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Research article

Myofiber-specific lipidomics unveil differential contributions to insulin sensitivity in individuals of African and European ancestry

Tova Eurén [a], Barbara Gower [c], Pär Steneberg [b], Andréa Wilson [a], Helena Edlund [b], Elin Chorell [a,*]

[a] Public Health and Clinical Medicine, Umeå University, Sweden
[b] Department of Medical and Translational Biology, Umeå University, Sweden
[c] Department of Nutrition Sciences, The University of Alabama at Birmingham, USA

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ABSTRACT

Aims: Individuals of African ancestry (AA) present with lower insulin sensitivity compared to their European counterparts (EA). Studies show ethnic differences in skeletal muscle fiber type (lower type I fibers in AA), muscle fat oxidation capacity (lower in AA), whilst no differences in total skeletal muscle lipids. However, skeletal muscle lipid subtypes have not been examined in this context. We hypothesize that lower insulin sensitivity in AA is due to a greater proportion of type II (non-oxidative) muscle fibers, and that this would result in an ancestry-specific association between muscle lipid subtypes and peripheral insulin sensitivity. To test this hypothesis, we examined the association between insulin sensitivity and muscle lipids in AA and EA adults, and in an animal model of insulin resistance with muscle-specific fiber types.

Methods: In this cross-sectional study, muscle biopsies were obtained from individuals with a BMI ranging from normal to overweight with AA (N = 24) and EA (N = 19). Ancestry was assigned via genetic admixture analysis; peripheral insulin sensitivity via hyperinsulinaemic–euglycemic clamp; and myofiber content via myosin heavy chain immunohistochemistry. Further, muscle types with high (soleus) and low (vastus lateralis) type I fiber content were obtained from high-fat diet-induced insulin resistant F1 mice and littermate controls. Insulin sensitivity in mice was assessed via intraperitoneal glucose tolerance test. Mass spectrometry (MS)-based lipidomics was used to measure skeletal muscle lipid.

Results: Compared to EA, AA had lower peripheral insulin sensitivity and lower oxidative type 1 myofiber content, with no differences in total skeletal muscle lipid content. Muscles with lower type I fiber content (AA and vastus from mice) showed lower levels of lipids associated with fat oxidation capacity, i.e., cardiolipins, triacylglycerols with low saturation degree and phospholipids, compared to muscles with a higher type 1 fiber content (EA and soleus from mice). Further, we found that muscle diacylglycerol content was inversely associated with insulin sensitivity in EA, who have more type I fiber, whereas no association was found in AA. Similarly, we found that insulin sensitivity in mice was associated with diacylglycerol content in the soleus (high in type I fiber), not in vastus (low in type I fiber).

Conclusions; Our data suggest that the lipid contribution to altered insulin sensitivity differs by ethnicity due to myofiber composition, and that this needs to be considered to increase our

* Corresponding author.
E-mail addresses: tova.euren@umu.se (T. Eurén), bgower@uab.edu (B. Gower), par.steneberg@umu.se (P. Steneberg), andreaawilson@me.com (A. Wilson), helena.edlund@umu.se (H. Edlund), elin.chorell@umu.se (E. Chorell).

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understanding of underlying mechanisms of altered insulin sensitivity in different ethnic populations.

1. Introduction

Type 2 diabetes disproportionately affects individuals of African ancestry compared to their European counterpart (diabetes prevalence in US 2021: 12.1 % versus 7.4 %) [1]. While lifestyle and socioeconomic factors contribute to this disparity, they do not fully explain the higher type 2 diabetes prevalence in individuals of African ancestry [2]. Interestingly, factors commonly associated with type 2 diabetes and insulin resistance (IR), such as visceral and hepatic fat accumulation, have been shown to be lower in individuals of African ancestry compared to those of European descent [3], suggesting a lipid paradox in populations of African ancestry with a higher type 2 diabetes risk despite a more favorable body fat distribution.

Numerous studies have highlighted the instrumental role of ectopic lipids, specifically diacylglycerols (DAGs) and ceramides, which accumulate in organs such as the pancreas, skeletal muscle, and liver, in the development and remission of type 2 diabetes [4].

