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ARTICLE

Physiology

Plasma lipid profiling of tissue-specific insulin resistance in human obesity

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Abstract

Background/Objectives Obesity-associated insulin resistance (IR) may develop in multiple organs, representing different aetiologies towards cardiometabolic diseases. This study aimed to identify distinct plasma lipid profiles in overweight/obese individuals who show muscle-IR and/or liver-IR.

Subjects/Methods Baseline data of the European multicenter DiOGenes project were used ($n = 640$; 401 women, non-diabetic BMI: 27-45 kg/m^2). Muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI) were derived from a 5-point oral glucose tolerance test. The 140 plasma lipids were quantified by liquid chromatography–mass spectrometry. Linear mixed models were used to evaluate associations between MISI, HIRI and plasma lipids.

Results MISI was comparable between sexes while HIRI and triacylglycerol (TAG) levels were lower in women than in men. MISI was associated with higher lysophosphatidylcholine (LPC) levels (standardized (std)$\beta = 0.126$; FDR-$p = 0.032$). Sex interactions were observed for associations between HIRI, TAG and diacylglycerol (DAG) lipid classes. In women, but not in men, HIRI was associated with higher levels of TAG (44 out of 55 species) and both DAG species (stdβ: 0.139–0.313; FDR-$p < 0.05$), a lower odd-chain/even-chain TAG ratio (std$\beta = -0.182$; FDR-$p = 0.005$) and a lower very-long-chain/long-chain TAG ratio (std$\beta = -0.156$; FDR-$p = 0.037$).

Conclusions In overweight/obese individuals, muscle insulin sensitivity is associated with higher plasma LPC concentrations. Women have less hepatic IR and lower TAG than men. Nevertheless, hepatic IR is associated with higher plasma TAG and DAG concentrations and a lower abundance of odd-chain and very-long-chain TAG in women, but not in men. This suggests a more pronounced worsening of plasma lipid profile in women with the progression of hepatic IR.

Introduction

Worldwide, over 2.1 billion people were overweight or obese in 2013 [1]. Overweight and obesity are major risk factors for a number of disorders including cardiovascular disease and type 2 diabetes [2]. Excess fat mass and lipid abnormalities are frequently associated with insulin

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resistance (IR) [3], but the relationship between obesity, IR and lipid metabolism is complex [4].

In the overweight/obese IR state, an impaired adipose tissue lipid buffering capacity may lead to an elevated lipid supply (increased non-esterified fatty acids and triacylglycerol (TAG)) to non-adipose tissues, for instance skeletal muscle and the liver [5]. This lipid overflow, in combination with an impaired capacity to adjust fat oxidation to the increased supply (metabolic inflexibility), leads to ectopic lipid accumulation in peripheral tissues [5]. In these organs, the accumulated lipids and lipid intermediates may interfere with insulin signalling through various mechanisms [6], which eventually are thought to contribute to the development or worsening of IR in skeletal muscle and liver.

In recent years, the plasma lipidome has gained special interest because it may reflect organ composition and metabolism. Circulating lipid species represent a read-out of different tissues, particularly the liver, but may also reflect muscle or adipose tissue lipid composition or metabolism [7]. Previous studies have identified lipid (sub)classes and species that are associated with obesity [8], IR [9] and type 2 diabetes [10–12]. In the San Antonio Family Heart Study the plasma lipid profile could explain a substantial part of the variability in glucose homoeostasis [13] and specific lipid species have been identified as potential biomarkers of glucose intolerance and type 2 diabetes [10]. In the Framingham Heart study with a 12-year follow-up, plasma TAG species with a low number of carbon atoms and double bonds were associated with the development of type 2 diabetes and whole-body IR [12]. Several studies also report that higher levels of lysophosphatidylcholines (LPC) are associated with a decreased risk of developing type 2 diabetes [7, 14, 15], but results are not fully consistent [16].

Whole-body IR reflects defective insulin action in major metabolic insulin-sensitive organs, such as skeletal muscle, liver and adipose tissue, and has long been known to precede the development of type 2 diabetes and cardiovascular disease [5]. IR can develop simultaneously in multiple organs, but severity may vary between different organs. It has been suggested that impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) represent distinct pathways towards type 2 diabetes. However, impaired hepatic and peripheral (muscle) insulin sensitivity might be the underlying disorders in IFG and IGT individuals, respectively [17]. IFG and IGT have been associated with different metabolic profiles. For instance, an altered skeletal muscle fatty acid handling is observed in IGT individuals when compared to IFG individuals [18]. These observations support the notion that the metabolic phenotype and disease risk are directly related to the severity of IR in different organs. In addition, there is evidence that interventions that increase insulin sensitivity are organ specific, at least for a part of their effects. For instance, physical activity was shown to mainly target muscle insulin sensitivity [19], while metformin treatment might have major effects on hepatic insulin sensitivity [20]. Moreover, a low-fat diet may be more beneficial for prediabetic individuals with hepatic IR, while the Mediterranean diet may target more specifically muscle insulin sensitivity [21].

