

Hepatic steatosis does not cause insulin resistance in people with familial hypobetalipoproteinaemia

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Abstract

Aims/hypothesis Hepatic steatosis is strongly associated with hepatic and whole-body insulin resistance. It has proved difficult to determine whether hepatic steatosis itself is a direct cause of insulin resistance. In patients with familial hypobetalipoproteinaemia (FHBL), hepatic steatosis is a direct consequence of impaired hepatic VLDL excretion, independently of metabolic derangements. Thus, patients with FHBL provide a unique opportunity to investigate the relation between increased liver fat and insulin sensitivity.

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Methods We included seven male participants with FHBL and seven healthy matched controls. Intrahepatic triacylglycerol content and intramyocellular lipid content were measured using localised proton magnetic resonance spectroscopy (¹H-MRS). A two-step hyperinsulinaemic–euglycaemic clamp, using stable isotopes, was assessed to determine hepatic and peripheral insulin sensitivity.

Results ¹H-MRS showed moderate to severe hepatic steatosis in patients with FHBL. Basal endogenous glucose production (EGP) and glucose levels did not differ between the two groups, whereas insulin levels tended to be higher in patients compared with controls. Insulin-mediated suppression of EGP during lower dose insulin infusion and insulin-mediated peripheral glucose uptake during higher dose insulin infusion were comparable between FHBL participants and controls. Baseline fatty acids and lipolysis (glycerol turnover) at baseline and during the clamp did not differ between groups. **Conclusions/interpretation** In spite of moderate to severe hepatic steatosis, people with FHBL do not display a reduction in hepatic or peripheral insulin sensitivity compared with healthy matched controls. These results indicate that hepatic steatosis per se is not a causal factor leading to insulin resistance.

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Keywords Endogenous glucose production · Familial hypobetalipoproteinaemia · FHBL · Glucose disposal · Hepatic insulin resistance · Hepatic steatosis · Hyperinsulinaemic–euglycaemic clamp · Insulin resistance · Non-alcoholic fatty liver disease · Peripheral insulin resistance

Abbreviations

ApoB	Apolipoprotein B
CAT	Computed axial tomography
EGP	Endogenous glucose production
FFM	Fat-free mass
FHBL	Familial hypobetalipoproteinaemia
HOMA-IR	HOMA-insulin resistance
¹ H-MRS	Proton magnetic resonance spectroscopy
IHTG	Intrahepatic triacylglycerol
IMCL	Intramyocellular lipid
NAFLD	Non-alcoholic fatty liver disease
R _d	Rate of disappearance
REE	Resting energy expenditure

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common feature of obesity and the metabolic syndrome [1, 2]. NAFLD is the result of hepatic fat accumulation due to an increased flux of NEFA through the portal vein, reduced hepatic fatty acid oxidation and increased hepatic de novo lipogenesis, all of which are associated with central obesity and insulin resistance [3]. Hyperinsulinaemic–euglycaemic clamp studies have shown that increased intrahepatic triacylglycerol (IHTG) content strongly correlates with insulin resistance in liver, skeletal muscle and adipose tissue across a large range of liver fat percentages. Therefore, even small amounts of IHTG content were associated with metabolic dysfunction [1, 4–8]. In addition, results of animal studies have shown that hepatic fat accumulation may interfere with insulin signalling in the liver through activation of protein kinase C ϵ [9, 10], suggesting a direct causal relationship between hepatic fat accumulation and insulin resistance. However, in obese humans it has been difficult to determine whether hepatic fat accumulation per se causes insulin resistance since both are features of metabolic derangements.