Skeletal muscle, responsible for 70–90 % of insulin-stimulated glucose disposal, is a major contributor to IR and a primary source of insulin desensitization. However, the molecular mechanisms by which lipids mediate alterations in skeletal muscle insulin sensitivity are widely debated, with contradictory findings partly due to the interpretation of total tissue lipid content rather than lipid subtypes. Indeed, the acyl composition [5] of lipid subtypes and their subcellular location [6] are critical to their biological activity and their ability to interfere with insulin signaling mechanisms.

Both DAGs and ceramides have been shown to block important enzymatic pathways that affect muscle responsiveness to insulin. Ceramides stimulate AKT/PKB and PKR/JNK signaling pathways [7], while DAGs have been shown to activate protein kinase C (PKC) family members, specifically PKCθ, in skeletal muscle [8]. Notably, only one specific DAG isomer, i.e., the 1.2-DAG, has been shown to activate PKCs [9], and the fatty acid composition of ceramides induces radically different effects on metabolic homeostasis [5]. Additionally, it has been reported that, in animal models, skeletal muscle fiber composition affects both PKC content and activation [10], and skeletal muscle fiber composition affects the association of DAGs and ceramides with insulin sensitivity, although no data exist in humans [11]. This underscores the importance of specific lipid subtypes rather than total tissue lipid content and the need to consider muscle fiber composition when unraveling the contribution of lipids to altered insulin signaling.

Furthermore, individuals of African ancestry have been shown to have lower rates of skeletal muscle fatty acid oxidation [12], which may be related to muscle fiber composition, as these individuals have been shown to have fewer type I oxidative muscle fibers and more type II glycolytic fibers compared to their European counterparts [13]. Existing literature suggests that total skeletal muscle lipid content does not differ between African and European ancestry [3], but data on lipid subtypes and muscle fiber type composition are sparse or nonexistent.

Based on these observations, we hypothesize that the link between skeletal muscle lipid subtypes, specifically DAGs and ceramides, and peripheral insulin sensitivity differs between African and European ancestry, and that muscle fiber composition contributes to this difference in association. Further research is warranted to investigate the role of lipid subtypes and muscle fiber composition in the context of insulin sensitivity, particularly in populations of African ancestry, to better understand the underlying mechanisms contributing to the higher prevalence of IR and type 2 diabetes in these populations. This knowledge may provide valuable insights for the development of targeted interventions to improve insulin sensitivity and prevent type 2 diabetes in individuals of African ancestry.

2. Methods

2.1. Participants

This study includes a subset of randomly selected individuals from a cross-sectional study that have been presented previously [14]. In brief, males and females (BMI 27 ± 6 kg/m^2) with African (AA, N = 24) and European ancestry (EA, N = 19) were included. Individuals with diabetes were excluded from participation following an oral glucose tolerance test. Individuals with diabetes were excluded from participation subsequent to an oral glucose tolerance test. Genotyping of ancestry information markers (AIMs) to measure genetic admixture was performed at the University of Minnesota using the Infinium Global Screening Array version 3.0 (Illumina, San Diego, CA), as described elsewhere. The proportion of an individual's genome of a given ancestral origin (e.g., African, or European) was obtained from the AIMs based on the maximum likelihood algorithm [15]. Their activity level was sedentary to moderately active (<2 h per week of moderate, intentional exercise). Participants were instructed to maintain their usual activity level, to avoid strenuous physical activity the day prior to testing, and to avoid all physical activity on the morning of testing. Body composition including total lean mass and total fat mass was assessed by dual-energy X-ray absorptiometry (iDXA instrument, GE Healthcare) using a GE Lunar iDXA densitometer and Core Scan software (encore 15 [SPT]) GE Lunar Corporation. Women were tested in the follicular phase of the menstrual cycle. Power calculation was performed on the original study design as described in Ref. [14]. No power calculation was conducted on the dataset as the lipidomics analyses were hypothesis-generating in nature. The sample size employed in this study aligns with the typical range observed in similar publications within the field. The study was conducted at the University of Alabama at Birmingham (UAB), US at the Center for Clinical and Translational Science. All participants provided written consent and the study was approved by the UAB Institutional Review Board.

2.1.1. Inclusion criteria

- Individuals without type 2 diabetes, confirmed through an oral glucose tolerance test.
- Participants with regular menstrual cycles.
- Weight stable individuals, with no more than a 2.3 kg change in weight in the previous 6 months.
- Not currently using oral contraceptives.
- Not actively following low-carbohydrate diets.