The information presented above emphasizes the relevance of tissue-specific IR in the development and treatment of cardiometabolic diseases. Thus far, lipidome analyses in large cohort studies have shown to be a powerful tool for identification of potential lipid contributors to endpoint type 2 diabetes [10–12]. However, studies that focus on early stages of disease, including development of IR in different tissues prior to type 2 diabetes development, are currently missing. Therefore, we investigated in healthy overweight/obese nondiabetic individuals who are at risk for developing cardiometabolic diseases whether distinct plasma lipid profiles can be identified that are associated with muscle insulin sensitivity or with hepatic IR. Quantification and identification of metabolic anomalies in organ-specific IR may provide directions for more personalized lifestyle or pharmacological interventions in the prevention and control of cardiometabolic diseases.

Materials and methods

Study design

The DiOGenes study is a multicentre, randomized, controlled dietary intervention study that involved 8 European countries. In total, 938 overweight or obese, nondiabetic adults free of cardiovascular disease (age 18–65 years, body mass index (BMI) 27–45 kg/m^2 and fasting blood glucose concentrations < 6.1 mmol/L) were recruited. More details on recruitment, inclusion and exclusion criteria, design and study procedures have been described previously [22]. The analyses described here include baseline data of 640 participants, prior to any intervention, for whom plasma lipidomics data and information of tissue-specific IR were available.

Local ethics committees approved the study and all participants gave written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Estimates of tissue-specific IR

Participants underwent a standard 5-point oral glucose tolerance test (OGTT) at baseline. In short, after an overnight fast, venous blood was sampled before (t0) and after a 75 g glucose load was ingested. Blood samples were taken at t0, t30, t60, t90 and t120 min to determine glucose and insulin

concentrations [22]. Muscle insulin sensitivity and hepatic IR were estimated using the methods of Abdul-Ghani et al. [23]. Both indexes were developed and validated against gold standard hyperinsulinaemic-euglycaemic clamp studies [23].

The muscle insulin sensitivity index (MISI) was calculated according to the following formula: MISI = (dG/dt)/mean plasma insulin concentration during OGTT. Here, dG/dt is the rate of decay of plasma glucose concentration during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir [23]. The decline in plasma glucose concentration after 60 min primarily reflects glucose uptake by peripheral tissues, mainly skeletal muscle.

The hepatic IR index (HIRI) was calculated using the square root of the product of the area under curves (AUCs) for glucose and insulin during the first 30 min of the OGTT; i.e., SQRT (glucose$^{0–30}$ [AUC in mg/d × h] × insulin$^{0–30}$ [AUC in μU/mL × h]). This index has been developed and validated against the product of fasting plasma insulin and endogenous glucose production in clamp studies [23].

Adipose tissue IR (ATIRI) was calculated as fasting insulin (μU/L) × fasting non-esterified fatty acids (μmol/L)/1000. Other indices of whole-body IR were: Matsuda insulin sensitivity index (ISI) = 10,000/√ [(fasting insulin [pmol/l] × fasting glucose [mmol/l]) × (mean OGTT insulin [pmol/l]) × (mean OGTT glucose [mmol/l])] and HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) calculated as fasting insulin [μU/L] × fasting glucose [mmol/L]/22.5.

Classification of participants into muscle-IR and liver-IR

For descriptive reasons, we created sex-specific tertiles according to the HIRI and MISI scores and tissue-specific IR groups were based on the top tertiles for the sexes. The lowest tertile of MISI represented individuals with muscle IR; the highest tertile of HIRI represented individuals with hepatic IR [21]. Accordingly, the participants were categorized in one of four groups: (1) no IR, (2) IR primarily in muscle (muscle-IR), (3) IR primarily in liver (liver-IR) and (4) IR in both muscle and liver (muscle/liver-IR).

Plasma lipidomics

Fasting plasma lipidome analysis was done using liquid chromatography–mass spectrometry as described previously [4]. Briefly, a mixture of internal standards and calibration standards was added to each sample and was followed by liquid–liquid extraction with a 2:1 mixture of dichloromethane and methanol [4]. The organic phase that contained most of the lipids was removed and prepared for further analysis. Extracted lipids were separated on a Ascentis Express C8 2.1 9 150-mm (2.7 mm particle size) column (Sigma-Aldrich) using an Acquity UPLC system (Waters) and analysed using quadrupole time-of-flight mass spectrometry (Agilent Technologies). Experimental conditions during the chromatographic analysis and detection by mass spectrometry were similar to Valsesia et al. [4].

Names and abbreviations were assigned to the identified lipids according to Lipid Maps nomenclature (http://www.lipidmaps.org). In total, 140 intact lipid species within 11 classes were measured: triacylglycerols (TAG; $n = 55$), diacylglycerols (DAG; $n = 2$), cholesterol esters (CE; $n = 3$), sphingomyelins (SM; $n = 20$), phosphatidylcholine (PC; $n = 25$), alkyl-phosphatidylcholine (PC(O−); $n = 15$), lysoalkylphosphatidylcholine (LPC(O−); $n = 2$), lysophosphatidylcholine (LPC; $n = 11$), phosphatidylethanolamine (PE; $n = 3$), alkylphosphatidylethanolamine (PE(O−); $n = 3$) and lysophosphatidylethanolamine (LPE; $n = 1$). Lipids were denoted by headgroup and total acyl-carbon and double-bond content. A detailed list of measured lipids, including within-class abundance in plasma, can be found in supplementary Table S1.

