BMC Gastroenterology

RESEARCH ARTICLE Open Access

Plasma Lipidome, *PNPLA3* polymorphism and hepatic steatosis in hereditary hemochromatosis

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Abstract

Background: Hereditary hemochromatosis (HH) is an autosomal recessive genetic disorder with increased intestinal iron absorption and therefore iron Overload*. iron* overload leads to increased levels of toxic non-transferrin bound iron which results in oxidative stress and lipid peroxidation. The impact of iron on lipid metabolism is so far not fully understood. The aim of this study was to investigate lipid metabolism including lipoproteins (HDL, LDL), neutral (triglycerides, cholesterol) and polar lipids (sphingo- and phospholipids), and *PNPLA3* polymorphism (rs738409/I148M) in HH.

Methods: We conducted a cohort study of 54 subjects with HH and 20 healthy subjects. Patients were analyzed for their iron status including iron, ferritin, transferrin and transferrin saturation and serum lipid profile on a routine follow-up examination.

Results: HH group showed significantly lower serum phosphatidylcholine (PC) and significantly higher phosphatidylethanolamine (PE) compared to healthy control group. The ratio of PC/PE was clearly lower in HH group indicating a shift from PC to PE. Triglycerides were significantly higher in HH group. No differences were seen for HDL, LDL and cholesterol. Hepatic steatosis was significantly more frequent in HH. *PNPLA3* polymorphism (CC vs. CG/GG) did not reveal any significant correlation with iron and lipid parameters including neutral and polar lipids, grade of steatosis and fibrosis.

Conclusion: Our study strengthens the hypothesis of altered lipid metabolism in HH and susceptibility to nonalcoholic fatty liver disease. Disturbed phospholipid metabolism may represent an important factor in pathogenesis of hepatic steatosis in HH.

Keywords: Hereditary hemochromatosis, Plasma lipidome, Phospholipids, Triglycerides, Nonalcoholic fatty liver disease, *PNPLA3* polymorphism

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Background

Hereditary hemochromatosis (HH) is an autosomal recessive genetic disorder with increased intestinal iron absorption which leads to iron overload and consequently to tissue damage and functional impairment of organs like liver, pancreas, and heart [1–3]. Iron is an essential metal involved in a wide spectrum of physiological functions such as oxygen transport and enzymatic reactions. Iron overload leads to increased levels of toxic non-transferrin bound iron (NTBI) which results in oxidative stress and lipid peroxidation [3, 4].

Iron overload has a direct effect on hepatic lipid metabolism [5]. Animal models with dietary iron overload showed marked changes in plasma lipid profiles with elevated triglycerides and total cholesterol, decreased high-density lipoprotein (HDL), altered composition of very-low density lipoprotein (VLDL) and hepatic sterol metabolism [6–8]. Iron treated HepG2 cells revealed a decrease of apolipoprotein B100 (ApoB100) and VLDL secretion [9] which was reversed by the iron chelator desferroxamine [10]. Additionally, iron overload increased intracellular lipid droplets, which was associated with increased phosphatidylserine (PS) in the outer leaflet of the plasma membrane [11]. This alteration was also observed in membranes of erythrocytes in HH [12]. Iron excess induces distinct changes in the serum concentrations of unsaturated long-chain fatty-acyl phosphatidylcholine (PC) including PC 40:2, PC 40:3, PC 40:4, and PC 42:1 in diabetes mellitus type 2 [13]. Inhibition of hepatic PC biosynthesis results in triglyceride accumulation and impairs the secretion of VLDL [14, 15]. In rat livers excess dietary iron intake causes pro-steatotic state which was associated with a decrease in ω-3 long-chain polyunsaturated fatty acid (PUFA) levels and an upregulation of the expression of lipogenic transcription factors [16].

