follow-up time

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27 60 2 Official registers used in event diagnoses - data is accurate and the classification is
28 61 systematic

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30 62 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
31 63 sensitivity
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35 65 **Introduction**

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38 66 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
39 67 accumulation, which exists in a spectrum ranging from steatosis with no inflammation to non-
40 68 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
41 69 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
42 70 countries, being the leading cause of liver disorders ². It is associated with obesity, type 2
43 71 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
44 72 manifestation of the metabolic syndrome and both conditions share several risk factors for
45 73 cardiovascular disease (CVD) ^{2,3}.
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3 75 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2%⁴. Every
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5 76 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%)⁵.
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7 77 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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9 78 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes⁶. In
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11 79 addition, total body fatness as well as abdominal fat accumulation increase independently the
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13 80 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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15 81 adiposity to cardiovascular risk⁷. Adipose tissue is now recognized as a significant endocrine
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17 82 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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19 83 mediators, such as adipokines, proinflammatory cytokines and hypofibrinolytic markers⁶ that
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21 84 may lead to oxidative stress and endothelial dysfunction, finally leading to atherosclerosis⁸.
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29 86 NAFLD and CVD share several molecular mechanisms^{9,10}. Fatty liver might play a part in
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31 87 the pathogenesis of CVD through the overexpression and systemic release of several
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33 88 inflammatory, hemostatic¹¹ and oxidative-stress mediators or via contributing to whole-body
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35 89 insulin resistance and atherogenic dyslipidemia². NAFLD has also been reported to be linked
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37 90 with circulatory endothelial dysfunction^{3,12}. Several investigators have reported that NAFLD
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39 91 is associated with coronary artery disease^{3,12} and increased carotid intima-media thickness¹³,
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49 94 It is known that subjects with fatty liver disease have an increased risk of suffering CVD³, but
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51 95 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
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53 96 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
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55 97 CVD. The aim of our study was to investigate if fatty liver could predict independently the
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98 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
99 follow-up after adjusting for all known conventional risk factors.

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101 **Materials and methods**

102 **Human subjects**

103 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
104 epidemiological prospective cohort study designed to address the risk factors and disease end
105 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
106 been described earlier ¹⁵. In short, a total of 520 men and 525 women participated: 259 control
107 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
108 Hypertensive participants were randomly selected from the national register for
109 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
110 age- and sex-matched control subject was randomly selected from the same register. Informed
111 consent in writing was obtained from each patient. The study protocol conformed to the
112 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
113 Ethical Committee of the Faculty of Medicine, University of Oulu.

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115 **Determination of liver adiposity**

116 The determination of liver adiposity was based on liver-kidney contrast measured with
117 ultrasonography ¹⁶ by one trained radiologist with extensive experience in abdominal
118 ultrasound examinations. The severity of liver adiposity was based on the brightness of the
119 liver and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating

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3 120 a non-fatty liver, 1 = medium bright, a moderate lipid accumulation and 2 = clearly bright, a
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5 121 severe lipid accumulation and fatty liver) ¹⁷.
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10 11 123 **Follow-up**

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14 124 Both the hypertensive and the control men were recruited during December 1990 to May
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16 125 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
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18 126 liver ultrasound result available at baseline. Mortality data were obtained from the National
19
20 127 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
21
22 128 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
23
24 129 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
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26 130 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
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28 131 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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36 133 CVD included a major coronary heart disease event (CHD) and stroke (excluding
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38 134 subarachnoid hemorrhage, SAH) - whichever of these happened first. The evidence of CHD
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40 135 was based on the following diagnosis: I20.0, I21, I22 [ICD-10, International Statistical
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42 136 Classification of Diseases and Related Health Problems] / 410, 4110 [ICD-8/9] as the main
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44 137 diagnosis (symptom or cause) and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis
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46 138 (symptom or cause) or second side diagnosis (symptom or cause) and third side diagnosis
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48 139 (ICD-8/9 only) or if a subject had undergone coronary artery bypass graft (CABG) surgery or
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50 140 angioplasty. CHD as a cause of death included I20–I25, I46, R96, R98 [ICD-10] / 410-414,
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52 141 798 (not 7980A) [ICD-8/9] as the underlying cause of death or immediate cause of death and
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54 142 I21 or I22 [ICD-10] / 410 [ICD-8/9] as first to third contributing cause of death. Stroke
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3 143 (excluding SAH) included I61, I63 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A,
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5 144 4340A, 4341A, 4349A, 436 [ICD-9] / 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as
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7 145 main diagnosis (symptom or cause) or as a first or second side diagnosis (symptom or cause)
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9 146 or as a third side diagnosis (ICD-8/9 only) or as an underlying cause of death or immediate
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11 147 cause of death or as a first to third contributing cause of death ¹⁸.

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149 **Laboratory analyses**

150 Waist circumference, body mass index (BMI) and blood pressure were measured as described
151 in previous study ¹⁵.

152

153 Blood insulin and glucose concentrations were analyzed at 0, 60, and 120 min after
154 administration of 75 g glucose ¹⁷. Insulin sensitivity was assessed using fasting plasma insulin
155 concentrations and a quantitative insulin sensitivity check index (QUICKI) {QUICKI=1/[log
156 (fasting insulin)+log (fasting glucose)]} ¹⁹.

157

158 Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density
159 lipoprotein (LDL) and hs-CRP concentrations ¹⁷ as well as alanine aminotransferase (ALT)
160 and gamma-glutamyltransferase (GGT) levels were measured as described previously ¹⁶.

161 Alcohol consumption and smoking history were determined by validated questionnaires ²⁰.

162 Alcohol consumption was divided into three groups: 0 (n=161) mean alcohol consumption

163 less than 1g/week in men and women, 1 (n=767) mean consumption less than 210g/week in

164 men and less than 140 g/week in women, 2 (n=76) mean alcohol consumption more than

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3 165 210g/week in men and more than 140g/week in women. Group 2 designates large-scale
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5 166 alcohol consumers according to the guidelines ²¹.
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10 11 168 **Statistical analysis**

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14 169 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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17 170 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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19 171 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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21 172 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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23 173 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
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25 174 investigate if liver brightness (fat) could predict the future risk for total mortality,
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27 175 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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34 177 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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36 178 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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38 179 improve normality before analysis of variance. We used three models with progressive
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40 180 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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42 181 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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44 182 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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46 183 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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48 184 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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50 185 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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53 186 when further adjustment with one covariate was performed. Model 4 included variables which
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3 187 were stable and were statistically significant in intermediate phases. Model 5 included stable
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5 188 and significant covariates without QUICKI (Table 2).
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11 190 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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13 191 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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15 192 to obtain 95% CI for c-index of each model.
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20 21 22 194 **Results**

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25 195 The main baseline characteristics of the study group are shown in Table 1.
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32 197 *Table 1 about here*
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36 37 38 199 **Incidence of cardiovascular disease**

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41 200 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
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43 201 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
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45 202 moderate liver fat accumulation and 29.2% (42/144) of the subjects having severe fatty liver
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47 203 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
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49 204 with non-fatty liver and 8.1% of the subjects with moderate liver fat accumulation, while
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51 205 12.5% of the subjects with severe fatty liver ($p < 0.001$).
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3 207 Severe liver fat accumulation predicted the risk for future risk of cardiovascular event when
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5 208 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
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7 209 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
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9 210 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
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11 211 statistically significant ($p < 0.01$). Statistical significance disappeared when further
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13 212 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
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15 213 CVD event sensitivity analyses, all covariates were added one by one and it was examined
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17 214 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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19 215 significant variables (including quick index) in the sensitivity analyses, the association
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21 216 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18, NS).
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23 217 When QUICKI was not added into Model 5, severe fatty liver did predict the risk for future
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25 218 risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index decreased
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27 219 when the risk factors were removed from the model (Table 3).
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36 221 *Tables 2 and 3 about here*

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42 223 The future risk of death from CVD in participants with severe fat accumulation was
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44 224 significant when age, gender and study group were added as covariates (Model 1: HR 2.95, CI
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46 225 1.58-5.51, $p < 0.01$). Even after further adjustments with other conventional risk factors
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48 226 (Model 2: HR 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI
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50 227 was added as the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43,
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52 228 NS) (Fig 1.).
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3 230 *Figure 1 about here*

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9 232 **Fatty liver and total mortality**

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12 233 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
13 234 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
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15 235 0.01). According to Model 1, severe fat accumulation predicted the risk for mortality from all
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17 236 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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19 237 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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21 238 not shown).
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30 240 We performed all Cox regression analyses after excluding the men consuming more than 210
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32 241 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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34 242 have any effect on the results (data not shown).
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38 243 We performed all Cox regression analyses after excluding patients with insulin treated
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40 244 diabetes mellitus ($n=9$), cortisone treatment at baseline ($n=41$) and previous diagnosis for
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42 245 liver disease ($n=15$) (e.g., virus, medications). This exclusion did not have any effect on the
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44 246 results (data not shown).
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47 247 **Discussion**

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50 248 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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52 249 continuously increasing in the Western world. The question if NAFLD is only a marker or
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54 250 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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56 251 results of the present study, which had an approximately 19-year follow-up fatty liver does
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3 252 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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5 253 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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7 254 development of cardiovascular events.
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13 256 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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15 257 with ultrasound-diagnosed fatty liver ^{22, 23}. There are a few follow-up-studies examining
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17 258 whether the fatty liver increases the risk for total mortality ^{24, 25}. Larger follow-up studies with
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19 259 ultrasound-diagnosed fatty liver investigating non-fatal and fatal cardiovascular endpoints are
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21 260 needed. An association between NAFLD and CVD has been reported ^{2, 22, 23, 26} however several
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23 261 earlier studies have used self-reported CVD history which may not be totally reliable.
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25 262 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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27 263 liver have performed some adjustments, the full range of well-known CVD risk factors have
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29 264 been rarely considered ²⁷. These studies have used biochemical, radiological and histological
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31 265 methodology for NAFLD diagnosis and staging, which leads to a challenging interpretation of
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33 266 the results ^{28, 29}.
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43 268 This study had an approximately 19-year follow-up time. When compared to earlier studies ^{27,}
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45 269 ²⁹ this study seems to be the first follow-up study with a large population-based randomly
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47 270 selected study group and a very long follow-up time and ultrasound-diagnosed fatty liver. The
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49 271 diagnosis of cardiovascular events was based on the registry of the National Institute for
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51 272 Health and Welfare and mortality data were obtained from the National Death Registry. The
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53 273 earlier verified FINRISK classification ¹⁸ was used to classify the events. Therefore, the
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55 274 reliability of event diagnosis data is accurate and the classification is systematic. All subjects
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57 275 who had myocardial infarction or stroke before baseline were excluded because a history of
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3 276 myocardial infarction is known to increase the risk for recurrent myocardial infarction or
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5 277 cardiovascular death³⁰ and medication as well as lifestyle secondary prevention strategies are
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7 278 intensive³¹.
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14 280 In the present study, severe fatty liver predicted the risk for overall mortality of any causes
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16 281 when age, gender and study group were added covariates, a result in line with an earlier report
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18 282³². In the published literature, NASH rather than simple steatosis has been stated to be linked
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20 283 with decreased overall survival³³ although one study with a large cohort found no association
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22 284 between NAFLD and overall mortality²⁵. In our study, the association between severe fatty
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24 285 liver and total mortality disappeared after further adjustment for BMI which means that
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26 286 severe fatty liver is not a strong predictor for overall mortality. In earlier studies NAFLD,
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28 287 especially NASH, has been reported to increase the risk for cardiovascular death²⁷. In the
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30 288 present study, severe fatty liver disease did predict the risk for cardiovascular death but the
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32 289 association seemed to be dependent on insulin sensitivity.
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40 291 The molecular mechanisms linking fatty liver with CVD have been investigated^{10, 34}.
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42 292 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD¹⁰. In
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44 293 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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46 294 fatty acid (NEFA) metabolism⁷ and the increasing NEFA flux to the liver may impair liver
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48 295 metabolism leading to increased glucose metabolism and liver dysfunction⁶. The liver is one
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50 296 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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52 297 factors², such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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54 298 cytokines¹⁰. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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3 299 in atherogenesis ¹⁰ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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5 300 and tumor necrosis factor- α ⁷ leading to chronic systemic inflammation. In addition, hepatic
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7 301 steatosis leads to overproduction of cholesterol-rich remnant particles ³.
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13 303 One limitation in this study may be that the grade of liver brightness was measured by
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15 304 ultrasound. The invasive diagnostic technique of liver biopsy is regarded as the golden
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17 305 standard, especially for the diagnosis of NASH ³⁵. Real time ultrasound using a combination
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19 306 of sonographic findings does have a high specificity but it underestimates the prevalence of
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21 307 hepatic steatosis when there is less than 20 % fat ³⁶. Nonetheless, the noninvasive ultrasound
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23 308 method was chosen because taking liver biopsies from large groups of symptomless subjects
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25 309 would have been ethically unjustifiable.
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33 311 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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35 312 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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37 313 any selection bias.
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40 314 **Conclusions**

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44 315 Severe liver fat accumulation increases the risk of a future cardiovascular event and mortality
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46 316 to cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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48 317 insulin sensitivity. Fatty liver also predicts the risk for overall mortality. However,
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50 318 conventional cardiovascular disease risk factors seem to play a major role in developing death
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52 319 from all causes. It would be beneficial to investigate larger cohorts and follow-up studies in
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54 320 order to validate this result.
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322 Figure legend

323 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
324 with no fat in the liver, moderate fat accumulation and severe fat accumulation.

325 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
326 of the subjects (10/124) with moderate liver fat accumulation, while 12.5% of the subjects
327 with severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model
328 1): adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
329 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
330 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
331 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
332 * $p < 0.05$.

333

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338 cooperation in organizing cardiovascular event and mortality data.

339 References

340 **1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-**
341 **31.**

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3 342 2. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with
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5 343 nonalcoholic fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.
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8 344 3. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of
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10 345 cardiovascular disease. *Atherosclerosis* 2007; Apr;191(2):235-40.
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14 346 4. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart
15
16 347 disease and stroke statistics--2011 update: a report from the American Heart
17
18 348 Association. *Circulation* 2011; Feb 1;123(4):e18-e209.
19
20
21
22 349 5. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A.
23
24 350 European cardiovascular disease statistics, 2008 ed. European Heart Network; 2008.
25
26
27 351 6. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec
28
29 352 14;444(7121):881-7.
30
31
32
33 353 7. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with
34
35 354 cardiovascular disease. *Nature* 2006; Dec 14;444(7121):875-80.
36
37
38 355 8. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J*
39
40 356 *Med* 2005; Apr 21;352(16):1685-95.
41
42
43
44 357 9. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in
45
46 358 the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.
47
48
49 359 10. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-
50
51 360 alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;
52
53 361 Nov;51(11):1947-53.
54
55
56
57
58
59
60

- 1
2
3 362 11. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic
4
5 363 hepatic steatosis and its relation to increased plasma biomarkers of inflammation and
6
7 364 endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med*
8
9 365 2005; Oct;22(10):1354-8.
- 11
12
13 366 12. Wong VW, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, et al. Coronary
14
15 367 artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver
16
17 368 disease. *Gut* 2011; Dec;60(12):1721-7.
- 19
20
21 369 13. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty
22
23 370 liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler*
24
25 371 *Thromb Vasc Biol* 2005; May;25(5):1045-50.
- 27
28
29 372 14. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with
30
31 373 carotid atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
- 33
34 374 15. Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA.
35
36 375 Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control
37
38 376 subjects. *J Intern Med* 1999; Feb;245(2):163-74.
- 40
41
42 377 16. Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma
43
44 378 immunoglobulin (Ig) A and low IgG antibody titers to oxidized low-density lipoprotein
45
46 379 are associated with markers of glucose metabolism. *J Clin Endocrinol Metab* 2010;
47
48 380 May;95(5):2467-75.
- 50
51
52 381 17. Pisto P, Ukkola O, Santaniemi M, Kesaniemi YA. Plasma adiponectin--an
53
54 382 independent indicator of liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
- 55
56
57
58
59
60

- 1
2
3 383 18. Pajunen P, Jousilahti P, Borodulin K, Harald K, Tuomilehto J, Salomaa V. Body fat
4
5 384 measured by a near-infrared interactance device as a predictor of cardiovascular
6
7 385 events: the FINRISK'92 cohort. *Obesity (Silver Spring)* 2011; Apr;19(4):848-52.
8
9
10
11 386 19. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al.
12
13 387 Quantitative insulin sensitivity check index: a simple, accurate method for assessing
14
15 388 insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; Jul;85(7):2402-10.
16
17
18 389 20. Kauma H, Savolainen MJ, Rantala AO, Lilja M, Kervinen K, Reunanen A, et al.
19
20 390 Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in
21
22 391 middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt 1):1334-43.
23
24
25
26 392 21. Bessebinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia
27
28 393 influenced by alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
29
30
31
32 394 22. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al.
33
34 395 Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J*
35
36 396 *Gastroenterol* 2007; Mar 14;13(10):1579-84.
37
38
39 397 23. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty
40
41 398 Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol*
42
43 399 *Hepatol* 2012; Jun;10(6):646-50.
44
45
46
47 400 24. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen
48
49 401 TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut*
50
51 402 2004; May;53(5):750-5.
52
53
54
55
56
57
58
59
60

- 1
2
3 403 **25. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-**
4
5 404 **alcoholic fatty liver disease and mortality among US adults: prospective cohort study.**
6
7 405 ***BMJ* 2011; Nov 18;343:d6891.**
8
9
10
11 406 **26. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty**
12
13 407 **liver disease and risk of future cardiovascular events among type 2 diabetic patients.**
14
15 408 ***Diabetes* 2005; Dec;54(12):3541-6.**
16
17
18
19 409 **27. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and**
20
21 410 **incident cardiovascular disease: a narrative review and clinical perspective of**
22
23 411 **prospective data. *Hepatology* 2010; Sep;52(3):1156-61.**
24
25
26 412 **28. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-**
27
28 413 **glutamyltransferase as a risk factor for cardiovascular disease mortality: an**
29
30 414 **epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;**
31
32 415 **Oct 4;112(14):2130-7.**
33
34
35
36 416 **29. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a**
37
38 417 **new and important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.**
39
40
41
42 418 **30. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,**
43
44 419 **pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.**
45
46
47
48 420 **31. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary**
49
50 421 **prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr***
51
52 422 ***Cardiol Rep* 2011; Aug;13(4):287-95.**
53
54
55
56
57
58
59
60

- 1
2
3 423 32. Calori G, Lattuada G, Ragogna F, Garancini MP, Crosignani P, Villa M, et al. Fatty
4
5 424 liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology*
6
7 425 2011; Jul;54(1):145-52.
8
9
10 426 33. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al.
11
12 427 Decreased survival of subjects with elevated liver function tests during a 28-year follow-
13
14 428 up. *Hepatology* 2010; Feb;51(2):595-602.
15
16
17
18 429 34. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk.
19
20 430 *Curr Opin Cardiol* 2012; Jul;27(4):420-8.
21
22
23
24 431 35. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur*
25
26 432 *J Gastroenterol Hepatol* 2003; May;15(5):539-43.
27
28
29 433 36. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity
30
31 434 of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J*
32
33 435 *Hepatol* 2009; Dec;51(6):1061-7.
34
35
36
37 436
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p	p	p
					(0-1)	(1-2)	(0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
(mmol/L)							
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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439 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
440 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
441 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
442 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat accumulation	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat accumulation	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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3 446 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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5 447 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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7 448 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat accumulation and 29.2%
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9 449 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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11 450 with different degrees of adjustments are presented. Alcohol consumption was divided into
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13 451 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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15 452 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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17 453 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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19 454 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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21 455 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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23 456 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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25 457 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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27 458 insulin sensitivity check index. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.
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Final model	Cardiovascular event c-index (95% CI)	Binary R ²	
			467
			468
			469
Model 3	0.729 (0.706-0.776)	0.153	470
Model 4	0.720 (0.689-0.763)	0.144	471
			472
Model 5	0.717 (0.686-0.758)	0.138	473
			474
Model 1	0.698 (0.656-0.742)	0.133	

Table 3. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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3 484 **Contributor statement:** All authors fulfill all three of the ICMJE guidelines for authorship
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11 487 **Merja Santaniemi:** Data acquisition, statistical analysis and data interpretation, critical
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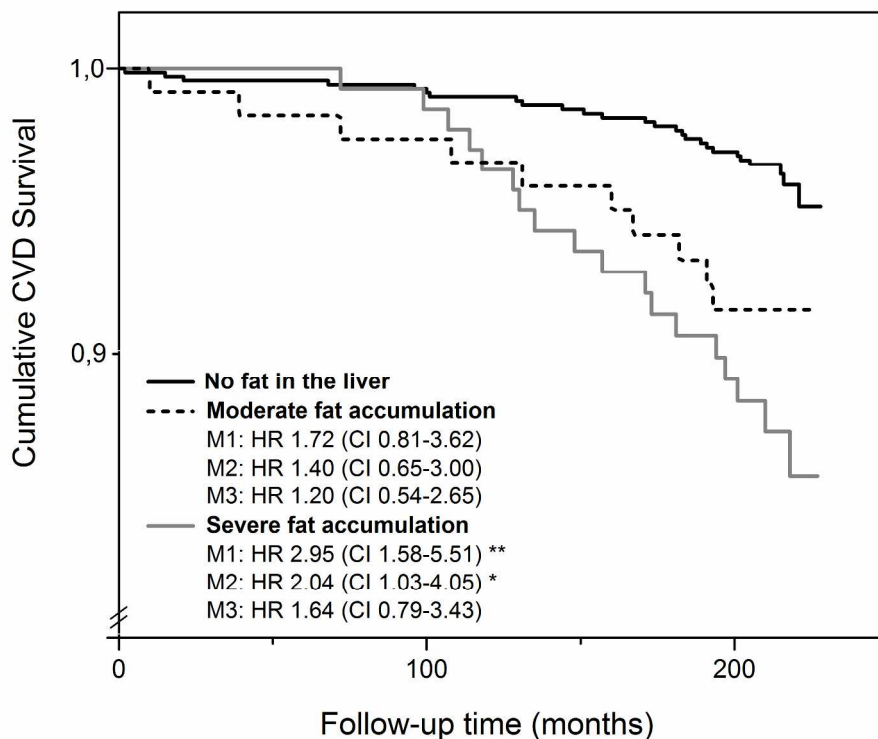
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37 496 **Data sharing statement:** Extra data is available by emailing pauliina.pisto(at)oulu.fi
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Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects with no fat in the liver, moderate fat accumulation and severe fat accumulation.

CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1% of the subjects (10/124) with moderate liver fat accumulation, while 12.5% of the subjects with severe fatty liver (18/144).

Cox regression analysis is used for adjustments. M1 (Model 1): adjusted for study group, age and gender.

M2 (Model 2): further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index. M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** p < 0.01, * p < 0.05.