Sum scores (e.g., sum of all TAG) were calculated per-lipid (sub)class. In addition, as saturation and chain length of fatty acids have been widely shown to be related to cardiometabolic risk factors [12, 24], sum scores were calculated for saturated TAG (TAG with 0 double bonds), unsaturated TAG (TAG with 1–3 double bonds) and polyunsaturated TAG (TAG with ≥4 double bonds). Moreover, ratios for TAG composition were calculated, i.e., ratio of the sum of all odd-chain TAG to the sum of all even-chain TAG and ratio of the sum of all very-long-chain (≥56 carbon atoms) TAG to the sum of all long-chain (<56 carbon atoms) TAG.

Statistical analyses

Differences in characteristics between tissue-specific IR groups and between sexes were assessed using one-way analysis of variance (ANOVA) and Bonferroni post hoc tests.

HIRI, MISI and most lipid species showed a right-skewed distribution, and therefore non-normal data were logarithmically transformed to approximate normality. To allow for direct comparison of the effect sizes, we additionally standardized HIRI, MISI and lipids by calculating *Z*-scores for each variable. Linear mixed model analyses were performed with plasma lipid species as the dependent variable (per-lipid analyses), HIRI or MISI (continuous variables) as fixed effect and study centre as random effect. In the adjusted analyses, sex, BMI and waist-to-hip ratio were included as covariates, because body composition is an important determinant in the development of

Table 1 Clinical characteristics of tissue-specific IR groups stratified by sex

	Women					Men				
	No IR (*n*=207)	*Muscle-IR* (*n*=70)	*Liver-IR* (*n*=57)	*Muscle/liver-IR* (*n*=67)	*P*	*No IR* (*n*=127)	*Muscle-IR* (*n*=37)	*Liver-IR* (*n*=32)	*Muscle/liver-IR* (*n*=43)	*P*
Age (years)	41 ± 6	41 ± 6	42 ± 7	41 ± 6	0.382	43 ± 6	44 ± 5	42 ± 5	42 ± 7	0.491
BMI (kg/m^2)	34 ± 4.8	34.6 ± 4.6	35.3 ± 5.0	35.2 ± 4.7	0.173	33.9 ± 4.8	34.5 ± 4.9	34.9 ± 3.8	35.3 ± 4.3	0.300
Waist-to-hip ratio	0.87 ± 0.07	0.87 ± 0.06	0.89 ± 0.07	0.91 ± 0.08*†	<0.001	1.00 ± 0.05	1.02 ± 0.05	1.01 ± 0.06	1.03 ± 0.05	0.046
Weight (kg)	94.0 ± 14.8	95.1 ± 15.5	99.8 ± 15.5	96.5 ± 16.6	0.076	108.1 ± 17.8	110.6 ± 16.8	110 ± 13.3	110.5 ± 17.3	0.777
Waist (cm)	102.2 ± 10.6	104.2 ± 11.5	107.0 ± 11.9*	108.3 ± 13.0*	<0.001	112.4 ± 12.5	115.6 ± 12.4	115.3 ± 9.2	116.8 ± 12.1	0.135
Systolic BP (mm Hg)	121 ± 15	123 ± 13	125 ± 16	125 ± 12	0.069	132 ± 13	131 ± 12	134 ± 12	130 ± 12	0.547
Diastolic BP (mm Hg)	75 ± 12	77 ± 9	78 ± 10	76 ± 9	0.242	81 ± 10	80 ± 11	83 ± 12	80 ± 10	0.573
Fat free mass (kg)	52.3 ± 6.9	52.6 ± 7.1	56.6 ± 9.1*†	53.6 ± 7.9	0.005	73.1 ± 9.8	72.8 ± 7.4	72.4 ± 9.4	71 ± 11.4	0.719
Fat mass (kg)	41.4 ± 9.8	42.8 ± 10.8	43.1 ± 9.4	44 ± 10.8	0.315	35.5 ± 12.3	37.5 ± 10.5	37.3 ± 11	38.3 ± 12.2	0.602
Body fat (%)	43.8 ± 4.9	44.4 ± 5.1	43.0 ± 4.8	44.7 ± 4.4	0.294	32.1 ± 6.8	33.5 ± 5.9	33.6 ± 7.3	34.6 ± 7.6	0.227
Cholesterol (mmol/L)	4.8 ± 0.9	4.6 ± 1.0	5.0 ± 0.9	4.9 ± 0.9	0.134	5.0 ± 1.1	5.0 ± 1.2	5.4 ± 1.3	4.9 ± 1.0	0.258
TAG (mmol/L)	1.1 ± 0.4	1.3 ± 0.6	1.5 ± 0.7*	1.5 ± 0.7*	<0.001	1.5 ± 0.7	1.6 ± 0.7	1.7 ± 0.7	1.7 ± 0.6	0.548
HDL (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.4*	0.006	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	0.216
LDL (mmol/L)	3.0 ± 0.9	2.8 ± 0.8	3.0 ± 0.7	3.0 ± 0.8	0.217	3.2 ± 0.9	3.2 ± 1.0	3.5 ± 1.2	3.1 ± 0.9	0.334
NEFA (μmol/L)	700 ± 333	686 ± 237	813 ± 472	679 ± 307	0.166	545 ± 264	546 ± 171	582 ± 367	560 ± 394	0.930
CRP (mg/L)	3.8 ± 3.6	4.8 ± 3.3	5.1 ± 4.6	5.9 ± 4.6*	<0.001	3.1 ± 3.1	3.6 ± 3.1	3.4 ± 3.6	3.1 ± 2.4	0.866
Glucose (mmol/L)	5.0 ± 0.7	4.9 ± 0.5	5.2 ± 0.6	5.2 ± 0.7*	0.002	5.2 ± 0.5	5.3 ± 0.7	5.3 ± 0.6	5.3 ± 0.6	0.735
Insulin (μU/mL)	7.4 ± 3.7	9.5 ± 3.1*	12.3 ± 4.6*†	17.8 ± 9.6*†‡	<0.001	10.1 ± 5.0	13.6 ± 5.4*	17.3 ± 10*	19.2 ± 9.3*†	<0.001
HOMA-IR (AU)	1.9 ± 1.1	2.4 ± 0.8	3.3 ± 1.4*†	4.8 ± 2.6*†‡	<0.001	2.8 ± 1.4	3.8 ± 1.8	4.9 ± 3.1*	5.3 ± 3.0*†	<0.001
ISI (AU)	7.3 ± 3.3	5.2 ± 2.2*	3.9 ± 1.5*†	2.7 ± 1.2*†	<0.001	5.3 ± 2.7	3.6 ± 1.7*	2.9 ± 1.3*	2.3 ± 1.0*	<0.001
MISI (AU)	0.08 ± 0.06	0.02 ± 0.01*	0.06 ± 0.03†	0.02 ± 0.01*‡	<0.001	0.09 ± 0.06	0.02 ± 0.01*‡	0.06 ± 0.02*†	0.02 ± 0.01*‡	<0.001
HIRI (AU)	24.7 ± 4.9	27.3 ± 4.1*	39.4 ± 6.6*†	43.7 ± 8.9*†‡	<0.001	29.3 ± 5.8	32.6 ± 5.0	48.8 ± 8.9*†	50.9 ± 9.5*†	<0.001
ATIRI (AU)	5.0 ± 3.7	6.4 ± 3.1	9.6 ± 6.2*†	12.4 ± 7.6*†‡	<0.001	5.7 ± 4.6	7.7 ± 4.2	9.7 ± 6.1*	11.3 ± 13.2*	<0.001
Oral anticonception use (%)	11.6	19.3	12.5	23.2	0.096					