Familial hypobetalipoproteinaemia (FHBL) is a rare disorder of lipoprotein metabolism (estimated prevalence ranges from 1 in 500 to 1 in 1,000) and is characterised by LDL-cholesterol and total apolipoprotein B (ApoB) levels below the 5th percentile [11, 12]. Approximately 50% of FHBL patients are carriers of a mutation in the *APOB* gene [11] leading to the formation of a dysfunctional form of ApoB. Since ApoB is the main component of VLDL, mutations in *APOB* gene give rise to a defective VLDL export system with a reduced capacity to export triacylglycerol from the liver. As a consequence, mean triacylglycerol content in the livers of FHBL participants, measured using localised proton magnetic resonance spectroscopy (¹H-MRS), is approximately three- to fivefold higher compared with that of controls [13]. Occasional reports on liver

biopsies in FHBL patients have revealed moderate to severe steatosis, in some patients associated with mild inflammation and fibrosis [11, 14]. However, hepatic triacylglycerol accumulation in FHBL, unlike in NAFLD, occurs predominantly independently of obesity-induced metabolic derangements [15]. For this reason FHBL patients provide a unique opportunity to investigate the relation between IHTG content and insulin sensitivity in humans.

In a previous study, hepatic steatosis in non-obese FHBL patients was associated with larger areas under the insulin curves of a 2 h glucose tolerance test when compared with healthy controls, although the difference was not statistically significant [13, 15]. In another study, insulin sensitivity, assessed using the HOMA index, was similar in non-obese patients with FHBL compared with healthy controls [14]. More recently, Amaro et al. [16] showed that, during a hyperinsulinaemic–euglycaemic clamp, hepatic and peripheral insulin sensitivity did not differ between three obese participants with FHBL and six obese controls without hepatic steatosis.

In the present report we describe the results of an extensive study of glucose and fat metabolism in patients with FHBL. We performed a two-step hyperinsulinaemic–euglycaemic clamp with stable isotopes to determine hepatic and peripheral insulin sensitivity and total triacylglycerol lipolysis. In addition we measured intrahepatic triacylglycerol (IHTG) content and intramyocellular lipid (IMCL) content by magnetic resonance spectroscopy (MRS) and determined body fat distribution using dual energy X-ray absorptiometry and abdominal computed axial tomography.

Methods

Subjects We included seven male patients with documented FHBL and hepatic steatosis and seven healthy controls. We were not able to recruit more participants because of the low prevalence of FHBL and the strict inclusion criteria. Four of the FHBL patients had the same mutation in the *APOB* gene (11712delC). Two of these patients were brothers. The mutations identified in the other FHBL patients were 2534delA, Q1309X and 2783delC, respectively [17]. Patients and controls were matched for age, sex, BMI and WHR. Persons who performed regular exercise above sedentary level and those who were regularly using >3 units of alcohol per day or any recreational drug during the last 30 days were excluded from participation. Participants did not have any somatic illnesses, nor did they use supplements or medication influencing glucose or lipid metabolism. Oral glucose tolerance tests were within the normal range (<7.8 mmol/l) according to the criteria of the ADA [18]. All control participants had a normal routine blood examination.

Written informed consent was obtained from all participants. The study protocol was approved by the local institutional review board. Patients were recruited from the outpatient clinic of the Academic Medical Center, Amsterdam, the Netherlands. Healthy volunteers were recruited via local advertisements.

Hyperinsulinaemic–euglycaemic clamp After an overnight fast, participants were admitted to the metabolic ward of the Academic Medical Center at 07:30 hours. Prior to the study day, all participants consumed at least 250 g of carbohydrates for 3 days and refrained from vigorous exercise for 1 week. A catheter was inserted into an antecubital vein for infusion of stable isotope tracers, insulin and glucose. Another catheter was inserted into a contralateral hand vein and kept in a thermoregulated (60°C) Plexiglas box for sampling of arterialised venous blood. Saline was infused as NaCl 0.9% at a rate of 50 ml/h to sustain catheter patency. [6,6-²H₂]Glucose and [1,1,2,3,3-²H₅]glycerol were infused as tracers (>99% enriched; Cambridge Isotopes, Andover, MA, USA) to study glucose kinetics and lipolysis (total triacylglycerol hydrolysis), respectively.