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STROBE Statement

Strengthening the reporting of observational studies in epidemiology

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional)
Authors	Contact details for the corresponding author
Study design	Description of the study design (e.g cohort, case-control, cross sectional)
Objective	Specific objectives or hypothesis
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007).
Participants	<i>Cohort study</i> —Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up
	<i>Case-control study</i> —Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection
	<i>Cross-sectional study</i> —Give the eligibility criteria, and the major sources and methods of selection of participants
	<i>Cohort study</i> —For matched studies, give matching and number of exposed and unexposed
	<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	Clearly define primary outcome for this report.
Statistical methods	Describe statistical methods, including those used to control for confounding
Results	
Participants	Report Number of participants at the beginning and end of the study
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
	Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals)
Conclusions	General interpretation of study results



Fatty liver predicts the risk for cardiovascular events in middle-aged population: a population-based cohort study

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4 1 **Fatty liver predicts the risk for cardiovascular events in middle-aged population: a**
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3 21 **Disclosure summary:** Authors report no conflict of interests.
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7 23 **ABSTRACT**
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10 24 **Objective:** We investigated if the differences in liver fat content would predict the
11
12 25 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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14 26 stroke).
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16 27 **Design, setting and participants:** Our study group is a population-based, randomly recruited
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18 28 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.
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20 29 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
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22 30 registry of the National Institute for Health and Welfare and the National death registry.
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24 31 **Main outcome measure:** The severity of hepatic steatosis was measured by ultrasound and
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26 32 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.
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29 33 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
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31 34 24.2% of subjects having moderate liver fat content and 29.2% of the subjects having severe
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33 35 fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$). Severe
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35 36 liver fat content predicted the risk for future risk of cardiovascular event even when adjusted
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37 37 for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When further adjustments
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39 40 for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic blood pressure were
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41 41 conducted, the risk still remained statistically significant (HR 1.74, CI 1.16-2.63, $p < 0.01$).
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43 42 Statistical significance disappeared with further adjustment for QUICKI.
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46 47 **Conclusions:** Liver fat content increases the risk of future cardiovascular disease event in
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48 48 long-term follow-up but it seems to be dependent on insulin sensitivity.
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3 46 **Article focus**
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6 47 1 To investigate if the differences in liver fat content predict the risk for development of fatal
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8 48 or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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14 50 **Key messages**
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18 51 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
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20 52 compared to the subjects without fat in the liver
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23 53 2 Severe liver fat content increases the risk of a future cardiovascular event and mortality to
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25 54 cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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27 55 insulin sensitivity
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31 56 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
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33 57 traditional metabolic risk factors
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36 58 **Strengths and limitations of the study**
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39 59 1 This is a follow-up study with a large population-based study group and a very long follow-
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41 60 up time
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45 61 2 Official registers used in event diagnoses - data is accurate and the classification is
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47 62 systematic
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50 63 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
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52 64 sensitivity
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57 66 **Introduction**
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3 67 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
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5 68 content, which exists in a spectrum ranging from steatosis with no inflammation to non-
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7 69 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
8
9 70 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
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11 71 countries, being the leading cause of liver disorders ^{2,3}. It is associated with obesity, type 2
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13 72 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
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15 73 manifestation of the metabolic syndrome and both conditions share several risk factors for
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17 74 cardiovascular disease (CVD) ^{3,4}.
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25 76 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2% ⁵. Every
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27 77 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%) ⁶.
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29 78 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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31 79 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes ⁷. In
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33 80 addition, total body fatness as well as abdominal fat accumulation increase independently the
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35 81 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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37 82 adiposity to cardiovascular risk ⁸. Adipose tissue is now recognized as a significant endocrine
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39 83 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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41 84 mediators ⁷. Adipokines, proinflammatory cytokines and hypofibrinolytic markers may lead to
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43 85 oxidative stress and endothelial dysfunction, finally leading to atherosclerosis ⁹.
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51 87 Hepatic steatosis has been discussed as a possible mechanism to explain CVD morbidity and
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53 88 mortality ¹⁰. NAFLD patients have been reported to have higher coronary heart disease (CHD)
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55 89 risk than the general population of the same age and gender ¹¹. According to previous study,
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3 90 liver dysfunction associated with CVD mortality in men¹² whereas another large study found
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5 91 no association between NAFLD and CVD in general population¹³. In addition, fatty liver did
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7 92 not predict CVD mortality and morbidity in patients with established coronary artery disease
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9 93¹⁴.

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16 95 The NAFLD and CVD share several molecular mechanisms^{15,16}. Fatty liver might play a part
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18 96 in the pathogenesis of CVD through the overexpression and systemic release of several
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20 97 inflammatory, hemostatic¹⁷ and oxidative-stress mediators or via contributing to whole-body
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22 98 insulin resistance and atherogenic dyslipidemia³. NAFLD has also been reported to be linked
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24 99 with circulatory endothelial dysfunction^{4,14}. Several investigators have reported that NAFLD
25
26 100 is associated with coronary artery disease^{4,14} and increased carotid intima-media thickness¹⁸,
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28 101¹⁹. Increased gamma-glutamyltransferase (GGT), which may be a marker of NAFLD, has
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30 102 been reported to be associated with stroke²⁰.

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38 104 It is known that subjects with fatty liver disease have an increased risk of suffering CVD⁴, but
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40 105 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
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42 106 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
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44 107 CVD. The aim of our study was to investigate if fatty liver could predict independently the
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46 108 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
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48 109 follow-up after adjusting for all known conventional risk factors.

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56 111 **Materials and methods**

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3 112 **Human subjects**
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6 113 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
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8 114 epidemiological prospective cohort study designed to address the risk factors and disease end
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10 115 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
11
12 116 been described earlier ²¹. In short, a total of 520 men and 525 women participated: 259 control
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14 117 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
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16 118 Hypertensive participants were randomly selected from the national register for
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18 119 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
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20 120 age- and sex-matched control subject was randomly selected from the same register. Informed
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22 121 consent in writing was obtained from each patient. The study protocol conformed to the
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24 122 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
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26 123 Ethical Committee of the Faculty of Medicine, University of Oulu.
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35 125 **Determination of hepatic steatosis**
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38 126 The determination of hepatic steatosis was based on liver-kidney contrast measured with
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40 127 ultrasonography ²² by one trained radiologist with 10 years' experience in abdominal
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42 128 ultrasound examinations. The severity of hepatic steatosis was based on the brightness of the
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44 129 liver and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating
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46 130 a non-fatty liver, 1 = medium bright, a moderate lipid content and 2 = clearly bright, a severe
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48 131 lipid content and fatty liver) ²³.
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55 133 **Follow-up**
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3 134 Both the hypertensive and the control men were recruited during December 1990 to May
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5 135 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
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7 136 liver ultrasound result available at baseline. Mortality data were obtained from the National
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9 137 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
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11 138 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
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13 139 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
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15 140 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
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17 141 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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25 143 CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage, SAH) -
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27 144 whichever of these happened first ²⁴. The evidence of CHD was based on the following
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29 145 diagnosis: I20.0, I21, I22 [ICD-10, International Statistical Classification of Diseases and
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31 146 Related Health Problems] / 410, 4110 [ICD-8/9] as the main diagnosis (symptom or cause)
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33 147 and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis (symptom or cause) or second
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35 148 side diagnosis (symptom or cause) and third side diagnosis (ICD-8/9 only) or if a subject had
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37 149 undergone coronary artery bypass graft (CABG) surgery or angioplasty. CHD as a cause of
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39 150 death included I20–I25, I46, R96, R98 [ICD-10] / 410-414, 798 (not 7980A) [ICD-8/9] as the
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41 151 underlying cause of death or immediate cause of death and I21 or I22 [ICD-10] / 410 [ICD-
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43 152 8/9] as first to third contributing cause of death. Stroke (excluding SAH) included I61, I63
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45 153 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436 [ICD-9] /
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47 154 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as main diagnosis (symptom or cause) or as
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49 155 a first or second side diagnosis (symptom or cause) or as a third side diagnosis (ICD-8/9 only)
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51 156 or as an underlying cause of death or immediate cause of death or as a first to third
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53 157 contributing cause of death ²⁵.
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6 159 **Laboratory analyses**
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9 160 Waist circumference, body mass index (BMI) and blood pressure were measured as described
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11 161 in previous study ²¹.
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18 163 All the laboratory test samples were obtained after an overnight fast. Blood insulin and
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20 164 glucose concentrations were analyzed at 0, 60, and 120 min after administration of 75 g
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22 165 glucose ²³. Insulin sensitivity was assessed using fasting plasma insulin concentrations and a
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24 166 quantitative insulin sensitivity check index (QUICKI) {QUICKI=1/[log (fasting insulin)+log
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26 167 (fasting glucose)]} ²⁶.
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33 169 Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density
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35 170 lipoprotein (LDL) and hs-CRP concentrations ²³ as well as alanine aminotransferase (ALT)
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37 171 and GGT levels were measured as described previously ²². Alcohol consumption and smoking
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39 172 history were determined by validated questionnaires ²⁷. Alcohol consumption was divided into
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41 173 three groups: 0 (n=161) mean alcohol consumption less than 1g/week in men and women, 1
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43 174 (n=767) mean consumption less than 210g/week in men and less than 140 g/week in women,
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45 175 2 (n=76) mean alcohol consumption more than 210g/week in men and more than 140g/week
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47 176 in women. Group 2 designates large-scale alcohol consumers according to the guidelines ²⁸.
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55 178 **Statistical analysis**
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3 179 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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5 180 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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7 181 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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9 182 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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11 183 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
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13 184 investigate if liver brightness (fat) could predict the future risk for total mortality,
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15 185 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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22 187 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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24 188 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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26 189 improve normality before analysis of variance. We used three models with progressive
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28 190 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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30 191 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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32 192 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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34 193 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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36 194 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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38 195 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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40 196 when further adjustment with one covariate was performed. Model 4 included variables which
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42 197 were stable and were statistically significant in intermediate phases. Model 5 included stable
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44 198 and significant covariates without QUICKI (Table 2).
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53 200 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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55 201 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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57 202 to obtain 95% CI for c-index of each model.
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6 204 **Results**
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9 205 The main baseline characteristics of the study group are shown in Table 1.
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16 207 *Table 1 about here*
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22 209 **Incidence of cardiovascular disease**
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25 210 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
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27 211 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
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29 212 moderate liver fat content and 29.2% (42/144) of the subjects having severe fatty liver
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31 213 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
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33 214 with non-fatty liver (26/720) and 8.1% of the subjects with moderate liver fat content
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35 215 (10/124), while 12.5% (18/144) of the subjects with severe fatty liver ($p < 0.001$).
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43 217 Severe liver fat content predicted the risk for future risk of cardiovascular event when
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45 218 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
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47 219 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
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49 220 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
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51 221 statistically significant ($p < 0.01$). Statistical significance disappeared when further
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53 222 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
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55 223 CVD event sensitivity analyses, all covariates were added one by one and it was examined
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3 224 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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5 225 significant variables (including quick index) in the sensitivity analyses, the association
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7 226 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18,
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9 227 $p=0.10$). When QUICKI was not added into Model 5, severe fatty liver did predict the risk for
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11 228 future risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index
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14 229 decreased when the risk factors were removed from the model (Table 3).

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20 231 *Tables 2 and 3 about here*

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27 233 The future risk of death from CVD in participants with severe fat content was significant
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29 234 when age, gender and study group were added as covariates (Model 1: HR 2.95, CI 1.58-5.51,
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31 235 $p < 0.01$). Even after further adjustments with other conventional risk factors (Model 2: HR
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33 236 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI was added as
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35 237 the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43, NS) (Fig 1.).

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42 239 *Figure 1 about here*

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46 47 48 241 **Fatty liver and total mortality**

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51 242 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
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53 243 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
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55 244 0.01). According to Model 1, severe fat content predicted the risk for mortality from all
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3 245 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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5 246 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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7 247 not shown).
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13 249 We performed all Cox regression analyses after excluding the men consuming more than 210
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15 250 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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17 251 have any effect on the results (data not shown).
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21 252 We performed all Cox regression analyses after excluding patients with insulin treated
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23 253 diabetes mellitus (n=9), cortisone treatment at baseline (n=41) and previous diagnosis for
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25 254 liver disease (n=15) (e.g., virus, medications). This exclusion did not have any effect on the
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27 255 results (data not shown).
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30 256 **Discussion**

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34 257 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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36 258 continuously increasing in the Western world. The question if NAFLD is only a marker or
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38 259 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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40 260 results of the present study, which had an approximately 19-year follow-up fatty liver does
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42 261 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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44 262 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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46 263 development of cardiovascular events.
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53 265 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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55 266 with ultrasound-diagnosed fatty liver^{29, 30} and larger studies with longer follow-up times are
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57 267 needed. An association between NAFLD and CVD has been reported^{3, 29-31} although contrary
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3 268 results also exist ^{13, 32}. An association between ultrasound-diagnosed fatty liver and CVD has
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5 269 been reported in general population ²⁹ and in subjects with T2DM ³¹. Furthermore, liver
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7 270 dysfunction has been reported to associate with CVD mortality ^{33, 34} and CHD risk ¹¹ in
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10 271 follow-up studies and especially survival of subjects with NASH is reported to be reduced ^{32,}
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12 272 ^{35, 36}. In the present study, severe fatty liver disease did predict the risk for cardiovascular
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14 273 death but the association seemed to be dependent on insulin sensitivity.

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19 275 Several earlier studies have used self-reported CVD history which may not be totally reliable.
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21 276 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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23 277 liver have performed some adjustments, the full range of well-known CVD risk factors have
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25 278 been rarely considered ³². We have performed adjustments with all so-called traditional risk
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27 279 factors for cardiovascular disease (i.e. age, gender, smoking, LDL concentration,
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29 280 hypertension, insulin resistance). Previous studies have used biochemical, radiological and
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31 281 histological methodology for NAFLD diagnosis and staging, which leads to a challenging
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33 282 interpretation of the results ^{34, 37}.

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40 284 This study had an approximately 19-year follow-up time, which is longer than in previous
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42 285 studies ¹¹⁻¹⁴. When compared to earlier studies ^{32, 37} this study seems to be the first follow-up
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44 286 study with a large population-based randomly selected study group and a very long follow-up
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46 287 time and ultrasound-diagnosed fatty liver. The diagnosis of cardiovascular events was based
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48 288 on the registry of the National Institute for Health and Welfare and mortality data were
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50 289 obtained from the National Death Registry. The earlier verified FINRISK classification ²⁵ was
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52 290 used to classify the events. Therefore, the reliability of event diagnosis data is accurate and
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54 291 the classification is systematic. All subjects who had myocardial infarction or stroke before
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3 292 baseline were excluded because a history of myocardial infarction is known to increase the
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5 293 risk for recurrent myocardial infarction or cardiovascular death³⁸ and medication as well as
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7 294 lifestyle secondary prevention strategies are intensive³⁹.
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13 296 There are a few follow-up-studies examining whether the fatty liver increases the risk for total
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15 297 mortality^{13,40}. In the present study, severe fatty liver predicted the risk for overall mortality of
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17 298 any causes when age, gender and study group were added covariates, a result in line with an
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19 299 earlier report⁴¹. In the published literature, NASH rather than simple steatosis has been stated
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21 300 to be linked with decreased overall survival³⁵ although one study with a large cohort found no
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23 301 association between NAFLD and overall mortality¹³. In our study, the association between
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25 302 severe fatty liver and total mortality disappeared after further adjustment for BMI which
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27 303 means that severe fatty liver is not a strong predictor for overall mortality.
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35 305 The molecular mechanisms linking fatty liver with CVD have been investigated^{10,16}.
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37 306 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD¹⁶. In
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39 307 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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41 308 fatty acid (NEFA) metabolism⁸ and the increasing NEFA flux to the liver may impair liver
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43 309 metabolism leading to increased glucose metabolism and liver dysfunction⁷. The liver is one
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45 310 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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47 311 factors³, such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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49 312 cytokines¹⁶. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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51 313 in atherogenesis¹⁶ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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3 314 and tumor necrosis factor- α ⁸ leading to chronic systemic inflammation. In addition, hepatic
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5 315 steatosis leads to overproduction of cholesterol-rich remnant particles⁴.
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9
10 317 One limitation in this study is that the grade of liver brightness was measured by ultrasound.
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12 318 The invasive diagnostic technique of liver biopsy is regarded as the “golden standard”,
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14 319 especially for the diagnosis of NASH⁴². Real time ultrasound using a combination of
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16 320 sonographic findings does have a high specificity but it underestimates the prevalence of
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18 321 hepatic steatosis when there is less than 20 % fat⁴³. Today, magnetic resonance spectroscopy
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20 322 is regarded as the best method for the quantification of liver fat, but this method is limited due
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22 323 to its availability⁴⁴. Nonetheless, the noninvasive ultrasound method was chosen because
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24 324 taking liver biopsies from large groups of symptomless subjects would have been ethically
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26 325 unjustifiable and magnetic resonance spectroscopy was not available at the baseline.
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35 327 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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37 328 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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39 329 any selection bias.
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41 42 330 **Conclusions**

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45 331 Severe liver fat content increased the risk of a future cardiovascular event and mortality to
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47 332 cardiovascular disease over the long-term follow-up but it seemed to be dependent on insulin
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49 333 sensitivity. Fatty liver also predicted the risk for overall mortality. However, conventional
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51 334 cardiovascular disease risk factors seemed to play a major role in developing death from all
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53 335 causes. It would be beneficial to investigate larger cohorts and follow-up studies in order to
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55 336 validate this result.
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56 338 **Figure legend**
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9 339 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
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11 340 with no fat in the liver, moderate fat content and severe fat content.
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14 341 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
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16 342 of the subjects (10/124) with moderate liver fat content, while 12.5% of the subjects with
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18 343 severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model 1):
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20 344 adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
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22 345 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
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24 346 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
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26 347 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
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28 348 * $p < 0.05$.
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40
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42
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45 354 cooperation in organizing cardiovascular event and mortality data.
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6
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8
9 361 thanked for the cooperation in organizing cardiovascular event and mortality data.

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12
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16 363 **Competing Interests** None

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377 **References**

378 **1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-**
379 **31.**

380 **2. Armstrong MJ, Houlihan DD, Bentham L, Shaw JC, Cramb R, Olliff S, et al.**
381 **Presence and severity of non-alcoholic fatty liver disease in a large prospective primary**
382 **care cohort. *J Hepatol* 2012; Jan;56(1):234-40.**

383 **3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with**
384 **nonalcoholic fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.**

385 **4. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of**
386 **cardiovascular disease. *Atherosclerosis* 2007; Apr;191(2):235-40.**

387 **5. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart**
388 **disease and stroke statistics--2011 update: a report from the American Heart**
389 **Association. *Circulation* 2011; Feb 1;123(4):e18-e209.**

390 **6. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A.**
391 **European cardiovascular disease statistics, 2008 ed. European Heart Network; 2008.**

392 **7. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec**
393 **14;444(7121):881-7.**

394 **8. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with**
395 **cardiovascular disease. *Nature* 2006; Dec 14;444(7121):875-80.**

- 1
2
3 396 **9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J***
4
5 397 ***Med* 2005; Apr 21;352(16):1685-95.**
6
7
8 398 **10. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk.**
9
10 399 ***Curr Opin Cardiol* 2012; Jul;27(4):420-8.**
11
12
13
14 400 **11. Treeprasertsuk S, Leverage S, Adams LA, Lindor KD, St Sauver J, Angulo P. The**
15
16 401 **Framingham risk score and heart disease in nonalcoholic fatty liver disease. *Liver Int***
17
18 402 **2012; Jul;32(6):945-50.**
19
20
21
22 403 **12. Haring R, Wallaschofski H, Nauck M, Dorr M, Baumeister SE, Volzke H.**
23
24 404 **Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated**
25
26 405 **serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; Nov;50(5):1403-11.**
27
28
29
30 406 **13. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-**
31
32 407 **alcoholic fatty liver disease and mortality among US adults: prospective cohort study.**
33
34 408 ***BMJ* 2011; Nov 18;343:d6891.**
35
36
37 409 **14. Wong VW, Wong GL, Yip GW, Lo AO, Limquiao J, Chu WC, et al. Coronary**
38
39 410 **artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver**
40
41 411 **disease. *Gut* 2011; Dec;60(12):1721-7.**
42
43
44
45 412 **15. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in**
46
47 413 **the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.**
48
49
50
51 414 **16. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-**
52
53 415 **alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;**
54
55 416 **Nov;51(11):1947-53.**
56
57
58
59
60

- 1
2
3 417 17. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic
4
5 418 hepatic steatosis and its relation to increased plasma biomarkers of inflammation and
6
7 419 endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med*
8
9 420 2005; Oct;22(10):1354-8.
- 11
12
13 421 18. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty
14
15 422 liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler*
16
17 423 *Thromb Vasc Biol* 2005; May;25(5):1045-50.
- 19
20
21 424 19. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with
22
23 425 carotid atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
- 24
25
26 426 20. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-
27
28 427 glutamyltransferase is associated with incident vascular events independently of alcohol
29
30 428 intake: analysis of the British Women's Heart and Health Study and Meta-Analysis.
31
32 429 *Arterioscler Thromb Vasc Biol* 2007; Dec;27(12):2729-35.
- 34
35
36 430 21. Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA.
37
38 431 Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control
39
40 432 subjects. *J Intern Med* 1999; Feb;245(2):163-74.
- 42
43
44 433 22. Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma
45
46 434 immunoglobulin (Ig) A and low IgG antibody titers to oxidized low-density lipoprotein
47
48 435 are associated with markers of glucose metabolism. *J Clin Endocrinol Metab* 2010;
49
50 436 May;95(5):2467-75.
- 52
53
54 437 23. Pisto P, Ukkola O, Santaniemi M, Kesaniemi YA. Plasma adiponectin--an
55
56 438 independent indicator of liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
57
58
59
60

- 1
2
3 439 24. Santaniemi M., Ukkola O., Malo E., Bloigu R., Kesaniemi YA. Metabolic syndrome
4
5 440 in the prediction of cardiovascular events: The potential additive role of hsCRP and
6
7 441 adiponectin. *Eur J Prev Cardiol* 2013; Jun 20.
- 8
9
10 442 25. Pajunen P, Jousilahti P, Borodulin K, Harald K, Tuomilehto J, Salomaa V. Body fat
11
12 443 measured by a near-infrared interactance device as a predictor of cardiovascular
13
14 444 events: the FINRISK'92 cohort. *Obesity (Silver Spring)* 2011; Apr;19(4):848-52.
- 15
16
17
18 445 26. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al.
19
20 446 Quantitative insulin sensitivity check index: a simple, accurate method for assessing
21
22 447 insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; Jul;85(7):2402-10.
- 23
24
25
26 448 27. Kauma H, Savolainen MJ, Rantala AO, Lilja M, Kervinen K, Reunanen A, et al.
27
28 449 Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in
29
30 450 middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt 1):1334-43.
- 31
32
33
34 451 28. Bessebinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia
35
36 452 influenced by alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
- 37
38
39
40 453 29. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al.
41
42 454 Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J*
43
44 455 *Gastroenterol* 2007; Mar 14;13(10):1579-84.
- 45
46
47 456 30. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty
48
49 457 Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol*
50
51 458 *Hepatol* 2012; Jun;10(6):646-50.
- 52
53
54
55 459

