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Citation for final published version:

Isokuortti, Elina, Zhou, You, Peltonen, Markku, Bugianesi, Elisabetta, Clement, Karine, Bonnefont-Rousselot, Dominique, Lacorte, Jean-Marc, Gastaldelli, Amalia, Schuppan, Detlef, Schattenberg, Jörn M., Hakkarainen, Antti, Lundbom, Nina, Jousilahti, Pekka, Männistö, Satu, Keinänen-Kiukaanniemi, Sirkka, Saltevo, Juha, Anstee, Quentin M. and Yki-Järvinen, Hannele 2017. Use of HOMA-IR to diagnose non-alcoholic fatty liver disease: a population-based and inter-laboratory study. Diabetologia 60 (10) , pp. 1873-1882. 10.1007/s00125-017-4340-1 file

Publishers page: http://dx.doi.org/10.1007/s00125-017-4340-1 <http://dx.doi.org/10.1007/s00125-017-4340-1>

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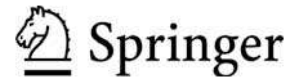

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Authors: **Isokuortti · Zhou · Peltonen · Bugianesi · Clement · Bonnefont-Rousselot · Lacorte · Gastaldelli · Schuppan · Schattenberg · Hakkarainen · Lundbom · Jousilahti · Mannisto · Keinanen-Kiukaanniemi · Saltevo · Anstee · Yki-Jarvinen**

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4	Journal Name	Diabetologia	
5	Corresponding Author	Family Name	**Isokuortti**
6		Particle	
7		Given Name	**Elina**
8		Suffix	
9		Organization	Minerva Foundation Institute for Medical Research
10		Division	
11		Address	Biomedicum Helsinki 2 U, Tukholmankatu 8, Helsinki FIN - 00290
12		Organization	University of Helsinki and Helsinki University Hospital
13		Division	Department of Medicine
14		Address	Helsinki
15		e-mail	elina.isokuortti@helsinki.fi
16	Author	Family Name	**Zhou**
17		Particle	
18		Given Name	**You**
19		Suffix	
20		Organization	Cardiff University
21		Division	Systems Immunity University Research Institute
22		Address	Cardiff
23		Organization	Cardiff University
24		Division	Division of Infection and Immunity, School of Medicine
25		Address	Cardiff
26		e-mail	
27	Author	Family Name	**Peltonen**

AUTHOR'S PROOF!

28		Particle	
29		Given Name	**Markku**
30		Suffix	
31		Organization	National Institute for Health and Welfare
32		Division	
33		Address	Helsinki
34		e-mail	
35		Family Name	**Bugianesi**
36		Particle	
37		Given Name	**Elisabetta**
38		Suffix	
39	Author	Organization	University of Torino
40		Division	Division of Gastroenterology, Department of Medical Sciences
41		Address	Torino
42		e-mail	
43		Family Name	**Clement**
44		Particle	
45		Given Name	**Karine**
46		Suffix	
47		Organization	Institute of Cardiometabolism and Nutrition (ICAN), Pitié-Salpêtrière Hospital
48	Author	Division	
49		Address	Paris
50		Organization	Sorbonne Université, UPMC University Paris 06, UMR_S 1166, Inserm
51		Division	
52		Address	Paris
53		e-mail	
54		Family Name	**Bonnefont-Rousselot**
55		Particle	
56		Given Name	**Dominique**
57		Suffix	
58	Author	Organization	La Pitié Salpêtrière-Charles Foix University Hospital (AP-HP)
59		Division	Department of Metabolic Biochemistry
60		Address	Paris
61		Organization	Paris Descartes University

AUTHOR'S PROOF!

62		Division	Department of Biochemistry, Faculty of Pharmacy
63		Address	Paris
64		Organization	Paris Descartes University
65		Division	CNRS UMR8258 – Inserm U1022, Faculty of Pharmacy
66		Address	Paris
67		e-mail	
68	Author	Family Name	**Lacorte**
69		Particle	
70		Given Name	**Jean-Marc**
71		Suffix	
72		Organization	Institute of Cardiometabolism and Nutrition (ICAN), Pitié-Salpêtrière Hospital
73		Division	
74		Address	Paris
75		Organization	Sorbonne Université, UPMC University Paris 06, UMR_S 1166, Inserm
76		Division	
77		Address	Paris
78		Organization	La Pitié Salpêtrière-Charles Foix University Hospital (AP-HP)
79		Division	Department of Endocrine and Oncological Biochemistry
80		Address	Paris
81		e-mail	
82	Author	Family Name	**Gastaldelli**
83		Particle	
84		Given Name	**Amalia**
85		Suffix	
86		Organization	Institute of Clinical Physiology, CNR
87		Division	Cardiometabolic Risk Laboratory
88		Address	Pisa
89		e-mail	
90	Author	Family Name	**Schuppan**
91		Particle	
92		Given Name	**Detlef**
93		Suffix	
94		Organization	Johannes Gutenberg University Mainz

95		Division	Institute of Translational Immunology, Research Center of Immune Therapy, University Medical Centre
96		Address	Mainz
97		e-mail	
98	Author	Family Name	**Schattenberg**
99		Particle	
100		Given Name	**Jörn M.**
101		Suffix	
102		Organization	Johannes Gutenberg University Mainz
103		Division	Department of Medicine I, University Medical Centre
104		Address	Mainz
105		e-mail	
106	Author	Family Name	**Hakkarainen**
107		Particle	
108		Given Name	**Antti**
109		Suffix	
110		Organization	University of Helsinki and Helsinki University Hospital
111		Division	Helsinki Medical Imaging Center
112		Address	Helsinki
113		e-mail	
114	Author	Family Name	**Lundbom**
115		Particle	
116		Given Name	**Nina**
117		Suffix	
118		Organization	University of Helsinki and Helsinki University Hospital
119		Division	Helsinki Medical Imaging Center
120		Address	Helsinki
121		e-mail	
122	Author	Family Name	**Jousilahti**
123		Particle	
124		Given Name	**Pekka**
125		Suffix	
126		Organization	National Institute for Health and Welfare
127		Division	
128		Address	Helsinki

AUTHOR'S PROOF!

129		e-mail	
130	Author	Family Name	**Männistö**
131		Particle	
132		Given Name	**Satu**
133		Suffix	
134		Organization	National Institute for Health and Welfare
135		Division	
136		Address	Helsinki
137		e-mail	
138	Author	Family Name	**Keinänen-Kiukaanniemi**
139		Particle	
140		Given Name	**Sirkka**
141		Suffix	
142		Organization	University of Oulu
143		Division	Institute of Health Sciences
144		Address	Oulu
145		e-mail	
146	Author	Family Name	**Saltevo**
147		Particle	
148		Given Name	**Juha**
149		Suffix	
150		Organization	Central Finland Central Hospital
151		Division	Department of Medicine
152		Address	Jyväskylä
153		e-mail	
154	Author	Family Name	**Anstee**
155		Particle	
156		Given Name	**Quentin M.**
157		Suffix	
158		Organization	Newcastle University
159		Division	Institute of Cellular Medicine, The Medical School
160		Address	Newcastle upon Tyne
161		Organization	Freeman Hospital
162		Division	Liver Unit, Newcastle Upon Tyne Hospitals NHS Trust
163		Address	Newcastle upon Tyne
164		e-mail	

AUTHOR'S PROOF!

165	Author	Family Name	**Yki-Järvinen**
166		Particle	
167		Given Name	**Hannele**
168		Suffix	
169		Organization	Minerva Foundation Institute for Medical Research
170		Division	
171		Address	Biomedicum Helsinki 2 U, Tukholmankatu 8, Helsinki FIN - 00290
172		Organization	University of Helsinki and Helsinki University Hospital
173		Division	Department of Medicine
174		Address	Helsinki
175		e-mail	

176	Schedule	Received	6 March 2017
177		Revised	
178		Accepted	15 May 2017

179 Abstract

Aims/hypothesis: Recent European guidelines for non-alcoholic fatty liver disease (NAFLD) call for reference values for HOMA-IR. In this study, we aimed to determine: (1) the upper limit of normal HOMA-IR in two population-based cohorts; (2) the HOMA-IR corresponding to NAFLD; (3) the effect of sex and *PNPLA3* genotype at rs738409 on HOMA-IR; and (4) inter-laboratory variations in HOMA-IR.