At time 0 (08:00 hours) blood samples were drawn for determination of background enrichments. A primed continuous infusion of isotopes was then started ([6,6-²H₂]glucose and [1,1,2,3,3-²H₅]glycerol, both at a rate of 0.11 μmol kg⁻¹ min⁻¹, with a priming dose equivalent to 80 min of infusion) and continued until the end of the study. After an equilibration time of 2.5 h, three blood samples were taken for the measurement of isotope enrichments and one for the measurement of glucoregulatory hormones and NEFA. Thereafter, a two-step hyperinsulinaemic–euglycaemic clamp was started. A continuous infusion of insulin (Actrapid 100 U/ml; Novo Nordisk Farma, Alphen aan de Rijn, the Netherlands) was started for 2 h and 10 min at the rate of 20 mU [m² body surface area]⁻¹ min⁻¹, followed by an infusion of insulin at a rate of 60 mU [m² body surface area]⁻¹ min⁻¹ for another 2 h and 10 min. Plasma glucose levels were measured every 5 min at the bedside. Glucose was infused as 20% glucose at a variable rate, to maintain a plasma glucose concentration of 5.0 mmol/l. [6,6-²H₂]Glucose was added to the 20% glucose solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimise changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose [19]. During the last 40 min of both hyperinsulinaemic periods, blood samples were drawn at 10 min intervals for determination of isotope enrichments, glucoregulatory hormones and NEFA. During the study day, participants remained fasted but were allowed to drink water.

Body composition and indirect calorimetry Body composition was measured by bioelectrical impedance analysis

(Maltron BF906; Maltron, Rayleigh, UK). Peripheral and trunk fat mass were quantified by dual-energy X-ray absorptiometry (QDR-4500W, software version whole body v8.26A: 5, Hologic, Bedford, MA, USA).

For determination of visceral and abdominal fat mass, a standardised single slice abdominal CAT-scan was performed through the level of the fourth lumbar vertebra (MX8000, Brilliance, Philips, Best, the Netherlands).

Oxygen consumption ($\dot{V}O_2$) and CO₂ production ($\dot{V}CO_2$) were measured with the ventilated hood technique (model 2900; SensorMedics, Anaheim, CA, USA). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured continuously during the final 30 min of the basal state and during the final 30 min of step 2 of the hyperinsulinaemic–euglycaemic clamp. The mean values of $\dot{V}O_2$ and $\dot{V}CO_2$ during the final 20 min were used for the calculation of glucose and fat oxidation.

In vivo ¹H-MRS IHTG content was measured by ¹H-MRS. We hypothesised that the reduced delivery of VLDL triacylglycerols to peripheral tissues in FHBL might be associated with reduced storage of triacylglycerols in skeletal muscle. Therefore, we also measured intramyocellular lipid content in the soleus muscle. Measurements were performed after an overnight fast, within a 2 week time frame before or after the clamp test. For logistical reasons, scanning was performed at two separate sites with two different scanners. ¹H-MRS spectra were acquired using a 3.0 T Magnetom Trio (Siemens, Erlangen, Germany) and a 3.0 T Intera (Philips, Best, the Netherlands). Identical scanning parameters were used in both situations. During the measurements, participants remained in the supine position within the MRI scanner.

For the measurement of IMCL content, the soleus muscle of the right leg was positioned within the homogeneous volume of the magnet. Scout images were acquired in order to position the volume of interest 15 cm beneath the tibia plateau. Two-dimensional chemical shift imaging MRS data were collected using a point-resolved spectroscopy sequence (PRESS) with the following parameters: repetition time (TR) 1,100 ms, echo time (TE) 30 ms, 10 mm slice thickness, 32×32 matrix size, field of view 16×16 cm, acquisition time 13 min, one acquisition. Spectra with and without water suppression were obtained. A number of voxels ranging from 5 to 27 inside the soleus were selected for further analysis and were processed using the freely available 3DiCSI package (version 1.9.11; Columbia University, New York, NY, USA). The number of voxels selected for analysis was similar between the two sites. Chemical shifts were reported using water as the internal standard at 4.65 ppm. Average spectra were then processed using specialised computer software (jMRUI 2.2) [20]. Three

peaks were line-fitted: IMCL (CH_2) and extramyocellular lipid content (CH_2 and CH_3) peaks. Lipid content was calculated from the peak areas of IMCL CH_2 (methylene) at 1.3 ppm. IMCL contents were then expressed as the percentage of water content. In one FHBL patient quantification of IMCL content was impossible because of a large extramyocellular lipid content.