- 1
2
3 460 **31. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty**
4
5 461 **liver disease and risk of future cardiovascular events among type 2 diabetic patients.**
6
7 462 ***Diabetes* 2005; Dec;54(12):3541-6.**
8
9
10
11 463 **32. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and**
12
13 464 **incident cardiovascular disease: a narrative review and clinical perspective of**
14
15 465 **prospective data. *Hepatology* 2010; Sep;52(3):1156-61.**
16
17
18 466 **33. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected**
19
20 467 **nonalcoholic fatty liver disease and mortality risk in a population-based cohort study.**
21
22 468 ***Am J Gastroenterol* 2008; Sep;103(9):2263-71.**
23
24
25
26 469 **34. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-**
27
28 470 **glutamyltransferase as a risk factor for cardiovascular disease mortality: an**
29
30 471 **epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;**
31
32 472 **Oct 4;112(14):2130-7.**
33
34
35
36 473 **35. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al.**
37
38 474 **Decreased survival of subjects with elevated liver function tests during a 28-year follow-**
39
40 475 **up. *Hepatology* 2010; Feb;51(2):595-602.**
41
42
43
44 476 **36. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et**
45
46 477 **al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology***
47
48 478 **2006; Oct;44(4):865-73.**
49
50
51
52 479 **37. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a**
53
54 480 **new and important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.**
55
56
57
58
59
60

- 1
2
3 481 **38. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,**
4
5 482 **pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.**
6
7
8
9 483 **39. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary**
10
11 484 **prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr***
12
13 485 ***Cardiol Rep* 2011; Aug;13(4):287-95.**
14
15
16 486 **40. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen**
17
18 487 **TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut***
19
20 488 **2004; May;53(5):750-5.**
21
22
23
24 489 **41. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty**
25
26 490 **liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology***
27
28 491 **2011; Jul;54(1):145-52.**
29
30
31
32 492 **42. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur***
33
34 493 ***J Gastroenterol Hepatol* 2003; May;15(5):539-43.**
35
36
37
38 494 **43. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity**
39
40 495 **of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J***
41
42 496 ***Hepatol* 2009; Dec;51(6):1061-7.**
43
44
45
46 497 **44. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et**
47
48 498 **al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence**
49
50 499 **of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;**
51
52 500 **Feb;288(2):E462-8.**
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p	p	p
					(0-1)	(1-2)	(0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
(mmol/L)							
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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504 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
505 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
506 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
507 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat content	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat content	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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3 511 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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5 512 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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7 513 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat content and 29.2%
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9 514 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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11 515 with different degrees of adjustments are presented. Alcohol consumption was divided into
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13 516 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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15 517 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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17 518 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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19 519 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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21 520 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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23 521 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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25 522 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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27 523 insulin sensitivity check index. *** p < 0.001, ** p < 0.01, * p < 0.05.
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Final model	Cardiovascular event c-index (95% CI)	Binary R ²	
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			534
Model 3	0.729 (0.706-0.776)	0.153	535
Model 4	0.720 (0.689-0.763)	0.144	536
			537
Model 5	0.717 (0.686-0.758)	0.138	538
			539
Model 1	0.698 (0.656-0.742)	0.133	

Table 3. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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For peer review only

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4 1 **Fatty liver predicts the risk for cardiovascular events in middle-aged population: a**
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6 2 **population-based cohort study**
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58 20 Research, dated 16 Apr, 2012.
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3 21 **Disclosure summary:** Authors report no conflict of interests.
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7 23 **ABSTRACT**
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10 24 **Objective:** We investigated if the differences in liver fat content would predict the
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12 25 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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14 26 stroke).
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16 27 **Design, setting and participants:** Our study group is a population-based, randomly recruited
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18 28 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.
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21 29 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
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23 30 registry of the National Institute for Health and Welfare and the National death registry.
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25 31 **Main outcome measure:** The severity of hepatic steatosis was measured by ultrasound and
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27 32 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.
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30 33 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
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32 34 24.2% of subjects having moderate liver fat content and 29.2% of the subjects having severe
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34 35 fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$). Severe
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36 36 liver fat content predicted the risk for future risk of cardiovascular event even when adjusted
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38 37 for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When further adjustments
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40 38 for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic blood pressure were
41
42 39 conducted, the risk still remained statistically significant (HR 1.74, CI 1.16-2.63, $p < 0.01$).
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44 40 Statistical significance disappeared with further adjustment for QUICKI.
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47 41 **Conclusions:** Liver fat content increases the risk of future cardiovascular disease event in
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49 42 long-term follow-up but it seems to be dependent on insulin sensitivity.
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3 46 **Article focus**
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6 47 1 To investigate if the differences in liver fat content predict the risk for development of fatal
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8 48 or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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14 50 **Key messages**
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18 51 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
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20 52 compared to the subjects without fat in the liver
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23 53 2 Severe liver fat content increases the risk of a future cardiovascular event and mortality to
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25 54 cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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27 55 insulin sensitivity
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31 56 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
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33 57 traditional metabolic risk factors
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36 58 **Strengths and limitations of the study**
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39 59 **1 This is a follow-up study with** a large population-based study group and a very long follow-
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41 60 up time
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45 61 2 Official registers used in event diagnoses - data is accurate and the classification is
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47 62 systematic
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50 63 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
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52 64 sensitivity
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57 66 **Introduction**
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3 67 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
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5 68 content, which exists in a spectrum ranging from steatosis with no inflammation to non-
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7 69 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
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10 70 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
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12 71 countries, being the leading cause of liver disorders ^{2, 3}. It is associated with obesity, type 2
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14 72 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
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16 73 manifestation of the metabolic syndrome and both conditions share several risk factors for
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18 74 cardiovascular disease (CVD) ^{3, 4}.

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25 76 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2% ⁵. Every
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27 77 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%) ⁶.
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29 78 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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31 79 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes ⁷. In
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33 80 addition, total body fatness as well as abdominal fat accumulation increase independently the
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35 81 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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37 82 adiposity to cardiovascular risk ⁸. Adipose tissue is now recognized as a significant endocrine
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39 83 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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41 84 mediators ⁷. Adipokines, proinflammatory cytokines and hypofibrinolytic markers may lead to
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43 85 oxidative stress and endothelial dysfunction, finally leading to atherosclerosis ⁹.

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51 87 Hepatic steatosis has been discussed as a possible mechanism to explain CVD morbidity and
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53 88 mortality ¹⁰. NAFLD patients have been reported to have higher coronary heart disease (CHD)
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55 89 risk than the general population of the same age and gender ¹¹. According to previous study,

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3 90 liver dysfunction associated with CVD mortality in men¹² whereas another large study found
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5 91 no association between NAFLD and CVD in general population¹³. In addition, fatty liver did
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7 92 not predict CVD mortality and morbidity in patients with established coronary artery disease
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16 95 The NAFLD and CVD share several molecular mechanisms^{15,16}. Fatty liver might play a part
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18 96 in the pathogenesis of CVD through the overexpression and systemic release of several
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20 97 inflammatory, hemostatic¹⁷ and oxidative-stress mediators or via contributing to whole-body
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22 98 insulin resistance and atherogenic dyslipidemia³. NAFLD has also been reported to be linked
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24 99 with circulatory endothelial dysfunction^{4,14}. Several investigators have reported that NAFLD
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26 100 is associated with coronary artery disease^{4,14} and increased carotid intima-media thickness¹⁸,
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28 101¹⁹. Increased gamma-glutamyltransferase (GGT), which may be a marker of NAFLD, has
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30 102 been reported to be associated with stroke²⁰.

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38 104 It is known that subjects with fatty liver disease have an increased risk of suffering CVD⁴, but
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40 105 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
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42 106 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
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44 107 CVD. The aim of our study was to investigate if fatty liver could predict independently the
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46 108 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
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48 109 follow-up after adjusting for all known conventional risk factors.

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56 111 **Materials and methods**

112 **Human subjects**

113 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
114 epidemiological prospective cohort study designed to address the risk factors and disease end
115 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
116 been described earlier ²¹. In short, a total of 520 men and 525 women participated: 259 control
117 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
118 Hypertensive participants were randomly selected from the national register for
119 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
120 age- and sex-matched control subject was randomly selected from the same register. Informed
121 consent in writing was obtained from each patient. The study protocol conformed to the
122 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
123 Ethical Committee of the Faculty of Medicine, University of Oulu.

124

125 **Determination of hepatic steatosis**

126 The determination of **hepatic steatosis** was based on liver-kidney contrast measured with
127 ultrasonography ²² by one trained radiologist **with 10 years' experience in abdominal**
128 **ultrasound examinations**. The severity of **hepatic steatosis** was based on the brightness of the
129 liver and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating
130 a non-fatty liver, 1 = medium bright, a moderate lipid content and 2 = clearly bright, a severe
131 lipid content and fatty liver) ²³.

132

133 **Follow-up**

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3 134 Both the hypertensive and the control men were recruited during December 1990 to May
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5 135 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
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7 136 liver ultrasound result available at baseline. Mortality data were obtained from the National
8
9 137 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
10
11 138 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
12
13 139 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
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15 140 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
16
17 141 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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25 143 CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage, SAH) -
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27 144 whichever of these happened first²⁴. The evidence of CHD was based on the following
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29 145 diagnosis: I20.0, I21, I22 [ICD-10, International Statistical Classification of Diseases and
30
31 146 Related Health Problems] / 410, 4110 [ICD-8/9] as the main diagnosis (symptom or cause)
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33 147 and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis (symptom or cause) or second
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35 148 side diagnosis (symptom or cause) and third side diagnosis (ICD-8/9 only) or if a subject had
36
37 149 undergone coronary artery bypass graft (CABG) surgery or angioplasty. CHD as a cause of
38
39 150 death included I20–I25, I46, R96, R98 [ICD-10] / 410-414, 798 (not 7980A) [ICD-8/9] as the
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41 151 underlying cause of death or immediate cause of death and I21 or I22 [ICD-10] / 410 [ICD-
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43 152 8/9] as first to third contributing cause of death. Stroke (excluding SAH) included I61, I63
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45 153 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436 [ICD-9] /
46
47 154 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as main diagnosis (symptom or cause) or as
48
49 155 a first or second side diagnosis (symptom or cause) or as a third side diagnosis (ICD-8/9 only)
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51 156 or as an underlying cause of death or immediate cause of death or as a first to third
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53 157 contributing cause of death²⁵.
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Laboratory analyses

Waist circumference, body mass index (BMI) and blood pressure were measured as described in previous study ²¹.

162

All the laboratory test samples were obtained after an overnight fast. Blood insulin and glucose concentrations were analyzed at 0, 60, and 120 min after administration of 75 g glucose ²³. Insulin sensitivity was assessed using fasting plasma insulin concentrations and a quantitative insulin sensitivity check index (QUICKI) $\{QUICKI=1/[\log(\text{fasting insulin})+\log(\text{fasting glucose})]\}$ ²⁶.

168

Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and hs-CRP concentrations ²³ as well as alanine aminotransferase (ALT) and GGT levels were measured as described previously ²². Alcohol consumption and smoking history were determined by validated questionnaires ²⁷. Alcohol consumption was divided into three groups: 0 (n=161) mean alcohol consumption less than 1g/week in men and women, 1 (n=767) mean consumption less than 210g/week in men and less than 140 g/week in women, 2 (n=76) mean alcohol consumption more than 210g/week in men and more than 140g/week in women. Group 2 designates large-scale alcohol consumers according to the guidelines ²⁸.

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Statistical analysis

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3 179 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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5 180 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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7 181 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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9 182 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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11 183 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
12
13 184 investigate if liver brightness (fat) could predict the future risk for total mortality,
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15 185 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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22 187 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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24 188 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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26 189 improve normality before analysis of variance. We used three models with progressive
27
28 190 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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30 191 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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32 192 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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34 193 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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36 194 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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38 195 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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40 196 when further adjustment with one covariate was performed. Model 4 included variables which
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42 197 were stable and were statistically significant in intermediate phases. Model 5 included stable
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44 198 and significant covariates without QUICKI (Table 2).
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53 200 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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55 201 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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57 202 to obtain 95% CI for c-index of each model.
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204 Results

205 The main baseline characteristics of the study group are shown in Table 1.

206

207 *Table 1 about here*

208

209 Incidence of cardiovascular disease

210 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
211 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
212 moderate liver fat content and 29.2% (42/144) of the subjects having severe fatty liver
213 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
214 with non-fatty liver (26/720) and 8.1% of the subjects with moderate liver fat content
215 (10/124), while 12.5% (18/144) of the subjects with severe fatty liver ($p < 0.001$).

216

217 Severe liver fat content predicted the risk for future risk of cardiovascular event when
218 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
219 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
220 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
221 statistically significant ($p < 0.01$). Statistical significance disappeared when further
222 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
223 CVD event sensitivity analyses, all covariates were added one by one and it was examined

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3 224 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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5 225 significant variables (including quick index) in the sensitivity analyses, the association
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7 226 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18,
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9 227 $p=0.10$). When QUICKI was not added into Model 5, severe fatty liver did predict the risk for
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11 228 future risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index
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13 229 decreased when the risk factors were removed from the model (Table 3).
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231 *Tables 2 and 3 about here*

232

233 The future risk of death from CVD in participants with severe fat content was significant
234 when age, gender and study group were added as covariates (Model 1: HR 2.95, CI 1.58-5.51,
235 $p < 0.01$). Even after further adjustments with other conventional risk factors (Model 2: HR
236 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI was added as
237 the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43, NS) (Fig 1.).
238

238

239 *Figure 1 about here*

240

241 **Fatty liver and total mortality**

242 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
243 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
244 0.01). According to Model 1, severe fat content predicted the risk for mortality from all

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3 245 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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5 246 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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7 247 not shown).
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13 249 We performed all Cox regression analyses after excluding the men consuming more than 210
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15 250 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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17 251 have any effect on the results (data not shown).
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21 252 We performed all Cox regression analyses after excluding patients with insulin treated
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23 253 diabetes mellitus (n=9), cortisone treatment at baseline (n=41) and previous diagnosis for
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25 254 liver disease (n=15) (e.g., virus, medications). This exclusion did not have any effect on the
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27 255 results (data not shown).
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30 31 256 **Discussion**

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34 257 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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36 258 continuously increasing in the Western world. The question if NAFLD is only a marker or
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38 259 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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40 260 results of the present study, which had an approximately 19-year follow-up fatty liver does
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42 261 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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44 262 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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46 263 development of cardiovascular events.
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53 265 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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55 266 with ultrasound-diagnosed fatty liver^{29, 30} and **larger studies with longer follow-up times are**
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57 267 **needed**. An association between NAFLD and CVD has been reported^{3, 29-31} **although contrary**
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3 268 results also exist^{13, 32}. An association between ultrasound-diagnosed fatty liver and CVD has
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5 269 been reported in general population²⁹ and in subjects with T2DM³¹. Furthermore, liver
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7 270 dysfunction has been reported to associate with CVD mortality^{33, 34} and CHD risk¹¹ in
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9 271 follow-up studies and especially survival of subjects with NASH is reported to be reduced^{32,}
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11 272^{35, 36}. In the present study, severe fatty liver disease did predict the risk for cardiovascular
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13 273 death but the association seemed to be dependent on insulin sensitivity.
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23 275 Several earlier studies have used self-reported CVD history which may not be totally reliable.
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25 276 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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27 277 liver have performed some adjustments, the full range of well-known CVD risk factors have
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29 278 been rarely considered³². We have performed adjustments with all so-called traditional risk
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31 279 factors for cardiovascular disease (i.e. age, gender, smoking, LDL concentration,
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33 280 hypertension, insulin resistance). Previous studies have used biochemical, radiological and
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35 281 histological methodology for NAFLD diagnosis and staging, which leads to a challenging
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37 282 interpretation of the results^{34, 37}.
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284 This study had an approximately 19-year follow-up time, which is longer than in previous
285 studies¹¹⁻¹⁴. When compared to earlier studies^{32, 37} this study seems to be the first follow-up
286 study with a large population-based randomly selected study group and a very long follow-up
287 time and ultrasound-diagnosed fatty liver. The diagnosis of cardiovascular events was based
288 on the registry of the National Institute for Health and Welfare and mortality data were
289 obtained from the National Death Registry. The earlier verified FINRISK classification²⁵ was
290 used to classify the events. Therefore, the reliability of event diagnosis data is accurate and
291 the classification is systematic. All subjects who had myocardial infarction or stroke before

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3 292 baseline were excluded because a history of myocardial infarction is known to increase the
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5 293 risk for recurrent myocardial infarction or cardiovascular death³⁸ and medication as well as
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7 294 lifestyle secondary prevention strategies are intensive³⁹.

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13 296 **There are a few follow-up-studies examining whether the fatty liver increases the risk for total**
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15 **mortality^{13, 40}.** In the present study, severe fatty liver predicted the risk for overall mortality of
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17 297
18 298 any causes when age, gender and study group were added covariates, a result in line with an
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20 299 earlier report⁴¹. In the published literature, NASH rather than simple steatosis has been stated
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22 300 to be linked with decreased overall survival³⁵ although one study with a large cohort found no
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24 301 association between NAFLD and overall mortality¹³. In our study, the association between
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26 302 severe fatty liver and total mortality disappeared after further adjustment for BMI which
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28 303 means that severe fatty liver is not a strong predictor for overall mortality.

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35 305 The molecular mechanisms linking fatty liver with CVD have been investigated^{10, 16}.
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37 306 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD¹⁶. In
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39 307 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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41 308 fatty acid (NEFA) metabolism⁸ and the increasing NEFA flux to the liver may impair liver
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43 309 metabolism leading to increased glucose metabolism and liver dysfunction⁷. The liver is one
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45 310 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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47 311 factors³, such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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49 312 cytokines¹⁶. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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51 313 in atherogenesis¹⁶ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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3 314 and tumor necrosis factor- α ⁸ leading to chronic systemic inflammation. In addition, hepatic
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5 315 steatosis leads to overproduction of cholesterol-rich remnant particles⁴.
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10 317 One limitation in this study is that the grade of liver brightness was measured by ultrasound.
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12 318 The invasive diagnostic technique of liver biopsy is regarded as the “golden standard”,
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14 319 especially for the diagnosis of NASH⁴². Real time ultrasound using a combination of
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16 320 sonographic findings does have a high specificity but it underestimates the prevalence of
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18 321 hepatic steatosis when there is less than 20 % fat⁴³. Today, magnetic resonance spectroscopy
19
20 322 is regarded as the best method for the quantification of liver fat, but this method is limited due
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22 323 to its availability⁴⁴. Nonetheless, the noninvasive ultrasound method was chosen because
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24 324 taking liver biopsies from large groups of symptomless subjects would have been ethically
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26 325 unjustifiable and magnetic resonance spectroscopy was not available at the baseline.
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35 327 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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37 328 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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39 329 any selection bias.
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41 42 330 **Conclusions**

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45 331 Severe liver fat content increased the risk of a future cardiovascular event and mortality to
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47 332 cardiovascular disease over the long-term follow-up but it seemed to be dependent on insulin
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49 333 sensitivity. Fatty liver also predicted the risk for overall mortality. However, conventional
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51 334 cardiovascular disease risk factors seemed to play a major role in developing death from all
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53 335 causes. It would be beneficial to investigate larger cohorts and follow-up studies in order to
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55 336 validate this result.
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337

338 Figure legend

339 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
340 with no fat in the liver, moderate fat content and severe fat content.

341 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
342 of the subjects (10/124) with moderate liver fat content, while 12.5% of the subjects with
343 severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model 1):
344 adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
345 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
346 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
347 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
348 * $p < 0.05$.

349

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354 cooperation in organizing cardiovascular event and mortality data.

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358 **References**

- 359 **1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-**
360 **31.**
- 361 **2. Armstrong MJ, Houlihan DD, Bentham L, Shaw JC, Cramb R, Olliff S, et al.**
362 **Presence and severity of non-alcoholic fatty liver disease in a large prospective primary**
363 **care cohort. *J Hepatol* 2012; Jan;56(1):234-40.**
- 364 **3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with**
365 **nonalcoholic fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.**
- 366 **4. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of**
367 **cardiovascular disease. *Atherosclerosis* 2007; Apr;191(2):235-40.**
- 368 **5. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart**
369 **disease and stroke statistics--2011 update: a report from the American Heart**
370 **Association. *Circulation* 2011; Feb 1;123(4):e18-e209.**
- 371 **6. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A.**
372 **European cardiovascular disease statistics, 2008 ed. European Heart Network; 2008.**
- 373 **7. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec**
374 **14;444(7121):881-7.**
- 375 **8. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with**
376 **cardiovascular disease. *Nature* 2006; Dec 14;444(7121):875-80.**
- 377 **9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J***
378 ***Med* 2005; Apr 21;352(16):1685-95.**

- 1
2
3 379 10. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk.
4
5 380 *Curr Opin Cardiol* 2012; Jul;27(4):420-8.
6
7
8
9 381 11. Treeprasertsuk S, Leverage S, Adams LA, Lindor KD, St Sauver J, Angulo P. The
10
11 382 Framingham risk score and heart disease in nonalcoholic fatty liver disease. *Liver Int*
12
13 383 2012; Jul;32(6):945-50.
14
15
16 384 12. Haring R, Wallaschofski H, Nauck M, Dorr M, Baumeister SE, Volzke H.
17
18 385 Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated
19
20 386 serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; Nov;50(5):1403-11.
21
22
23
24 387 13. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-
25
26 388 alcoholic fatty liver disease and mortality among US adults: prospective cohort study.
27
28 389 *BMJ* 2011; Nov 18;343:d6891.
29
30
31
32 390 14. Wong VW, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, et al. Coronary
33
34 391 artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver
35
36 392 disease. *Gut* 2011; Dec;60(12):1721-7.
37
38
39
40 393 15. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in
41
42 394 the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.
43
44
45 395 16. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-
46
47 396 alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;
48
49 397 Nov;51(11):1947-53.
50
51
52
53 398 17. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic
54
55 399 hepatic steatosis and its relation to increased plasma biomarkers of inflammation and
56
57
58
59
60

1
2
3 400 endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med*
4
5 401 2005; Oct;22(10):1354-8.
6
7

8
9 402 18. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty
10
11 403 liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler*
12
13 404 *Thromb Vasc Biol* 2005; May;25(5):1045-50.
14

15
16 405 19. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with
17
18 406 carotid atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
19

20
21
22 407 20. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-
23
24 408 glutamyltransferase is associated with incident vascular events independently of alcohol
25
26 409 intake: analysis of the British Women's Heart and Health Study and Meta-Analysis.
27
28 410 *Arterioscler Thromb Vasc Biol* 2007; Dec;27(12):2729-35.
29

30
31
32 411 21. Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA.
33
34 412 Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control
35
36 413 subjects. *J Intern Med* 1999; Feb;245(2):163-74.
37

38
39 414 22. Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma
40
41 415 immunoglobulin (Ig) A and low IgG antibody titers to oxidized low-density lipoprotein
42
43 416 are associated with markers of glucose metabolism. *J Clin Endocrinol Metab* 2010;
44
45 417 May;95(5):2467-75.
46
47

48
49 418 23. Pisto P, Ukkola O, Santaniemi M, Kesaniemi YA. Plasma adiponectin--an
50
51 419 independent indicator of liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
52
53
54
55
56
57
58
59
60