2.1.2. Exclusion criteria

- Use of medications known to influence carbohydrate or lipid metabolism, or energy expenditure.
- Use of antihypertensive agents that impact glucose tolerance, such as thiazide diuretics at doses exceeding 25 mg/day or angiotensin-converting-enzyme inhibitors.

2.2. Human skeletal muscle biopsies

Skeletal muscle biopsies were collected at rest after an overnight fast from the lateral lower portion of the *m. vastus lateralis* under local anesthesia using a 5 mm Bergstrom biopsy needle with suction. The tissue was cleaned of adipose and connective tissue. Muscle fiber composition was determined via myosin heavy chain immunohistochemistry as described previously [16].

Visible fat, connective tissue, and blood clots in the biopsy samples were immediately removed under a dissection microscope and muscle biopsy samples were flash frozen in liquid nitrogen and stored at −80 °C for lipidomics analysis.

2.3. Human peripheral insulin sensitivity

Insulin sensitivity, normalized to lean mass, was assessed after a ≥10 h overnight fast using a hyperinsulinemic euglycemic clamp with a continuous infusion of insulin at 120 mU/m^2/min to capture predominantly skeletal muscle glucose uptake, according to Tam et al. [17].

An intravenous catheter was placed in the antecubital vein for insulin and glucose infusions, and another catheter in the contralateral arm for blood collection every 5 min using a Glucose Analyzer (YSI 2300 STAT Plus, YSI, Inc.). A 20 % dextrose solution was adjusted to maintain blood glucose at fasting levels during a 3 h clamp.

A steady state period, defined as 30 min occurring 1 h after insulin infusion, had blood glucose, serum insulin and glucose infusion rate with less than 5 % variation. Serum analyses for glucose (SIRRUS analyzer, Stanbio Laboratories) and insulin (TOSOH AIA-II immunoassay analyzer, TOSCHCorp) were conducted at UAB's Diabetes Research Center.

Insulin sensitivity (SI_{Clamp},10^{-4} dL. kg^{-1}.min^{-1}/[μU/ml]) was defined as M/(G × ΔI), where M is the steady state glucose infusion rate (mg/kg lean mass/min), G is the steady-state serum glucose concentration (mg/dl), and ΔI is the difference between basal and steady-state serum insulin concentrations (μU/mL).

2.4. Animals and diet

F1 mice were obtained from breeding male C57BL/6 J mice (#000664, The Jackson Laboratory, US) with female CBA/CaCrl (#609, Charles River, UK). Both CBA and C57BL/6 J mice are phenotypically stable strains that are susceptible to diet-induced obesity and insulin resistance, which makes them relevant for studying metabolic disorders related to altered insulin sensitivity. The F1 mouse is the first filial generation resulting from crossbreeding of these two different parental strains, which will mimic the genetic diversity of natural populations. 10 weeks old F1 mice were randomly divided into two groups (n = 7 per group) fed either a standard chow diet (SC, Special Diet Service #801730) or a high fat diet (HFD, 21.92 kJ/g chow, D12492, Research Diets, Inc., New Brunswick, NJ, USA), with free access to water. The mice were fed ad libitum for nine weeks, which is a duration that has shown to induce profound metabolic difference in insulin resistance and fat incorporation in target tissue [18,19]. After 9 weeks of diet, mice were killed at fed state and skeletal muscle vastus lateralis and soleus were isolated. The tissues were cleaned from blood vessels and blood and flash-frozen in liquid nitrogen and stored in −80 °C until mass spectrometry analyses.

Animal experiments were performed in accordance with Guidelines for the Care and Use of Laboratory animals and approved by the Animal Review Board at the Court of Appeal of Northern Norrland in Umeå 190219 (approval numbers Dnr: A73–15 and A11-17).

2.5. Animal glucose and insulin measurements

Intraperitoneal glucose tolerance tests were performed at baseline and after 9 weeks HFD/SC feeding. Fasted (16 h) mice were subjected to Hypnorm (Veta Pharma) and midazolam prior to intraperitoneal glucose injection (2 g/kg body weight; Gibco). Blood glucose levels were monitored from the tail tip using a glucometer (Ultra 2, One Touch, Accu Check, Roche, Germany) in the basal state and at 10, 30, 60, 90, and 120 min after glucose administration.