IHTG content was obtained using single-voxel ^1H -MRS, using a body array coil as the transmitter and phased surface coils as receivers. MRS measurements were acquired during breathhold, using single-voxel stimulated acquisition mode (TE/TR 20/3,000 ms, six acquisitions). Volumes of interest in the liver were located away from major vascular structures and bile ducts. Voxel size was 27 mm^3 . The water and fat resonance peaks, located at 4.65 and 1.3 ppm, were integrated using jMRUI software [20] and relative fat content was expressed as the ratio of the fat peak area over the cumulative water and fat peak areas. Calculated peak areas of water and fat were corrected for T2 relaxation ($T_{2,\text{water}}$, 34 ms; $T_{2,\text{fat}}$, 68 ms) [21] and the percentage hepatic fat content was calculated according to Szczepaniak et al. [22].

Glucose and lipid metabolism measurements Plasma glucose concentrations were measured with the glucose oxidase method using a Biosen C-line plus glucose analyser (EKF Diagnostics, Barleben/Magdeburg, Germany). Plasma NEFA concentrations were determined with an enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals, Neuss, Germany) with intra-assay variation of 1%, inter-assay variation of 4–15% and a detection limit of 0.02 mmol/l. $[6,6\text{-}^2\text{H}_2]$ Glucose enrichment (tracer-to-tracee ratio) was measured as described by Ackermans et al. [23]. $[6,6\text{-}^2\text{H}_2]$ Glucose enrichment intra-assay variation was 0.5–1% with an inter-assay variation of 1% and a detection limit of 0.04%. $[1,1,2,3,3\text{-}^2\text{H}_5]$ Glycerol enrichment was determined as described earlier [24]. Intra-assay variation was 1–3% for glycerol and 4% for $[1,1,2,3,3\text{-}^2\text{H}_5]$ glycerol; inter-assay variation was 2–3% for glycerol and 7% for $[1,1,2,3,3\text{-}^2\text{H}_5]$ glycerol.

Glucoregulatory hormones Insulin and cortisol were determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA). Insulin was measured with a chemiluminescent immunometric assay with intra-assay variation of 3–6%, inter-assay variation of 4–6% and detection limit of 15 pmol/l. Cortisol was measured with a chemiluminescent immunoassay with intra-assay variation of 7–8%, inter-assay variation of 7–8% and a detection limit of 50 nmol/l. Glucagon was determined with the Linco 125I RIA (Linco Research, St Charles, MO, USA) with an intra-assay variation of 9–10%, inter-assay variation of 5–7% and detection limit of 15 ng/l.

Calculations and statistics HOMA of insulin resistance (HOMA-IR) was calculated using the formula described previously by Matthews et al. [25]. Endogenous glucose production (EGP) and peripheral glucose uptake (rate of disappearance [R_d]) were calculated using the modified forms of the Steele equations as described previously [19, 26]. EGP and R_d were expressed as $\mu\text{mol}(\text{kg FFM})^{-1}\text{min}^{-1}$ (FFM, fat-free mass). Insulin clearance was calculated as the rate of insulin infusion ($\text{mU}[\text{m}^2\text{ body surface area}]^{-1}\text{min}^{-1}$) divided by the mean plasma insulin concentration during the clamp [27]. Total triacylglycerol hydrolysis/lipolysis (glycerol turnover) was calculated using formulas for steady-state kinetics adapted for stable isotopes [24, 26] and was expressed as $\mu\text{mol kg}^{-1}\text{min}^{-1}$.

Resting energy expenditure (REE), glucose oxidation and fat oxidation rates were calculated from $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ as reported previously [28]. Non-oxidative glucose disposal was calculated as the difference between total glucose disposal and glucose oxidation.

All data were analysed with non-parametric tests. Comparisons between groups were performed using the Mann–Whitney U test. SPSS version 14.0.2 (SPSS, Chicago, IL, USA) was used for statistical analysis. Data are presented as median (minimum–maximum). In the [Electronic supplementary material](#) (ESM) data are also presented as mean \pm SEM.