In humans iron overload causes elevated triglycerides [17]. Genetic predisposition for HH is associated with primary hypertriglyceridemia [18]. Hypertriglyceridemia was found in almost a third of subjects with HH which was significantly decreased by phlebotomies [19] whereas in non-HH subjects with hyperferritinemia and hypertriglyceridemia repeated phlebotomies did not reduce triglyceride concentrations [20]. Reduced levels of LDL at baseline [10] and an increase of HDL under phlebotomy in HH patients were shown [21], but in their study no effect of phlebotomy was seen on total cholesterol, low density lipoprotein (LDL) and triglyceride levels [21]. Hence, iron has effects on hepatocellular metabolism of phospholipids and lipoproteins, and on unsaturation of fatty acids in experimental and clinical settings.

Obesity-related steatosis is already identified as cofactor in liver injury in HH [22], but HH is also associated with increased susceptibility to nonalcoholic fatty liver disease (NAFLD) despite lower BMI and lower triglycerides levels [23, 24]. Nevertheless the role of HFE polymorphism in the occurrence of NAFLD is not fully understood.

PNPLA3 (human patatin like phospholipase domain-containing 3) belongs to a group of lipid metabolizing enzymes [25]. In humans, *PNPLA3* has the highest expression in the hepatic stellate cells, retina, and hepatocytes [26]. The rs738409 CG variant in *PNPLA3* is considered the major genetic determinant of NAFLD [27]. SNPs rs738409 C > G in the *PNPLA3* gene encodes for the isoleucine to methionine substitution at position 148 (I148M). It has been shown that *PNPLA3* has a triglyceride hydrolase activity [28]. The I148M mutation causes a loss of function of the enzymes activity leading to impairment of lipid catabolism, lipid droplets remodeling, and VLDL secretions in hepatocytes with reduced fatty acid hydrolysis, increased triglyceride accumulation and a decrease of VLDL secretion thereby contributing to hepatic steatosis, inflammation and a greater risk for developing fibrosis [26, 29–32]. These alterations were observed with and without symptoms of metabolic syndrome like insulin resistance and obesity [33, 34]. In HH the I148M variant was linked with the severity of fibrosis [35]. The mechanism underlying the progression of liver disease is still under investigation. It is hence surmised that the role of iron overload on hepatocellular lipid metabolism may involve *PNPLA3*.

Therefore, the aim of our study was to evaluate alterations in lipid metabolism including analyses of blood neutral and polar lipids depending on iron status and *PNPLA3* polymorphism (rs738409/I148M) in our HH cohort in comparison to healthy control group.

Methods

Study population

A total of 54 subjects with HH were retrospectively examined at Internal Medicine IV, University Heidelberg Hospital from 2012 to 2019. Written informed consent was obtained from each patient. Ethical approval was given by the Ethics Committee of University of Heidelberg. Results of HH patients were compared with 20 healthy control subjects. The study protocol was approved by the ethical committee of the University of Heidelberg. Patients serum samples were collected on a routine follow-up examination for HbA1c, BMI, albumin, bilirubin, GGT, AST, ALT, prothrombin time (P), ferritin, transferrin, transferrin saturation (TS), iron, HFE-genotype, and I148M *PNPLA3* polymorphism (rs738409/I148M) as well as serum lipids and lipoproteins including triglycerides, cholesterol, HDL, LDL and phospho- and sphingolipids. Patients with alcohol consumption > 20 g/day and statin therapy or any other lipid

lowering therapy were excluded from the study. Ultrasound and evaluation for liver fibrosis using noninvasive method (Fibroscan) was performed by experienced medical doctors. The duration of experience was more than six months as a fulltime job in the department of sonography. Sonographic features to evaluate patients for hepatic steatosis were increased echogenicity of the background liver parenchyma, difference in echogenicity between the liver and right kidney, obscuration of the margins of the portal triads within the liver, and attenuation of the sound beam with diminished visualization of the deep right lobe. These criteria were used in the assessment of the presence and graduation of hepatic steatosis as normal, mild, moderate and severe. Diagnosis of HH was done by genetic analysis (C282Y) in the hospital central laboratory according to standard methods and determination of iron parameters (iron, ferritin, transferrin, TS). Patients with HH were treated by phlebotomy. The therapy was monitored by the determination of ferritin which should be less than 100 μg/l.