Methods: We identified healthy individuals in two population-based cohorts (FINRISK 2007 [*n* = 5024] and the Programme for Prevention of Type 2 Diabetes in Finland [FIN-D2D; *n* = 2849]) to define the upper 95th percentile of HOMA-IR. Non-obese individuals with normal fasting glucose levels, no excessive alcohol use, no known diseases and no use of any drugs were considered healthy. The optimal HOMA-IR cut-off for NAFLD (liver fat ≥5.56%, based on the Dallas Heart Study) was determined in 368 non-diabetic individuals (35% with NAFLD), whose liver fat was measured using proton magnetic resonance spectroscopy (^{1}H-MRS). Samples from ten individuals were simultaneously analysed for HOMA-IR in seven European laboratories.

Results: The upper 95th percentiles of HOMA-IR were 1.9 and 2.0 in healthy individuals in the FINRISK (*n* = 1167) and FIN-D2D (*n* = 459) cohorts. Sex or *PNPLA3* genotype did not influence these values. The optimal HOMA-IR cut-off for NAFLD was 1.9 (sensitivity 87%, specificity 79%). A HOMA-IR of 2.0 corresponded to normal liver fat (<5.56% on ^{1}H-MRS) in linear regression analysis. The 2.0 HOMA-IR measured in Helsinki corresponded to 1.3, 1.6, 1.8, 1.8, 2.0 and 2.1 in six other laboratories. The inter-laboratory CV% of

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HOMA-IR was 25% due to inter-assay variation in insulin (25%) rather than glucose (5%) measurements.
Conclusions/interpretation: The upper limit of HOMA-IR in population-based cohorts closely corresponds to that of normal liver fat. Standardisation of insulin assays would be the first step towards definition of normal values for HOMA-IR.

180	Keywords separated by ' - '	Insulin - Liver fat - NAFLD - *PNPLA3* - Reference values
181	Foot note information	The online version of this article (doi:10.1007/s00125-017-4340-1) contains supplementary material, which is available to authorized users.

Electronic supplementary material

ESM 1
(PDF 777 kb)

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JrnlID 125_ArtID 4340_Proof# 1 - 17/06/2017

Diabetologia
DOI 10.1007/s00125-017-4340-1

1 3 2 ARTICLE

4 # Use of HOMA-IR to diagnose non-alcoholic fatty liver disease:
5 a population-based and inter-laboratory study

6 **Elina Isokuortti[1,2] · You Zhou[3,4] · Markku Peltonen[5] · Elisabetta Bugianesi[6] ·**
7 **Karine Clement[7,8] · Dominique Bonnefont-Rousselot[9,10,11] · Jean-Marc Lacorte[7,8,12] ·**
8 **Amalia Gastaldelli[13] · Detlef Schuppan[14] · Jörn M. Schattenberg[15] ·**
9 **Antti Hakkarainen[16] · Nina Lundbom[16] · Pekka Jousilahti[5] · Satu Männistö[5] ·**
10 **Sirkka Keinänen-Kiukaanniemi[17] · Juha Saltevo[18] · Quentin M. Anstee[19,20] ·**
11 **Hannele Yki-Järvinen[1,2]**
12

13 Received: 6 March 2017 / Accepted: 15 May 2017
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15 **Abstract**
16 *Aims/hypothesis* Recent European guidelines for non-
17 alcoholic fatty liver disease (NAFLD) call for reference values
18 for HOMA-IR. In this study, we aimed to determine: (1) the
19 upper limit of normal HOMA-IR in two population-based
20 cohorts; (2) the HOMA-IR corresponding to NAFLD; (3)
21 the effect of sex and *PNPLA3* genotype at rs738409 on
22 HOMA-IR; and (4) inter-laboratory variations in HOMA-IR.
23 *Methods* We identified healthy individuals in two
24 population-based cohorts (FINRISK 2007 [n = 5024]
25 and the Programme for Prevention of Type 2 Diabetes in
26 Finland [FIN-D2D; n = 2849]) to define the upper 95th

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Electronic supplementary material The online version of this article (doi:10.1007/s00125-017-4340-1) contains supplementary material, which is available to authorized users.

✉ Elina Isokuortti
elina.isokuortti@helsinki.fi

[1] Minerva Foundation Institute for Medical Research, Biomedicum Helsinki 2 U, Tukholmankatu 8, FIN - 00290 Helsinki, Finland

[2] Department of Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[3] Systems Immunity University Research Institute, Cardiff University, Cardiff, UK

[4] Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, UK

[5] National Institute for Health and Welfare, Helsinki, Finland

[6] Division of Gastroenterology, Department of Medical Sciences, University of Torino, Torino, Italy

[7] Institute of Cardiometabolism and Nutrition (ICAN), Pitié-Salpêtrière Hospital, Paris, France

[8] Sorbonne Université, UPMC University Paris 06, UMR_S 1166, Inserm, Paris, France

[9] Department of Metabolic Biochemistry, La Pitié Salpêtrière-Charles Foix University Hospital (AP-HP), Paris, France

[10] Department of Biochemistry, Faculty of Pharmacy, Paris Descartes University, Paris, France

[11] CNRS UMR8258 – Inserm U1022, Faculty of Pharmacy, Paris Descartes University, Paris, France

[12] Department of Endocrine and Oncological Biochemistry, La Pitié Salpêtrière-Charles Foix University Hospital (AP-HP), Paris, France

[13] Cardiometabolic Risk Laboratory, Institute of Clinical Physiology, CNR, Pisa, Italy

[14] Institute of Translational Immunology, Research Center of Immune Therapy, University Medical Centre, Johannes Gutenberg University Mainz, Mainz, Germany

[15] Department of Medicine I, University Medical Centre, Johannes Gutenberg University Mainz, Mainz, Germany

[16] Helsinki Medical Imaging Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[17] Institute of Health Sciences, University of Oulu, Oulu, Finland

[18] Department of Medicine, Central Finland Central Hospital, Jyväskylä, Finland

[19] Institute of Cellular Medicine, The Medical School, Newcastle University, Newcastle upon Tyne, UK

[20] Liver Unit, Newcastle Upon Tyne Hospitals NHS Trust, Freeman Hospital, Newcastle upon Tyne, UK

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percentile of HOMA-IR. Non-obese individuals with normal fasting glucose levels, no excessive alcohol use, no known diseases and no use of any drugs were considered healthy. The optimal HOMA-IR cut-off for NAFLD (liver fat ≥5.56%, based on the Dallas Heart Study) was determined in 368 non-diabetic individuals (35% with NAFLD), whose liver fat was measured using proton magnetic resonance spectroscopy (^{1}H-MRS). Samples from ten individuals were simultaneously analysed for HOMA-IR in seven European laboratories.

Results The upper 95th percentiles of HOMA-IR were 1.9 and 2.0 in healthy individuals in the FINRISK (n = 1167) and FIN-D2D (n = 459) cohorts. Sex or *PNPLA3* genotype did not influence these values. The optimal HOMA-IR cut-off for NAFLD was 1.9 (sensitivity 87%, specificity 79%). A HOMA-IR of 2.0 corresponded to normal liver fat (<5.56% on ^{1}H-MRS) in linear regression analysis. The 2.0 HOMA-IR measured in Helsinki corresponded to 1.3, 1.6, 1.8, 1.8, 2.0 and 2.1 in six other laboratories. The inter-laboratory CV% of HOMA-IR was 25% due to inter-assay variation in insulin (25%) rather than glucose (5%) measurements.

Conclusions/interpretation The upper limit of HOMA-IR in population-based cohorts closely corresponds to that of normal liver fat. Standardisation of insulin assays would be the first step towards definition of normal values for HOMA-IR.