Insulin concentrations were measured in blood plasma collected at the basal state and at 2,5, 5, 10, 30, and 60 min after glucose administration using an UltraSensitive Mouse Insulin ELISA kit (Chrystal Chem Inc. USA #90080). Insulin sensitivity, the MATSUDA index, was calculated via [10000/sqrt (insulin (0 min) x glucose (0 min) x insulin mean (0–60 min) x glucose mean (0–60 min)] [20].

2.6. Human and mice skeletal muscle lipidomics

Skeletal muscle samples from humans and mice were subjected to lipidomics analyses using liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS, Agilent Technologies Inc., Santa Clara, CA, USA). Skeletal muscle tissues were prepared according to Nygren et al. [21] using a 70/30, v/v chloroform:methanol for lipid extraction. A detailed description of sample preparations and analyses are provided in the Electronic Supplementary Material (ESM). All sample analyses were performed according to a study design to circumvent methodological biases to interfere with the interpretation of results.

The LC-MS data were processed using Agilent Masshunter Profinder version B.08.00 (Agilent Technologies Inc., Santa Clara, CA, USA). All lipids were annotated according to standard lipid nomenclature set by *Lipid Maps Lipidomics Gateway* (lipidmaps.org), i.e., lipid class, total number of carbons in the attached fatty acids, and total number of double bonds.

2.7. Statistical analysis

The statistical analysis included both univariate and multivariate analyses and was carried out using SIMCA 17.0 software (Sartorius, Umeå, Sweden) and GraphPad Prism v 9.5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Principal component analysis (PCA) was used for data inspection to evaluate groupings, outliers, and trends in the data sets. Next, orthogonal partial least squares (OPLS) analyses were applied to study the relationships between lipidomic profiles and peripheral insulin sensitivity and ethnicity. To ensure the suitability of the dataset for statistical analysis, rigorous data preprocessing steps were performed. For univariate analysis, the assumptions underlying the *t*-test were evaluated, including normality, homogeneity of variances, and independence of observations. Normality was assessed using Shapiro-Wilk test and independence of observations was confirmed through study design and standardized data analytical protocol as described above. For non-normally distributed data, the Mann-Whitney *U* test was applied. Prior to multivariate analysis, i.e., PCA and OPLS, appropriate transformation was applied if necessary. The fulfillment of these assumptions was confirmed ensuring the reliability and validity of the dataset for subsequent statistical analysis. The multivariate models were validated based on analysis of variance of the cross-validated OPLS scores (CV-ANOVA) for significance testing [22]. Further significance testing of highlighted findings from the OPLS models was carried out via the Mann Whitney Wilcoxon test and univariate linear regression using Spearman correlation. A variable was considered significant based on univariate *p*-value and a significant jack knifing-based confidence interval from the obtained OPLS models [23]. A 95 % significance level was applied throughout this work.

3. Results

3.1. Clinical data

Anthropometric data, body composition, and metabolic parameters, including insulin sensitivity and ectopic fat, for the full study have been published previously [14]. Estimated genetic admixture was 85 % African ancestry for the AA group and 99 % European ancestry for the EA group (Table 1). A summary of participant characteristics included in this material are presented in Table 1. Healthy (without type 2 diabetes) individuals of African ancestry (AA) and European ancestry (EA) of both sexes were included in this study. BMI ranged from 21.5 to 33.1, spanning the categories of normal-weight, overweight, and obese. AA had significantly higher BMI ($P = 0.009$) and percentage lean mass ($P = 0.028$) compared to EA. No difference was observed between AA and EA in body fat percentage (Table 1). Peripheral insulin sensitivity (pIS) was significantly lower in AA compared to EA ($P < 0.001$), Fig. 1a). The skeletal muscle fiber composition also differed; AA had significantly lower oxidative type I muscle fiber content than EA ($P = 0.02$, Fig. 1b).

Table 1
Subject characteristics.

Variable	African American (n = 24)	European American (n = 19)	P (Mann-Whitney U)
Sex, males/females	12/12	10/9	
Age (y)	28 ± 8	28 ± 8	
AA ADM	0.85 ± 0.07	0.004 ± 0.007	<0.001[b]
EA ADM	0.14 ± 0.065	0.99 ± 0.009	<0.001[b]
BMI (kg/m^2)	28.3 ± 4.8	24.7 ± 3.2	0.009[a]
Triglycerides (mg/dL)	90.7 ± 97.2 (n = 21)	67.3 ± 24.9(n = 17)	0.637
Dual-Energy X-ray Absorptiometry			
Fat Mass (kg)	26.8 ± 10.2	21.8 ± 8.3	0.104
Fat Mass (%)	31.3 ± 8.0	30.1 ± 8.8	0.851
Lean Mass (kg)	54.3 ± 12.0	47.1 ± 8.2	0.028[a]

Data reported as mean ± standard deviation. Significant difference between the groups.
Abbreviations: ADM, Admixture coefficients.
[a] $p < 0.05$.
[b] $p < 0.001$.