Lipidomic analysis

Serum samples were subjected to lipid extraction according to Folch methods. The levels of phospho- and sphingolipids in lipid extracts were determined with a triple-quadrupole Micro Mass Quattro Premier mass spectrometer coupled with a liquid-chromatography system using the running conditions as previously described [36]. The following polar lipids (phospho- and sphingolipids) were measured: phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), phosphatidylserine, phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), and sphingomyelin (SM). Serum polar lipids were quantified as ng/μl serum.

Statistical methods

Variables were described by mean ± standard deviation (SD) or median (interquartile range IQR) or frequencies, respectively. By means of detailed summary statistics and visual inspection of the distribution normality assumption was checked. Statistical differences in means between two groups (healthy vs. HH and CC vs. CG/GG) were evaluated using Welch's t-test (due to unequal sample size homogeneity of variances cannot be assumed) or the nonparametric Mann-Whitney U test in case normal distribution cannot be assumed. Statistical differences in proportions were assessed by chi-square test or Fisher's exact test in case of small expectancy counts. All analyses were performed with SPSS version 24 (IBM SPSS Statistic, Chicago, IL, USA). Two-sided *p*-values were interpreted descriptively and a p-value of < 0.05 was considered statistically significant.

Results

Baseline characteristics of study population and stratified by low and high transferrin saturation

Patient characteristics are outlined in Table 1. A total of 54 subjects with HH were analyzed and compared with 20 healthy subjects. The two groups were significantly different in serum iron contents (Healthy subjects vs. HH: transferrin 2.1 vs. 1.8 g/l, $p < 0.0001$, transferrin saturation 26.0% vs. 70.1%, $p < 0.0001$, iron 13.5 μmol/l vs. 31.8 μmol/l, $p < 0.0001$, ferritin 133.0 μ/l vs. 261.0 μ/l, $p = 0.02$). Triglycerides (74.0 vs. 103.5 mg/dl, $p = 0.01$) were significantly higher in HH group. Cholesterol, HDL and LDL did not differ between the groups. GGT (12.5 mg/dl vs. 23.0 mg/dl, $p = 0.001$) and AST (22.0 U/l vs. 29.0 U/l, $p = 0.01$) were in the normal range but significantly increased in HH group. Sonography of the liver showed that 29.6% of subjects with HH had normal liver without hepatic steatosis while 33.3, 22.2 and 9.3% had mild, moderate and severe steatosis, respectively. Significant more hepatic steatosis in HH compared to the control group was found ($p < 0.0001$). Fibroscan measured liver stiffness of 6.7 kPa (± 4.8 kPa) in HH.

Serum phospho- and sphingolipids of study population

In addition to serum neutral lipids and lipoproteins, we also determined serum contents of polar lipids (phospho- and sphingolipids) in 53 subjects with HH which were compared with those of healthy control group (Table 2). A significant difference was observed for total ($p = 0.005$) and polyunsaturated fatty acids (PUFA, $p = 0.008$) containing PE and total ($p = 0.04$) and monounsaturated fatty acids (MUFA, $p = 0.01$) containing PI with higher values in subjects with HH. No difference was observed for total, MUFA and PUFA containing PS between the two groups. In HH group, significant higher levels were observed PUFA containing LPE ($p = 0.045$). In LPC, a significant difference was seen in MUFA ($p = 0.02$) and PUFA ($p = 0.004$) containing LPC. No significance was reached for total and saturated LPC. Saturated ($p < 0.0001$), PUFA ($p = 0.003$), alkyl containing ($p = 0.045$) and total PC ($p = 0.04$) showed significant differences between the groups. Saturated ($p = 0.03$), total ($p = 0.03$) and MUFA ($p = 0.045$) containing SM were significantly elevated in HH group. PC/PE ratio was clearly lower in HH group indicating a shift towards PE and therefore indicating a disturbance in PC metabolism.