Keywords Insulin · Liver fat · NAFLD · *PNPLA3* · Reference values

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUROC	Area under the receiver operating characteristic (curve)
DHS	Dallas Heart Study
DILGOM	Dietary Lifestyle and Genetic Determinants of the Development of Obesity and Metabolic Syndrome study
FIN-D2D	Programme for Prevention of Type 2 Diabetes in Finland
GGT	γ-Glutamyltransferase
^{1}H-MRS	Proton magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver disease
NPV	Negative predictive value
PNPLA3	Patatin-like phospholipase domain-containing protein 3
PPV	Positive predictive value
ROC	Receiver operating characteristic

Introduction

Insulin resistance in individuals with non-alcoholic fatty liver disease (NAFLD) is characterised by reduced whole body, hepatic and adipose tissue insulin sensitivity [1, 2]. The liver, once insulin resistant, overproduces glucose that stimulates insulin secretion, resulting in mild hyperglycaemia and hyperinsulinaemia. Therefore, the product of fasting glucose and fasting insulin divided by a constant (i.e. HOMA-IR) [3] is a good surrogate for insulin sensitivity in non-diabetic individuals [3–5]. Once the glucose concentration reaches the diagnostic threshold for type 2 diabetes, the insulin concentration starts to decline relative to glucose [6–9] and HOMA-IR no longer exclusively reflects insulin sensitivity.

A recent joint European practice guideline for NAFLD [10] concluded: 'HOMA-IR provides a surrogate estimate of insulin resistance in persons without diabetes and can therefore be recommended, provided proper reference values have been established.' A reference value can be defined as the mean + 2 SDs for normally distributed variables or the 95th percentile for non-normally distributed variables of a population-based sample [11, 12]. Definition of health then becomes dependent on the underlying population. This is particularly relevant for HOMA-IR, as obesity is highly prevalent and perhaps the single most important cause of variation in insulin [13]. Thus, for HOMA-IR, it would seem wiser to use reference values derived from healthy individuals, although definitions of health can also vary [14–16]. Pre-analytical causes of variation should also be considered [3, 4], and the inter-assay variation of insulin [17] and glucose should be known.

Normal liver fat content, measured using proton magnetic resonance spectroscopy (^{1}H-MRS), was determined in the population-based Dallas Heart Study (DHS) [18]. In healthy individuals (BMI <25 kg/m^{2}, no diabetes, normal fasting glucose levels, low alcohol consumption, no known liver disease or risk factors for liver disease and normal alanine aminotransferase [ALT] levels; n = 345), the 95th percentile for liver fat content was 5.56%. It is unknown how HOMA-IR relates to this amount of liver fat and whether this definition of normal liver fat reflects what normal liver fat is elsewhere. The I148M variant of the gene encoding patatin-like phospholipase domain-containing protein 3 (PNPLA3) (rs738409 c.444 C>G, p.I148M) has a prevalence of 30–50% [19]. It increases the risk of NAFLD, but not of insulin resistance [20]. The impact of this gene variant on reference values for HOMA-IR has not been studied.

In the present study, we aimed to determine: (1) the upper 95th percentile of HOMA-IR in two population-based cohorts; (2) the HOMA-IR that best distinguishes between NAFLD and normal liver fat content, as quantified by ^{1}H-MRS in a cohort of non-diabetic individuals; (3) whether sex or the *PNPLA3* genotype at rs738409 influences reference values for HOMA-IR; and (4) the inter-laboratory variation in

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142 HOMA-IR among European centres participating in the
143 Elucidating Pathways of Steatohepatitis (EPoS) consortium
144 (www.epos-nafld.eu).

145 Methods

146 Study designs

147 **Population-based cohorts for the determination of normal**
148 **HOMA-IR** To determine normal HOMA-IR, we studied non-
149 pregnant adults in two population-based cohorts: the National
150 FINRISK 2007/ Dietary Lifestyle and Genetic Determinants
151 of the Development of Obesity and Metabolic Syndrome
152 study (DILGOM) study (n = 5024), conducted by the
153 National Institute for Health and Welfare in Finland between
154 January and July 2007 [21]; and the Programme for
155 Prevention of Type 2 Diabetes in Finland (FIN-D2D)
156 (n = 2849), conducted between October and December 2007
157 [22] (see electronic supplementary material [ESM] Methods
158 and ESM Fig. 1). The definition of healthy was as in the
159 population-based DHS [18]: (1) alcohol use <30 g/day in
160 men and <20 g/day in women; (2) no diabetes, based on his-
161 tory and normal fasting plasma glucose levels (<6.1 mmol/l);
162 (3) BMI <25 kg/m^2; (4) no regular use of drugs; and (5) no
163 clinical or biochemical evidence of liver or other disease, as
164 defined by history and biochemical examinations.

165 **Liver fat cohort** Participants for the liver fat cohort were
166 recruited using newspaper advertisements, by contacting oc-
167 cupational health services and from individuals referred to the
168 Department of Gastroenterology, Helsinki University Hospital
169 (Helsinki, Finland), because of chronically elevated serum
170 transaminase concentrations using the following inclusion
171 criteria: (1) age 18–75 years; (2) no known acute or chronic
172 disease except obesity, hypertension or NAFLD based on
173 medical history, physical examination, standard laboratory
174 tests and ECG; (3) non-diabetic based on a fasting plasma
175 glucose level of ≤6.9 mmol/l; and (4) alcohol consumption
176 of ≤20 g per day in women and ≤30 g in men [23]. Study
177 physicians assessed alcohol intake using the same question-
178 naire as in the population-based studies. Exclusion criteria
179 included: (1) pregnancy; (2) serologic evidence of hepatitis
180 B/C or autoimmune hepatitis; (3) clinical signs or symptoms
181 of inborn errors of metabolism; (4) a history of predisposition
182 to toxins; (5) use of drugs associated with liver steatosis; and
183 (6) use of antihypertensive drugs or other drugs possibly
184 influencing glucose metabolism. The study protocol was ap-
185 proved by the ethics committee of the Helsinki University
186 Central Hospital and was carried out in accordance with the
187 Declaration of Helsinki. Each participant provided written in-
188 formed consent.

Inter-laboratory variation in insulin assays Ten non- 189
diabetic individuals covering a wide range of insulin sensitiv- 190
ities were recruited. The participants were healthy based on 191
medical history, physical examination and standard laboratory 192
tests, but eight of them were overweight or obese (BMI 193
≥25 kg/m^2). Blood was drawn in Helsinki after a 12 h fast 194
for measurement of fasting insulin, glucose, HDL-cholesterol, 195
LDL-cholesterol, total cholesterol, triacylglycerol, aspartate 196
aminotransferase (AST), ALT, γ-glutamyltransferase (GGT), 197
ferritin and albumin. Measurements of laboratory variables 198
other than insulin or glucose were performed for comparison 199
to estimate their inter-assay CVs. The fresh samples were 200
analysed immediately in Helsinki. To study the effect of freez- 201
ing, another set of samples were immediately frozen to −80°C 202
and then melted and assayed on the same day in Helsinki. To 203
study the effect of time, a third set of samples were frozen to 204
−80°C and assayed after 2 weeks in Helsinki. At this same 205
time point, six additional sets of samples, which had been 206
shipped in dry ice, were assayed in Newcastle (UK), Paris 207
(France), Pisa (clinical and research laboratories; Italy), 208
Torino (Italy) and Mainz (Germany). The study protocol was 209
approved by the ethics committee of the Helsinki University 210
Central Hospital and was carried out in accordance with the 211
Declaration of Helsinki. Each participant provided written in- 212
formed consent. 213

Biochemical measurements 214

FINRISK/DILGOM and FIN-D2D Biochemical assays 215
were performed in the Laboratory of Analytical 216
Biochemistry of the Institute of Health and Welfare 217
(Helsinki, Finland) using an Architect ci8200 analyser 218
(Abbott Laboratories, Abbott Park, IL, USA). Plasma glucose 219
was determined using the hexokinase method (Abbott 220
Laboratories) and serum insulin using a chemiluminescent 221
microparticle immunoassay (Abbott Laboratories). Serum to- 222
tal cholesterol, HDL-cholesterol and triacylglycerol concen- 223
trations were measured using enzymatic kits (Abbott 224
Laboratories), and the LDL-cholesterol concentration was cal- 225
culated using the Friedewald formula [24]. Total cholesterol 226
was measured using the CHOD-PAP assay (Abbott 227
Laboratories). Samples were stored at −80°C before analysis. 228
In the FIN-D2D study, HbA_{1c} was measured using an 229
immunoturbidimetric method (Abbott Laboratories), and se- 230
rum ALT, AST and GGT concentrations were measured using 231
International Federation of Clinical Chemistry photometric 232
methods (Abbott Laboratories). In the liver fat cohort, plasma 233
glucose was measured using the hexokinase method in an 234
autoanalyser (Roche Diagnostics Hitachi 917; Hitachi, 235
Tokyo, Japan) and serum insulin was measured in fresh serum 236
samples using a time-resolved fluoroimmunoassay with 237
AutoDELFIA kits (Wallac, Turku, Finland). HbA_{1c} was mea- 238
sured using HPLC using the fully automated analyser system 239