Data are mean ± SD unless otherwise indicated. Differences between tissue-specific IR groups within sex were assessed using one-way ANOVAs and Bonferroni post hoc tests

BMI body mass index, *BP* blood pressure, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *NEFA* non-esterified fatty acids, *CRP* C-reactive protein, *HOMA-IR* homoeostatic model for assessment of insulin resistance, *MISI* muscle insulin sensitivity index, *HIRI* hepatic insulin resistance index, *ISI* (Matsuda) insulin sensitivity index, *ATIRI* adipose tissue insulin resistance index

*$P < 0.05$ for the difference with no IR group

†$P < 0.05$ for the difference with muscle-IR group

‡$P < 0.05$ for difference with liver-IR group

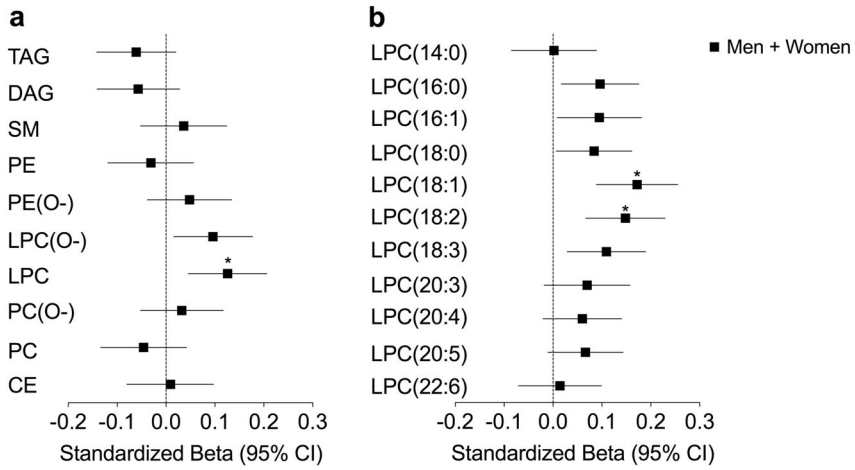

Fig. 1 Associations of MISI with plasma lipid (sub-)classes and individual lysophosphatidylcholine lipid species. **a** Standardized coefficients (βs) in linear mixed models with the sum scores of plasma lipid (sub-)classes as dependent variable, MISI as fixed effect and study centre as random effect, adjusted for sex, BMI, waist-to-hip ratio and HIRI. **b** Standardized coefficients (βs) in linear mixed models with the individual LPC lipid species as dependent variable, MISI as fixed effect and study centre as random effect, adjusted for sex, BMI, waist-to-hip ratio and HIRI. No significant sex interactions were observed in the associations between MISI and lipids, and therefore squares represent all individuals ($n = 640$). Error bars denote 95% confidence intervals. Asterisks indicate a significant association after Benjamini and Hochberg FDR corrections for multiple testing (FDR $p < 0.05$)

obesity-related IR [25]. In the fully adjusted models, HIRI was included as a covariate in analyses on MISI and MISI was included in analyses on HIRI to assess the independent effects of tissue-specific IR. Benjamini and Hochberg false discovery rate (FDR) correction was applied to adjust for multiple testing. Since sex differences in lipid metabolism are well established [26], we also evaluated sex interactions in each comparison by including interaction terms for sex and HIRI or sex and MISI, as applicable. As similar associations were found in all models, only the results of the fully adjusted models are shown. Additional linear mixed model analyses were performed with the individual plasma lipid species as dependent variable (per-lipid analyses), ATIRI as fixed effect and study centre as random effect, adjusted for sex, BMI, waist-to-hip ratio, MISI and HIRI.