PNPLA3 genotype of study population

54 subjects with HH were analyzed according to their I148M *PNPLA3* polymorphism (rs738409). 48.2% were wildtype (CC), 44.4% were heterozygous (CG) and 7.4% homozygous (GG) for the *PNPLA3* polymorphism I148M (Table 1). Analysis of CC in comparison to CG/

Table 1 Clinical characteristics of study population. Mean ± SD or median (IQR) or frequencies *n* (%) are shown with *p*-values for the group differences based on Welch's t-test (a), Mann-Whitney U test (b), chi-square test (c) or Fisher's exact test (d)

	Limits of normal	Healthy $n = 20$	HH $n = 54$	***p*-value**
gender (male)		11 (55%)	39 (72.2%)	0.98[c]
Age (years)		48.9 ± 11.8	51.3 ± 15.0	0.47[a]
Type II diabetes mellitus		0 (0%)	6 (11.1%)	0.18[d]
BMI	kg/m^2	24.4 ± 2.9	26.0 ± 3.4	0.05[a]
Cholesterol	mg/dl	186.6 ± 35.5	188.0 ± 38.3	0.89[a]
HDL	> 40 mg/dl	60.0 ± 17.3	55.3 ± 19.2	0.34[a]
LDL	< 160 mg/dl	109.4 ± 31.1	111.7 ± 26.8	0.79[a]
Triglycerides	< 150 mg/dl	74.0 (48.0)	103.5 (83.0)	**0.01[b]**
Albumin	30–50 g/l	44.6 ± 1.5	44.8 ± 2.6	0.75[a]
Bilirubin	< 1.0 mg/dl	0.6 (0.2)	0.8 (0.6)	0.38[b]
GGT	< 60 U/l	12.5 (18.5)	23.0 (30.3)	**0.001[b]**
AST	< 46 U/l	22.0 (14.0)	29.0 (19.5)	**0.01[b]**
ALT	< 50 U/l	25.0 (8.0)	26.0 (17.5)	0.25[b]
PT	70–125%	101.9 ± 11.0	98.6 ± 16.2	0.32[a]
Ferritin	30–300 μg/l	133.0 (150.5)	261.0 (731.3)	**0.02[b]**
Transferrin	2.0–3.6 g/l	2.1 ± 0.3	1.8 ± 0.3	**< 0.0001[a]**
TS	16–45%	26.0 ± 9.6	70.1 ± 24.9	**< 0.0001[a]**
Iron	14–32 μmol/l	13.5 ± 4.7	31.8 ± 10.2	**< 0.0001[a]**
Hepatic steatosis	none	18 (90%)	16 (29.6%)	**< 0.0001[c]**
	mild	0 (0%)	18 (33.3%)	
	moderate	2 (10%)	12 (22.2%)	
	severe	0 (0%)	5 (9.3%)	
FibroScan	< 6 kPa	–	6.7 ± 4.8	–
HFE-genotype	C282Y	–	54 (100%)	–
***PNPLA3* genotype**	CC	–	26 (48.2%)	–
	CG	–	24 (44.4%)	–
	GG	–	4 (7.4%)	–

ALT alanine aminotransferase; *AST* aspartate aminotransferase; *BMI* body mass index; *HDL* high-density lipoprotein; *HH* hereditary hemochromatosis; *IQR* interquartile range; *LDL* low-density lipoprotein; *PNPLA3* human patatin like phospholipase domain-containing 3; *PT* prothrombin time; *SD* Standard deviation; *TS* transferrin saturation;

GG showed also no significant difference between the groups, especially in lipid (cholesterol, HDL, LDL, triglycerides) and iron parameters (transferrin, iron, TS, ferritin), grade of hepatic steatosis and fibrosis (Table 3). Phospho- and sphingolipids as well demonstrated no significant difference between these two groups (Table 4).

Discussion

In our study, we analyzed lipid metabolism and hepatic steatosis in HH. This study is to our knowledge the first study in HH which investigated in addition to lipoproteins (HDL, LDL) and neutral lipids (cholesterol, triglycerides) phospho- and sphingolipid metabolism. Our results strengthen the hypothesis of altered lipid metabolism in HH, especially in phospholipid metabolism and susceptibility to NAFLD and this was independent of *PNPLA3* polymorphism.