- 1
2
3 420 24. Santaniemi M., Ukkola O., Malo E., Bloigu R., Kesaniemi YA. Metabolic syndrome
4
5 421 in the prediction of cardiovascular events: The potential additive role of hsCRP and
6
7 422 adiponectin. *Eur J Prev Cardiol* 2013; Jun 20.
- 8
9
10 423 25. Pajunen P, Jousilahti P, Borodulin K, Harald K, Tuomilehto J, Salomaa V. Body fat
11
12 424 measured by a near-infrared interactance device as a predictor of cardiovascular
13
14 425 events: the FINRISK'92 cohort. *Obesity (Silver Spring)* 2011; Apr;19(4):848-52.
- 15
16
17
18 426 26. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al.
19
20 427 Quantitative insulin sensitivity check index: a simple, accurate method for assessing
21
22 428 insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; Jul;85(7):2402-10.
- 23
24
25
26 429 27. Kauma H, Savolainen MJ, Rantala AO, Lilja M, Kervinen K, Reunanen A, et al.
27
28 430 Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in
29
30 431 middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt 1):1334-43.
- 31
32
33
34 432 28. Bessebinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia
35
36 433 influenced by alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
- 37
38
39
40 434 29. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al.
41
42 435 Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J*
43
44 436 *Gastroenterol* 2007; Mar 14;13(10):1579-84.
- 45
46
47 437 30. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty
48
49 438 Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol*
50
51 439 *Hepatol* 2012; Jun;10(6):646-50.
- 52
53
54
55 440

- 1
2
3 441 31. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty
4
5 442 liver disease and risk of future cardiovascular events among type 2 diabetic patients.
6
7 443 *Diabetes* 2005; Dec;54(12):3541-6.
8
9
10
11 444 32. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and
12
13 445 incident cardiovascular disease: a narrative review and clinical perspective of
14
15 446 prospective data. *Hepatology* 2010; Sep;52(3):1156-61.
16
17
18 447 33. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected
19
20 448 nonalcoholic fatty liver disease and mortality risk in a population-based cohort study.
21
22 449 *Am J Gastroenterol* 2008; Sep;103(9):2263-71.
23
24
25
26 450 34. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-
27
28 451 glutamyltransferase as a risk factor for cardiovascular disease mortality: an
29
30 452 epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;
31
32 453 Oct 4;112(14):2130-7.
33
34
35
36 454 35. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al.
37
38 455 Decreased survival of subjects with elevated liver function tests during a 28-year follow-
39
40 456 up. *Hepatology* 2010; Feb;51(2):595-602.
41
42
43
44 457 36. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et
45
46 458 al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*
47
48 459 2006; Oct;44(4):865-73.
49
50
51
52 460 37. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a
53
54 461 new and important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.
55
56
57
58
59
60

- 1
2
3 462 **38. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,**
4
5 463 **pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.**
6
7
8
9 464 **39. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary**
10
11 465 **prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr***
12
13 466 ***Cardiol Rep* 2011; Aug;13(4):287-95.**
14
15
16 467 **40. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen**
17
18 468 **TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut***
19
20 469 **2004; May;53(5):750-5.**
21
22
23
24 470 **41. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty**
25
26 471 **liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology***
27
28 472 **2011; Jul;54(1):145-52.**
29
30
31
32 473 **42. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur***
33
34 474 ***J Gastroenterol Hepatol* 2003; May;15(5):539-43.**
35
36
37
38 475 **43. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity**
39
40 476 **of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J***
41
42 477 ***Hepatol* 2009; Dec;51(6):1061-7.**
43
44
45 478 **44. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et**
46
47 479 **al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence**
48
49 480 **of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;**
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51 481 **Feb;288(2):E462-8.**
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p	p	p
					(0-1)	(1-2)	(0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
(mmol/L)							
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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485 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
486 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
487 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
488 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat content	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat content	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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3 492 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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5 493 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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7 494 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat content and 29.2%
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9 495 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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11 496 with different degrees of adjustments are presented. Alcohol consumption was divided into
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13 497 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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15 498 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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17 499 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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19 500 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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21 501 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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23 502 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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25 503 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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27 504 insulin sensitivity check index. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.
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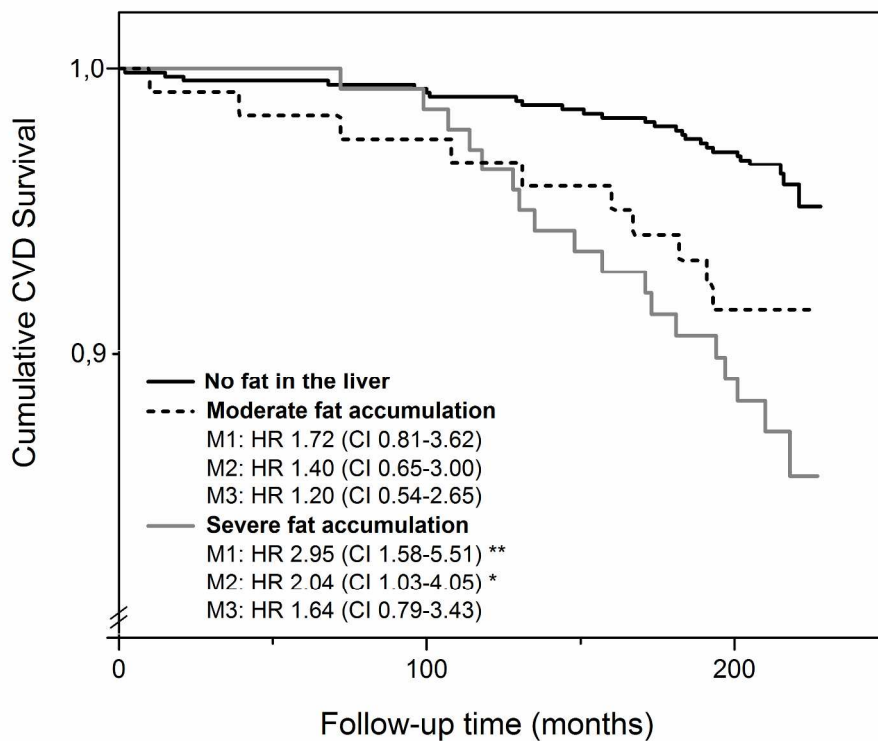
Final model	Cardiovascular event c-index (95% CI)	Binary R ²	
			513
			514
			515
Model 3	0.729 (0.706-0.776)	0.153	516
Model 4	0.720 (0.689-0.763)	0.144	517
			518
Model 5	0.717 (0.686-0.758)	0.138	519
			520
Model 1	0.698 (0.656-0.742)	0.133	

Table 3. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects with no fat in the liver, moderate fat accumulation and severe fat accumulation.

CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1% of the subjects (10/124) with moderate liver fat accumulation, while 12.5% of the subjects with severe fatty liver (18/144).

Cox regression analysis is used for adjustments. M1 (Model 1): adjusted for study group, age and gender.

M2 (Model 2): further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index. M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** p < 0.01, * p < 0.05.

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STROBE Statement

Strengthening the reporting of observational studies in epidemiology

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional)
Authors	Contact details for the corresponding author
Study design	Description of the study design (e.g cohort, case-control, cross sectional)
Objective	Specific objectives or hypothesis
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007).
Participants	<i>Cohort study</i> —Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up
	<i>Case-control study</i> —Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection
	<i>Cross-sectional study</i> —Give the eligibility criteria, and the major sources and methods of selection of participants
Variables	<i>Cohort study</i> —For matched studies, give matching and number of exposed and unexposed
	<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Statistical methods	Clearly define primary outcome for this report.
Statistical methods	Describe statistical methods, including those used to control for confounding
Results	
Participants	Report Number of participants at the beginning and end of the study
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
	Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals)
Conclusions	General interpretation of study results



Fatty liver predicts the risk for cardiovascular events in middle-aged population: a population-based cohort study

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4 1 **Fatty liver predicts the risk for cardiovascular events in middle-aged population: a**
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6 2 **population-based cohort study**
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9 3 Pauliina Pisto¹, Merja Santaniemi¹, Risto Bloigu², Olavi Ukkola¹, Y. Antero Kesäniemi¹
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51 17 **Word count:** 6093
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58 20 Research, dated 16 Apr, 2012.
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3 21 **Disclosure summary:** Authors report no conflict of interests.
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7 23 **ABSTRACT**
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10 24 **Objective:** We investigated if the differences in liver fat content would predict the
11
12 25 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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14 26 stroke).
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16 27 **Design, setting and participants:** Our study group is a population-based, randomly recruited
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18 28 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.
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20 29 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
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22 30 registry of the National Institute for Health and Welfare and the National death registry.
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24 31 **Main outcome measure:** The severity of hepatic steatosis was measured by ultrasound and
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26 32 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.
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29 33 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
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31 34 24.2% of subjects having moderate liver fat content and 29.2% of the subjects having severe
32
33 35 fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$). Severe
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35 36 liver fat content predicted the risk for future risk of cardiovascular event even when adjusted
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37 37 for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When further adjustments
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39 40 for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic blood pressure were
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41 42 conducted, the risk still remained statistically significant (HR 1.74, CI 1.16-2.63, $p < 0.01$).
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43 44 Statistical significance disappeared with further adjustment for QUICKI.
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46 47 **Conclusions:** Liver fat content increases the risk of future cardiovascular disease event in
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48 49 long-term follow-up but it seems to be dependent on insulin sensitivity.
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3 46 **Article focus**
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6 47 1 To investigate if the differences in liver fat content predict the risk for development of fatal
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8 48 or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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11 49 **Key messages**
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14 50 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
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16 51 compared to the subjects without fat in the liver
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20 52 2 Severe liver fat content increases the risk of a future cardiovascular event and mortality to
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22 53 cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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24 54 insulin sensitivity
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28 55 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
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30 56 traditional metabolic risk factors
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33 57 **Strengths and limitations of the study**
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36 58 1 This is a follow-up study with a large population-based study group and a very long follow-
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38 59 up time
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42 60 2 Official registers used in event diagnoses - data is accurate and the classification is
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44 61 systematic
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47 62 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
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49 63 sensitivity
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54 65 **Introduction**
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3 66 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
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5 67 content, which exists in a spectrum ranging from steatosis with no inflammation to non-
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7 68 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
8
9 69 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
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11 70 countries, being the leading cause of liver disorders ^{2,3}. It is associated with obesity, type 2
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13 71 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
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15 72 manifestation of the metabolic syndrome and both conditions share several risk factors for
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17 73 cardiovascular disease (CVD) ^{3,4}.

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25 75 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2% ⁵. Every
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27 76 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%) ⁶.
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29 77 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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31 78 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes ⁷. In
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33 79 addition, total body fatness as well as abdominal fat accumulation increase independently the
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35 80 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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37 81 adiposity to cardiovascular risk ⁸. Adipose tissue is now recognized as a significant endocrine
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39 82 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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41 83 mediators ⁷. Adipokines, proinflammatory cytokines and hypofibrinolytic markers may lead to
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43 84 oxidative stress and endothelial dysfunction, finally leading to atherosclerosis ⁹.

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51 86 Hepatic steatosis has been discussed as a possible mechanism to explain CVD morbidity and
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53 87 mortality ¹⁰. NAFLD patients have been reported to have higher coronary heart disease (CHD)
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55 88 risk than the general population of the same age and gender ¹¹. According to previous study,
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89 liver dysfunction associated with CVD mortality in men¹² whereas another large study found
90 no association between NAFLD and CVD in general population¹³. In addition, fatty liver did
91 not predict CVD mortality and morbidity in patients with established coronary artery disease
92¹⁴.

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94 The NAFLD and CVD share several molecular mechanisms^{15,16}. Fatty liver might play a part
95 in the pathogenesis of CVD through the overexpression and systemic release of several
96 inflammatory, hemostatic¹⁷ and oxidative-stress mediators or via contributing to whole-body
97 insulin resistance and atherogenic dyslipidemia³. NAFLD has also been reported to be linked
98 with circulatory endothelial dysfunction^{4,14}. Several investigators have reported that NAFLD
99 is associated with coronary artery disease^{4,14} and increased carotid intima-media thickness¹⁸,
100¹⁹. Increased gamma-glutamyltransferase (GGT), which may be a marker of NAFLD, has
101 been reported to be associated with stroke²⁰.

102

103 It is known that subjects with fatty liver disease have an increased risk of suffering CVD⁴, but
104 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
105 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
106 CVD. The aim of our study was to investigate if fatty liver could predict independently the
107 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
108 follow-up after adjusting for all known conventional risk factors.

109

110 **Materials and methods**

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3 111 **Human subjects**
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6 112 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
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8 113 epidemiological prospective cohort study designed to address the risk factors and disease end
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10 114 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
11
12 115 been described earlier ²¹. In short, a total of 520 men and 525 women participated: 259 control
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14 116 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
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16 117 Hypertensive participants were randomly selected from the national register for
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18 118 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
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20 119 age- and sex-matched control subject was randomly selected from the same register. Informed
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22 120 consent in writing was obtained from each patient. The study protocol conformed to the
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24 121 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
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26 122 Ethical Committee of the Faculty of Medicine, University of Oulu.
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34 124 **Determination of hepatic steatosis**
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38 125 The determination of hepatic steatosis was based on liver-kidney contrast ²² measured with
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40 126 ultrasonography ²³ by one trained radiologist with 10 years' experience in abdominal
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42 127 ultrasound examinations. Normal liver parenchyma should be slightly more echogenic (brighter)
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44 128 than the kidney parenchyma. In a case of increased liver echogenicity an ultrasound diagnosis of
45
46 129 bright liver was settled. The severity of hepatic steatosis was based on the brightness of the liver
47
48 130 and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating a
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50 131 non-fatty liver, 1 = medium bright, a moderate lipid content and 2 = clearly bright, a severe
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52 132 lipid content and fatty liver) ²⁴.
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3 134 **Follow-up**
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6 135 Both the hypertensive and the control men were recruited during December 1990 to May
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8 136 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
9
10 137 liver ultrasound result available at baseline. Mortality data were obtained from the National
11
12 138 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
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14 139 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
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16 140 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
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18 141 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
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20 142 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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28 144 CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage, SAH) -
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30 145 whichever of these happened first ²⁵. The evidence of CHD was based on the following
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32 146 diagnosis: I20.0, I21, I22 [ICD-10, International Statistical Classification of Diseases and
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34 147 Related Health Problems] / 410, 4110 [ICD-8/9] as the main diagnosis (symptom or cause)
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36 148 and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis (symptom or cause) or second
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38 149 side diagnosis (symptom or cause) and third side diagnosis (ICD-8/9 only) or if a subject had
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40 150 undergone coronary artery bypass graft (CABG) surgery or angioplasty. CHD as a cause of
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42 151 death included I20–I25, I46, R96, R98 [ICD-10] / 410-414, 798 (not 7980A) [ICD-8/9] as the
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44 152 underlying cause of death or immediate cause of death and I21 or I22 [ICD-10] / 410 [ICD-
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46 153 8/9] as first to third contributing cause of death. Stroke (excluding SAH) included I61, I63
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48 154 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436 [ICD-9] /
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50 155 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as main diagnosis (symptom or cause) or as
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52 156 a first or second side diagnosis (symptom or cause) or as a third side diagnosis (ICD-8/9 only)
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3 157 or as an underlying cause of death or immediate cause of death or as a first to third
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5 158 contributing cause of death ²⁶.

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11 160 **Laboratory analyses**

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14 161 Waist circumference, body mass index (BMI) and blood pressure were measured as described
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16 162 in previous study ²¹.

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23 164 All the laboratory test samples were obtained after an overnight fast. Blood insulin and
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25 165 glucose concentrations were analyzed at 0, 60, and 120 min after administration of 75 g
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27 166 glucose ²⁴. Insulin sensitivity was assessed using fasting plasma insulin concentrations and a
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29 167 quantitative insulin sensitivity check index (QUICKI) {QUICKI=1/[log (fasting insulin)+log
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31 168 (fasting glucose)]} ²⁷.

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38 170 Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density
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40 171 lipoprotein (LDL) and hs-CRP concentrations ²⁴ as well as alanine aminotransferase (ALT)
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42 172 and GGT levels were measured as described previously ²³. Alcohol consumption and smoking
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44 173 history were determined by validated questionnaires ²⁸. Alcohol consumption was divided into
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46 174 three groups: 0 (n=161) mean alcohol consumption less than 1g/week in men and women, 1
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48 175 (n=767) mean consumption less than 210g/week in men and less than 140 g/week in women,
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50 176 2 (n=76) mean alcohol consumption more than 210g/week in men and more than 140g/week
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53 177 in women. Group 2 designates large-scale alcohol consumers according to the guidelines ²⁹.

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3 179 **Statistical analysis**
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6 180 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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8 181 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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10 182 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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12 183 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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14 184 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
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16 185 investigate if liver brightness (fat) could predict the future risk for total mortality,
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18 186 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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25 188 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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27 189 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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29 190 improve normality before analysis of variance. We used three models with progressive
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31 191 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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33 192 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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35 193 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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37 194 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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39 195 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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41 196 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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43 197 when further adjustment with one covariate was performed. Model 4 included variables which
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45 198 were stable and were statistically significant in intermediate phases. Model 5 included stable
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47 199 and significant covariates without QUICKI (Table 2).
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3 201 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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5 202 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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7 203 to obtain 95% CI for c-index of each model.
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13 205 **Results**

16 206 The main baseline characteristics of the study group are shown in Table 1.
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23 208 *Table 1 about here*
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28 209 29 210 **Incidence of cardiovascular disease**

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32 211 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
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34 212 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
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36 213 moderate liver fat content and 29.2% (42/144) of the subjects having severe fatty liver
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38 214 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
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40 215 with non-fatty liver (26/720) and 8.1% of the subjects with moderate liver fat content
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42 216 (10/124), while 12.5% (18/144) of the subjects with severe fatty liver ($p < 0.001$) (Table 3).
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50 218 Severe liver fat content predicted the risk for future risk of cardiovascular event when
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52 219 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
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54 220 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
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56 221 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
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3 222 statistically significant ($p < 0.01$). Statistical significance disappeared when further
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5 223 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
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7 224 CVD event sensitivity analyses, all covariates were added one by one and it was examined
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9 225 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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11 226 significant variables (including quick index) in the sensitivity analyses, the association
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14 227 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18,
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16 228 $p=0.10$). When QUICKI was not added into Model 5, severe fatty liver did predict the risk for
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18 229 future risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index
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20 230 decreased when the risk factors were removed from the model (Table 4).
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27 232 *Tables 2, 3 and 4 about here*
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33 234 The future risk of death from CVD in participants with severe fat content was significant
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35 235 when age, gender and study group were added as covariates (Model 1: HR 2.95, CI 1.58-5.51,
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37 236 $p < 0.01$). Even after further adjustments with other conventional risk factors (Model 2: HR
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39 237 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI was added as
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41 238 the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43, NS) (Fig 1.).
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49 240 *Figure 1 about here*
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55 242 **Fatty liver and total mortality**
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3 243 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
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5 244 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
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7 245 0.01). According to Model 1, severe fat content predicted the risk for mortality from all
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9 246 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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11 247 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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13 248 not shown).

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20 250 We performed all Cox regression analyses after excluding the men consuming more than 210
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22 251 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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24 252 have any effect on the results (data not shown).

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28 253 We performed all Cox regression analyses after excluding patients with insulin treated
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30 254 diabetes mellitus ($n=9$), cortisone treatment at baseline ($n=41$) and previous diagnosis for
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32 255 liver disease ($n=15$) (e.g., virus, medications). This exclusion did not have any effect on the
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34 256 results (data not shown).

35 36 37 38 257 **Discussion**

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41 258 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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43 259 continuously increasing in the Western world. The question if NAFLD is only a marker or
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45 260 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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47 261 results of the present study, which had an approximately 19-year follow-up fatty liver does
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49 262 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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51 263 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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53 264 development of cardiovascular events.

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3 266 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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5 267 with ultrasound-diagnosed fatty liver^{30, 31} and larger studies with longer follow-up times are
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7 268 needed. An association between NAFLD and CVD has been reported^{3, 30-32} although contrary
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9 269 results also exist^{13, 33}. A previous large population-based prospective cohort study found no
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11 270 association between NAFLD and CVD, however they categorized the degree of steatosis as a
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14 271 two level variable: none to mild and moderate to severe¹³. An association between
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16 272 ultrasound-diagnosed fatty liver and CVD has been reported in general population³⁰ and in
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18 273 subjects with T2DM³². Furthermore, liver dysfunction has been reported to associate with
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21 274 CVD mortality^{34, 35} and CHD risk¹¹ in follow-up studies and especially survival of subjects
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23 275 with NASH is reported to be reduced^{33, 36, 37}. In the present study, severe fatty liver disease did
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25 276 predict the risk for cardiovascular death but the association seemed to be dependent on insulin
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27 277 sensitivity.

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32 279 Several earlier studies have used self-reported CVD history which may not be totally reliable.
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34 280 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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36 281 liver have performed some adjustments, the full range of well-known CVD risk factors have
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38 282 been rarely considered³³. We have performed adjustments with all so-called traditional risk
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40 283 factors for cardiovascular disease (i.e. age, gender, smoking, LDL concentration,
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42 284 hypertension, insulin resistance). Previous studies have used biochemical, radiological and
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44 285 histological methodology for NAFLD diagnosis and staging, which leads to a challenging
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46 286 interpretation of the results^{35, 38}.

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54 288 This study had an approximately 19-year follow-up time, which is longer than in previous
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56 289 studies¹¹⁻¹⁴. When compared to earlier studies^{33, 38} this study seems to be the first follow-up
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3 290 study with a large population-based randomly selected study group and a very long follow-up
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5 291 time and ultrasound-diagnosed fatty liver. The diagnosis of cardiovascular events was based
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7 292 on the registry of the National Institute for Health and Welfare and mortality data were
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9 293 obtained from the National Death Registry. The earlier verified FINRISK classification ²⁶ was
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11 294 used to classify the events. Therefore, the reliability of event diagnosis data is accurate and
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13 295 the classification is systematic. All subjects who had myocardial infarction or stroke before
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15 296 baseline were excluded because a history of myocardial infarction is known to increase the
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17 297 risk for recurrent myocardial infarction or cardiovascular death ³⁹ and medication as well as
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19 298 lifestyle secondary prevention strategies are intensive ⁴⁰.

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27 300 There are a few follow-up-studies examining whether the fatty liver increases the risk for total
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29 301 mortality ^{13, 41}. In the present study, severe fatty liver predicted the risk for overall mortality of
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31 302 any causes when age, gender and study group were added covariates, a result in line with an
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33 303 earlier report ⁴². In the published literature, NASH rather than simple steatosis has been stated
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35 304 to be linked with decreased overall survival ³⁶ although one study with a large cohort found no
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37 305 association between NAFLD and overall mortality ¹³. In our study, the association between
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39 306 severe fatty liver and total mortality disappeared after further adjustment for BMI which
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41 307 means that severe fatty liver is not a strong predictor for overall mortality.