The data were analysed using SPSS for Mac version 22.0 (SPSS, Chicago, IL, USA) and R statistical programming system (http://www.r-project.org/). The statistical significance was set at (FDR-adjusted) $p < 0.05$.

Results

Clinical characteristics of study population

Table 1 shows the demographic and metabolic characteristics of the participants according to their sex and IR phenotype. Overall, in women, plasma TAG concentrations were lower ($p < 0.001$) and high-density lipoprotein (HDL) cholesterol ($p < 0.001$) and C-reactive protein (CRP; $p < 0.001$) were higher than in men. Furthermore, MISI was not different between sexes ($p = 0.154$), but HIRI was higher in men than in women ($p < 0.001$).

In both sexes, age and BMI did not differ significantly between the groups, while in women, but not in men, waist-to-hip ratio was significantly larger in muscle/liver-IR group compared to the no IR group. In addition, in women, but not in men, plasma TAG levels were significantly higher in the liver-IR and muscle/liver-IR groups than in the no IR group, while HDL cholesterol was lowest in the muscle/liver-IR group. In both sexes, muscle-IR, liver-IR and muscle/liver-IR groups all showed significantly higher fasting insulin concentrations and lower levels of ISI than those with no IR.

Muscle insulin sensitivity is associated with higher lysophosphatidylcholine concentrations

No significant sex interactions were observed for the associations between MISI and lipid classes and species (Table S2). Therefore, associations between MISI and lipids were assessed in men and women combined.

MISI was positively associated with the sum score for LPC (standardized β (stdβ) = 0.126; FDR $p = 0.032$), but not with any of the other lipid classes after correction for multiple testing (Fig. 1a, Table S3). This indicates that higher levels of LPC were associated with better muscle insulin sensitivity. MISI showed a positive and nominal significant association with 6 out of the 11 individual LPC species, of which LPC(18:1) (stdβ = 0.172; FDR $p = 0.008$) and LPC(18:2) (stdβ = 0.148; FDR $p = 0.025$) remained statistically significant after correction for multiple testing (Fig. 1b). In addition, because LPC constitute a

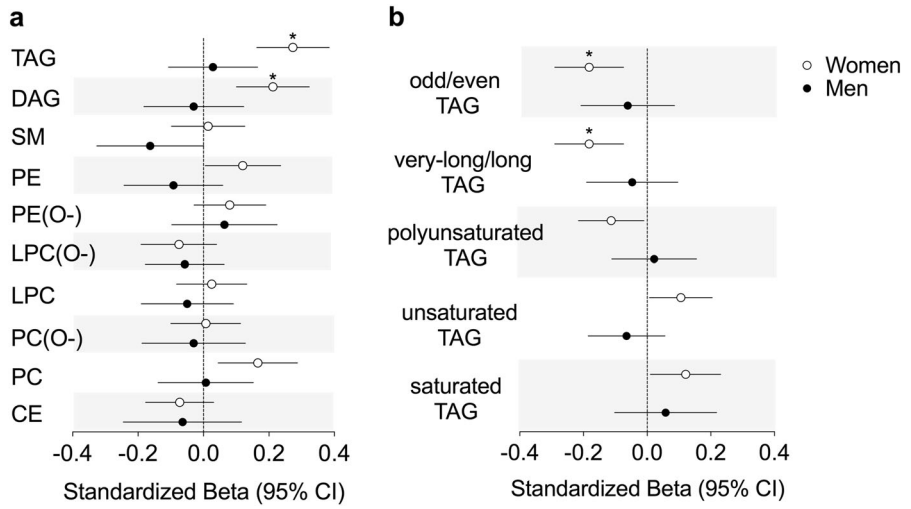

Fig. 2 Associations of HIRI with plasma lipid (sub-)classes and TAG composition. Significant sex interactions were observed in the associations between HIRI and TAG, DAG and PE; therefore, all analyses presented are stratified by sex. **a** Standardized coefficients (βs) in linear mixed models with the sum scores of plasma lipid (sub-)classes as dependent variable, HIRI as fixed effect and study centre as random effect, adjusted for BMI, waist-to-hip ratio and MISI. **b** Standardized βs in linear mixed models with TAG composition as dependent variable, HIRI as fixed effect and study centre as random effect, adjusted for BMI, waist-to-hip ratio and MISI. White circles represent women ($n = 401$) and black circles represent men ($n = 239$). Error bars denote 95% confidence intervals. Asterisks indicate a significant association after Benjamini and Hochberg FDR corrections for multiple testing (FDR $p < 0.05$). Saturated TAG: 0 double bonds; unsaturated TAG: 1–3 double bonds, polyunsaturated TAG: ≥4 double bonds; Odd/even TAG: odd-chain to even-chain TAG ratio; Very-long/long TAG: very-long-chain (≥56 carbon atoms) to long-chain (<56 carbon atoms) TAG ratio

quantitatively important subclass of phospholipids in HDL [27], we verified whether the associations between MISI and LPC were not merely a reflection of low HDL cholesterol levels. The associations between MISI and LPC did not change after additional adjustment for HDL (data not shown). MISI was not associated with any other individual lipid species (Table S3).