It is known that hepatic steatosis is a cofactor in liver injury in HH [22], and HH-dependent susceptibility to NAFLD is independent of BMI and triglyceride levels [23, 24]. Clinical significant liver disease in HH is also associated with risk factors like heavy alcohol abuse and concomitant chronic liver diseases like chronic hepatitis C [37]. As HH subjects with high (> 20 g/day) alcohol consumption and concomitant chronic liver diseases were excluded from our study, it has to be assumed that these risk factors do not play a role in the observed alterations in our cohort.

In our cohort, BMI was as well not affected in HH, and this indicates non-obese NAFLD in HH pathogenesis. However, our cohort shows that blood triglycerides

Table 2 Phospho- and sphingolipids (ng/μl) of study population. Serum samples were quantified in ng/μl serum. Mean ± SD or median (IQR) are shown with *p*-values for the group differences based on Welch's t-test (a) or Mann-Whitney U test (b)

		Healthy *n* = 20	HH *n* = 53	***p*-value**
Phospholipids				
PC	Saturated	996.2 ± 369.3	750.6 ± 30.6	**< 0.0008[a]**
	MUFA	2327.0 ± 866.7	2401.8 ± 976.2	0.75[a]
	PUFA	6163.9 ± 2104.8	8001.3 ± 2334.6	**0.003[a]**
	Alkyl	521.6 ± 169.7	619.3 ± 203.1	**0.045[a]**
	Total	10,008.8 ± 3154.7	11,773.0 ± 3339.3	**0.04[a]**
LPC	Saturated	51.5 ± 11.3	56.9 ± 14.6	0.10[a]
	MUFA	12.5 ± 3.4	10.5 ± 2.7	**0.02[a]**
	PUFA	19.6 ± 4.2	16.2 ± 4.2	**0.004[a]**
	Total	83.7 ± 17.6	83.6 ± 19.7	0.98[a]
PI	MUFA	14.4 ± 6.5	19.7 ± 10.0	**0.01[a]**
	PUFA	137.4 ± 50.1	165.0 ± 61.0	0.06[a]
	Total	152.1 ± 55.7	184.7 ± 69.1	**0.04[a]**
PS	MUFA	0.4 (2.6)	0.0 (4.0)	0.71[b]
	PUFA	17.7 (17.9)	24.6 (52.0)	0.15[b]
	Total	18.4 (18.3)	26.5 (59.5)	0.18[b]
PE	MUFA	75.2 (55.4)	91.9 (329.7)	0.05[b]
	PUFA	198.6 (103.5)	289.2 (257.0)	**0.005[b]**
	Total	276.4 (123.2)	407.8 (573.2)	**0.008[b]**
LPE	Saturated	1.2 (0.9)	1.3 (0.8)	0.87[b]
	MUFA	0.5 (0.4)	0.5 (0.9)	0.95[b]
	PUFA	1.6 (0.8)	2.3 (3.0)	**0.045[b]**
	Total	3.6 (1.7)	4.5 (4.8)	0.10[b]
Sphingolipids				
SM	Saturated	711.3 ± 271.3	876.1 ± 279.5	**0.03[a]**
	MUFA	2168.3 ± 1339.6	2879.7 ± 1173.2	**0.045[a]**
	PUFA	1537.9 ± 517.6	1728.4 ± 380.7	0.15[a]
	Alkyl	344.3 ± 256.5	465.4 ± 236.7	0.08[a]
	Total	4761.8 ± 2104.7	5949.6 ± 1743.3	**0.03[a]**
Ratio PC/PE		34.7	22.2	