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240 (Bio-Rad, Richmond, CA, USA). Serum triacylglycerol, total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations were measured with enzymatic kits from Roche Diagnostics using an autoanalyser (Roche Diagnostics Hitachi 917; Hitachi). Serum ALT, AST and GGT activities were determined as recommended by the European Committee for Clinical Laboratory Standards using the Roche Diagnostics Hitachi 917 (Hitachi). HOMA-IR was calculated as described by Matthews et al [3]. The methods used by the seven participating centres for HOMA-IR and the other laboratory variables are shown in ESM Methods. 250

251 Genotyping of *PNPLA3* at rs738409

252 **FINRISK/DILGOM** The *PNPLA3* genotype was determined from 1000G imputed genome-wide association study data consisting of three subsets genotyped using the Illumina HumanCoreExome, Illumina OmniExpress and Illumina 610K (Illumina, San Diego, CA, USA). 256

257 **FIN-D2D** Genomic DNA was extracted from whole blood using automated Chemagen DNA extraction equipment (PerkinElmer, Waltham, MA, USA) or a QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) following the protocol of the kit with slight modifications. Genotyping was performed using a TaqMan assay (Applied Biosystems, Paisley, UK). 263

264 **Liver fat cohort** DNA was isolated from whole blood and the *PNPLA3* genotype at rs738409 was determined as previously described using a TaqMan assay (Applied Biosystems) [25]. 266

267 Measurement of liver fat content by ^{1}H-MRS

268 Liver fat was measured by using ^{1}H-MRS as previously described [23]. Liver fat content was expressed as a mass fraction in percentage units [23]. NAFLD was defined as in the DHS (liver fat ≥5.56% by ^{1}H-MRS) [18]. 271

272 Other measurements

273 In all cohorts, body weight, height, BMI and waist and hip circumferences were measured as previously described [23, 26, 27]. 275

276 Statistical analysis

277 Distribution of continuous variables was analysed for normality using the Kolmogorov–Smirnov test. Data are shown as means ± SD for normally distributed data and as medians (25–75%) for non-normally distributed data. To compare characteristics among groups, the unpaired *t* test and the Mann–Whitney *U* test were used for continuous variables, and Fisher's exact test and the χ^2 test were used for categorical variables, where appropriate. Logarithmic transformation was performed for non-normally distributed data if needed. Correlation analyses were performed using Pearson's correlation coefficient. 287

288 Healthy individuals in the FINRISK/DILGOM (n = 1167) and FIN-D2D (n = 459) cohorts were identified. HOMA-IR was not normally distributed, and therefore the 95th percentile (90% CI) rather than the mean + 2 SD was used to determine the upper reference value for HOMA-IR [12]. After $\log_2$ transformation, HOMA-IR values were adjusted in a generalised linear model, using age and BMI as covariates. 294

295 We used two methods to identify a cut-off value of HOMA-IR for NAFLD. First, we calculated the HOMA-IR value that corresponded to the normal liver fat content based on the DHS (liver fat <5.56% [18]) using linear regression analysis. We tested whether the slopes and intercepts in linear regression analysis differed between men and women, and carriers and non-carriers of the PNPLA3 I148M variant. The 95th percentile was used to define normal liver fat content in healthy individuals in the liver fat cohort, as in the DHS [18]. Second, we determined the receiver operating characteristic (ROC) curve to calculate the area under the ROC curve (AUROC [95% CI]). The Youden index [28] was used to identify the optimal cut-off of HOMA-IR. For this, individuals were randomly divided into discovery (two-thirds of the individuals) and validation (one-third) groups. The discovery group was used to determine the ROC curve for HOMA-IR. The validation group and all individuals were used for validation. For additional validation, we generated 1000 random sets of samples and used the bootstrap method to validate the model in the sample sets. The AUROC of each set was estimated, and the average of these estimates provided the overall prediction accuracy of the model. Power analysis was conducted to estimate the appropriate sample size for correlation analysis and ROC analysis. To detect a correlation coefficient of 0.2 between HOMA-IR and liver fat content with a power of 0.8, a sample size of at least 193 was required. By setting the ratio of sample sizes between negative and positive groups at 2, at least 23 cases and 46 control participants were needed to reach a statistical power of 0.8 to detect the minimum AUROC of 0.7. 324

325 The inter-laboratory CVs of fasting insulin, glucose, HOMA-IR, lipids, liver enzymes, ferritin and albumin among laboratories were calculated. Linear regression analyses were performed to compare insulin, glucose and HOMA-IR measurements in Helsinki to those in other centres. The HOMA-IR in each centre corresponding to the upper limit of normal HOMA-IR in Helsinki was defined from linear regression equations. 332

333 We considered a p value of <0.05 to be statistically significant. Calculations were made using R Project for Statistical Computing version 3.1.1 (www.r-project.org, Vienna, 335

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336 Austria) and GraphPad Prism version 6.00 for Mac OS X
337 (GraphPad Software, San Diego, CA, USA).

338 Results

339 Reference values for HOMA-IR in two population-based 340 cohorts

341 Characteristics of the healthy individuals in the two
342 population-based cohorts (n = 1167 in FINRISK/DILGOM,
343 n = 459 in FIN-D2D) are shown in Table 1. Characteristics of
344 these individuals subgrouped based on their *PNPLA3* geno-
345 type at rs738409 are shown in ESM Table 1. The upper limit
346 of normal (95th percentile [90% CI]) HOMA-IR was 1.9 (1.8,
347 2.0) in the FINRISK/DILGOM cohort and 2.0 (1.9, 2.2) in the
348 FIN-D2D cohort (Fig. 1).
349 There was no sex difference among HOMA-IRs in either
350 cohort (Fig. 1). Since there were slight differences in age and
351 BMI between men and women in the two studies (Table 1) we
352 also calculated age- and BMI-adjusted HOMA-IRs, which
353 were very similar to the unadjusted values (Table 1). The
354 *PNPLA3* genotype did not influence HOMA-IR in either co-
355 hort (Fig. 1).
356 The 95th percentile of serum ALT in the FIN-D2D cohort
357 was 31 U/l in women and 43 U/l in men. In the FINRISK/
358 DILGOM cohort aged 25–74 years, age weakly inversely cor-
359 related with HOMA-IR ($\rho = -0.16$, $p < 0.001$). No significant
360 relationship was observed between age and HOMA-IR in the
361 FIN-D2D cohort aged 45–74 years ($\rho = 0.06$, $p = 0.21$).