Results

Study participants We included seven male FHBL participants and seven healthy male controls. All patients were matched to controls for age, BMI and WHR. The baseline characteristics of the participants are listed in Table 1 and ESM Table 1. As expected, there was a significant difference in plasma levels of ApoB, LDL-cholesterol and triacylglycerol. In addition, plasma levels of alanine aminotransferase and aspartate aminotransferase were above the reference value, of 45 and 40 U/l respectively, in some FHBL patients but not in controls. The HOMA index tended to be higher in FHBL patients compared with controls, although the difference was not statistically significant.

Body composition and ^1H -MRS Participants with FHBL did not differ in body composition from their matched controls (Table 2 and ESM Table 2). ^1H -MRS measurements showed moderate hepatic steatosis in all participants with FHBL and mild steatosis in two control patients. As expected, IHTG content was significantly higher in participants with FHBL (29.8% [15.9–38.0%]) compared with controls (0.8% [0.0–12.4%]) ($p < 0.005$; Fig. 1a). One FHBL patient showed a substantially higher IMCL content compared with all other

Table 1 Baseline characteristics of FHBL patients and healthy controls

Characteristic	FHBL (n=7)	Control (n=7)	p value
Age (years)	47 (22–61)	45 (22–60)	NS
BMI (kg/m ²)	27 (22–32)	26 (20–36)	NS
WHR (cm)	1.02 (0.97–1.05)	1.01 (0.94–1.12)	NS
Total cholesterol (mmol/l)	1.94 (1.39–2.81)	4.75 (3.47–5.23)	<0.01
Triacylglycerol (mmol/l)	0.14 (0.05–0.49)	0.76 (0.40–1.07)	<0.01
ApoB (g/l)	0.24 (0.12–0.4)	0.83 (0.54–0.90)	<0.01
HDL (mmol/l)	1.19 (0.7–1.53)	1.3 (0.79–1.83)	NS
HOMA index	1.58 (0.21–2.35)	0.76 (0.23–1.58)	NS
Aspartate aminotransferase (U/l)	39 (22–49)	28 (14–34)	<0.05
Alanine aminotransferase (U/l)	62 (22–106)	23 (10–32)	0.05
C-reactive protein (mg/l)	1.4 (0.50–3.10)	1.0 (0.50–6.20)	NS

Data are median (range)

participants but median IMCL concentrations did not significantly differ between groups (patients 7.7% [4.5–10.1%], controls 5.1% [3.3–21.4%]; not significant) (Fig. 1b).

Glucose metabolism Data on glucose metabolism are summarised in Table 3 and ESM Table 3.

At baseline, plasma glucose was comparable between groups, whereas insulin levels tended to be higher in participants with FHBL compared with controls.

EGP at baseline was 11.5 (10.1–13.2) $\mu\text{mol (kg FFM)}^{-1} \text{min}^{-1}$ in patients and 13.0 (11.4–14.0) $\mu\text{mol (kg FFM)}^{-1} \text{min}^{-1}$ in controls ($p=0.3$). Insulin infusion during the first step of the clamp procedure increased plasma insulin concentrations to 199 (177–302) pmol/l in FHBL participants and to 162 (132–294) pmol/l in healthy controls ($p=0.2$). During the second step of the clamp procedure insulin concentrations increased to 615 (572–836) pmol/l in FHBL and to 504 (426–712) pmol/l in control participants ($p=0.1$).

Plasma levels for other glucoregulatory hormones, e.g. cortisol and glucagon, were comparable between groups both in the basal state and during the clamp (data not shown).

The ability of insulin to suppress endogenous glucose production was not impaired in FHBL patients compared with controls. At lower levels, insulin-mediated suppression of EGP was 68.6% (59.3–82.8%) in patients and 72.9% (56.8–86.9%) in controls ($p=0.9$; Fig. 2a). During higher dose insulin infusion EGP was completely suppressed in both groups.

The ability of insulin to increase peripheral glucose disposal was also not significantly impaired in patients with FHBL compared with controls (Fig. 2b). Correction of R_d for plasma insulin did not have an effect on our results (data not shown). Insulin clearance did not differ significantly between patients and controls during either step of the clamp.