HH hereditary hemochromatosis; *IQR* interquartile range; *LPC* lysophosphatidylcholine; *LPE* lysophosphatidylethanoletamine; *MUFA* monounsaturated fatty acid; *PC* phosphatidylcholine; *PE* phosphatidylethanolamine; *PS* phosphatidylserine; *PUFA* polyunsaturated fatty acid; *SD* standard deviation; *SM* sphingomyelin;

were significantly higher and hepatic steatosis was significantly more frequent in HH group. While the role of HFE polymorphism in the occurrence of NAFLD still remains unclear, our findings may suggest that iron-overload and subsequent oxidative stress in HH could be due to the alteration of hepatic triglyceride metabolism associated with hepatic steatosis. Accordingly, it is known that iron-overload increases serum triglycerides associated with fatty liver in experimental animals [38]. NAFLD patients also show an increase of both triglycerides and iron in the liver [39], and that iron stores as ferritin in NAFLD is associated with hypertriglyceridemia [40]. Iron overload can also affect visceral adipose tissue metabolism by a mechanism involving hepcidin up-regulation [41]. In addition to NAFLD, hepatocellular iron deposition is associated with an increased risk of hepatic fibrosis [42] suggesting a critical role of iron in NAFLD progression to NASH. Taken together, increased blood triglycerides observed in our HH cohort is a pathologic marker of exaggerated hepatic steatosis caused by iron-overload. The alteration of adipose tissue metabolism by iron-overload could result in the lack of any increase of BMI in HH, despite of increased hepatic steatosis.

Together with increased hepatic triglycerides, hepatic steatosis is associated with the depletion of liver

Table 3 *PNPLA3* genotype CC vs. CG/GG of hereditary hemochromatosis (HH) group. Mean ± SD or median (IQR) or frequencies n (%) are shown with p-values for the group differences based on Welch's t-test (a), Mann-Whitney U test (b) or chi-square test (c)

	limits of normal	CC $n = 26$	CG/GG $n = 28$	***p*-value**
Cholesterol	mg/dl	181.6 ± 35.2	193.9 ± 40.8	0.26[a]
HDL	> 40 mg/dl	58.8 ± 20.4	51.1 ± 18.1	0.18[a]
LDL	< 160 mg/dl	105.8 ± 22.7	116.3 ± 29.3	0.20[a]
Triglycerides	< 150 mg/dl	97.0 (58.8)	128.5 (125.0)	0.17[b]
Ferritin	μg/l	153.5 (465.5)	562.0 (1064.5)	0.18[b]
Transferrin	2.0–3.6 g/l	1.9 ± 0.3	1.8 ± 0.3	0.40[a]
TS	16–45%	73.8 ± 22.8	68.4 ± 26.7	0.44[a]
Iron	14–32 μmol/l	33.1 ± 9.2	30.6 ± 11.1	0.34[a]
Albumin	30–50 g/l	44.7 ± 2.7	44.9 ± 2.6	0.79[a]
Bilirubin	< 1.0 mg/dl	0.6 (0.5)	0.8 (0.7)	0.20[b]
GGT	< 60 U/l	23.0 (39.5)	26.0 (25.3)	0.94[b]
AST	< 46 U/l	25.0 (15.0)	35.5 (20.3)	**0.04[b]**
ALT	< 50 U/l	23.5 (10.5)	31.5 (16.5)	0.19[b]
BMI	kg/m^2	26.4 ± 3.9	25.7 ± 2.7	0.44[a]
FibroScan	< 6 kPa	6.8 ± 5.4	6.5 ± 4.1	0.79[a]
Hepatic steatosis	none	9 (34.6%)	7 (25%)	0.62[c]
	mild	9 (34.6%)	9 (32.1%)	
	moderate	4 (15.4%)	8 (28.6%)	
	severe	3 (3.8%)	2 (7.1%)	