362 Relationship between HOMA-IR and liver fat content

363 Characteristics of the non-diabetic individuals in the liver fat
364 cohort (n = 368) are shown in Table 1. Of them, 35% had
365 NAFLD as evaluated by ^{1}H-MRS. Liver fat percentage posi-
366 tively correlated with HOMA-IR ($r = 0.67$, $p < 0.001$)
367 (Fig. 2a). Normal liver fat, defined as in the DHS (<5.56%),
368 corresponded to a HOMA-IR of 2.0 (95% CI 1.9, 2.1)
369 (Fig. 2a) in non-diabetic individuals, with a HOMA-IR of
370 1.9 (1.8, 2.1) in women and 2.1 (1.9, 2.2) in men ($p = 0.29$).
371 The HOMA-IR corresponding to the normal liver fat content
372 (<5.56%) was significantly higher in non-carriers (2.1 [2.0,
373 2.2]) than carriers (1.8 [1.6, 1.9], $p = 0.007$) of the PNPLA3
374 I148M variant (Fig. 2b) (i.e. the variant allele carriers had a
375 higher liver fat content for any given HOMA-IR than non-
376 carriers). The upper 95th percentile for liver fat in the 96
377 healthy individuals was 5.9%.
378 The discovery and validation groups for defining the
379 HOMA-IR cut-off for NAFLD were similar with respect to
380 clinical and biochemical characteristics (ESM Table 2). The
381 AUROC for HOMA-IR was 0.88 (95% CI 0.84, 0.92) in the
382 discovery group (Fig. 3). The optimal HOMA-IR cut-off for
383 NAFLD, based on the Youden index, the point of optimal
384 sensitivity and specificity, was 1.9. This cut-off had a sensi-
385 tivity of 87%, specificity of 79%, negative predictive value
386 (NPV) of 92% and positive predictive value (PPV) of 67%.
387 The results were similar for the validation group (AUROC
388 0.80 [0.70, 0.88], sensitivity 68%, specificity 82%, NPV
389 81% and PPV 70%) and for all individuals (AUROC 0.85
390 [0.80, 0.89] sensitivity 80%, specificity 80%, NPV 88% and
391 PPV 68%) (Fig. 3). The AUROC for bootstrap samples was
392 0.88 (0.82, 0.92) and the overall estimate of optimism was
393 0.00079. Neither sex ($p = 0.22$) nor *PNPLA3* genotype
394 ($p = 0.18$) significantly influenced the AUROC.

395 Inter-laboratory variation in insulin assays 396 and HOMA-IR

397 The ten individuals (three men, seven women) recruited to
398 investigate inter-laboratory variations ranged in age from 22
399 to 62 years and in BMI from 21.3 to 42.4 kg/m^2. Among the
400 seven laboratories, the mean values ranged from 18.0 to
401 91.2 pmol/l for insulin, from 4.7 to 6.1 mmol/l for glucose
402 and from 0.69 to 4.0 for HOMA-IR. Freezing and thawing
403 the serum on the same day had no impact on fasting insulin
404 (52.8 ± 28.8 vs 54.0 ± 29.4 pmol/l, $p = 0.077$). Serum insulin
405 concentrations decreased over time when stored at −80°C de-
406 grees for 2 weeks (54.0 ± 29.4 vs 45.6 ± 25.8 pmol/l,
407 $p = 0.005$).
408 The CV of fasting insulin measured in the seven participat-
409 ing laboratories after 2 weeks of storage at −80°C averaged
410 25.4%. The CV of fasting glucose was significantly lower and
411 averaged 4.6%. The CV of HOMA-IR was 25.0%. The
412 HOMA-IR value of 2.0, as measured in Helsinki,
413 corresponded to HOMA-IRs of 1.3, 1.6, 1.8, 1.8, 2.0 and 2.1
414 in the six other centres (Fig. 4). The relationships between
415 insulin and glucose measurements in Helsinki vs the other
416 centres are shown in ESM Fig. 2, 3.
417 The inter-laboratory CVs for the other analytes were as
418 follows: total cholesterol 7.4%, LDL-cholesterol 12.8%,
419 HDL-cholesterol 7.0%, triacylglycerol 8.3%, AST 11.7%,
420 ALT 11.6%, GGT 11.3%, ferritin 19.1% and albumin 7.7%.
421 All of these CVs, with the exception of ferritin, were signifi-
422 cantly lower than the CV for fasting insulin ($p < 0.01$).

423 Discussion

424 The present studies were undertaken to determine whether a
425 single value of HOMA-IR could be used to clearly identify
426 individuals with NAFLD, and how HOMA-IRs determined
427 by different laboratories in European countries compare with
428 each other. In two population-based cohorts, the upper limits
429 of normal HOMA-IR were 1.9 and 2.0. In individuals whose
430 liver fat content was determined using ^{1}H-MRS, a HOMA-IR

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Table 1 Participant characteristics

Variable	FINRISK/DILGOM			FIN-D2D			Liver fat cohort		
	All (n = 1167)	Women (n = 798)	Men (n = 369)	All (n = 459)	Women (n = 308)	Men (n = 151)	All (n = 368)	Women (n = 221)	Men (n = 147)
Age (years)	44 (35–56)	43 (33–53)	47 (36–56)**	55 (50–62)	55 (50–62)	57 (50–65)	42 (28–52)	42 (32–52)	41 (27–51)
Weight (kg)	63.4 (57.8–69.9)	60.2 (55.6–64.8)	72.2 (67.0–76.6)***	63.6 (58.3–70.0)	61.0 (55.7–64.9)	72.7 (67.9–76.7)***	83.4 (24.5–33.2)	81.4 (68.0–94.7)	86.3 (76–2–100.1)**
BMI (kg/m^2)	22.7 (21.4–24.0)	22.5 (21.0–23.7)	23.4 (22.1–24.3)***	22.9 (21.6–24.1)	22.7 (21.3–23.9)	23.3 (22.1–24.3)**	28.8 (24.5–33.2)	29.8 ± 6.2	27.6 (24.5–31.1)*
Waist circumference (cm)	78.0 (73.0–83.0)	75.5 (71.5–80.0)	84.5 (80.0–88.5)***	81.0 ± 7.2	78.2 ± 5.9	87.1 ± 5.7***	96.9 ± 15.2	96.0 ± 16.3	98.4 ± 13.4
fS total cholesterol (mmol/l)	4.9 (4.4–5.6)	4.9 (4.3–5.5)	5.1 ± 0.9**	5.4 (4.8–6.0)	5.4 ± 0.9	5.3 ± 0.9	4.9 (4.3–5.6)	4.8 (4.3–5.7)	4.9 (4.4–5.5)
fS HDL-cholesterol (mmol/l)	1.5 (1.4–1.8)	1.6 (1.4–1.9)	1.4 (1.2–1.6)***	1.6 (1.4–1.8)	1.7 ± 0.3	1.4 (1.3–1.6) ***	1.4 (1.2–1.7)	1.5 (1.2–1.8)	1.3 (1.1–1.6)***
fS LDL-cholesterol (mmol/l)	3.0 (2.4–3.5)	2.8 (2.4–3.4)	3.2 ± 0.8***	3.3 (2.8–3.9)	3.3 (2.8–3.9)	3.5 ± 0.8	3.0 (2.4–3.6)	2.9 (2.3–3.5)	3.1 ± 0.9
fS triacylglycerol (mmol/l)	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.9 (0.7–1.1)***	0.9 (0.7–1.1)	0.9 (0.7–1.2)	0.9 (0.7–1.2)	1.1 (0.8–1.6)	1.0 (0.7–1.5)	1.2 (0.9–1.6)*
fP glucose (mmol/l)	5.5 (5.2–5.7)	5.4 ± 0.3	5.6 (5.4–5.8)***	5.6 (5.4–5.8)	5.6 (5.3–5.8)	5.7 ± 0.2***	5.4 (5.0–5.8)	5.4 ± 0.6	5.5 ± 0.5*
fS insulin (pmol/l)	24.6 (19.8–31.8)	25.2 (20.4–32.4)	24.0 (19.2–30.6)*	27.0 (20.4–33.6)	27.0 (21.6–33.6)	27.0 (19.8–33.6)	40.2 (22.2–66.0)	38.4 (21.6–66.0)	42.0 (22.8–72.0)
HOMA-IR	1.0 (0.8–1.3)	1.0 (0.8–1.3)	1.0 (0.8–1.3)	1.1 (0.8–1.4)	1.1 (0.8–1.4)	1.1 (0.8–1.4)	1.6 (0.8–2.7)	1.5 (0.8–2.5)	1.8 (0.9–3.2)
Adjusted HOMA-IR[a]	1.0 (0.9–1.1)	1.0 (0.9–1.1)	1.0 (1.0–1.1)**	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.1 (1.1–1.2)**	–	–	–
HbA_{1c} (%)	–	–	–	5.1 (4.9–5.3)	5.1 (4.9–5.3)	5.2 (5.0–5.4)**	5.5 (5.3–5.7)	5.5 (5.3–5.7)	5.5 (5.2–5.7)
HbA_{1c} (mmol/mol)	–	–	–	32 (30–34)	32 (30–34)	33 (31–36)**	37 (34–39)	37 (34–39)	37 (33–39)
fS ALT (U/l)	–	–	–	18 (15–23)	17 (14–22)	22 (16–28)***	25 (18–39)	22 (16–31)	34 (23–53)***
fS AST (U/l)	–	–	–	22 (18–26)	21 (17–25)	24 (20–28)***	26 (22–32)	24 (21–30)	29 (24–37)***
fS GGT (U/l)	–	–	–	19 (14–28)	17 (13–23)	23 (17–33)***	23 (15–40)	19 (14–33)	30 (20–50)***
PNPLA3^{I148M} (non-carrier/carrier, %)	59/41	59/41	60/40	60/40	63/37	53/47*	60/40	53/47	65/35
Liver fat (%)	–	–	–	–	–	–	2.7 (0.9–8.6)	2.4 (0.8–7.1)	3.3 (1.1–12.4)*