Hepatic IR is associated with TAG and DAG levels in women, but not in men

Significant sex interactions were observed in the associations between HIRI and the lipid classes TAG and DAG (Table S2). Therefore, all statistical analyses for associations between HIRI and TAG or DAG were stratified by sex (Table S4).

In women, HIRI was significantly associated with the sum score for TAG (stdβ = 0.273; FDR $p < 0.001$) and DAG (stdβ = 0.212; FDR $p < 0.001$) (Fig. 2a). The positive associations of HIRI with total TAG and DAG indicate that higher plasma TAG and DAG levels were associated with a more severe hepatic IR. Importantly, both DAG species and 44 out of 55 TAG species contributed significantly to this effect (stdβs: 0.139–0.313; FDR $p < 0.05$, Table S4). In men, no associations of HIRI with TAG and DAG were found (Fig. 2a, Table S4).

Next, TAG composition was analysed in more detail. Associations between HIRI and variables that reflect TAG composition, i.e., fatty acid saturation and chain length (Table S2), were statistically significant in women but not in men, even though the interaction term for sex and HIRI did not reach statistical significance. In women, HIRI was inversely associated with the odd-to-even-chain TAG ratio (stdβ = −0.182; FDR $p = 0.005$) as well as with the very-long-chain to long-chain TAG ratio (stdβ = −0.156; FDR $p = 0.037$). This indicates that TAG with a relative lower abundance of odd-chain and very-long-chain fatty acids was associated with worse hepatic IR (Fig. 2b, Table S4). In men, no association between HIRI and TAG composition was found (Fig. 2b, Table S4).

Hepatic IR and other lipid (sub-)classes

Sex interactions were significant in the association between HIRI and the lipid class PE, but not with the other lipid classes (Table S2). Likewise, we performed sex-stratified analyses (Table S4).

A trend for a positive association of HIRI with PE was observed in women (stdβ = 0.120; FDR $p = 0.070$), but not in men (stdβ = −0.092; FDR $p = 0.780$). The association between HIRI and the sum score for PC was significant in women (stdβ = 0.166; FDR $p = 0.028$), but not in men (stdβ = 0.007; FDR $p = 0.927$), although the interaction term for sex and HIRI was not significant in these analyses ($p = 0.226$). Likewise, in men and women combined, HIRI was significantly associated with 9 out of the 28 individual

PC species (stdβs: 0.098–0.195; FDR $p < 0.05$), but stratified analyses showed that, again, these were primarily driven by the associations in women (Table S4). Lastly, in men and women combined, significant associations were observed between HIRI and four individual lipid species from the other lipid classes, i.e., a positive association for LPC(14:0) (stdβ = 0.157; FDR $p = 0.003$), and inverse associations for PCO(42:6) (stdβ = −0.110; FDR $p = 0.015$), PCO(44:5) (stdβ = 0.110; FDR $p = 0.033$) and SM (d18:0/24:2) (stdβ = −0.156; FDR $p = 0.002$).

Adipose tissue IR and the plasma lipidome

Adipose tissue IR might be related to alterations in the plasma lipidome as well, although it was not the primary interest of the current study. Therefore, associations of ATIRI with the plasma lipidome were additionally evaluated, which were adjusted for HIRI and MISI (Table S5). No significant sex interactions were observed for the associations between ATIRI and the sum scores of lipid classes (Table S5).

ATIRI was positively associated with the sum score for lipid classes TAG (stdβ = 0.249; FDR $p < 0.001$), DAG (stdβ = 0.251; FDR $p < 0.001$) and PC (stdβ = 0.183; FDR $p = 0.004$) (Table S5). In addition, ATIRI was inversely associated with the odd-to-even-chain TAG ratio (stdβ = −0.207; FDR $p = 0.001$). The positive associations of ATIRI with total TAG and DAG indicate that higher plasma TAG and DAG levels were associated with a more severe adipose tissue IR in both sexes.

Discussion

The role of the plasma lipidome in IR and type 2 diabetes has been the subject of intense research in recent years. The present study focussed on the relationship between the plasma lipidome and tissue-specific IR. In a cross-sectional evaluation of overweight and obese individuals without cardiometabolic disorders, we showed that a higher muscle insulin sensitivity was associated with higher plasma LPC concentrations. Furthermore, in women, but not in men, we observed positive associations between hepatic IR and several lipid classes including TAG and DAG, while a similar tendency was observed for PC and PE.