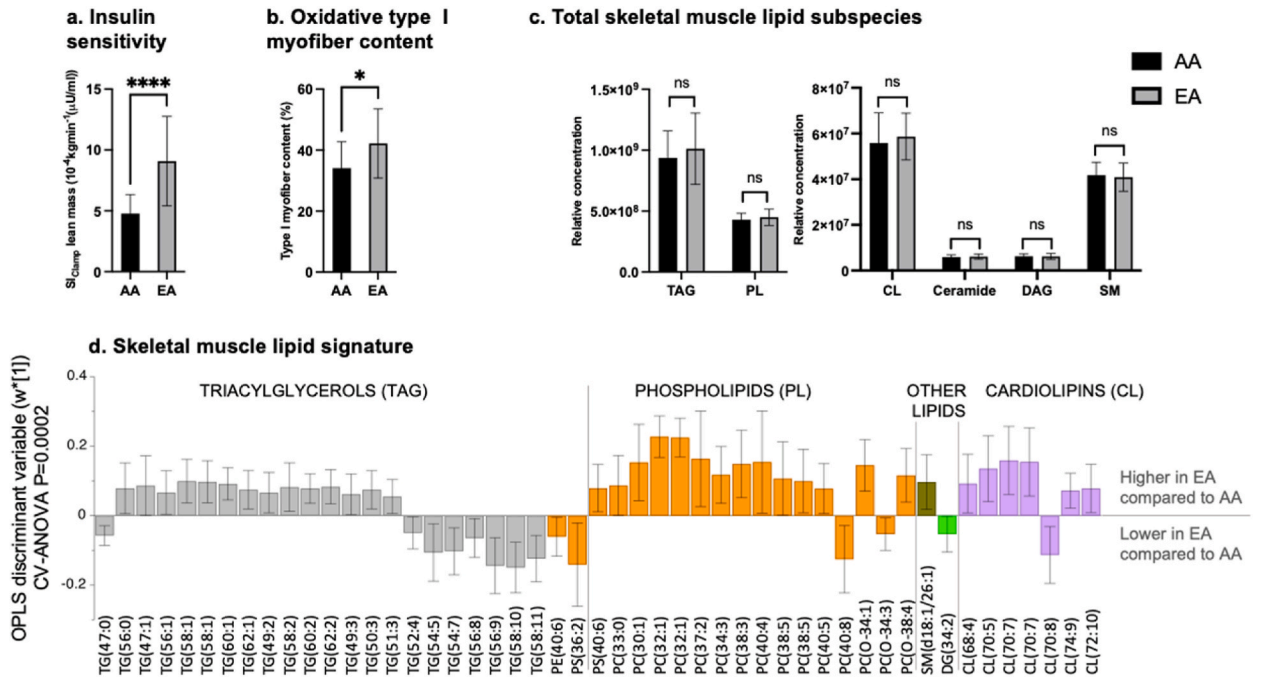

Fig. 1. Skeletal muscle characteristics in individuals with African (AA) and European (EA) ancestry. **(a)** Peripheral insulin sensitivity established via hyperinsulinemic euglycemic clamp in AA (black bars) and EA (grey bars) individuals adjusted for lean body mass. **(b)** Type 1 skeletal muscle fiber content (%) determined by myosin heavy chain immunohistochemistry. **(c)** Total concentration of skeletal muscle lipid subclasses detected by mass spectrometry-based lipidomics analysis. **(d)** Skeletal muscle lipid signature that differed significantly between individuals with EA and AA (OPLS CV-ANOVA P = 0.0002). *All data reported as median (interquartile range). Significant difference between AA and EA P<0.05, ***P<0.001, Triacylglycerol (TAG); Phospholipids (PL); Cardiolipins (CL); Ceramide (Cer); Diacylglycerol (DAG); Sphingomyelin (SM); Orthogonal Partial Least Squares (OPLS). a-c: data are expressed as the mean ± SD.*