Data are means ± SD (variables with normal distribution) or median (25–75%) (variables with non-normal distribution), unless otherwise stated

[a] Adjusted for BMI and age

*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ for men vs women within cohorts

fP, fasting plasma; fS, fasting serum

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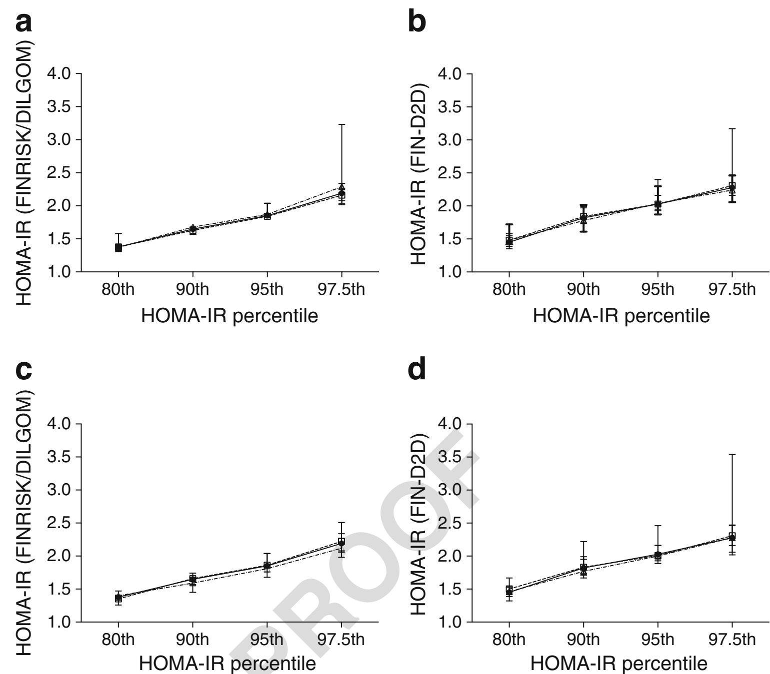

Fig. 1 Percentiles (90% CI) of HOMA-IR in healthy men (triangles and dotted/dashed lines), women (squares and dashed lines) and all individuals (circles and solid lines) in the population-based FINRISK/DILGOM (**a**) and FIN-D2D cohorts (**b**), and in I148M variant non-carriers (PNPLA3^{I148II}; squares and dashed lines) and carriers (PNPLA3$^{I148IM/MM}$; triangles and dotted/dashed lines) and all individuals (circles and solid lines) in the population-based FINRISK/DILGOM (**c**) and FIN-D2D cohorts (**d**)

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431 cut-off of 1.9 was optimal for diagnosing NAFLD based on 432 the Youden index. A HOMA-IR of 2.0 corresponded to the 433 upper limit of normal liver fat content of 5.56%, as defined in 434 the DHS. A HOMA-IR value of 2.0 corresponded to HOMA-435 IRs between 1.3 and 2.1 in six other laboratories, with an inter-436 laboratory CV of 25%. These data show that the upper limit of 437 normal HOMA-IR closely corresponds to the upper limit of 438 liver fat defined as in the DHS, and that there is large inter-439 laboratory variation in insulin measurements.

440 The upper limit of a reference value is usually defined in 441 population-based samples of healthy individuals as the mean + 442 2 SD in normally distributed samples and as the 95th percen-443 tile in non-normally distributed samples [12]. In this study, the 444 95th percentiles were 1.9 and 2.0 in the healthy individuals of 445 two population-based cohorts. Three previous studies have 446 been performed in healthy individuals. These studies were 447 smaller (161 Japanese, 161 Italian and 312 Brazilian individ-448 uals) than the present study (459–1167 Finnish individuals) 449 [14–16]. In the Japanese study [14], the 90th percentile of 450 HOMA-IR was 1.7, which is comparable with that found in 451 present study. In the Italian study, however, the participants 452 were not healthy as they included diabetic and hypertensive 453 individuals. The 80th percentile of HOMA-IR was 2.77 [16]. 454 This study used a non-specific RIA from Linco Research (St. 455 Charles, MO, USA), which has produced the highest insulin 456 concentrations of several insulin assays tested [17, 29]. 457 Similarly, the Brazilian study also used this RIA, and the 458 90th percentile of HOMA-IR was equally high (2.71) [15]. 459 Thus, the higher HOMA-IR in these studies compared with 460 the present study could be due to the inclusion of diabetic and 461 hypertensive individuals in the Italian study, and to the use of 462 an RIA that is no longer used in most laboratories [17].

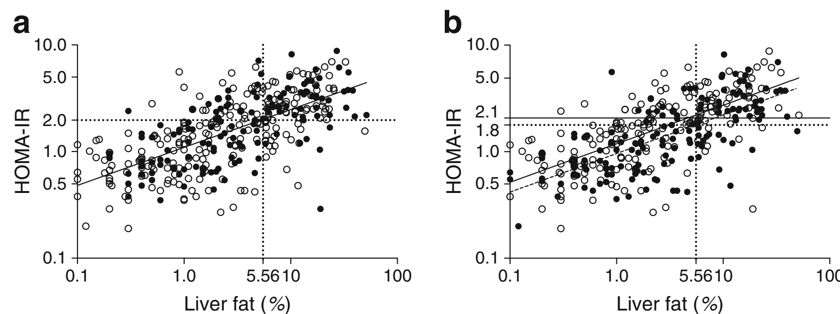

Fig. 2 Relationship between liver fat measured by ^{1}H-MRS ($\log_{10}$) and HOMA-IR ($\log_{10}$). (**a**) The relationship was similar (slopes $p = 0.79$, elevations $p = 0.75$) in men (black circles) ($r = 0.67$, $p < 0.001$) and in women (white circles) ($r = 0.66$, $p < 0.001$). The HOMA-IR corresponding to normal liver fat (<5.56%), as defined in the DHS [18], was 2.0. (**b**) There was a significant difference in the intercepts of the regression lines ($p = 0.007$) between carriers (PNPLA3$^{I148IM/MM}$) ($r = 0.69$, $p < 0.001$) (black circles) and non-carriers (PNPLA3^{I148II}) ($r = 0.68$, $p < 0.001$) (white circles) of the I148M variant, showing that HOMA-IR was lower for any given liver fat content in carriers than non-carriers. No significant difference between the slopes was observed ($p = 0.99$)

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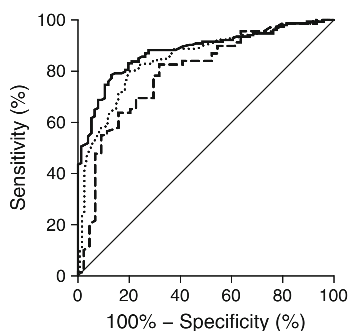

Fig. 3 AUROC for HOMA-IR and NAFLD. The AUROC for HOMA-IR was 0.88 (95% CI 0.84, 0.92) in the discovery group (solid line), 0.80 (0.70, 0.88) in the validation group (dashed line) and 0.85 (0.80, 0.89) in all individuals (dotted line)

463 We found no significant differences in HOMA-IR percen-
464 tiles between men and women among the healthy individuals
465 in either population-based cohort (Fig. 1). The men were,
466 however, slightly more obese and older than the women, and
467 therefore we also calculated age- and BMI-adjusted HOMA-
468 IRs. After adjustment, men had slightly higher HOMA-IRs
469 than women in both studies, but the differences in absolute
470 units were trivial (0.02 in FINRISK/DILGOM and 0.05 in the
471 FIN-D2D study; Table 1). Previous population-based studies
472 including healthy individuals have not reported HOMA-IRs
473 separately for men and women [14–16].
474 In keeping with the 95th percentile in healthy individuals in
475 the population-based cohorts, we found a HOMA-IR of 1.9 to
476 best distinguish non-diabetic individuals with and without
477 NAFLD. This value is similar to that found in 204 Brazilian
478 individuals [30]. As in the present study, in ROC analysis, a
479 HOMA-IR of 2.0 (AUROC 0.84) best distinguished between
480 NAFLD and non-NAFLD diagnosed by ultrasound or biopsy.