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49 309 The molecular mechanisms linking fatty liver with CVD have been investigated ^{10, 16}.
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51 310 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD ¹⁶. In
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53 311 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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55 312 fatty acid (NEFA) metabolism ⁸ and the increasing NEFA flux to the liver may impair liver

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3 313 metabolism leading to increased glucose metabolism and liver dysfunction ⁷. The liver is one
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5 314 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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7 315 factors ³, such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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9 316 cytokines ¹⁶. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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11 317 in atherogenesis ¹⁶ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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13 318 and tumor necrosis factor- α ⁸ leading to chronic systemic inflammation. In addition, hepatic
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15 319 steatosis leads to overproduction of cholesterol-rich remnant particles ⁴.
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22 321 One limitation in this study is that the grade of liver brightness was measured by ultrasound.
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24 322 The invasive diagnostic technique of liver biopsy is regarded as the “golden standard”,
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26 323 especially for the diagnosis of NASH ⁴³. Real time ultrasound using a combination of
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28 324 sonographic findings does have a high specificity but it underestimates the prevalence of
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30 325 hepatic steatosis when there is less than 20 % fat ⁴⁴. Today, magnetic resonance spectroscopy
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32 326 is regarded as the best method for the quantification of liver fat, but this method is limited due
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34 327 to its availability ⁴⁵. Nonetheless, the noninvasive ultrasound method was chosen because
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36 328 taking liver biopsies from large groups of symptomless subjects would have been ethically
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38 329 unjustifiable and magnetic resonance spectroscopy was not available at the baseline.
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46 331 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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48 332 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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50 333 any selection bias.
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53 334 **Conclusions**
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3 335 Severe liver fat content increased the risk of a future cardiovascular event and mortality to
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5 336 cardiovascular disease over the long-term follow-up but it seemed to be dependent on insulin
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7 337 sensitivity. Fatty liver also predicted the risk for overall mortality. However, conventional
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9 338 cardiovascular disease risk factors seemed to play a major role in developing death from all
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11 339 causes. It would be beneficial to investigate larger cohorts and follow-up studies in order to
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13 340 validate this result.
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22 342 **Figure legend**

23
24 343 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
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26 344 with no fat in the liver, moderate fat content and severe fat content.
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29 345 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
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31 346 of the subjects (10/124) with moderate liver fat content, while 12.5% of the subjects with
32
33 347 severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model 1):
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35 348 adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
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37 349 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
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39 350 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
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41 351 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
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43 352 * $p < 0.05$.
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5 358 cooperation in organizing cardiovascular event and mortality data.
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12 360 **Contributor statement:** All authors fulfill all three of the ICMJE guidelines for authorship
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14
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17 362 writing, final approval of the version to be published
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23 364 revision of the manuscript, final approval of the version to be published
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26 365 **Risto Bloigu:** Data analysis, interpretation of data, critical revision of the manuscript, final
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28 366 approval of the version to be published
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34 368 manuscript, final approval of the version to be published
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37 369 **Y.A Kesäniemi:** Study design, data acquisition, data interpretation, critical revision of the
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39 370 manuscript, final approval of the version to be published
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46 372 **Data sharing statement:** Extra data is available by emailing pauliina.pisto(at)oulu.fi
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50 373 **Competing Interests:** None
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3 376 **References**
4
5

6 377 **1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-**
7
8 378 **31.**

9
10
11 379 **2. Armstrong MJ, Houlihan DD, Bentham L, Shaw JC, Cramb R, Olliff S, et al.**
12 **Presence and severity of non-alcoholic fatty liver disease in a large prospective primary**
13 **care cohort. *J Hepatol* 2012; Jan;56(1):234-40.**
14
15
16
17

18
19
20 382 **3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with**
21 **nonalcoholic fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.**
22
23

24
25 384 **4. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of**
26 **cardiovascular disease. *Atherosclerosis* 2007; Apr;191(2):235-40.**
27
28

29
30
31 386 **5. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart**
32 **disease and stroke statistics--2011 update: a report from the American Heart**
33 **Association. *Circulation* 2011; Feb 1;123(4):e18-e209.**
34
35
36

37
38
39 389 **6. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A.**
40 **European cardiovascular disease statistics, 2008 ed. European Heart Network; 2008.**
41
42

43
44 391 **7. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec**
45 **14;444(7121):881-7.**
46
47

48
49
50 393 **8. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with**
51 **cardiovascular disease. *Nature* 2006; Dec 14;444(7121):875-80.**
52
53

54
55 395 **9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J***
56 ***Med* 2005; Apr 21;352(16):1685-95.**
57
58
59
60

- 1
2
3 397 **10. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk.**
4
5 398 ***Curr Opin Cardiol* 2012; Jul;27(4):420-8.**
6
7
8
9 399 **11. Treeprasertsuk S, Leverage S, Adams LA, Lindor KD, St Sauver J, Angulo P. The**
10
11 400 **Framingham risk score and heart disease in nonalcoholic fatty liver disease. *Liver Int***
12
13 401 **2012; Jul;32(6):945-50.**
14
15
16 402 **12. Haring R, Wallaschofski H, Nauck M, Dorr M, Baumeister SE, Volzke H.**
17
18 403 **Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated**
19
20 404 **serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; Nov;50(5):1403-11.**
21
22
23
24 405 **13. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-**
25
26 406 **alcoholic fatty liver disease and mortality among US adults: prospective cohort study.**
27
28 407 ***BMJ* 2011; Nov 18;343:d6891.**
29
30
31
32 408 **14. Wong VW, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, et al. Coronary**
33
34 409 **artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver**
35
36 410 **disease. *Gut* 2011; Dec;60(12):1721-7.**
37
38
39
40 411 **15. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in**
41
42 412 **the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.**
43
44
45 413 **16. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-**
46
47 414 **alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;**
48
49 415 **Nov;51(11):1947-53.**
50
51
52
53 416 **17. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic**
54
55 417 **hepatic steatosis and its relation to increased plasma biomarkers of inflammation and**
56
57
58
59
60

1
2
3 418 endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med*
4
5 419 2005; Oct;22(10):1354-8.
6
7

8
9 420 18. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty
10
11 421 liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler*
12
13 422 *Thromb Vasc Biol* 2005; May;25(5):1045-50.
14

15
16 423 19. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with
17
18 424 carotid atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
19

20
21
22 425 20. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-
23
24 426 glutamyltransferase is associated with incident vascular events independently of alcohol
25
26 427 intake: analysis of the British Women's Heart and Health Study and Meta-Analysis.
27
28 428 *Arterioscler Thromb Vasc Biol* 2007; Dec;27(12):2729-35.
29

30
31
32 429 21. Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA.
33
34 430 Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control
35
36 431 subjects. *J Intern Med* 1999; Feb;245(2):163-74.
37

38
39
40 432 22. Yajima Y, Ohta K, Narui T, Abe R, Suzuki H, Ohtsuki M. Ultrasonographical
41
42 433 diagnosis of fatty liver: significance of the liver-kidney contrast. *Tohoku J Exp Med*
43
44 434 1983; Jan;139(1):43-50.
45

46
47 435 23. Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma
48
49 436 immunoglobulin (Ig) A and low IgG antibody titers to oxidized low-density lipoprotein
50
51 437 are associated with markers of glucose metabolism. *J Clin Endocrinol Metab* 2010;
52
53 438 May;95(5):2467-75.
54
55
56
57
58
59
60

- 1
2
3 439 24. Pisto P, Ukkola O, Santaniemi M, Kesaniemi YA. Plasma adiponectin--an
4
5 440 independent indicator of liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
6
7
8
9 441 25. Santaniemi M., Ukkola O., Malo E., Bloigu R., Kesaniemi YA. Metabolic syndrome
10
11 442 in the prediction of cardiovascular events: The potential additive role of hsCRP and
12
13 443 adiponectin. *Eur J Prev Cardiol* 2013; Jun;20.
14
15
16 444 26. Pajunen P, Jousilahti P, Borodulin K, Harald K, Tuomilehto J, Salomaa V. Body fat
17
18 445 measured by a near-infrared interactance device as a predictor of cardiovascular
19
20 446 events: the FINRISK'92 cohort. *Obesity (Silver Spring)* 2011; Apr;19(4):848-52.
21
22
23
24 447 27. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al.
25
26 448 Quantitative insulin sensitivity check index: a simple, accurate method for assessing
27
28 449 insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; Jul;85(7):2402-10.
29
30
31
32 450 28. Kauma H, Savolainen MJ, Rantala AO, Lilja M, Kervinen K, Reunanen A, et al.
33
34 451 Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in
35
36 452 middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt 1):1334-43.
37
38
39
40 453 29. Bessebinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia
41
42 454 influenced by alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
43
44
45 455 30. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al.
46
47 456 Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J*
48
49 457 *Gastroenterol* 2007; Mar 14;13(10):1579-84.
50
51
52
53 458 31. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty
54
55 459 Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol*
56
57 460 *Hepatol* 2012; Jun;10(6):646-50.
58
59
60

- 1
2
3 461 32. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty
4
5 462 liver disease and risk of future cardiovascular events among type 2 diabetic patients.
6
7 463 *Diabetes* 2005; Dec;54(12):3541-6.
8
9
10
11 464 33. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and
12
13 465 incident cardiovascular disease: a narrative review and clinical perspective of
14
15 466 prospective data. *Hepatology* 2010; Sep;52(3):1156-61.
16
17
18 467 34. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected
19
20 468 nonalcoholic fatty liver disease and mortality risk in a population-based cohort study.
21
22 469 *Am J Gastroenterol* 2008; Sep;103(9):2263-71.
23
24
25
26 470 35. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-
27
28 471 glutamyltransferase as a risk factor for cardiovascular disease mortality: an
29
30 472 epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;
31
32 473 Oct 4;112(14):2130-7.
33
34
35
36 474 36. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al.
37
38 475 Decreased survival of subjects with elevated liver function tests during a 28-year follow-
39
40 476 up. *Hepatology* 2010; Feb;51(2):595-602.
41
42
43
44 477 37. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et
45
46 478 al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*
47
48 479 2006; Oct;44(4):865-73.
49
50
51
52 480 38. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a
53
54 481 new and important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.
55
56
57
58
59
60

- 1
2
3 482 39. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,
4
5 483 pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.
6
7
8
9 484 40. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary
10
11 485 prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr*
12
13 486 *Cardiol Rep* 2011; Aug;13(4):287-95.
14
15
16 487 41. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen
17
18 488 TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut*
19
20 489 2004; May;53(5):750-5.
21
22
23
24 490 42. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty
25
26 491 liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology*
27
28 492 2011; Jul;54(1):145-52.
29
30
31
32 493 43. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur*
33
34 494 *J Gastroenterol Hepatol* 2003; May;15(5):539-43.
35
36
37
38 495 44. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity
39
40 496 of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J*
41
42 497 *Hepatol* 2009; Dec;51(6):1061-7.
43
44
45 498 45. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et
46
47 499 al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence
48
49 500 of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;
50
51 501 Feb;288(2):E462-8.
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p	p	p
					(0-1)	(1-2)	(0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
(mmol/L)							
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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505 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
506 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
507 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
508 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat content	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat content	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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2
3 512 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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5 513 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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7 514 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat content and 29.2%
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9 515 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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11 516 with different degrees of adjustments are presented. Alcohol consumption was divided into
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13 517 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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15 518 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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17 519 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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19 520 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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21 521 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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23 522 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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25 523 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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27 524 insulin sensitivity check index. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.
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Grade of liver bightness	Total	0 (n=720)	1 (n=124)	2 (n=144)	p
Non-fatal events					
CVD	11.6% (115)	9.9% (71)	16.1% (20)	16.7% (24)	< 0.05
CHD	7.8% (77)	6.5% (47)	11.3% (14)	11.1% (16)	NS
Stroke	5.0% (49)	4.2% (30)	8.1% (10)	6.2% (9)	NS
Fatal events					
CVD	5.5% (54)	3.6% (26)	8.1% (10)	12.5% (18)	< 0.001
CHD	4.8% (47)	3.2% (23)	7.3% (9)	10.4% (15)	< 0.01
Stroke	0.8% (8)	0.6% (4)	0.8% (1)	2.1% (3)	NS

532 **Table 3.** CVD, CHD and stroke follow-up data of the study group as percentages (number of
533 events). CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage) -
534 whichever of these happened first. N=number of subjects. CHD, coronary heart disease,
535 CVD, cardiovascular disease.

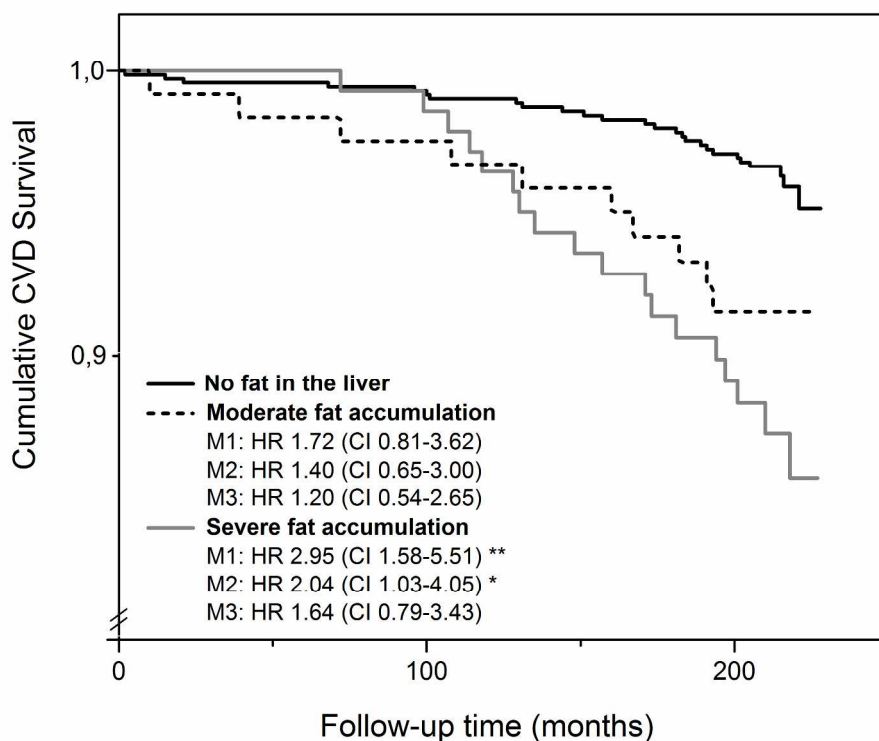
Final model	Cardiovascular event c-index (95% CI)	Binary R ²	
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			537
			538
Model 3	0.729 (0.706-0.776)	0.153	539
			540
Model 4	0.720 (0.689-0.763)	0.144	
			541
			542
Model 5	0.717 (0.686-0.758)	0.138	
			543
Model 1	0.698 (0.656-0.742)	0.133	

Table 4. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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35 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects with no fat in the
 36 liver, moderate fat content and severe fat content.

37 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1% of the subjects
 38 (10/124) with moderate liver fat content, while 12.5% of the subjects with severe fatty liver (18/144). Cox
 39 regression analysis is used for adjustments. M1 (Model 1): adjusted for study group, age and gender. M2
 40 (Model 2): further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol
 41 level and body mass index. M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI,
 42 confidence interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$, * p
 43 < 0.05 .

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STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) page 1
Authors	Contact details for the corresponding author page 1
Study design	Description of the study design (e.g cohort, case-control, cross sectional) page 6
Objective	Specific objectives or hypothesis page 5
Methods	page 5
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). page 7
Participants	<p><i>Cohort study</i>—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up page 6</p> <p><i>Case-control study</i>—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection</p> <p><i>Cross-sectional study</i>—Give the eligibility criteria, and the major sources and methods of selection of participants</p>
<i>Cohort study</i> —For matched studies, give matching and number of exposed and unexposed page 7	
<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Variables	Clearly define primary outcome for this report. page 10
Statistical methods	Describe statistical methods, including those used to control for confounding page 9
Results	
Participants	Report Number of participants at the beginning and end of the study page 7
Main results	<p>Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</p> <p>Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals) page 10</p>
Conclusions	General interpretation of study results page 12

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Fatty liver predicts the risk for cardiovascular events in middle-aged population: a population-based cohort study

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4 1 **Fatty liver predicts the risk for cardiovascular events in middle-aged population: a**
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6 2 **population-based cohort study**
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50
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52 18 **Keywords:** coronary disease, fatty liver, insulin resistance, risk factors, stroke
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2
3 21 **ABSTRACT**
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5 22 **Objective:** We investigated if the differences in liver fat content would predict the
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7 23 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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9 24 stroke).

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11 25 **Design, setting and participants:** Our study group is a population-based, randomly recruited
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13 26 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.

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15 27 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
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17 28 registry of the National Institute for Health and Welfare and the National death registry.

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19 29 **Main outcome measure:** The severity of hepatic steatosis was measured by ultrasound and
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21 30 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.

22
23 31 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
24
25 32 24.2% of subjects having moderate liver fat content and 29.2% of the subjects having severe
26
27 33 fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$). Severe
28
29 34 liver fat content predicted the risk for future risk of cardiovascular event even when adjusted
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31 35 for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When further adjustments
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33 36 for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic blood pressure were
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35 37 conducted, the risk still remained statistically significant (HR 1.74, CI 1.16-2.63, $p < 0.01$).
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37 38 Statistical significance disappeared with further adjustment for QUICKI.

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39 39 **Conclusions:** Liver fat content increases the risk of future cardiovascular disease event in
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41 40 long-term follow-up but it seems to be dependent on insulin sensitivity.
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3 45 **Article focus**
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6 46 1 To investigate if the differences in liver fat content predict the risk for development of fatal
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8 47 or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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11 48 **Key messages**
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14 49 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
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16 50 compared to the subjects without fat in the liver
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20 51 2 Severe liver fat content increases the risk of a future cardiovascular event and mortality to
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22 52 cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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24 53 insulin sensitivity
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27 54 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
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29 55 traditional metabolic risk factors
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33 56 **Strengths and limitations of the study**
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36 57 1 This is a follow-up study with a large population-based study group and a very long follow-
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38 58 up time
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41 59 2 Official registers used in event diagnoses - data is accurate and the classification is
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43 60 systematic
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46 61 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
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48 62 sensitivity
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8 68 **Introduction**
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10 69 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
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12 70 content, which exists in a spectrum ranging from steatosis with no inflammation to non-
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14 71 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
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16 72 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
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18 73 countries, being the leading cause of liver disorders ^{2,3}. It is associated with obesity, type 2
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20 74 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
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22 75 manifestation of the metabolic syndrome and both conditions share several risk factors for
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24 76 cardiovascular disease (CVD) ^{3,4}.
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32 78 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2% ⁵. Every
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34 79 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%) ⁶.
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36 80 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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38 81 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes ⁷. In
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40 82 addition, total body fatness as well as abdominal fat accumulation increase independently the
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42 83 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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44 84 adiposity to cardiovascular risk ⁸. Adipose tissue is now recognized as a significant endocrine
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46 85 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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48 86 mediators ⁷. Adipokines, proinflammatory cytokines and hypofibrinolytic markers may lead to
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50 87 oxidative stress and endothelial dysfunction, finally leading to atherosclerosis ⁹.
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3 89 Hepatic steatosis has been discussed as a possible mechanism to explain CVD morbidity and
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5 90 mortality¹⁰. NAFLD patients have been reported to have higher coronary heart disease (CHD)
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7 91 risk than the general population of the same age and gender¹¹. According to previous study,
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9 92 liver dysfunction associated with CVD mortality in men¹² whereas another large study found
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11 93 no association between NAFLD and CVD in general population¹³. In addition, fatty liver did
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13 94 not predict CVD mortality and morbidity in patients with established coronary artery disease
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23 97 The NAFLD and CVD share several molecular mechanisms^{15,16}. Fatty liver might play a part
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25 98 in the pathogenesis of CVD through the overexpression and systemic release of several
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27 99 inflammatory, hemostatic¹⁷ and oxidative-stress mediators or via contributing to whole-body
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29 100 insulin resistance and atherogenic dyslipidemia³. NAFLD has also been reported to be linked
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31 101 with circulatory endothelial dysfunction^{4,14}. Several investigators have reported that NAFLD
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33 102 is associated with coronary artery disease^{4,14} and increased carotid intima-media thickness¹⁸,
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36 103¹⁹. Increased gamma-glutamyltransferase (GGT), which may be a marker of NAFLD, has
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38 104 been reported to be associated with stroke²⁰.

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45 106 It is known that subjects with fatty liver disease have an increased risk of suffering CVD⁴, but
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47 107 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
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49 108 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
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51 109 CVD. The aim of our study was to investigate if fatty liver could predict independently the
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53 110 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
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55 111 follow-up after adjusting for all known conventional risk factors.
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56 113 **Materials and methods**
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9 114 **Human subjects**
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12 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
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14 epidemiological prospective cohort study designed to address the risk factors and disease end
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16 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
17
18 been described earlier²¹. In short, a total of 520 men and 525 women participated: 259 control
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20 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
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22 Hypertensive participants were randomly selected from the national register for
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24 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
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26 age- and sex-matched control subject was randomly selected from the same register. Informed
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28 consent in writing was obtained from each patient. The study protocol conformed to the
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30 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
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32 Ethical Committee of the Faculty of Medicine, University of Oulu.
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52 127 **Determination of hepatic steatosis**
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55 The determination of hepatic steatosis was based on liver-kidney contrast²² measured with
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57 ultrasonography²³ by one trained radiologist with 10 years' experience in abdominal
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59 ultrasound examinations. Normal liver parenchyma should be slightly more echogenic (brighter)
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than the kidney parenchyma. In a case of increased liver echogenicity an ultrasound diagnosis of
bright liver was settled. The severity of hepatic steatosis was based on the brightness of the liver
and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating a

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3 134 non-fatty liver, 1 = medium bright, a moderate lipid content and 2 = clearly bright, a severe
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5 135 lipid content and fatty liver) ²⁴.
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137 **Follow-up**

138 Both the hypertensive and the control men were recruited during December 1990 to May
139 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
140 liver ultrasound result available at baseline. Mortality data were obtained from the National
141 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
142 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
143 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
144 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
145 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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147 CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage, SAH) -
148 whichever of these happened first ²⁵. The evidence of CHD was based on the following
149 diagnosis: I20.0, I21, I22 [ICD-10, International Statistical Classification of Diseases and
150 Related Health Problems] / 410, 4110 [ICD-8/9] as the main diagnosis (symptom or cause)
151 and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis (symptom or cause) or second
152 side diagnosis (symptom or cause) and third side diagnosis (ICD-8/9 only) or if a subject had
153 undergone coronary artery bypass graft (CABG) surgery or angioplasty. CHD as a cause of
154 death included I20–I25, I46, R96, R98 [ICD-10] / 410-414, 798 (not 7980A) [ICD-8/9] as the
155 underlying cause of death or immediate cause of death and I21 or I22 [ICD-10] / 410 [ICD-
156 8/9] as first to third contributing cause of death. Stroke (excluding SAH) included I61, I63

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3 157 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436 [ICD-9] /
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5 158 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as main diagnosis (symptom or cause) or as
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7 159 a first or second side diagnosis (symptom or cause) or as a third side diagnosis (ICD-8/9 only)
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10 160 or as an underlying cause of death or immediate cause of death or as a first to third
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12 161 contributing cause of death ²⁶.

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163 **Laboratory analyses**

164 Waist circumference, body mass index (BMI) and blood pressure were measured as described
165 in previous study ²¹.

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168 All the laboratory test samples were obtained after an overnight fast. Blood insulin and
169 glucose concentrations were analyzed at 0, 60, and 120 min after administration of 75 g
170 glucose ²⁴. Insulin sensitivity was assessed using fasting plasma insulin concentrations and a
171 quantitative insulin sensitivity check index (QUICKI) {QUICKI=1/[log (fasting insulin)+log
(fasting glucose)]} ²⁷.