ALT alanine aminotransferase; *AST* aspartate aminotransferase; *BMI* body mass index; *HDL* high-density lipoprotein; *IQR* interquartile range; *LDL* low-density lipoprotein; *PNPLA3*. human patatin like phospholipase domain-containing 3; *SD* standard deviation; *TS* transferrin saturation;

phospholipids including PC and PE in morbidly obese mice [43] and NAFLD patients [44]. Studies in *PEMT*-deficient mice have indicated that a decrease in hepatic PC/PE ratio is linked to NAFLD [45], and variants of *PEMT* gene is associated with non-obese NAFLD [46]. HH subjects in our study had significantly higher serum PUFA-containing and total PE species and lower saturated-containing PC resulting in a decrease of PC/PE ratio. Thus, hepatic metabolism in steatotic HH may be dominated by a shift of metabolism towards PE as seen in *PEMT*-deficient mice [45] and non-obese NAFLD [46]. In support of our results, patients with NAFLD and NASH show higher hepatic PE and lower erythrocyte PC [47]. Furthermore, serum PE is shown to be significantly increased in NASH but not in NAFLD [48] Thus, a decrease of serum PC/PE ratio appears to be a hallmark biomarker of HH with iron-overload being critical in the progression to NASH [49]. While iron deficiency is reported to increase hepatic PC and PE in rats [50], conversely, iron-overload in HH liver may induce a decrease of PC and PE differently by an unknown mechanism. As liver X receptor (LXR) regulates PUFA metabolism in phospholipids [51] particularly PE [52], we speculate that LXR could be a target of iron-induced oxidative stress [53] that could lead to increased hepatic and serum PE in steatotic HH.

While carriers of *PNPLA3* polymorphism (rs738409/I148M) are associated with hepatic steatosis and impaired lipid metabolism [25, 28–30, 32] and hepatic steatosis was frequently seen in our HH cohort, we analyzed *PNPLA3* polymorphism. In our cohort, *PNPLA3* polymorphism did not show any significant alterations on iron and lipid metabolism parameters including lipoproteins (HDL, LDL), neutral lipids (cholesterol, triglycerides), phospho- and sphingolipid metabolism. In a recent study, the I148M variant in HH was also not associated with altered lipid levels, but with the presence of hepatic steatosis and severity of fibrosis and therefore maybe representing a potential factor for fibrosis progression [34]. In our cohort, no effect on grade of hepatic steatosis and fibrosis for risk allele G was seen indicating that it does not have any impact on NAFLD pathophysiology in HH. Hepatic steatosis in HH is therefore rather related to iron-overload and subsequent oxidative stress with impaired apolipoprotein B100 (ApoB100) and VLDL secretion [9, 10]. Our study is to our knowledge the first study which investigated phospholipid metabolism in HH depending on *PNPLA3* polymorphism. Our HH cohort revealed significantly lower serum PC and significantly higher serum PE when compared to healthy controls, but this was independent of *PNPLA3* polymorphism suggesting that it does not

Table 4 Phospho- and sphingolipids (ng/μl) of hereditary hemochromatosis (HH) group stratified by *PNPLA3* genotype. Serum samples were quantified in ng/μl serum. Mean ± SD or median (IQR) are shown with p-values for the group differences based on Welch's t-test (a) or Mann-Whitney U test (b)