In keeping with these data, a study comprising 263 Columbian 481
men found a HOMA-IR of 1.7 (AUROC 0.78) to be the cut- 482
off for NAFLD diagnosed by ultrasound [31]. In a recent 483
population-based study in Iran, the best cut-off for NAFLD 484
diagnosed by ultrasound was 2.0 in women and 1.8 in men 485
[32]. Even though these results in different ethnic groups seem 486
consistent, it will be important to perform studies in ethnic 487
groups other than Finns. 488
In linear regression analysis (Fig. 2), HOMA-IRs of 1.9 489
and 2.0 corresponded to liver fat contents of 5.0% and 490
5.56%. The latter value is identical to that defined as the upper 491
limit of normal liver fat measured by ^{1}H-MRS in the DHS 492
[18]. The prevalence of NAFLD in the population-based 493
DHS was 31% [33], which is comparable with that in our 494
cohort of research volunteers (35%). In our cohort, the 95th 495
percentile of liver fat in healthy individuals was 5.9%. This 496
value is close to the 5.56% in the DHS [18]. However, as our 497
liver fat cohort was not population-based, the 5.56% in the 498
DHS can be considered more accurate than our estimate of 499
5.9%. 500
The PNPLA3 I148M variant predisposes to NAFLD but 501
not to features of insulin resistance [34, 35]. Thus, despite an 502
increased liver fat content in PNPLA3 I148M variant carriers, 503
HOMA-IR has been reported to be similar in carriers and non- 504
carriers of similar age, sex and BMI [36–39]. Consistent with 505
these data, in the present two healthy population-based co- 506
horts, no difference existed in clinical characteristics between 507
carriers and non-carriers of the PNPLA3 I148M variant. The 508
upper limit of normal HOMA-IR was the same for both 509
groups. In the liver fat cohort, in which 35% of individuals 510
had NAFLD, the optimal cut-off for distinguishing NAFLD 511
from non-NAFLD was also not affected by genotype. 512

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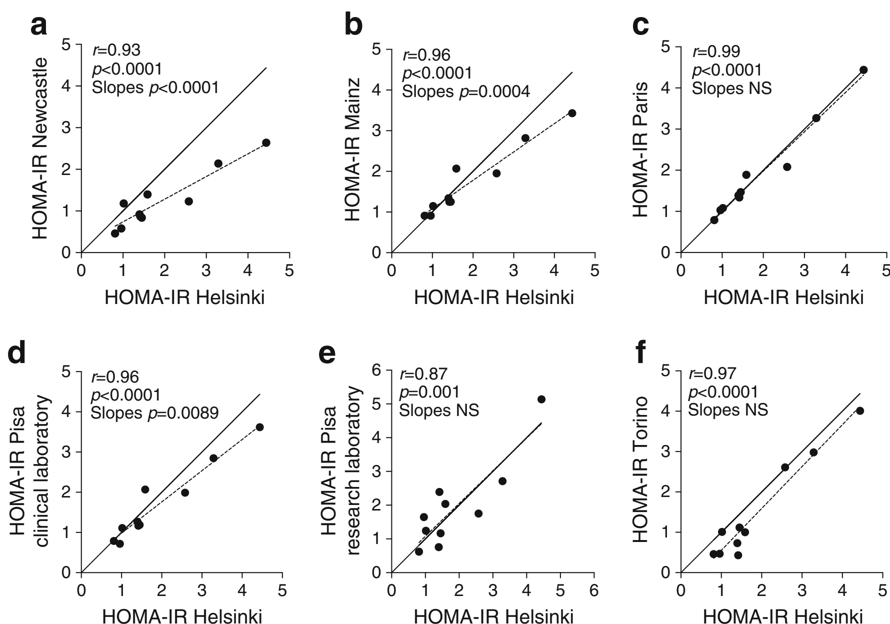

Fig. 4 Linear regression between HOMA-IR measured in Helsinki by chemiluminescent immunoassay using Liaison XL (DiaSorin, Saluggia, Italy) for insulin measurement and HOMA-IR measured in six other laboratories (**a**–**f**) using insulin assays as described in ESM Fig. 2. (**f**) Intercepts $p = 0.0005$

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However, when comparing carriers and non-carriers at a similar liver fat content, carriers were found to have lower HOMA-IR than non-carriers (Fig. 2b). These data imply that HOMA-IR cannot be used to diagnose individuals with NAFLD due to the PNPLA3 I148M variant, and that they can only be identified by genotyping for this gene variant [10].

A limitation of HOMA-IR is that it is valid only as long as serum insulin concentrations reflect merely insulin sensitivity, not secretion [40–42]. In individuals with non-diabetic glucose tolerance, fasting glucose and insulin concentrations are closely positively correlated [43]. Once glucose tolerance becomes diabetic, insulin concentrations start to decline and their relationship to glucose is inverse rather than positive [6]. Under such conditions HOMA-IR underestimates insulin resistance-associated NAFLD, although in a recent study including 56 participants with type 2 diabetes, a HOMA-IR of 4.5 was estimated to be the optimal threshold for distinguishing NAFLD diagnosed by ultrasound or computed tomography [44]. The method used to measure insulin concentrations was not specified. The extreme example is type 1 diabetes, where there is no endogenous insulin. HOMA-IR is also influenced by insulin clearance, unlike direct measurements of insulin sensitivity. However, this may not be a problem as the decrease in insulin clearance closely parallels that in hepatic insulin sensitivity [45].

Use of HOMA-IR in the clinic assumes the degree of inter-laboratory variation in insulin assays is known [29]. In the present study, we analysed fasting blood samples obtained from ten individuals covering a wide range of HOMA-IRs after a similar period of freezing and thawing and time of storage. From the regression lines relating assay results between two laboratories (Fig. 4), the upper limit of normal HOMA-IR was similar in Helsinki and Paris using the same insulin assay (2.0), but was 1.3, 1.6, 1.8, 1.8 and 2.1 in the five other laboratories using different assays. The inter-laboratory CV was 25%. In contrast, the inter-laboratory CVs for other analytes, with the exception of ferritin, were much lower and ranged from 5% to 13%. This implies that every laboratory should establish its own reference value for HOMA-IR, or at least understand how its insulin assay compares with other laboratories. Furthermore, reference values for HOMA-IR, even in healthy individuals, and the relationship between HOMA-IR and liver fat may be population-specific.

We conclude that the upper limit of HOMA-IR, defined based on the identification of healthy individuals in two population-based Finnish cohorts, closely corresponds to the upper limit of normal liver fat content (<5.56%) found in the DHS. This finding supports the use of HOMA-IR in identifying individuals with ‘metabolic NAFLD’. The use of HOMA-IR has, however, several limitations. HOMA-IR varies considerably and more than other routine analytes among laboratories, particularly due to the use of different insulin assays. If HOMA-IR were to be used as a surrogate for insulin resistance and NAFLD, insulin assays would need to be standardised. In addition, HOMA-IR underestimates liver fat content in individuals with NAFLD associated with the PNPLA3 I148M variant and, although not examined in this study, in individuals with defective insulin secretion.