REE, the basal rate of glucose oxidation and the insulin-stimulated increase in glucose oxidation and non-oxidative glucose disposal were similar between groups (Table 3 and ESM Table 3).

NEFA and lipolysis In the basal state, circulating concentrations of NEFAs were not different between patients and control participants (Table 3 and ESM Table 3). Likewise, lipolysis (glycerol turnover) and lipid oxidation were

Table 2 Body fat composition

Variable	FHBL	Controls	p value
Body weight (kg)	86 (70–110)	87 (71–114)	NS
Body fat (%)	26 (19–30)	23 (12–35)	NS
Lean body mass (kg)	61 (49–77)	64 (58–67)	NS
Peripheral fat mass (kg)	8.4 (5.7–14.1)	8.6 (3.9–16.6)	NS
Trunk fat mass (kg)	11.7 (6.1–16.7)	10.8 (3.6–19.9)	NS
Abdomen			
Subcutaneous adipose tissue (cm ²)	189.31 (148.43–429.06)	203.35 (47.76–504.59)	NS
Visceral adipose tissue (cm ²)	124.20 (38.71–180.71)	106.61 (26.46–218.99)	NS
Visceral:subcutaneous adipose tissue ratio	0.62 (0.26–1.19)	0.55 (0.22–1.08)	NS

Data are median (range)

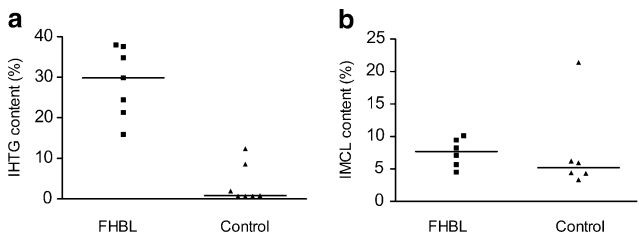


Fig. 1 IHTG and IMCL content assessed by MRS in participants with FHBL and healthy controls. The horizontal line represents the median. **a** Intrahepatic triacylglycerol (IHTG) content ($p < 0.005$). **b** Intramyocellular lipid content (IMCL) content (NS)

comparable between patients and controls both in the basal state and during the clamp.

Baseline lipolysis corrected for REE did not have an effect on the results (data not shown). Furthermore, lipolysis tended to be less suppressed by insulin in patients 60% (37–73%) compared with controls 66% (55–75%), although the difference was not statistically significant ($p = 0.09$; Fig. 2c).

Discussion

In the present study we show that patients with FHBL, despite the presence of moderate to severe hepatic steatosis, do not display a decrease in hepatic or peripheral insulin sensitivity compared with unaffected, matched controls. This indicates that hepatic triacylglycerol accumulation in itself is not causally related to hepatic or peripheral insulin resistance.

In the basal state, HOMA-IR tended to be higher in FHBL patients compared with controls. Since glucose levels were similar between patients and controls, the difference in HOMA-IR is probably explained by higher basal insulin levels in patients than in controls and is most likely caused by reduced insulin clearance and not by increased insulin secretion. This would be in line with earlier observations in non-diabetic participants, in whom hepatic fat accumulation was shown to be associated with impaired insulin clearance, independently of obesity [27]. A tendency towards higher insulin levels in FHBL patients was also observed during the hyperinsulinaemic clamp. Although calculation of insulin clearance during the clamp failed to show a significant difference between patients and controls, this finding further supports the concept that insulin clearance may be decreased in patients with FHBL.

It should be noted that when R_d was corrected for circulating plasma insulin levels, peripheral insulin sensitivity in FHBL patients did not differ from that in controls. Thus, despite a possible difference in insulin clearance, insulin-

mediated peripheral glucose uptake was not impaired in patients with FHBL.

Two of the seven patients in the control group showed mild hepatic steatosis, of 12% and 8%, respectively. One could argue that increased IHTG content in these patients might, to some extent, have skewed the data. However, since patients and controls were matched, individuals could not be excluded from analysis.