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174 Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density
175 lipoprotein (LDL) and hs-CRP concentrations ²⁴ as well as alanine aminotransferase (ALT)
176 and GGT levels were measured as described previously ²³. Alcohol consumption and smoking
177 history were determined by validated questionnaires ²⁸. Alcohol consumption was divided into
178 three groups: 0 (n=161) mean alcohol consumption less than 1g/week in men and women, 1
(n=767) mean consumption less than 210g/week in men and less than 140 g/week in women,

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3 179 2 (n=76) mean alcohol consumption more than 210g/week in men and more than 140g/week
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5 180 in women. Group 2 designates large-scale alcohol consumers according to the guidelines²⁹.
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10 11 182 **Statistical analysis**

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14 183 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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16 184 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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18 185 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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20 186 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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22 187 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
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24 188 investigate if liver brightness (fat) could predict the future risk for total mortality,
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26 189 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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34 191 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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36 192 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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38 193 improve normality before analysis of variance. We used three models with progressive
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40 194 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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42 195 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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44 196 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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46 197 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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48 198 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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50 199 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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53 200 when further adjustment with one covariate was performed. Model 4 included variables which
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3 201 were stable and were statistically significant in intermediate phases. Model 5 included stable
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5 202 and significant covariates without QUICKI (Table 2).
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11 204 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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13 205 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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15 206 to obtain 95% CI for c-index of each model.
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22 208 **Results**

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25 209 The main baseline characteristics of the study group are shown in Table 1.
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32 211 *Table 1 about here*
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38 213 **Incidence of cardiovascular disease**

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41 214 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
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43 215 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
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45 216 moderate liver fat content and 29.2% (42/144) of the subjects having severe fatty liver
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47 217 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
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49 218 with non-fatty liver (26/720) and 8.1% of the subjects with moderate liver fat content
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51 219 (10/124), while 12.5% (18/144) of the subjects with severe fatty liver ($p < 0.001$) (Table 3).
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3 221 Severe liver fat content predicted the risk for future risk of cardiovascular event when
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5 222 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
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7 223 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
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9 224 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
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11 225 statistically significant ($p < 0.01$). Statistical significance disappeared when further
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13 226 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
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15 227 CVD event sensitivity analyses, all covariates were added one by one and it was examined
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17 228 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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19 229 significant variables (including quick index) in the sensitivity analyses, the association
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21 230 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18,
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23 231 $p=0.10$). When QUICKI was not added into Model 5, severe fatty liver did predict the risk for
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25 232 future risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index
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27 233 decreased when the risk factors were removed from the model (Table 4).
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36 235 *Tables 2, 3 and 4 about here*

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42 237 The future risk of death from CVD in participants with severe fat content was significant
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44 238 when age, gender and study group were added as covariates (Model 1: HR 2.95, CI 1.58-5.51,
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46 239 $p < 0.01$). Even after further adjustments with other conventional risk factors (Model 2: HR
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48 240 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI was added as
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50 241 the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43, NS) (Fig 1.).
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6 245 **Fatty liver and total mortality**
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9 246 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
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11 247 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
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13 248 0.01). According to Model 1, severe fat content predicted the risk for mortality from all
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15 249 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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17 250 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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19 251 not shown).
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27 253 We performed all Cox regression analyses after excluding the men consuming more than 210
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29 254 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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31 255 have any effect on the results (data not shown).
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34 256 We performed all Cox regression analyses after excluding patients with insulin treated
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36 257 diabetes mellitus ($n=9$), cortisone treatment at baseline ($n=41$) and previous diagnosis for
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38 258 liver disease ($n=15$) (e.g., virus, medications). This exclusion did not have any effect on the
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40 259 results (data not shown).
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44 260 **Discussion**
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47 261 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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49 262 continuously increasing in the Western world. The question if NAFLD is only a marker or
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51 263 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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53 264 results of the present study, which had an approximately 19-year follow-up fatty liver does
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55 265 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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3 266 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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5 267 development of cardiovascular events.
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10 269 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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12 270 with ultrasound-diagnosed fatty liver ^{30,31} and larger studies with longer follow-up times are
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14 271 needed. An association between NAFLD and CVD has been reported ^{3,30-32} although contrary
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16 272 results also exist ^{13,33}. A previous large population-based prospective cohort study found no
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18 273 association between NAFLD and CVD, however they categorized the degree of steatosis as a
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20 274 two level variable: none to mild and moderate to severe ¹³. An association between
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22 275 ultrasound-diagnosed fatty liver and CVD has been reported in general population ³⁰ and in
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24 276 subjects with T2DM ³². Furthermore, liver dysfunction has been reported to associate with
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26 277 CVD mortality ^{34,35} and CHD risk ¹¹ in follow-up studies and especially survival of subjects
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28 278 with NASH is reported to be reduced ^{33,36,37}. In the present study, severe fatty liver disease did
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30 279 predict the risk for cardiovascular death but the association seemed to be dependent on insulin
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32 280 sensitivity.
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39 282 Several earlier studies have used self-reported CVD history which may not be totally reliable.
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41 283 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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43 284 liver have performed some adjustments, the full range of well-known CVD risk factors have
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45 285 been rarely considered ³³. We have performed adjustments with all so-called traditional risk
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47 286 factors for cardiovascular disease (i.e. age, gender, smoking, LDL concentration,
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49 287 hypertension, insulin resistance). Previous studies have used biochemical, radiological and
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51 288 histological methodology for NAFLD diagnosis and staging, which leads to a challenging
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53 289 interpretation of the results ^{35,38}.
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6 291 This study had an approximately 19-year follow-up time, which is longer than in previous
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8 292 studies ¹¹⁻¹⁴. When compared to earlier studies ^{33, 38} this study seems to be the first follow-up
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10 293 study with a large population-based randomly selected study group and a very long follow-up
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12 294 time and ultrasound-diagnosed fatty liver. The diagnosis of cardiovascular events was based
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14 295 on the registry of the National Institute for Health and Welfare and mortality data were
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16 296 obtained from the National Death Registry. The earlier verified FINRISK classification ²⁶ was
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18 297 used to classify the events. Therefore, the reliability of event diagnosis data is accurate and
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20 298 the classification is systematic. All subjects who had myocardial infarction or stroke before
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22 299 baseline were excluded because a history of myocardial infarction is known to increase the
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24 300 risk for recurrent myocardial infarction or cardiovascular death ³⁹ and medication as well as
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26 301 lifestyle secondary prevention strategies are intensive ⁴⁰.

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34 303 There are a few follow-up-studies examining whether the fatty liver increases the risk for total
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36 304 mortality ^{13, 41}. In the present study, severe fatty liver predicted the risk for overall mortality of
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38 305 any causes when age, gender and study group were added covariates, a result in line with an
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40 306 earlier report ⁴². In the published literature, NASH rather than simple steatosis has been stated
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42 307 to be linked with decreased overall survival ³⁶ although one study with a large cohort found no
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44 308 association between NAFLD and overall mortality ¹³. In our study, the association between
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46 309 severe fatty liver and total mortality disappeared after further adjustment for BMI which
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48 310 means that severe fatty liver is not a strong predictor for overall mortality.

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3 312 The molecular mechanisms linking fatty liver with CVD have been investigated ^{10, 16}.
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5 313 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD ¹⁶. In
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7 314 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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9 315 fatty acid (NEFA) metabolism ⁸ and the increasing NEFA flux to the liver may impair liver
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11 316 metabolism leading to increased glucose metabolism and liver dysfunction ⁷. The liver is one
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13 317 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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15 318 factors ³, such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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17 319 cytokines ¹⁶. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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19 320 in atherogenesis ¹⁶ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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21 321 and tumor necrosis factor- α ⁸ leading to chronic systemic inflammation. In addition, hepatic
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23 322 steatosis leads to overproduction of cholesterol-rich remnant particles ⁴.
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33 324 One limitation in this study is that the grade of liver brightness was measured by ultrasound.
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35 325 The invasive diagnostic technique of liver biopsy is regarded as the “golden standard”,
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37 326 especially for the diagnosis of NASH ⁴³. Real time ultrasound using a combination of
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39 327 sonographic findings does have a high specificity but it underestimates the prevalence of
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41 328 hepatic steatosis when there is less than 20 % fat ⁴⁴. Today, magnetic resonance spectroscopy
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43 329 is regarded as the best method for the quantification of liver fat, but this method is limited due
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45 330 to its availability ⁴⁵. Unfortunately quantitative measurement of liver fat by ultrasound is
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47 331 subject to several limitations compared to more validated and standardized methods for
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49 332 diagnosing NAFLD and the analysis of intra-observer reproducibility could have been more
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51 333 accurate in the present study. Nonetheless, the noninvasive ultrasound method was chosen
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53 334 because taking liver biopsies from large groups of symptomless subjects would have been
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55 335 ethically unjustifiable and magnetic resonance spectroscopy was not available at the baseline.
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6 337 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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8 338 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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10 339 any selection bias.
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13 340 **Conclusions**

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17 341 Severe liver fat content increased the risk of a future cardiovascular event and mortality to
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19 342 cardiovascular disease over the long-term follow-up but it seemed to be dependent on insulin
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21 343 sensitivity. Fatty liver also predicted the risk for overall mortality. However, conventional
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23 344 cardiovascular disease risk factors seemed to play a major role in developing death from all
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25 345 causes. It would be beneficial to investigate larger cohorts and follow-up studies in order to
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27 346 validate this result.
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34 348 **Figure legend**

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37 349 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
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39 350 with no fat in the liver, moderate fat content and severe fat content.
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43 351 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
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45 352 of the subjects (10/124) with moderate liver fat content, while 12.5% of the subjects with
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47 353 severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model 1):
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49 354 adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
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51 355 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
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53 356 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
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3 357 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
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5 358 * $p < 0.05$.

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42 371 writing, final approval of the version to be published

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11 379 manuscript, final approval of the version to be published
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17 381 **Data sharing statement:** Extra data is available by emailing pauliina.pisto(at)oulu.fi
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21 382 **Competing Interests:** None
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394 **References**

- 395 1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-31.
- 396 2. Armstrong MJ, Houlihan DD, Bentham L, et al. Presence and severity of non-alcoholic
397 fatty liver disease in a large prospective primary care cohort. *J Hepatol* 2012; Jan;56(1):234-
398 40.
- 399 3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic
400 fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.
- 401 4. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular
402 disease. *Atherosclerosis* 2007; Apr;191(2):235-40.
- 403 5. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2011 update:
404 a report from the American Heart Association. *Circulation* 2011; Feb 1;123(4):e18-e209.
- 405 6. Allender S, Scarborough P, Peto V, et al. European cardiovascular disease statistics, 2008
406 ed. European Heart Network; 2008.
- 407 7. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec
408 14;444(7121):881-7.
- 409 8. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular
410 disease. *Nature* 2006; Dec 14;444(7121):875-80.
- 411 9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*
412 2005; Apr 21;352(16):1685-95.

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3 413 10. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk. *Curr*
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5 414 *Opin Cardiol* 2012; Jul;27(4):420-8.
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9 415 11. Treeprasertsuk S, Leverage S, Adams LA, et al. The Framingham risk score and heart
10
11 416 disease in nonalcoholic fatty liver disease. *Liver Int* 2012; Jul;32(6):945-50.
12
13
14 417 12. Haring R, Wallaschofski H, Nauck M, et al. Ultrasonographic hepatic steatosis increases
15
16 418 prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels.
17
18 419 *Hepatology* 2009; Nov;50(5):1403-11.
19
20
21
22 420 13. Lazo M, Hernaez R, Bonekamp S, et al. Non-alcoholic fatty liver disease and mortality
23
24 421 among US adults: prospective cohort study. *BMJ* 2011; Nov 18;343:d6891.
25
26
27 422 14. Wong VW, Wong GL, Yip GW, et al. Coronary artery disease and cardiovascular
28
29 423 outcomes in patients with non-alcoholic fatty liver disease. *Gut* 2011; Dec;60(12):1721-7.
30
31
32
33 424 15. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in the
34
35 425 pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.
36
37
38
39 426 16. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-
40
41 427 alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;
42
43 428 Nov;51(11):1947-53.
44
45
46 429 17. Targher G, Bertolini L, Scala L, et al. Non-alcoholic hepatic steatosis and its relation to
47
48 430 increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic
49
50 431 men. Role of visceral adipose tissue. *Diabet Med* 2005; Oct;22(10):1354-8.
51
52
53
54
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57
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- 1
2
3 432 18. Brea A, Mosquera D, Martin E, et al. Nonalcoholic fatty liver disease is associated with
4
5 433 carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol* 2005;
6
7 434 May;25(5):1045-50.
8
9
10 435 19. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid
11
12 436 atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
13
14
15
16 437 20. Fraser A, Harris R, Sattar N, et al. Gamma-glutamyltransferase is associated with incident
17
18 438 vascular events independently of alcohol intake: analysis of the British Women's Heart and
19
20 439 Health Study and Meta-Analysis. *Arterioscler Thromb Vasc Biol* 2007; Dec;27(12):2729-35.
21
22
23
24 440 21. Rantala AO, Kauma H, Lilja M, et al. Prevalence of the metabolic syndrome in drug-
25
26 441 treated hypertensive patients and control subjects. *J Intern Med* 1999; Feb;245(2):163-74.
27
28
29 442 22. Yajima Y, Ohta K, Narui T, et al. Ultrasonographical diagnosis of fatty liver: significance
30
31 443 of the liver-kidney contrast. *Tohoku J Exp Med* 1983; Jan;139(1):43-50.
32
33
34
35 444 23. Sampi M, Veneskoski M, Ukkola O, et al. High plasma immunoglobulin (Ig) A and low
36
37 445 IgG antibody titers to oxidized low-density lipoprotein are associated with markers of glucose
38
39 446 metabolism. *J Clin Endocrinol Metab* 2010; May;95(5):2467-75.
40
41
42
43 447 24. Pisto P, Ukkola O, Santaniemi M, et al. Plasma adiponectin--an independent indicator of
44
45 448 liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
46
47
48
49 449 25. Santaniemi M., Ukkola O., Malo E., et al. Metabolic syndrome in the prediction of
50
51 450 cardiovascular events: The potential additive role of hsCRP and adiponectin. *Eur J Prev*
52
53 451 *Cardiol* 2013; Jun;20.
54
55
56
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2
3 452 26. Pajunen P, Jousilahti P, Borodulin K, et al. Body fat measured by a near-infrared
4
5 453 interactance device as a predictor of cardiovascular events: the FINRISK'92 cohort. *Obesity*
6
7 454 (*Silver Spring*) 2011; Apr;19(4):848-52.
- 8
9
10 455 27. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple,
11
12 456 accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000;
13
14 457 Jul;85(7):2402-10.
- 15
16
17 458 28. Kauma H, Savolainen MJ, Rantala AO, et al. Apolipoprotein E phenotype determines the
18
19 459 effect of alcohol on blood pressure in middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt
20
21 460 1):1334-43.
- 22
23
24 461 29. Bessebinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia influenced by
25
26 462 alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
- 27
28
29 463 30. Hamaguchi M, Kojima T, Takeda N, et al. Nonalcoholic fatty liver disease is a novel
30
31 464 predictor of cardiovascular disease. *World J Gastroenterol* 2007; Mar 14;13(10):1579-84.
- 32
33
34 465 31. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty Liver
35
36 466 Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol Hepatol* 2012;
37
38 467 Jun;10(6):646-50.
- 39
40
41 468 32. Targher G, Bertolini L, Poli F, et al. Nonalcoholic fatty liver disease and risk of future
42
43 469 cardiovascular events among type 2 diabetic patients. *Diabetes* 2005; Dec;54(12):3541-6.
- 44
45
46 470 33. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident
47
48 471 cardiovascular disease: a narrative review and clinical perspective of prospective data.
49
50 472 *Hepatology* 2010; Sep;52(3):1156-61.
- 51
52
53
54
55
56
57
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2
3 473 34. Dunn W, Xu R, Wingard DL, et al. Suspected nonalcoholic fatty liver disease and
4
5 474 mortality risk in a population-based cohort study. *Am J Gastroenterol* 2008; Sep;103(9):2263-
6
7 475 71.
8
9
10 476 35. Ruttman E, Brant LJ, Concin H, et al. Gamma-glutamyltransferase as a risk factor for
11
12 477 cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944
13
14 478 Austrian adults. *Circulation* 2005; Oct 4;112(14):2130-7.
15
16
17
18 479 36. Soderberg C, Stal P, Askling J, et al. Decreased survival of subjects with elevated liver
19
20 480 function tests during a 28-year follow-up. *Hepatology* 2010; Feb;51(2):595-602.
21
22
23
24 481 37. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with
25
26 482 NAFLD and elevated liver enzymes. *Hepatology* 2006; Oct;44(4):865-73.
27
28
29
30 483 38. Bhatia LS, Curzen NP, Calder PC, et al. Non-alcoholic fatty liver disease: a new and
31
32 484 important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.
33
34
35 485 39. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,
36
37 486 pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.
38
39
40
41 487 40. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary
42
43 488 prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr*
44
45 489 *Cardiol Rep* 2011; Aug;13(4):287-95.
46
47
48
49 490 41. Dam-Larsen S, Franzmann M, Andersen IB, et al. Long term prognosis of fatty liver: risk
50
51 491 of chronic liver disease and death. *Gut* 2004; May;53(5):750-5.
52
53
54 492 42. Calori G, Lattuada G, Ragogna F, et al. Fatty liver index and mortality: the Cremona
55
56 493 study in the 15th year of follow-up. *Hepatology* 2011; Jul;54(1):145-52.
57
58
59
60

- 1
2
3 494 43. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur J*
4
5 495 *Gastroenterol Hepatol* 2003; May;15(5):539-43.
6
7
8 496 44. Dasarathy S, Dasarathy J, Khiyami A, et al. Validity of real time ultrasound in the
9
10 497 diagnosis of hepatic steatosis: a prospective study. *J Hepatol* 2009; Dec;51(6):1061-7.
11
12
13 498 45. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to
14
15 499 measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population.
16
17 500 *Am J Physiol Endocrinol Metab* 2005; Feb;288(2):E462-8.
18
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p (0-1)	p (1-2)	p (0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose (mmol/L)	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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504 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
505 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
506 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
507 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat content	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat content	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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3 511 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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5 512 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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7 513 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat content and 29.2%
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9 514 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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11 515 with different degrees of adjustments are presented. Alcohol consumption was divided into
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13 516 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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15 517 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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17 518 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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19 519 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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21 520 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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23 521 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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25 522 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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27 523 insulin sensitivity check index. *** p < 0.001, ** p < 0.01, * p < 0.05.
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Grade of liver bightness	Total	0 (n=720)	1 (n=124)	2 (n=144)	p
Non-fatal events					
CVD	11.6% (115)	9.9% (71)	16.1% (20)	16.7% (24)	< 0.05
CHD	7.8% (77)	6.5% (47)	11.3% (14)	11.1% (16)	NS
Stroke	5.0% (49)	4.2% (30)	8.1% (10)	6.2% (9)	NS
Fatal events					
CVD	5.5% (54)	3.6% (26)	8.1% (10)	12.5% (18)	< 0.001
CHD	4.8% (47)	3.2% (23)	7.3% (9)	10.4% (15)	< 0.01
Stroke	0.8% (8)	0.6% (4)	0.8% (1)	2.1% (3)	NS

531 **Table 3.** CVD, CHD and stroke follow-up data of the study group as percentages (number of
532 events). Statistical significances between percentages were measured by using χ^2 test. CVD
533 included a major CHD event and stroke (excluding subarachnoid hemorrhage) - whichever of
534 these happened first. N=number of subjects. CHD, coronary heart disease, CVD,
535 cardiovascular disease.

Final model	Cardiovascular event c-index (95% CI)	Binary R ²	
			536
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			538
Model 3	0.729 (0.706-0.776)	0.153	539
			540
Model 4	0.720 (0.689-0.763)	0.144	
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Model 5	0.717 (0.686-0.758)	0.138	
			542
Model 1	0.698 (0.656-0.742)	0.133	
			543

Table 4. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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For peer review only

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7 **1 Fatty liver predicts the risk for cardiovascular events in middle-aged population: a**
8 **2 population-based cohort study**

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53 20 Research, dated 16 Apr, 2012.
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7 21 **Disclosure summary:** Authors report no conflict of interests.
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10 23 **ABSTRACT**

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12 24 **Objective:** We investigated if the differences in liver fat content would predict the
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14 25 development of non-fatal and fatal atherosclerotic endpoints (coronary heart disease and
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16 26 stroke).
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18 27 **Design, setting and participants:** Our study group is a population-based, randomly recruited
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20 28 cohort (OPERA), initiated in 1991. The cohort consisted of 988 middle-aged Finnish subjects.
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22 29 **Intervention:** Total mortality and hospital events were followed up to 2009 based on the
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24 30 registry of the National Institute for Health and Welfare and the National death registry.
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26 31 **Main outcome measure:** The severity of hepatic steatosis was measured by ultrasound and
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28 32 divided into three groups (0-2). Cox regression analysis was used in the statistical analysis.
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30 33 **Results:** In the follow-up of years 1991-2009, 13.5% of the subjects with non-fatty liver,
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32 34 24.2% of subjects having moderate liver fat content and 29.2% of the subjects having severe
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34 35 fatty liver experienced a cardiovascular event during the follow-up time ($p < 0.001$). Severe
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36 36 liver fat content predicted the risk for future risk of cardiovascular event even when adjusted
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38 37 for age, gender and study group (HR 1.92, CI 1.32-2.80, $p < 0.01$). When further adjustments
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40 38 for smoking, alcohol consumption, LDL-cholesterol, BMI and systolic blood pressure were
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42 39 conducted, the risk still remained statistically significant (HR 1.74, CI 1.16-2.63, $p < 0.01$).
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44 40 Statistical significance disappeared with further adjustment for QUICKI.

45 41 **Conclusions:** Liver fat content increases the risk of future cardiovascular disease event in
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47 42 long-term follow-up but it seems to be dependent on insulin sensitivity.
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7 46 **Article focus**

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9 47 1 To investigate if the differences in liver fat content predict the risk for development of fatal
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11 48 or nonfatal atherosclerotic endpoints such as coronary heart disease and stroke.
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14 49 **Key messages**

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17 50 1 Subjects with ultrasound-diagnosed fatty liver have cardiovascular disease more often
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19 51 compared to the subjects without fat in the liver
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21 52 2 Severe liver fat content increases the risk of a future cardiovascular event and mortality to
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23 53 cardiovascular disease over the long-term follow-up but it does seem to be dependent on
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25 54 insulin sensitivity
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28 55 3 Severe fatty liver predicts the risk for overall mortality but the association is dependent on
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30 56 traditional metabolic risk factors
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33 57 **Strengths and limitations of the study**

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35 58 1 This is a follow-up study with a large population-based study group and a very long follow-
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37 59 up time
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40 60 2 Official registers used in event diagnoses - data is accurate and the classification is
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42 61 systematic
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45 62 3 Grade of liver brightness was measured by ultrasound, which has a high specificity but low
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51 65 **Introduction**
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7 66 Non-alcoholic fatty liver disease (NAFLD) refers to liver disorders such as abnormal fat
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9 67 content, which exists in a spectrum ranging from steatosis with no inflammation to non-
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11 68 alcoholic steatohepatitis (NASH), which can ultimately lead to liver cirrhosis ¹. The
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13 69 prevalence of NAFLD is estimated to range from 20 to 30% of population in Western
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15 70 countries, being the leading cause of liver disorders ^{2,3}. It is associated with obesity, type 2
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17 71 diabetes mellitus (T2DM) and hyperlipidemia ¹. NAFLD is commonly regarded as a hepatic
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19 72 manifestation of the metabolic syndrome and both conditions share several risk factors for
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21 73 cardiovascular disease (CVD) ^{3,4}.