		CC *n* = 26	CG/GG *n* = 28	***p***-value
Phospholipids				
PC	Saturated	753.7 ± 37.6	747.8 ± 23.0	0.50[a]
	MUFA	2524.5 ± 1078.8	2292.3 ± 880.0	0.40[a]
	PUFA	8352.1 2 ± 467.5	7688.1 ± 2206.7	0.31[a]
	Alkyl	619.1 ± 204.9	619.4 ± 205.3	0.99[a]
	Total	12,249.3 ± 3624.0	11,347.7 ± 3066.5	0.34[a]
LPC	Saturated	54.0 ± 12.4	59.5 ± 16.0	0.17[a]
	MUFA	10.2 ± 2.5	10.8 ± 2.9	0.44[a]
	PUFA	15.8 ± 4.0	16.6 ± 4.5	0.52[a]
	Total	80.0 ± 16.5	86.8 ± 21.9	0.21[a]
PI	MUFA	18.80 ± 9.5	20.5 ± 10.5	0.55[a]
	PUFA	155.1 ± 57.6	173.9 ± 63.7	0.26[a]
	Total	173.9 ± 64.9	194.3 ± 72.4	0.28[a]
PS	MUFA	3.2 (5.1)	0.0 (2.6)	0.16[b]
	PUFA	24.6 (98.3)	23.4 (39.4)	0.55[b]
	Total	27.2 (104.1)	23.6 (49.9)	0.43[b]
PE	MUFA	85.7 (363.4)	92.8 (271.3)	0.99[b]
	PUFA	279.1 (236.7)	302.5 (278.7)	0.48[b]
	total	370.9 (607.0)	409.1 (545.4)	0.64[b]
LPE	Saturated	1.3 (0.8)	1.3 (0.8)	0.94[b]
	MUFA	0.4 (1.1)	0.6 (0.9)	0.46[b]
	PUFA	2.4 (4.7)	2.2 (2.5)	0.76[b]
	Total	3.9 (5.1)	4.6 (4.5)	0.78[b]
Sphingolipids				
SM	Saturated	892.4 ± 269.5	861.6 ± 292.2	0.69[a]
	MUFA	3074.6 ± 1122.1	2705.7 ± 1210.4	0.26[a]
	PUFA	1702.8 ± 409.3	1751.3 ± 359.3	0.65[a]
	Alkyl	461.2 ± 228.5	469.10 ± 248.0	0.90[a]
	Total	6130.94 ± 1688.2	5787.7 ± 1806.0	0.48[a]
Ratio PC/PE		23.1	21.4	

HH hereditary hemochromatosis; *IQR* interquartile range; *LPC* lysophosphatidylcholine; *LPE* lysophosphatidylethanoletamine; *PC* phosphatidylcholine; *PE* phosphatidylethanolamine; *PS* phosphatidylserine; *SD* standard deviation; *SM* sphingomyelin;

have any effect on altered phospholipid metabolism in HH.

Limitations of our study are a small cohort size. For further investigations, it is important to analyze liver lipidome, measure VLDL secretion and phospholipid profiles of VLDL of HH patients to clarify the role of altered VLDL secretion and composition in pathogenesis of susceptibility to NAFLD in HH.

Conclusion

Our study supports the hypothesis of altered lipid metabolism and susceptibility to NAFLD in HH. Disturbed phospholipid metabolism is likely an important factor in pathogenesis of hepatic steatosis in HH.

Abbreviations

ALT: Alanine aminotransferase; ApoB100: Apolipoprotein B100; AST: Aspartate aminotransferase; BMI: Body mass index; FFA: Free fatty acids; FP-1: Ferroportin-1; GGT: Gamma-glutamyltransferase; HH: Hereditary hemochromatosis; HDL: High-density lipoprotein; LCPUFA: Long-chain polyunsaturated fatty acids; LDL: Low-density lipoprotein; LPC: Lysophosphatidylcholine; LPE: Lysophosphatidylethanolamine; LXR: Liver X receptor; MUFA: Monounsaturated fatty acid; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PEMT: Phosphatidylethanolamine N-methyltransferase; PI: Phosphatidylinositol; PNPLA3: Human patatin like phospholipase domain-containing 3; PS: Phosphatidylserine; PT: Prothrombin time; PUFA: Polyunsaturated fatty acid; SM: Sphingomyelin; TS: Transferrin saturation; VLDL: Very-low density lipoprotein

Acknowledgments

J.S. was a recipient of Rahel Goitein Straus award from Medical Faculty of University of Heidelberg. We thank Petra Klöters-Plachky for technical assistance.

Authors' contributions

UM, WS, JS were involved in study concept and design, acquisition of data, analysis, interpretation of data and statistical analysis. MK was involved in interpretation of data and statistical analysis. UM, WC, JS were involved in drafting of manuscript, study supervision, revision of manuscript for intellectual content. HGS carried out liquid-chromatography measurement and analyses. All authors read and approved final version of manuscript.

Funding

None.

Availability of data and materials

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval for this prospective, observational single center study was given by the Ethics Committee of University of Heidelberg. Written informed consent was obtained from each patient at study inclusion.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 28 December 2019 Accepted: 22 April 2020
Published online: 17 July 2020

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