Acknowledgements We gratefully acknowledge A. Salo and A. Karioja-Kallio (Department of Medicine, Helsinki University Hospital), P. Pölönen (HUS Medical Imaging Center, Radiology, Helsinki University Hospital), M. L. Abate and C. Rosso (Department of Medical Sciences, University of Torino), M. Gaggini and E. Buzzigoli (Institute of Clinical Physiology, CNR Pisa, Italy), S. Depoix (I. Department of Medicine, University Medical Center Mainz), and D. Neeley and S. McLellen (Newcastle Hospitals NHS Foundation Trust) for skilful technical assistance; and volunteers for their help.

Compliance with ethical standards

Funding This study was supported by research grants from the Academy of Finland (HY-J), EU/EFPIA Innovative Medicines Initiative Joint Undertaking (EMIF grant no. 115372, HY-J), the Sigrid Juselius (HY-J), the Novo Nordisk Foundations (HY-J), EVO (HY-J), The Paulo Foundation (EI) and the Finnish Medical Foundation (EI). The authors are members of the EPoS (Elucidating Pathways of Steatohepatitis) consortium, which is funded by the Horizon 2020 Framework Program of the European Union under grant agreement 634413.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement EI and YZ analysed the data. EI and HY-J interpreted the data and wrote the main text. EI, HY-J, EB, KC, AG, DS and QMA designed and EI, HY-J, EB, KC, DB-R, J-ML, AG, DS, JMS and QMA acquired data in and supervised the inter-laboratory insulin assay study. AH and NL performed and interpreted the ^{1}H-MRS studies. MP, PJ, SM, SK-K and JS designed, performed and supervised the population-based FINRISK/DILGOM (PJ, SM) and FIN-D2D (SK-K, JS, MP) studies. HY-J designed and supervised the project. All authors have revised the manuscript critically for important intellectual content and approved the final version of the manuscript. HY-J is the guarantor of this work.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Marchesini G, Brizi M, Bianchi G et al (2001) Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 50: 1844–1850
2. Bugianesi E, Gastaldelli A, Vanni E et al (2005) Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 48:634–642
3. Matthews DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
4. Wallace TM, Levy JC, Matthews DR (2004) Use and abuse of HOMA modeling. Diabetes Care 27:1487–1495

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619 5. Radziuk J (2000) Insulin sensitivity and its measurement: structural commonalities among the methods. J Clin Endocrinol Metab 85: 4426–4433
622 6. Lillioja S, Mott DM, Howard BV et al (1988) Impaired glucose tolerance as a disorder of insulin action. N Engl J Med 318:1217–1225
624 7. Lillioja S, Mott DM, Spraul M et al (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. N Engl J Med 329:1988–1992
628 8. Defronzo RA (2009) From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58:773–795
631 9. Gastaldelli A (2011) Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. Diabetes Res Clin Pract 93:S60–S65
634 10. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO) (2016) EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. Diabetologia 59:1121–1140
639 11. Gräsbeck R, Alström T (1981) Reference values in laboratory medicine. The current state of the art. Wiley, Ann Arbor
641 12. Horn PS, Pesce AJ (2003) Reference intervals: an update. Clin Chim Acta 334:5–23
643 13. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G (1997) Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Invest 100:1166–1173
647 14. Nakai Y, Fukushima M, Nakaishi S et al (2002) The threshold value for insulin resistance on homeostasis model assessment of insulin sensitivity. Diabet Med 19:346–347
650 15. Geloneze B, Repetto EM, Geloneze SR, Tambascia MA, Ermetice MN (2006) The threshold value for insulin resistance (HOMA-IR) in an admixtured population IR in the Brazilian Metabolic Syndrome Study. Diabetes Res Clin Pract 72:219–220
654 16. Bonora E, Kiechl S, Willeit J et al (1998) Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 47: 1643–1649
657 17. Manley SE, Stratton IM, Clark PM, Luzio SD (2007) Comparison of 11 human insulin assays: implications for clinical investigation and research. Clin Chem 53:922–932
660 18. Szczepaniak LS, Nurenberg P, Leonard D et al (2005) Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 288:E462–E468
664 19. Anstee QM, Day CP (2015) The genetics of nonalcoholic fatty liver disease: spotlight on PNPLA3 and TM6SF2. Semin Liver Dis 35: 270–290
667 20. Yki-Järvinen H (2014) Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2:901–910
670 21. Vartiainen E, Laatikainen T, Peltonen M et al (2010) Thirty-five-year trends in cardiovascular risk factors in Finland. Int J Epidemiol 39:504–518
673 22. Saaristo T, Peltonen M, Keinänen-Kiukaanniemi S et al (2007) National type 2 diabetes prevention programme in Finland: FIN-D2D. Int J Circumpolar Health 66:101–112
676 23. Kotronen A, Peltonen M, Hakkarainen A et al (2009) Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 137:865–872
679 24. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
682 25. Kotronen A, Johansson LE, Johansson LM et al (2009) A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia 52:1056–1060
685 26. Männistö S, Harald K, Kontto J et al (2014) Dietary and lifestyle characteristics associated with normal-weight obesity: the National FINRISK 2007 Study. Br J Nutr 111:887–894
688 27. Hyysalo J, Männistö VT, Zhou Y et al (2014) A population-based study on the prevalence of NASH using scores validated against liver histology. J Hepatol 60:839–846
691 28. Greiner M, Pfeiffer D, Smith RD (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45:23–41
694 29. Manley SE, Luzio SD, Stratton IM, Wallace TM, Clark PMS (2008) Preanalytical, analytical, and computational factors affect homeostasis model assessment estimates. Diabetes Care 31:1877–1883
697 30. Salgado AL, Carvalho LD, Oliveira AC, Santos VN, Vieira JG, Parise ER (2010) Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol 47:165–169
701 31. Perez M, Gonzáles L, Olarte R et al (2011) Nonalcoholic fatty liver disease is associated with insulin resistance in a young Hispanic population. Prev Med 52:174–177
704 32. Motamed N, Miresmail SJH, Rabiee B et al (2016) Optimal cutoff points for HOMA-IR and QUICKI in the diagnosis of metabolic syndrome and non-alcoholic fatty liver disease: a population based study. J Diabetes Complicat 30:269–274
708 33. Browning JD, Szczepaniak LS, Dobbins R et al (2004) Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40:1387–1395
711 34. Sookoian S, Pirola CJ (2011) Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 53:1883–1894
715 35. Petäjä EM, Yki-Järvinen H (2016) Definitions of normal liver fat and the association of insulin sensitivity with acquired and genetic NAFLD—a systematic review. Int J Mol Sci 17:E633
718 36. Verrijken A, Beckers S, Francque S et al (2013) A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. Obesity 21:2138–2145
721 37. Romeo S, Sentinelli F, Cambuli VM et al (2010) The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. J Hepatol 53:335–338
724 38. Valenti L, Alisi A, Galmozzi E et al (2010) I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology 52:1274–1280
728 39. Del Ben M, Polimeni L, Brancorsini M et al (2014) Non-alcoholic fatty liver disease, metabolic syndrome and patatin-like phospholipase domain-containing protein3 gene variants. Eur J Intern Med 25:566–570
732 40. Singh B, Saxena A (2010) Surrogate markers of insulin resistance: a review. World J Diabetes 1:36–47
734 41. Borai A, Livingstone C, Farzal A, Kholeif M, Wang T, Ferns G (2010) Reproducibility of HOMA and QUICKI among individuals with variable glucose tolerance. Diabetes Metab 36:247–249
737 42. Bonora E, Targher G, Alberiche M et al (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23: 57–63
742 43. Defronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM: a balanced overview. Diabetes Care 15:318–368
744 44. Gutierrez-Buey G, Núñez-Córdoba JM, Llavero-Valero M, Gargallo J, Salvador J, Escalada J (2017) Is HOMA-IR a potential screening test for non-alcoholic fatty liver disease in adults with type 2 diabetes? Eur J Intern Med. doi:10.1016/j.ejim.2017.03.006
748 45. Kotronen A, Vehkavaara S, Seppälä-Lindroos A et al (2007) Effect of liver fat on insulin clearance. Am J Physiol Endocrinol Metab 293:E1709–E1715
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