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26 75 In 2008, the prevalence of CVD in adults (≥ 20 years) in United States was 36.2% ⁵. Every
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28 76 year, 4.3 million subjects die for CVD in Europe causing nearly half of the all deaths (48%) ⁶.
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30 77 So-called traditional risk factors for cardiovascular disease are age, gender, smoking, high
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32 78 low-density lipoprotein (LDL) cholesterol concentration, hypertension and diabetes ⁷. In
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34 79 addition, total body fatness as well as abdominal fat accumulation increase independently the
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36 80 risk of CVD and insulin resistance is regarded to be an important factor linking visceral
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38 81 adiposity to cardiovascular risk ⁸. Adipose tissue is now recognized as a significant endocrine
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40 82 organ as adipocytes and macrophages infiltrating adipocytes secrete a number of bioactive
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42 83 mediators ⁷. Adipokines, proinflammatory cytokines and hypofibrinolytic markers may lead to
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44 84 oxidative stress and endothelial dysfunction, finally leading to atherosclerosis ⁹.

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49 86 Hepatic steatosis has been discussed as a possible mechanism to explain CVD morbidity and
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51 87 mortality ¹⁰. NAFLD patients have been reported to have higher coronary heart disease (CHD)
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53 88 risk than the general population of the same age and gender ¹¹. According to previous study,

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7 89 liver dysfunction associated with CVD mortality in men ¹² whereas another large study found
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9 90 no association between NAFLD and CVD in general population ¹³. In addition, fatty liver did
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11 91 not predict CVD mortality and morbidity in patients with established coronary artery disease
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13 92 ¹⁴.

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18 94 The NAFLD and CVD share several molecular mechanisms ^{15,16}. Fatty liver might play a part
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20 95 in the pathogenesis of CVD through the overexpression and systemic release of several
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22 96 inflammatory, hemostatic ¹⁷ and oxidative-stress mediators or via contributing to whole-body
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24 97 insulin resistance and atherogenic dyslipidemia ³. NAFLD has also been reported to be linked
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26 98 with circulatory endothelial dysfunction ^{4,14}. Several investigators have reported that NAFLD
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28 99 is associated with coronary artery disease ^{4,14} and increased carotid intima-media thickness ¹⁸,
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30 100 ¹⁹. Increased gamma-glutamyltransferase (GGT), which may be a marker of NAFLD, has
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32 101 been reported to be associated with stroke ²⁰.

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37 103 It is known that subjects with fatty liver disease have an increased risk of suffering CVD ⁴, but
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39 104 whether NAFLD is an independent indicator of cardiovascular disease is still far from clear.
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41 105 Long-term follow-up studies are needed to clarify the correlation between fatty liver and
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43 106 CVD. The aim of our study was to investigate if fatty liver could predict independently the
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45 107 risk for total mortality as well as non-fatal and fatal cardiovascular endpoints with a 19-year
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47 108 follow-up after adjusting for all known conventional risk factors.

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52 110 **Materials and methods**
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7 111 **Human subjects**

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9 112 OPERA (Oulu Project Elucidating Risk of Atherosclerosis) is a population-based,
10 113 epidemiological prospective cohort study designed to address the risk factors and disease end
11 114 points of atherosclerotic cardiovascular diseases. Selection criteria of the study subjects have
12 115 been described earlier²¹. In short, a total of 520 men and 525 women participated: 259 control
13 116 men, 261 hypertensive men, 267 control women and 258 hypertensive women aged 40-59.
14 117 Hypertensive participants were randomly selected from the national register for
15 118 reimbursement of the costs of antihypertensive medication. For each hypertensive subject, an
16 119 age- and sex-matched control subject was randomly selected from the same register. Informed
17 120 consent in writing was obtained from each patient. The study protocol conformed to the
18 121 ethical guidelines of the 1975 Declaration of Helsinki and this study was approved by the
19 122 Ethical Committee of the Faculty of Medicine, University of Oulu.
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34 124 **Determination of hepatic steatosis**

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37 125 The determination of hepatic steatosis was based on liver-kidney contrast²² measured with
38 126 ultrasonography²³ by one trained radiologist with 10 years' experience in abdominal
39 127 ultrasound examinations. Normal liver parenchyma should be slightly more echogenic (brighter)
40 128 than the kidney parenchyma. In a case of increased liver echogenicity an ultrasound diagnosis of
41 129 bright liver was settled. The severity of hepatic steatosis was based on the brightness of the liver
42 130 and it was classified into three groups ranging from 0 to 2 (0 = normal bright, indicating a
43 131 non-fatty liver, 1 = medium bright, a moderate lipid content and 2 = clearly bright, a severe
44 132 lipid content and fatty liver)²⁴.
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7 134 **Follow-up**
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9 135 Both the hypertensive and the control men were recruited during December 1990 to May
10 136 1992 and the women approximately one year later (n=1045). In total, 1023 subjects had a
11 137 liver ultrasound result available at baseline. Mortality data were obtained from the National
12 138 Death Registry and the diagnoses of cardiovascular events were based on the registry of the
13 139 National Institute for Health and Welfare. The follow-up time ended December 31, 2009 or
14 140 whenever the first event occurred. Cardiovascular events included fatal and non-fatal
15 141 endpoints. Subjects with a previous hospital-diagnosed myocardial infarction or stroke (n=41)
16 142 at baseline were excluded. In total, 988 subjects participated in this part of the study.
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28 144 CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage, SAH) -
29 145 whichever of these happened first²⁵. The evidence of CHD was based on the following
30 146 diagnosis: I20.0, I21, I22 [ICD-10, International Statistical Classification of Diseases and
31 147 Related Health Problems] / 410, 4110 [ICD-8/9] as the main diagnosis (symptom or cause)
32 148 and I21, I22 [ICD-10] / 410 [ICD-8/9] as a first side diagnosis (symptom or cause) or second
33 149 side diagnosis (symptom or cause) and third side diagnosis (ICD-8/9 only) or if a subject had
34 150 undergone coronary artery bypass graft (CABG) surgery or angioplasty. CHD as a cause of
35 151 death included I20–I25, I46, R96, R98 [ICD-10] / 410-414, 798 (not 7980A) [ICD-8/9] as the
36 152 underlying cause of death or immediate cause of death and I21 or I22 [ICD-10] / 410 [ICD-
37 153 8/9] as first to third contributing cause of death. Stroke (excluding SAH) included I61, I63
38 154 (not I636), I64 [ICD -10] / 431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436 [ICD-9] /
39 155 431 (except 43101, 43191) 433, 434, 436 [ICD-8] as main diagnosis (symptom or cause) or as
40 156 a first or second side diagnosis (symptom or cause) or as a third side diagnosis (ICD-8/9 only)
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7 157 or as an underlying cause of death or immediate cause of death or as a first to third
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9 158 contributing cause of death ²⁶.

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14 160 **Laboratory analyses**

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17 161 Waist circumference, body mass index (BMI) and blood pressure were measured as described
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19 162 in previous study ²¹.

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24 164 All the laboratory test samples were obtained after an overnight fast. Blood insulin and
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26 165 glucose concentrations were analyzed at 0, 60, and 120 min after administration of 75 g
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28 166 glucose ²⁴. Insulin sensitivity was assessed using fasting plasma insulin concentrations and a
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30 167 quantitative insulin sensitivity check index (QUICKI) {QUICKI=1/[log (fasting insulin)+log
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32 168 (fasting glucose)]} ²⁷.

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38 170 Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density
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40 171 lipoprotein (LDL) and hs-CRP concentrations ²⁴ as well as alanine aminotransferase (ALT)
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42 172 and GGT levels were measured as described previously ²³. Alcohol consumption and smoking
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44 173 history were determined by validated questionnaires ²⁸. Alcohol consumption was divided into
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46 174 three groups: 0 (n=161) mean alcohol consumption less than 1g/week in men and women, 1
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48 175 (n=767) mean consumption less than 210g/week in men and less than 140 g/week in women,
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50 176 2 (n=76) mean alcohol consumption more than 210g/week in men and more than 140g/week
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52 177 in women. Group 2 designates large-scale alcohol consumers according to the guidelines ²⁹.

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7 179 **Statistical analysis**
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9 180 Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 20.0
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11 181 (Armonk, NY: IBM Corp.). Analysis of variance was used to compare the means of the
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13 182 variables measured. Post hoc tests were performed using the Tukey method. Statistical
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15 183 significances between percentages were measured by using χ^2 test. Cumulative survival rates
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17 184 were estimated using Kaplan-Meier method. Cox regression analysis was performed to
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19 185 investigate if liver brightness (fat) could predict the future risk for total mortality,
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21 186 cardiovascular death or hospital events. A p value < 0.05 was regarded as significant.
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26 188 Skewed variables (smoking, alcohol consumption, fasting insulin, fasting glucose,
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28 189 triglyceride, ALT, GGT concentration, hs-CRP level) were logarithmically transformed to
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30 190 improve normality before analysis of variance. We used three models with progressive
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32 191 degrees of adjustments. Model 1 included study group (subjects with medicine-treated
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34 192 hypertension and their age- and sex-matched controls), age and gender. Model 2 included
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36 193 further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-
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38 194 cholesterol level and body mass index. Model 3 included further adjustment for QUICKI. We
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40 195 carried out sensitivity analyses: in the analyses of cardiovascular events, we added all
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42 196 covariates one by one and investigated if the hazard ratios (HR) changed or remained stable
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44 197 when further adjustment with one covariate was performed. Model 4 included variables which
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46 198 were stable and were statistically significant in intermediate phases. Model 5 included stable
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48 199 and significant covariates without QUICKI (Table 2).
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7 201 C-index was calculated for the model 1, model 3, model 4 and model 5 to assess the
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9 202 discrimination of the risk markers. The analyses were performed in 250 bootstrap resamplings
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11 203 to obtain 95% CI for c-index of each model.
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16 205 **Results**

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19 206 The main baseline characteristics of the study group are shown in Table 1.
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24 208 *Table 1 about here*
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30 210 **Incidence of cardiovascular disease**

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32 211 The median follow-up time was 212 (maximum 228) months. During the follow-up time,
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34 212 13.5% of the subjects with no fat in the liver (97/720), 24.2% (30/124) of subjects having
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36 213 moderate liver fat content and 29.2% (42/144) of the subjects having severe fatty liver
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38 214 experienced a CVD event ($p < 0.001$). CVD was the cause of death in 3.6% of the subjects
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40 215 with non-fatty liver (26/720) and 8.1% of the subjects with moderate liver fat content
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42 216 (10/124), while 12.5% (18/144) of the subjects with severe fatty liver ($p < 0.001$) (Table 3).
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48 218 Severe liver fat content predicted the risk for future risk of cardiovascular event when
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50 219 adjusted for age, gender and study group (Model 1: HR 1.92, CI 1.32-2.80, $p < 0.01$) (Table
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52 220 2). When further adjustments were made for smoking, alcohol consumption, LDL-cholesterol,
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54 221 BMI and systolic blood pressure (Model 2: HR 1.74, CI 1.16-2.63), the risk still remained
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7 222 statistically significant ($p < 0.01$). Statistical significance disappeared when further
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9 223 adjustment for QUICKI was performed (Model 3: HR 1.49, CI 0.97-2.30, $p=0.071$). In the
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11 224 CVD event sensitivity analyses, all covariates were added one by one and it was examined
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13 225 whether the hazard ratios would change or remain stable. After adjusting for the statistically
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15 226 significant variables (including quick index) in the sensitivity analyses, the association
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17 227 between severe fatty liver was no longer significant (Model 4: HR 1.43, CI 0.93-2.18,
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19 228 $p=0.10$). When QUICKI was not added into Model 5, severe fatty liver did predict the risk for
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21 229 future risk for CVD event (HR 1.76, CI 1.21- 2.56, $p < 0.001$) (Table 2). The c-index
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23 230 decreased when the risk factors were removed from the model (Table 4).
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27 232 *Tables 2, 3 and 4 about here*
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33 234 The future risk of death from CVD in participants with severe fat content was significant
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35 235 when age, gender and study group were added as covariates (Model 1: HR 2.95, CI 1.58-5.51,
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37 236 $p < 0.01$). Even after further adjustments with other conventional risk factors (Model 2: HR
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39 237 2.04, CI 1.03-4.05), statistical significance remained ($p < 0.05$). When QUICKI was added as
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41 238 the covariate, then significance disappeared (Model 3: HR 1.64, CI 0.79-3.43, NS) (Fig 1.).
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46 240 *Figure 1 about here*
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52 242 **Fatty liver and total mortality**
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7 243 In total, 11.9% of the participants not having fatty liver, 18.5% of the subjects having
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9 244 moderate fatty liver and 22.2% of the subjects with severe fatty liver died from all causes ($p <$
10 245 0.01). According to Model 1, severe fat content predicted the risk for mortality from all
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12 246 causes when age, gender and study group were added as covariates (HR 1.60, CI 1.05-2.43, p
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14 247 < 0.05). The significance disappeared when body mass index was added as a covariate (data
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16 248 not shown).

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21 250 We performed all Cox regression analyses after excluding the men consuming more than 210
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23 251 g alcohol and the women drinking more than 140 g alcohol per week. This exclusion did not
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25 252 have any effect on the results (data not shown).

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28 253 We performed all Cox regression analyses after excluding patients with insulin treated
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30 254 diabetes mellitus ($n=9$), cortisone treatment at baseline ($n=41$) and previous diagnosis for
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32 255 liver disease ($n=15$) (e.g., virus, medications). This exclusion did not have any effect on the
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34 256 results (data not shown).

35 36 37 257 **Discussion**

38
39 258 The incidences of non-alcoholic fatty liver disease and cardiovascular disease are
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41 259 continuously increasing in the Western world. The question if NAFLD is only a marker or
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43 260 also an early mediator of cardiovascular disease is still largely unanswered. According to the
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45 261 results of the present study, which had an approximately 19-year follow-up fatty liver does
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47 262 predict the future risk for death from all causes, death from cardiovascular disease and risk of
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49 263 cardiovascular events. Insulin sensitivity seems to play a more dominant role in the
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51 264 development of cardiovascular events.

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7 266 Only a few studies have investigated the risk for future cardiovascular risk among subjects
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9 267 with ultrasound-diagnosed fatty liver ^{30, 31} and larger studies with longer follow-up times are
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11 268 needed. An association between NAFLD and CVD has been reported ^{3, 30-32} although contrary
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13 269 results also exist ^{13, 33}. A previous large population-based prospective cohort study found no
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15 270 association between NAFLD and CVD, however they categorized the degree of steatosis as a
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17 271 two level variable: none to mild and moderate to severe ¹³. An association between
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19 272 ultrasound-diagnosed fatty liver and CVD has been reported in general population ³⁰ and in
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21 273 subjects with T2DM ³². Furthermore, liver dysfunction has been reported to associate with
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23 274 CVD mortality ^{34, 35} and CHD risk ¹¹ in follow-up studies and especially survival of subjects
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25 275 with NASH is reported to be reduced ^{33, 36, 37}. In the present study, severe fatty liver disease did
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27 276 predict the risk for cardiovascular death but the association seemed to be dependent on insulin
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29 277 sensitivity.

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32 279 Several earlier studies have used self-reported CVD history which may not be totally reliable.
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34 280 Although earlier studies on the risk for future cardiovascular risk among subjects with fatty
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36 281 liver have performed some adjustments, the full range of well-known CVD risk factors have
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38 282 been rarely considered ³³. We have performed adjustments with all so-called traditional risk
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40 283 factors for cardiovascular disease (i.e. age, gender, smoking, LDL concentration,
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42 284 hypertension, insulin resistance). Previous studies have used biochemical, radiological and
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44 285 histological methodology for NAFLD diagnosis and staging, which leads to a challenging
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46 286 interpretation of the results ^{35, 38}.

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51 288 This study had an approximately 19-year follow-up time, which is longer than in previous
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53 289 studies ¹¹⁻¹⁴. When compared to earlier studies ^{33, 38} this study seems to be the first follow-up
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7 290 study with a large population-based randomly selected study group and a very long follow-up
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9 291 time and ultrasound-diagnosed fatty liver. The diagnosis of cardiovascular events was based
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11 292 on the registry of the National Institute for Health and Welfare and mortality data were
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13 293 obtained from the National Death Registry. The earlier verified FINRISK classification ²⁶ was
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15 294 used to classify the events. Therefore, the reliability of event diagnosis data is accurate and
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17 295 the classification is systematic. All subjects who had myocardial infarction or stroke before
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19 296 baseline were excluded because a history of myocardial infarction is known to increase the
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21 297 risk for recurrent myocardial infarction or cardiovascular death ³⁹ and medication as well as
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23 298 lifestyle secondary prevention strategies are intensive ⁴⁰.

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28 300 There are a few follow-up-studies examining whether the fatty liver increases the risk for total
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30 301 mortality ^{13,41}. In the present study, severe fatty liver predicted the risk for overall mortality of
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32 302 any causes when age, gender and study group were added covariates, a result in line with an
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34 303 earlier report ⁴². In the published literature, NASH rather than simple steatosis has been stated
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36 304 to be linked with decreased overall survival ³⁶ although one study with a large cohort found no
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38 305 association between NAFLD and overall mortality ¹³. In our study, the association between
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40 306 severe fatty liver and total mortality disappeared after further adjustment for BMI which
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42 307 means that severe fatty liver is not a strong predictor for overall mortality.

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47 309 The molecular mechanisms linking fatty liver with CVD have been investigated ^{10, 16}.
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49 310 Enlarged visceral adipose tissue may explain why NAFLD associates with CVD ¹⁶. In
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51 311 individuals with visceral obesity, insulin resistance may contribute to impaired non-esterified
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53 312 fatty acid (NEFA) metabolism ⁸ and the increasing NEFA flux to the liver may impair liver

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7 313 metabolism leading to increased glucose metabolism and liver dysfunction ⁷. The liver is one
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9 314 of the targets of the resulting systemic abnormalities and the source of several proatherogenic
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11 315 factors ³, such as CRP, fibrinogen, plasminogen activator inhibitor-1 and other inflammatory
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13 316 cytokines ¹⁶. Furthermore, visceral adipose tissue and ectopic fat overexpress factors involved
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15 317 in atherogenesis ¹⁶ such as NEFAs and proinflammatory cytokines, for instance interleukin-6
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17 318 and tumor necrosis factor- α ⁸ leading to chronic systemic inflammation. In addition, hepatic
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19 319 steatosis leads to overproduction of cholesterol-rich remnant particles ⁴.

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23 321 One limitation in this study is that the grade of liver brightness was measured by ultrasound.
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25 322 The invasive diagnostic technique of liver biopsy is regarded as the “golden standard”,
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27 323 especially for the diagnosis of NASH ⁴³. Real time ultrasound using a combination of
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29 324 sonographic findings does have a high specificity but it underestimates the prevalence of
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31 325 hepatic steatosis when there is less than 20 % fat ⁴⁴. Today, magnetic resonance spectroscopy
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33 326 is regarded as the best method for the quantification of liver fat, but this method is limited due
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35 327 to its availability ⁴⁵. Unfortunately quantitative measurement of liver fat by ultrasound is
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37 328 subject to several limitations compared to more validated and standardized methods for
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39 329 diagnosing NAFLD and the analysis of intra-observer reproducibility could have been more
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41 330 accurate in the present study. Nonetheless, the noninvasive ultrasound method was chosen
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43 331 because taking liver biopsies from large groups of symptomless subjects would have been
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45 332 ethically unjustifiable and magnetic resonance spectroscopy was not available at the baseline.

Comment [PP1]: Sentence added

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50 334 The OPERA study group consists of subjects with drug-treated hypertension and randomly
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52 335 selected sex- and age-matched controls. Study group was added as a covariate to minimize
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54 336 any selection bias.

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7 337 **Conclusions**

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9 338 Severe liver fat content increased the risk of a future cardiovascular event and mortality to
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11 339 cardiovascular disease over the long-term follow-up but it seemed to be dependent on insulin
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13 340 sensitivity. Fatty liver also predicted the risk for overall mortality. However, conventional
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15 341 cardiovascular disease risk factors seemed to play a major role in developing death from all
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17 342 causes. It would be beneficial to investigate larger cohorts and follow-up studies in order to
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19 343 validate this result.

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23
24 345 **Figure legend**

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27 346 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects
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29 347 with no fat in the liver, moderate fat content and severe fat content.

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32 348 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1%
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34 349 of the subjects (10/124) with moderate liver fat content, while 12.5% of the subjects with
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36 350 severe fatty liver (18/144). Cox regression analysis is used for adjustments. M1 (Model 1):
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38 351 adjusted for study group, age and gender. M2 (Model 2): further adjustments for smoking,
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40 352 alcohol consumption, systolic blood pressure, LDL-cholesterol level and body mass index.
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42 353 M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI, confidence
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44 354 interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$,
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46 355 * $p < 0.05$.