In a recent small study it was shown that both the hepatic insulin sensitivity index (inverse of the product of the basal EGP and fasting plasma insulin concentration) and the insulin-mediated increase in R_d did not differ between three obese participants with FHBL and six obese controls without hepatic steatosis [16]. In that study only a high-dose insulin infusion (50 mU/m^2) was used during the clamp. In the present study we extended these findings in a larger number of patients, including both lean and obese FHBL patients, and carefully matched controls. Moreover, by using both a high- and a low-dose insulin infusion we were able to unambiguously demonstrate that FHBL does not affect hepatic insulin sensitivity. In addition, we show that FHBL does not lead to impaired sensitivity in other target pathways of insulin, e.g. glucose oxidation, non-oxidative glucose disposal, lipolysis and lipid oxidation. Thus, our data provide strong evidence to show that in patients with FHBL, hepatic steatosis is a determinant of neither hepatic nor peripheral (muscle or adipose tissue) insulin resistance.

The lack of hepatic insulin resistance in FHBL in spite of severe steatosis may be surprising. Absence of insulin resistance has, however, been observed previously in animal models of hepatic steatosis [29]. In these animals, overabundance of hepatic diacylglycerol acyltransferase, an enzyme catalysing the final step in triacylglycerol synthesis [30]; deletion of long-chain fatty acid elongase family member 6 (ELOVL6), a microsomal enzyme involved in the elongation of fatty acids [31]; deletion of microsomal triacylglycerol transfer protein, responsible for the assembly of triacylglycerol-rich lipoproteins [32]; and pharmacological blockade of hepatic fatty acid β -oxidation [33], have all been associated with the induction of hepatic steatosis without hepatic or peripheral insulin resistance.

If hepatic steatosis in itself does not cause insulin resistance, factors other than hepatic triacylglycerol accumulation must be responsible for the close relation between these two entities in epidemiological studies [1, 4, 6–8]. Thus, hepatic steatosis and insulin resistance could represent two separate manifestations of the same metabolic derangements, such as chronic inflammation, endoplasmic reticulum stress or stress caused by other, as yet unidentified, metabolites [34, 35]. Recent studies have put forward the concept that lipid metabolites such as fatty acids, long-

Table 3 Glucose and fat metabolism at baseline and during the clamp

Variable	Basal state			Clamp 1			Clamp 2		
	FHBL	Control	<i>p</i> value	FHBL	Control	<i>p</i> value	FHBL	Control	<i>p</i> value
	Glucose (mmol/l)	5.5 (4.2–5.6)	5.0 (4.0–5.5)	NS	5.1 (4.8–5.2)	4.9 (4.6–5.1)	NS	4.9 (4.7–5.2)	4.9 (4.8–5.3)
Insulin (pmol/l)	53 (8–71)	24 (8–83)	NS	199 (177–302)	162 (132–294)	NS	615 (572–836)	504 (426–712)	NS
EGP ($\mu\text{mol}[\text{kg FFM}]^{-1}\text{min}^{-1}$)	11.5 (10.1–13.2)	13.0 (11.4–14.0)	NS	3.1 (1.5–5.2)	2.2 (1.3–6.4)	NS	^a	^a	NS
R_d ($\mu\text{mol}[\text{kg FFM}]^{-1}\text{min}^{-1}$)	–	–	–	22.4 (16.2–32.6)	22.5 (18.8–42.9)	NS	57.5 (48.2–73.7)	65.0 (44.3–82.9)	NS
Insulin clearance ^b	–	–	–	0.10 (0.07–0.15)	0.10 (0.08–0.14)	NS	0.12 (0.07–0.15)	0.12 (0.08–0.14)	NS
Glucose oxidation ($\mu\text{mol}[\text{kg FFM}]^{-1}\text{min}^{-1}$)	6.8 (5.1–11.1)	9.1 (5.2–11.9)	NS	–	–	–	22.6 (16.4–25.7)	26.1 (16.9–37.1)	NS
NOGD ($\mu\text{mol}[\text{kg FFM}]^{-1}\text{min}^{-1}$)	3.2 (2.1–8.1)	3.7 (0.0–6.3)	NS	–	–	–	33.1 (28.2–55.5)	42.0 (26.5–52.1)	NS
REE (kJ/day)	7,125(6,113–9,874)	7,280 (6,653–8,786)	NS	–	–	–	7,908 (6,5147–9,945)	7,862 (6,991–9,803)	NS
NEFA (mmol/l)	0.47 (0.18–0.49)	0.40 (0.29–0.82)	NS	0.08 (0.03–0.18)	0.05 (0.03–0.18)	NS	0.03 (0.01–0.06)	0.01 (0.01–0.05)	NS
Lipolysis ($\mu\text{mol kg}^{-1}\text{min}^{-1}$)	1.6 (1.5–1.9)	1.7 (1.2–2.5)	NS	0.8 (0.4–1.0)	0.6 (0.4–1.1)	NS	0.6 (0.3–0.9)	0.4 (0.3–0.8)	NS
Fat oxidation ($\mu\text{mol}[\text{kg FFM}]^{-1}\text{min}^{-1}$)	1.8 (1.2–2.1)	1.7 (1.0–1.9)	NS	–	–	–	0.6 (0.5–1.2)	0.4 (0.0–1.0)	NS