Our current analyses showed that muscle insulin sensitivity (as reflected by a higher MISI) was positively associated with the sum score for plasma LPC. Moreover, muscle insulin sensitivity showed strong and positive associations with two individual LPC species, LPC(18:1) and LPC(18:2). Several previous studies showed associations between LPC and general indices of insulin sensitivity. For instance, lower plasma concentrations of numerous individual LPC species have been reported in human obesity [9, 14] and IR [7, 28], and have also been associated with an increased risk of type 2 diabetes [15, 28, 29]. Floegel et al. [15] identified LPC(18:2) to be positively associated with the Matsuda ISI in the Tübingen Family study for type 2 diabetes, while Barber et al [14]. reported lower LPC(18:1) and LPC(18:2) in obesity. The latter study suggested that obesity-related factors, such as diet and adiposity, rather than IR and diabetes per se, contributed to the changes in the LPC profile. Notably, in our study, adjustment for body composition (i.e., BMI and waist-to-hip ratio) did not affect the positive association between muscle insulin sensitivity and LPCs. This might imply that IR, and more specifically muscle insulin resistance, rather than obesity per se is associated with lower plasma LPC levels. In addition, it was recently shown that fasting plasma levels of several LPCs, including LPC(18:1) and LPC (18:2), were increased in healthy male participants after completion of a 10-week exercise programme [30]. Furthermore, in human myotubes, it was shown that extracellular LPC(16:0) and LPC(18:1) can act as lipid signalling molecules and, as such, activate peroxisome proliferator-activated receptor-δ (PPARδ)-dependent gene expression and reduce lipid-induced inflammation and IR [31]. These in vivo and in vitro results are in line with our current findings and imply that LPCs may indeed be specifically related to muscle insulin sensitivity.

In contrast to the other LPCs, LPC(14:0) was the only LPC that was positively and significantly associated with hepatic IR (as reflected by HIRI), while it was not associated with muscle insulin sensitivity. Several reports showed associations of LPC(14:0) with general markers of whole-body IR. Rauschert et al. [9] reported that increased LPC(14:0) was significantly associated with high HOMA-IR in normal-weight young adults. Another study in infants found elevated LPC(14:0) levels to be predictive of obesity onset at early school age [32]. Moreover, obese men had lower levels of LPC(18:1) and higher levels of LPC(14:0) than normal-weight controls [33]. In our study, adjustment for body composition did not affect the positive association between hepatic IR and LPC(14:0). This suggests that LPC (14:0) might be specifically associated with hepatic IR, independent of obesity. However, more research is needed to further unravel the potential role of LPC(14:0) in hepatic IR.

In women, hepatic IR was positively associated with the sum of plasma TAG and DAG, with a similar tendency for PC and PE. These associations were not observed in men. Importantly, the associations between hepatic IR and TAG/DAG species were not affected by adjustments for body composition and body fat distribution. Thus, these observed differences between men and women were unlikely to result from the known differences in body composition. Healthy,

premenopausal women generally have a more favourable cardiometabolic plasma lipid profile than men [26]. In our current evaluations we indeed observed that, in general, women had less hepatic IR, lower plasma TAG and higher HDL concentrations as compared to men. However, our data also suggest that in women, the presence of hepatic IR is prominently related to a more pronounced worsening of lipid profile. A potential mechanism might be related to very-low-density lipoprotein (VLDL) production in the liver. Women have lower plasma TAG concentrations, and produce fewer, but larger, TAG-rich VLDL particles than men [34]. Previous studies have shown that IR attenuates insulin-mediated hepatic suppression of VLDL production and insulin-mediated hepatic de novo lipogenesis and thereby enhance total VLDL production [35]. Notably, these studies did not differentiate between sexes. Given our current observations, it is tempting to speculate that under hepatic IR conditions, VLDL production might be differentially affected in men and women, leading to an accelerated VLDL production in women. Furthermore, sex hormones are known to influence VLDL production in women [26]. For instance, VLDL-TAG turnover is faster in postmenopausal women compared with well-matched premenopausal women despite similar plasma (VLDL)-TAG concentrations [36]. In the current study, we could not directly explain our observations by hormonal state, as most women were most likely in a premenopausal status. There may be more subtle effects of sex hormones in the context of hepatic IR, but we could not address that point as no information was available on sex hormones or phase in menstrual cycle. Together, these associations between hepatic IR and TAG species are intriguing findings and might imply that women with hepatic IR 'catch-up' with men with respect to cardiovascular disease risk.

TAG composition was also analysed in more detail, including saturation and chain length of TAGs. Here, we show that hepatic IR, rather than muscle insulin sensitivity, was associated with a relatively lower abundance of odd-chain fatty acids in plasma TAGs. In line, in the EPIC (European Prospective Investigation into Cancer and Nutrition) study a relatively lower abundance of odd-chain fatty acids in plasma phospholipids was shown to be inversely associated with incidence of type 2 diabetes and coronary heart disease [24]. Moreover, the odd-chain fatty acid C15:0 was positively associated with insulin sensitivity and β-cell function, as measured by an intravenous glucose tolerance test, as well as with a 27% decreased risk of incident diabetes after 5 years [37]. The biological mechanisms underlying the associations between hepatic IR and individual odd-chain fatty acids have not yet been elucidated, but some pathways can be suggested. Enhanced de novo lipogenesis in hepatic IR increases levels of even-chain saturated fatty acids and may, as such, contribute to a lower ratio of odd-to-even-chain saturated fatty acids in the liver. Eventually this novo lipogenesis might increase hepatic steatosis and hepatic IR [38]. Lastly, alterations in membrane fluidity have previously been linked to insulin sensitivity [39]. Odd-chain fatty acids might have a lower melting point than their next lower even-numbered homologue, which may affect fluidity [40]. In addition, the fatty acid composition of circulating TAG might also represent fatty acid composition of, for example, glycerophospholipids in cell membranes [41]. However, how these TAG characteristics specifically attribute to hepatic IR remains to be studied.