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51 357 **Acknowledgements**

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12
13 361 cooperation in organizing cardiovascular event and mortality data.
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18 363 **Contributor statement:** All authors fulfill all three of the ICMJE guidelines for authorship
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20
21 364 **Pauliina Pisto:** Data acquisition, statistical analysis and interpretation of data, manuscript
22
23 365 writing, final approval of the version to be published
24

25
26 366 **Merja Santaniemi:** Data acquisition, statistical analysis and data interpretation, critical
27
28 367 revision of the manuscript, final approval of the version to be published
29

30
31 368 **Risto Bloigu:** Data analysis, interpretation of data, critical revision of the manuscript, final
32
33 369 approval of the version to be published
34

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36 370 **Olavi Ukkola:** Study design, data acquisition, data interpretation, critical revision of the
37
38 371 manuscript, final approval of the version to be published
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41 372 **Y.A Kesäniemi:** Study design, data acquisition, data interpretation, critical revision of the
42
43 373 manuscript, final approval of the version to be published
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48 375 **Data sharing statement:** Extra data is available by emailing pauliina.pisto(at)oulu.fi
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379 **References**

380 **1. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; Apr 18;346(16):1221-**
381 **31.**

382 **2. Armstrong MJ, Houlihan DD, Bentham L, Shaw JC, Cramb R, Olliff S, et al.**
383 **Presence and severity of non-alcoholic fatty liver disease in a large prospective primary**
384 **care cohort. *J Hepatol* 2012; Jan;56(1):234-40.**

385 **3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with**
386 **nonalcoholic fatty liver disease. *N Engl J Med* 2010; Sep 30;363(14):1341-50.**

387 **4. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of**
388 **cardiovascular disease. *Atherosclerosis* 2007; Apr;191(2):235-40.**

389 **5. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart**
390 **disease and stroke statistics--2011 update: a report from the American Heart**
391 **Association. *Circulation* 2011; Feb 1;123(4):e18-e209.**

392 **6. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A.**
393 **European cardiovascular disease statistics, 2008 ed. European Heart Network; 2008.**

394 **7. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; Dec**
395 **14;444(7121):881-7.**

396 **8. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with**
397 **cardiovascular disease. *Nature* 2006; Dec 14;444(7121):875-80.**

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2
3
4
5
6
7 398 9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J*
8
9 399 *Med* 2005; Apr 21;352(16):1685-95.
10
11 400 10. Bhatia LS, Curzen NP, Byrne CD. Nonalcoholic fatty liver disease and vascular risk.
12
13 401 *Curr Opin Cardiol* 2012; Jul;27(4):420-8.
14
15
16 402 11. Treeprasertsuk S, Leverage S, Adams LA, Lindor KD, St Sauver J, Angulo P. The
17
18 403 Framingham risk score and heart disease in nonalcoholic fatty liver disease. *Liver Int*
19
20 404 2012; Jul;32(6):945-50.
21
22
23 405 12. Haring R, Wallaschofski H, Nauck M, Dorr M, Baumeister SE, Volzke H.
24
25 406 Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated
26
27 407 serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; Nov;50(5):1403-11.
28
29
30 408 13. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-
31
32 409 alcoholic fatty liver disease and mortality among US adults: prospective cohort study.
33
34 410 *BMJ* 2011; Nov 18;343:d6891.
35
36
37 411 14. Wong VW, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, et al. Coronary
38
39 412 artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver
40
41 413 disease. *Gut* 2011; Dec;60(12):1721-7.
42
43
44 414 15. Loria P, Lonardo A, Targher G. Is liver fat detrimental to vessels?: intersections in
45
46 415 the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 2008; Jul;115(1):1-12.
47
48
49 416 16. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-
50
51 417 alcoholic fatty liver disease: causal effect or epiphenomenon?. *Diabetologia* 2008;
52 418 Nov;51(11):1947-53.
53
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7 419 17. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic
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9 420 hepatic steatosis and its relation to increased plasma biomarkers of inflammation and
10
11 421 endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. *Diabet Med*
12
13 422 2005; Oct;22(10):1354-8.
- 14
15 423 18. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty
16
17 424 liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler*
18
19 425 *Thromb Vasc Biol* 2005; May;25(5):1045-50.
- 20
21
22 426 19. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with
23
24 427 carotid atherosclerosis: a systematic review. *J Hepatol* 2008; Oct;49(4):600-7.
- 25
26
27 428 20. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-
28
29 429 glutamyltransferase is associated with incident vascular events independently of alcohol
30
31 430 intake: analysis of the British Women's Heart and Health Study and Meta-Analysis.
32
33 431 *Arterioscler Thromb Vasc Biol* 2007; Dec;27(12):2729-35.
- 34
35
36 432 21. Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA.
37
38 433 Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control
39
40 434 subjects. *J Intern Med* 1999; Feb;245(2):163-74.
- 41
42
43 435 22. Yajima Y, Ohta K, Narui T, Abe R, Suzuki H, Ohtsuki M. Ultrasonographical
44
45 436 diagnosis of fatty liver: significance of the liver-kidney contrast. *Tohoku J Exp Med*
46
47 437 1983; Jan;139(1):43-50.
- 48
49 438 23. Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma
50
51 439 immunoglobulin (Ig) A and low IgG antibody titers to oxidized low-density lipoprotein
52
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6
7 440 are associated with markers of glucose metabolism. *J Clin Endocrinol Metab* 2010;
8
9 441 May;95(5):2467-75.
- 10
11 442 24. Pisto P, Ukkola O, Santaniemi M, Kesaniemi YA. Plasma adiponectin--an
12
13 443 independent indicator of liver fat accumulation. *Metabolism* 2011; Nov;60(11):1515-20.
- 14
15
16 444 25. Santaniemi M, Ukkola O, Malo E., Bloigu R., Kesaniemi YA. Metabolic syndrome
17
18 445 in the prediction of cardiovascular events: The potential additive role of hsCRP and
19
20 446 adiponectin. *Eur J Prev Cardiol* 2013; Jun;20.
- 21
22
23 447 26. Pajunen P, Jousilahti P, Borodulin K, Harald K, Tuomilehto J, Salomaa V. Body fat
24
25 448 measured by a near-infrared interactance device as a predictor of cardiovascular
26
27 449 events: the FINRISK'92 cohort. *Obesity (Silver Spring)* 2011; Apr;19(4):848-52.
- 28
29
30 450 27. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al.
31
32 451 Quantitative insulin sensitivity check index: a simple, accurate method for assessing
33
34 452 insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; Jul;85(7):2402-10.
- 35
36
37 453 28. Kauma H, Savolainen MJ, Rantala AO, Lilja M, Kervinen K, Reunanen A, et al.
38
39 454 Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in
40
41 455 middle-aged men. *Am J Hypertens* 1998; Nov;11(11 Pt 1):1334-43.
- 42
43
44 456 29. Bessembinders K, Wielders J, van de Wiel A. Severe hypertriglyceridemia
45
46 457 influenced by alcohol (SHIBA). *Alcohol Alcohol* 2011; Mar-Apr;46(2):113-6.
- 47
48
49 458 30. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al.
50
51 459 Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J*
52
53 460 *Gastroenterol* 2007; Mar 14;13(10):1579-84.
- 54
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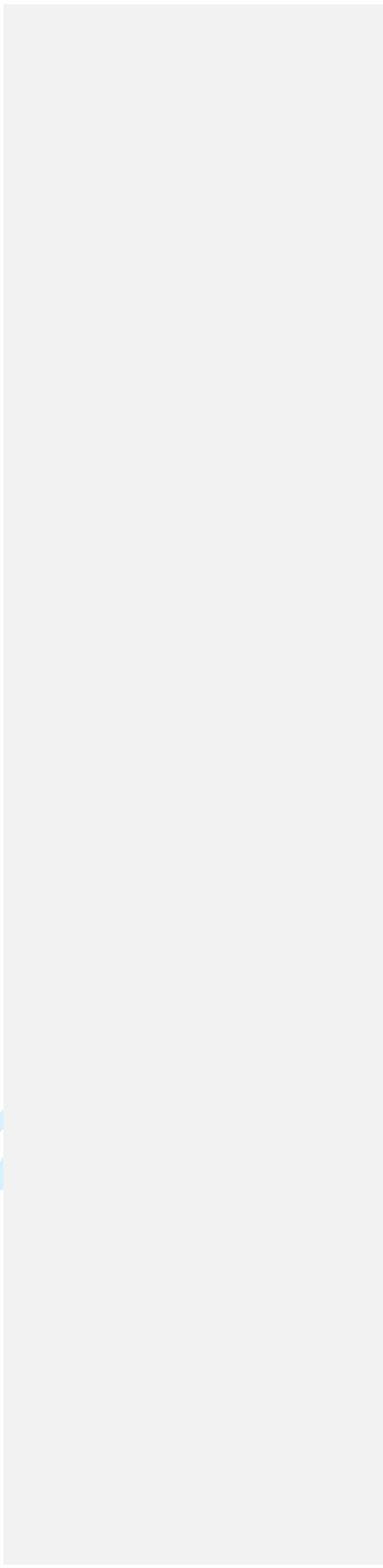
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5
6
7 461 31. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty
8
9 462 Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol*
10
11 463 *Hepatol* 2012; Jun;10(6):646-50.
12
13
14 464 32. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty
15
16 465 liver disease and risk of future cardiovascular events among type 2 diabetic patients.
17
18 466 *Diabetes* 2005; Dec;54(12):3541-6.
19
20 467 33. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and
21
22 468 incident cardiovascular disease: a narrative review and clinical perspective of
23
24 469 prospective data. *Hepatology* 2010; Sep;52(3):1156-61.
25
26
27 470 34. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected
28
29 471 nonalcoholic fatty liver disease and mortality risk in a population-based cohort study.
30
31 472 *Am J Gastroenterol* 2008; Sep;103(9):2263-71.
32
33
34 473 35. Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-
35
36 474 glutamyltransferase as a risk factor for cardiovascular disease mortality: an
37
38 475 epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005;
39
40 476 Oct 4;112(14):2130-7.
41
42
43 477 36. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al.
44
45 478 Decreased survival of subjects with elevated liver function tests during a 28-year follow-
46
47 479 up. *Hepatology* 2010; Feb;51(2):595-602.
48
49
50 480 37. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et
51
52 481 al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*
53
54 482 2006; Oct;44(4):865-73.
55
56
57
58
59
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2
3
4
5
6
7 483 38. Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a
8
9 484 new and important cardiovascular risk factor?. *Eur Heart J* 2012; May;33(10):1190-200.
10
11 485 39. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology,
12
13 486 pathophysiology, and management. *JAMA* 2002; May 15;287(19):2570-81.
14
15
16 487 40. Joseph P, Teo K. Optimal medical therapy, lifestyle intervention, and secondary
17
18 488 prevention strategies for cardiovascular event reduction in ischemic heart disease. *Curr*
19
20 489 *Cardiol Rep* 2011; Aug;13(4):287-95.
21
22
23 490 41. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen
24
25 491 TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut*
26
27 492 2004; May;53(5):750-5.
28
29
30 493 42. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty
31
32 494 liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology*
33
34 495 2011; Jul;54(1):145-52.
35
36 496 43. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary?. *Eur*
37
38 497 *J Gastroenterol Hepatol* 2003; May;15(5):539-43.
39
40
41 498 44. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity
42
43 499 of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J*
44
45 500 *Hepatol* 2009; Dec;51(6):1061-7.
46
47
48 501 45. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et
49
50 502 al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence
51
52 503 of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;
53
54 504 Feb;288(2):E462-8.
55
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Grade of liver bightness	0 (n=720)	1 (n=124)	2 (n=144)	p	p (0-1)	p (1-2)	p (0-2)
Age (years)	50.9 (6.0)	51.9 (6.1)	51.5 (5.5)	NS	NS	NS	NS
Males	44.3 % (n=319)	65.3 % (n=81)	59.9 % (n=82)	< 0.001	-	-	-
Hypertensives	41.4 % (n=298)	66.1 % (n=82)	71.5 % (n=103)	< 0.001	-	-	-
BMI (kg/m ²)	26.4 (3.9)	29.8 (5.0)	31.9 (4.9)	< 0.001	< 0.001	< 0.001	< 0.001
Waist circumference (cm)	86.8 (11.9)	97.7 (12.0)	102.3 (11.8)	< 0.001	< 0.001	< 0.01	< 0.001
Smoking (pack years)	10.6 (13.3)	14.3 (14.9)	14.0 (14.6)	< 0.05	NS	NS	NS
Alcohol consumption (g/week)	51.1 (83.0)	95.1 (117.0)	82.6 (105.1)	< 0.01	< 0.05	NS	NS
Total serum cholesterol (mmol/L)	5.6 (1.0)	5.8 (1.1)	5.8 (1.1)	NS	NS	NS	NS
LDL (mmol/L)	3.5 (0.9)	3.7 (1.1)	3.5 (0.9)	NS	NS	NS	NS
Triglycerides (mmol/L)	1.4 (0.8)	1.9 (0.8)	2.2 (1.4)	< 0.001	< 0.001	< 0.05	< 0.001
Systolic blood pressure	145.2 (21.5)	152.7 (20.3)	157.1 (22.2)	< 0.001	< 0.01	NS	< 0.001
Fasting insulin (mmol/L)	10.8 (7.7)	18.2 (10.3)	23.8 (17.6)	< 0.001	< 0.001	< 0.001	< 0.001

Fasting glucose (mmol/L)	4.4 (0.7)	5.0 (1.4)	6.1 (2.8)	< 0.001	< 0.001	< 0.001	< 0.001
QUICKI	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)	< 0.001	< 0.001	< 0.001	< 0.001
hs-CRP (ng/mL)	3039.4 (6758.3)	3981.4 (6068.2)	6122.0 (6630.8)	< 0.001	< 0.001	< 0.01	< 0.001
ALT U/L	26.2 (15.5)	37.8 (17.1)	55.4 (37.7)	< 0.001	< 0.001	< 0.001	< 0.001
GGT U/L	35.1 (33.5)	69.7 (116.3)	76.8 (92.4)	< 0.001	< 0.001	< 0.01	< 0.001
Anti-hypertensive treatment	43.6% (n=314)	66.9% (n=83)	72.9% (n=105)	< 0.001	-	-	-
Lipid-lowering treatment	2.2% (n=16)	1.6% (n=2)	6.2% (n=9)	< 0.05	-	-	-
Hypoglycaemic drug	1.1% (n=8)	1.6% (n=2)	10.4% (n=15)	< 0.001	-	-	-
Type 2 diabetes	2.4% (n=17)	12.1% (n=15)	36.8% (n=53)	< 0.001	-	-	-

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508 **Table 1.** Baseline characteristics of the study group as means (standard deviations) or
509 percentages. N= number of subjects. ALT, alanine aminotransferase, BMI, body mass index,
510 GGT, gamma-glutamyltransferase, hs-CRP, high-sensitivity C-reactive protein, LDL, low-
511 density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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	Model 1	Model 2	Model 3	Model 4	Model 5
Moderate fat content	1.51 (0.99-2.29)	1.44 (0.93-2.23)	1.31 (0.84-2.05)	1.30 (0.84-2.01)	1.49 (0.99-2.26)
Severe fat content	1.92 (1.32-2.80)**	1.74 (1.16-2.63) **	1.49 (0.97-2.30)	1.43 (0.93-2.18)	1.76 (1.21- 2.56) **
Study group	1.34 (0.98-1.85)	1.29 (0.92-1.80)	1.28 (0.92-1.78)		
Age	1.06 (1.03-1.09)***	1.05(1.02-1.08)**	1.05 (1.02-1.08)**	1.05 (1.02-1.07)**	1.05 (1.02-1.08) **
Gender	2.39 (1.71-3.34)*	1.91 (1.34-2.71)***	1.80 (1.26-2.57)**	1.83 (1.29-2.60) **	1.92 (1.36-2.72) ***
LDL-cholesterol		1.17 (0.99-1.39)	1.15 (0.97-1.37)		
Smoking (pack-years)		1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03)***	1.02 (1.01-1.03) ***
Alcohol consumption (gr1)		0.93 (0.59-1.45)	0.92(0.59-1.44)		
Alcohol consumption (gr2)		0.84 (0.44-1.60)	0.81(0.42-1.54)		
Systolic blood pressure		1.01 (1.00-1.02)**	1.01 (1.00-1.02)*	1.01 (1.00-1.02)**	1.01 (1.00-1.02) **
Body mass index		0.99 (0.96-1.03)	0.97 (0.93-1.01)		
QUICKI			0.12 (0.02-0.90)*	0.16 (0.03-0.99)*	

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7 515 **Table 2.** Multivariate analysis for cardiovascular events with different degrees of adjustments
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9 516 (Cox regression analysis). CVD event occurred in 13.5% of the subjects with no fat in the
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11 517 liver (97/720), 24.2% (30/124) of subjects having moderate liver fat content and 29.2%
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13 518 (42/144) of the subjects having severe fatty liver. Hazard ratios with 95% confidence interval
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15 519 with different degrees of adjustments are presented. Alcohol consumption was divided into
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17 520 groups (reference group: less than 1g/week in men and women, group 1: less than 210g/week
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19 521 in men and less than 140 g/week in women, group 2: more than 210g/week in men and more
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21 522 than 140g/week in women). Model 1: adjustment for study group, age and gender. Model 2:
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23 523 further adjustments for LDL-cholesterol, smoking, alcohol consumption, systolic blood
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25 524 pressure and body mass index. Model 3: further adjustment for QUICKI. Model 4:
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27 525 adjustments with statistically significant covariates. Model 5: adjustments with statistically
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29 526 significant covariates without QUICKI. LDL, low-density lipoprotein, QUICKI, quantitative
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31 527 insulin sensitivity check index. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

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Grade of liver brightness	Total	0 (n=720)	1 (n=124)	2 (n=144)	p
Non-fatal events					
CVD	11.6% (115)	9.9% (71)	16.1% (20)	16.7% (24)	< 0.05
CHD	7.8% (77)	6.5% (47)	11.3% (14)	11.1% (16)	NS
Stroke	5.0% (49)	4.2% (30)	8.1% (10)	6.2% (9)	NS
Fatal events					
CVD	5.5% (54)	3.6% (26)	8.1% (10)	12.5% (18)	< 0.001
CHD	4.8% (47)	3.2% (23)	7.3% (9)	10.4% (15)	< 0.01
Stroke	0.8% (8)	0.6% (4)	0.8% (1)	2.1% (3)	NS

Table 3. CVD, CHD and stroke follow-up data of the study group as percentages (number of events). **Statistical significances between percentages were measured by using χ^2 test.** CVD included a major CHD event and stroke (excluding subarachnoid hemorrhage) - whichever of these happened first. N=number of subjects. CHD, coronary heart disease, CVD, cardiovascular disease.

Comment [PP2]: Sentence added

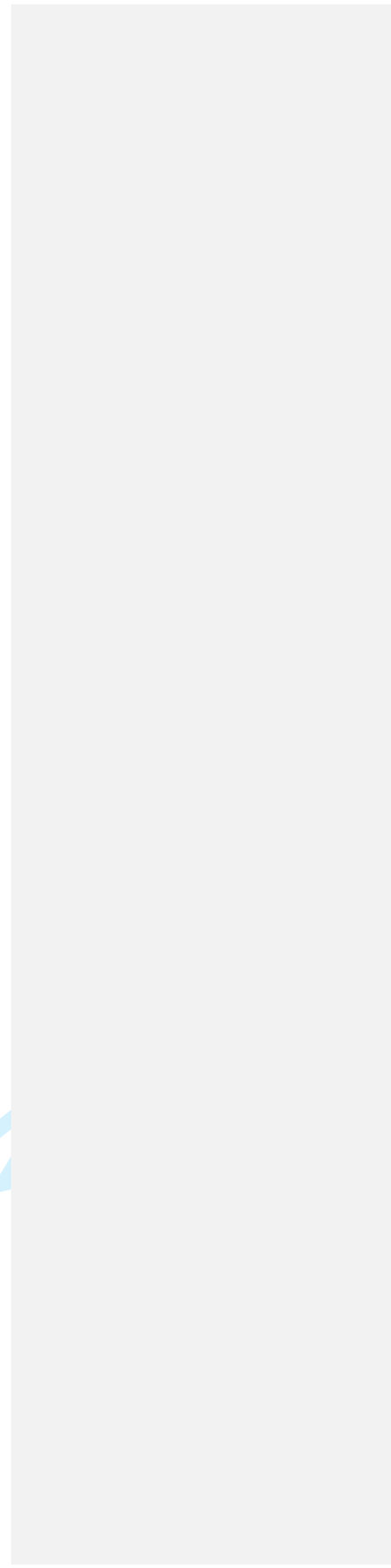
	Cardiovascular event	Binary R ²	
			540
Final model	c-index (95% CI)		541
			542
Model 3	0.729 (0.706-0.776)	0.153	543
			544
Model 4	0.720 (0.689-0.763)	0.144	
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Model 5	0.717 (0.686-0.758)	0.138	
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Model 1	0.698 (0.656-0.742)	0.133	
			547

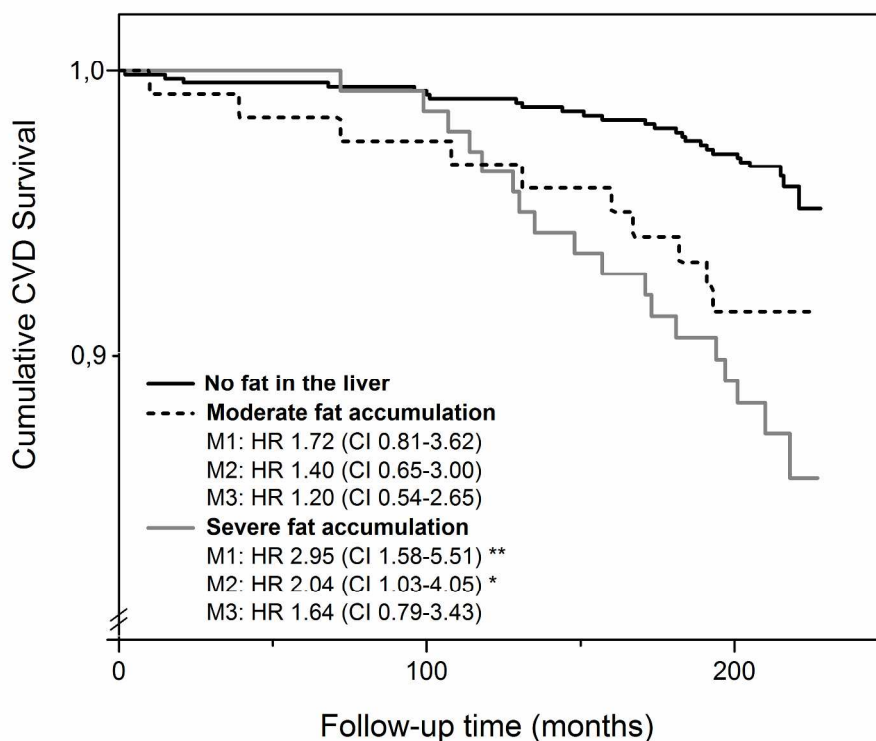
Table 4. Multivariate analysis for cardiovascular events (logistic regression analysis). Cardiovascular disease risk factors have been removed from the models step by step. Model 3 included liver brightness, study group, age, gender, smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol level, body mass index and QUICKI. Model 4 included liver brightness, age, gender, smoking, blood pressure and QUICKI. Model 5 included liver brightness, age, gender, smoking, blood pressure. Model 1 included liver brightness, study group, age and gender. C-index with confidence intervals obtained from 250 bootstrap resamplings and binary R² was used. LDL, low-density lipoprotein, QUICKI, quantitative insulin sensitivity check index.

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35 Title: Kaplan Meier cumulative survival rates censored for cardiovascular death in subjects with no fat in the
 36 liver, moderate fat content and severe fat content.

37 CVD was the cause of death in 3.6% of the subjects (26/720) with non-fatty liver and 8.1% of the subjects
 38 (10/124) with moderate liver fat content, while 12.5% of the subjects with severe fatty liver (18/144). Cox
 39 regression analysis is used for adjustments. M1 (Model 1): adjusted for study group, age and gender. M2
 40 (Model 2): further adjustments for smoking, alcohol consumption, systolic blood pressure, LDL-cholesterol
 41 level and body mass index. M3 (Model 3): further adjustment for QUICKI. CVD, cardiovascular disease, CI,
 42 confidence interval, HR, hazard ratio, QUICKI, quantitative insulin sensitivity check index. ** $p < 0.01$, * p
 43 < 0.05 .

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STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) page 1
Authors	Contact details for the corresponding author page 1
Study design	Description of the study design (e.g cohort, case-control, cross sectional) page 6
Objective	Specific objectives or hypothesis page 5
Methods	page 5
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). page 7
Participants	<p><i>Cohort study</i>—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up page 6</p> <p><i>Case-control study</i>—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection</p> <p><i>Cross-sectional study</i>—Give the eligibility criteria, and the major sources and methods of selection of participants</p>
<i>Cohort study</i> —For matched studies, give matching and number of exposed and unexposed page 7	
<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Variables	Clearly define primary outcome for this report. page 10
Statistical methods	Describe statistical methods, including those used to control for confounding page 9
Results	
Participants	Report Number of participants at the beginning and end of the study page 7
Main results	<p>Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</p> <p>Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals) page 10</p>
Conclusions	General interpretation of study results page 12

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