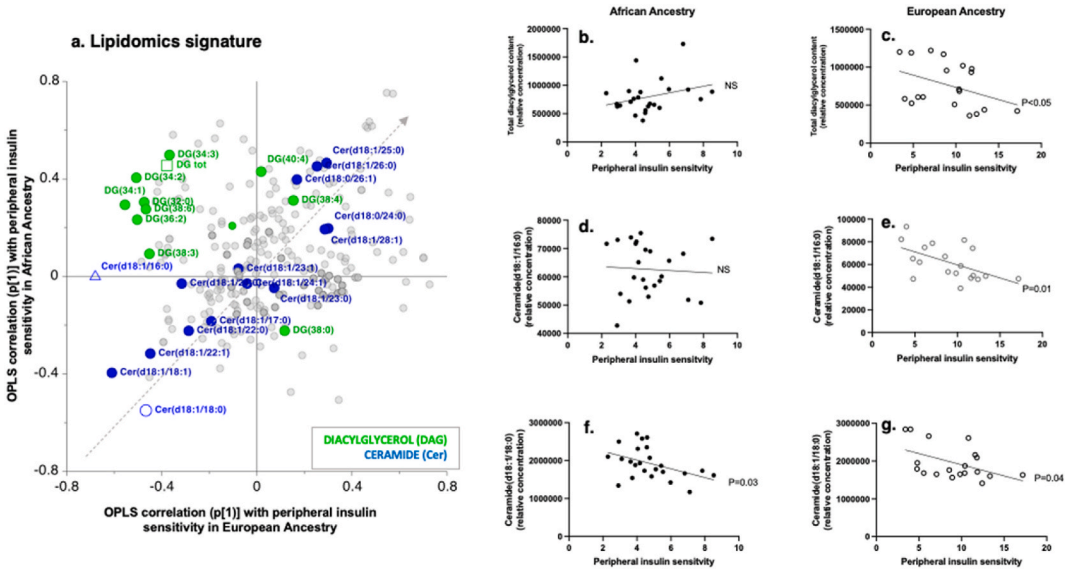

Fig. 2. Skeletal muscle lipids association with peripheral insulin sensitivity. **a)** Multivariate association between skeletal muscle lipidomics and peripheral insulin sensitivity (pIS) in individuals with European ancestry (x-axis) and African Ancestry (y-axis). Group specific spearman rank correlations between pIS and total skeletal muscle diacylglycerol **(b–c)**, skeletal muscle ceramide(d18:1/16:0) **(d–e)**, and skeletal muscle ceramide (d18:1/18:0) **(f–g)**. *Orthogonal Partial Least Squares (OPLS); Ceramide (Cer); Diacylglycerol (DAG).*

3.2. Human skeletal muscle lipidomics

A total of 253 putative lipid compounds, from 11 different lipid classes, were detected in the obtained skeletal muscle biopsies. If not stated otherwise, all discussed lipids levels are significant according to criteria in the Methods section. All variables were scaled to normal distribution prior to analysis.

We observed no differences in total levels of skeletal muscle lipid subtypes between AA and EA (Fig. 1c). Instead, specific skeletal muscle lipids subtypes differed between individuals with AA and EA (OPLS, CV-ANOVA $P = 0.0002$, Fig. 1d). More specifically, AA showed lower levels of mitochondria-specific cardiolipins (CL), phosphatidylcholines (PC) and triacylglycerols (TAG) with low number of saturations ($N < 4$) on their attached acyl chains compared to EA (Fig. 1d). In addition, TAGs and PC with higher saturation degree ($N > 8$) were higher in AA muscle compared to EA. We also found higher levels of specific diacylglycerol, phosphatidylserine (PS), phosphatidylethanolamine (PE) and CL, i.e., DAG(34:2), PS(36:2), PE(40:6) and CL(70:8), in AA compared to EA ($P < 0.05$, Fig. 1d).

3.3. Skeletal muscle lipid signatures that describe peripheral insulin sensitivity

We found a significant association between skeletal muscle lipids and pIS in both EA and AA (Fig. 2a, OPLS CV-ANOVA $P < 0.05$), but the associations differed. Diacylglycerols (DAGs) were positively associated with pIS in AA, whilst the opposite was observed for EA (Fig. 2a). Further, spearman rank correlations confirmed the observed OPLS pattern and showed a significant inverse correlation between total skeletal muscle DAG and pIS in EA ($P < 0.05$, Fig. 2c). However, the positive association, seen in the OPLS model, between DAG and pIS in AA was not significant (Fig. 2b).