Data are median (range)

To convert from micromoles per kilogram per minute to milligrams per kilogram per minute, multiply by 0.18

NOGD, non-oxidative glucose disposal

^a Endogenous glucose production was completely suppressed in both groups during high-dose insulin infusion

^b $\text{mU}[\text{m}^2\text{ body surface area}]^{-1}\text{min}^{-1}$ [$\text{pmol}/\text{l}^{-1}$]

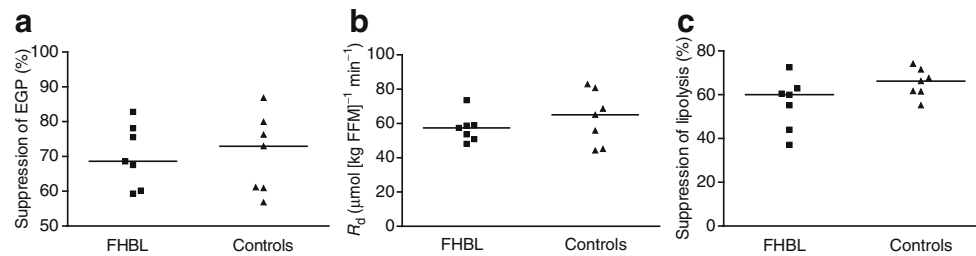


Fig. 2 Clamp results in participants with FHBL and healthy controls. The horizontal line represents the median. **a** Suppression of EGP during low-dose insulin infusion ($p=0.40$). **b** R_d of glucose during

high-dose insulin infusion ($p=0.34$). **c** Suppression of lipolysis during low-dose insulin infusion ($p=0.09$)

chain acyl-CoAs, diacylglycerol and ceramides rather than triacylglycerols themselves [36, 37] are determinants of the onset of insulin resistance [29, 38–40]. In this scenario, hepatic steatosis in FHBL may be the result of a harmless accumulation of triacylglycerols, whereas NAFLD is the result of an accumulation of toxic lipid metabolites leading to insulin resistance. In the present study, circulating concentrations of NEFA were similar in FHBL patients compared with controls.

Furthermore, the association between hepatic steatosis and insulin resistance may have a genetic basis. For example, polymorphisms in *APOC3* have recently been shown to be associated with both NAFLD and insulin resistance, whereas the single-nucleotide polymorphism rs738409 in *PNPLA3* was associated with increased liver fat but not with insulin resistance [41, 42]. Unfortunately, the results of the present study do not provide answers to explain the true mechanism underlying the relationship between hepatic steatosis and insulin resistance.

Hepatic steatosis has been suggested to be causally related to hepatic as well as peripheral insulin resistance. In the present study, we convincingly show in a unique human model of severe fatty liver disease that hepatic steatosis is not associated with hepatic or peripheral insulin resistance. Whereas the results of the present study do not unravel the exact mechanisms underlying the complex relationship between these two highly prevalent metabolic disorders, further studies focusing on the comparison of different hepatic steatosis models in large cohorts are required.

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

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