Our primary interest in the present study was to compare effects of IR in the liver and in the muscle on the plasma lipidome profile. Nevertheless, we are well aware that other organs, including e.g. adipose tissue, pancreas and brain, can also develop IR. Of these, adipose tissue IR may lead to an increased release of non-esterified fatty acids into the circulation, which may contribute to liver and/or muscle IR, as well as provide fuel for further lipid synthesis and oxidation in these tissues [5]. Interestingly, while hepatic IR showed a positive relationship with TAG and DAG concentrations in females and not in males (as discussed above), adipose tissue IR showed a positive relationship with TAG and DAG lipid species in both males and females. These data indicate that hepatic and adipose tissue IR are related to distinct plasma lipidome profiles, of which the underlying mechanisms need further elucidation.

The major strength of our study is the availability of a 5-point OGTT in a large and well-phenotyped cohort to estimate muscle insulin sensitivity as well as hepatic IR in each participant. Importantly, the calculated MISI and HIRI represent different responses to the OGTT and they have been validated against gold standard hyperinsulinaemic-euglycaemic clamp studies [23]. For the first time, information on tissue-specific IR was combined with comprehensive plasma lipidome analysis, hence adding novel information to deepen our understanding of the aetiology of tissue-specific IR. Furthermore, these data were generated in healthy overweight and obese individuals without cardiometabolic diseases, thus representing early events in the progression towards these diseases. The main limitation of our study is its cross-sectional design which prohibits causal inferences. Therefore, experimental studies should follow-up on these outcomes to fully elucidate biological mechanisms.

In conclusion, we showed that the previously reported positive associations between LPC levels and whole-body insulin sensitivity were related to muscle insulin sensitivity, rather than hepatic IR. Furthermore, we identified sex differences in the associations between hepatic IR and the plasma lipidome. In men, the degree of hepatic IR did not

relate to alterations in the plasma lipidome. In contrast, in women, worse hepatic IR was associated with higher levels of plasma TAG and DAG, with a similar tendency for PC and PE. In addition, a lower abundance of odd-chain and very-long-chain TAG was observed in worse hepatic IR in women. Combined with the observation that overweight/obese women had less hepatic IR and lower TAG levels than men, this suggests that, in women, the plasma lipidome may be more responsive to worsening of hepatic IR, or vice versa. Notably, such an effect was not observed for muscle insulin sensitivity. These distinct plasma lipid profiles in tissue-specific IR may provide directions for more personalized lifestyle or pharmacological interventions in the prevention of cardiometabolic disease that can, at least at the group level, be tailored towards a specific sex, or a specific type of insulin resistance. Notwithstanding, the underlying mechanisms that drive the observed differences require further study.

Author contributions WHMS and AA designed the DiOGenes clinical study; AV, AA, WHMS and TH designed the lipidomics studies; EEB designed and led the present study; NV and BWvdK conducted the statistical analyses; JWEJ, NV and MMJvG supervised the lipidomics data analyses; BWvdK wrote the manuscript. All authors contributed to revising the article critically for important intellectual content and gave their final approval of the version to be published. EEB is the guarantor of this work.

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Compliance with ethical standards

Conflict of interest AV is a full-time employee at Nestlé Institute of Health Sciences SA. WHMS reports having received research support from several food companies such as Nestlé, DSM, Unilever, Nutrition et Sante and Danone as well as Pharmaceutical companies such as GSK, Novartis and Novo Nordisk; he is an unpaid scientific advisor for the International Life Science Institute, ILSI Europe. AA reports grants and personal fees from Global Dairy Platform, personal fees from McCain Foods, McDonald's, Arena Pharmaceuticals Inc., Basic Research, Dutch Beer Knowledge Institute, Netherlands, Gelesis, Novo Nordisk, Denmark, Orexigen Therapeutics Inc., S-Biotek, Denmark, Twinlab and Vivus Inc., grants from Arla Foods, Denmark, Danish Dairy Research Council and Nordea Foundation, Denmark, outside the submitted work, and royalties received for the book first published in Danish as 'Verdens Bedste Kur' (Politiken; Copenhagen, Denmark), and subsequently published in Dutch as 'Het beste dieet ter wereld' (Kosmos Uitgevers; Utrecht/Antwerpen, Netherlands), in Spanish as 'Plan DIOGENES para el control del peso. La dieta personalizada inteligente' (Editorial Evergra´ficas; Léon, Spain) and in English as 'World's Best Diet' (Penguin, Australia). EEB receives grant support from food industry such as DSM, Danone, Friesland Campina, Avebe and Sensus, partly within the context of public–private consortia and has received funding from pharmaceutical companies like Novartis. She is involved in several task forces/expert groups related to the International Life Science Institute, ILSI Europe.

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