In contrast to DAGs, most ceramide subtypes were similarly associated with pIS for both AA and EA; where total ceramide content and C18-ceramides, i.e., Cer(d18:1/18:0) and Cer(d18:1/18:1), were inversely associated with pIS; whereas ceramides with longer acyl chains, i.e., Cer(d18:1/25:0) and Cer(d18:1/26:0), were positively associated with pIS (Fig. 2a). Univariate analysis confirmed a significant inverse correlation between Cer(d18:1/18:0) and pIS in both groups ($P < 0.05$, Fig. 2f and g). However, no significant univariate correlation was found between pIS and ceramides with longer acyl chains, i.e., 25/26-ceramides (data not shown), or with total skeletal muscle ceramide content (Fig. 2f and g).

In addition, OPLS analyses highlighted an inverse association between skeletal muscle C16-ceramide, i.e., Cer(d18:1/16:0) and pIS in EA but not in AA (Fig. 2a), which was confirmed via spearman rank analyses ($P < 0.05$, Fig. 2d and e).

To explore the relationship between skeletal muscle lipids, insulin sensitivity and muscle fiber composition, we examined mouse skeletal muscle types with varying levels of oxidative type 1 muscle fiber content.

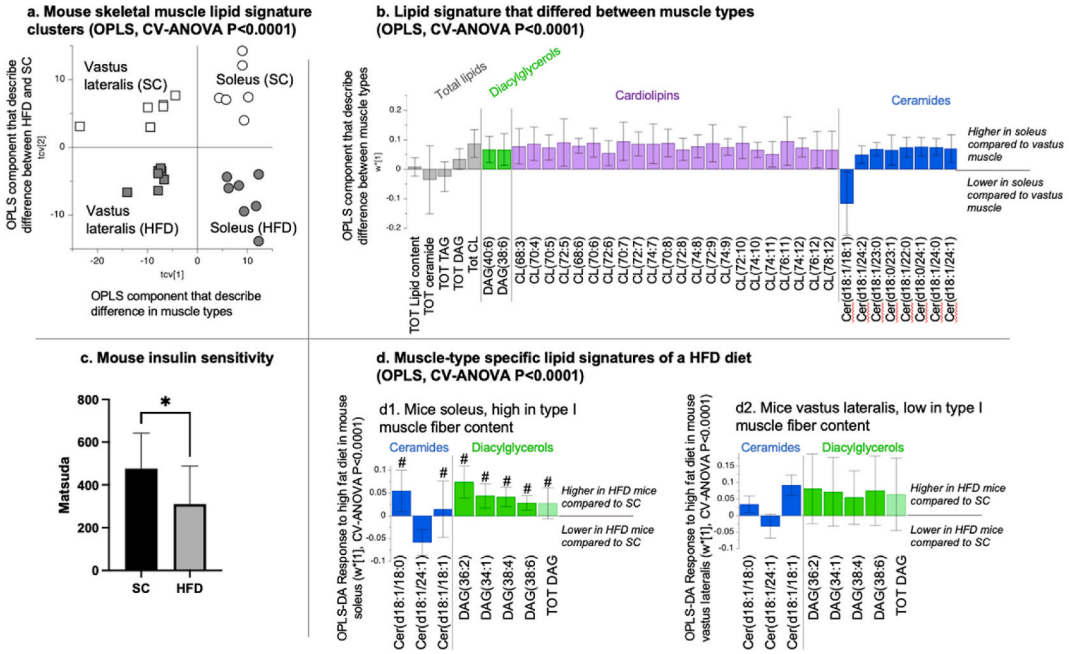

Fig. 3. Mouse OPLS models of lipid signatures that describe differences in muscle types and high fat diet induced decrease in insulin sensitivity. **a)** Skeletal muscle lipid signatures in the defined mouse muscle types (tcv[1]) of mice fed a high fat diet (HFD, $n = 7$) compared to standard chow (SC, $n = 7$) (tcv[2]) calculated from skeletal muscle lipids (OPLS, CV-ANOVA $P < 0.0001$). **b)** Lipid signature that differ between soleus and vastus lateralis muscle harvested from SC fed mice (OPLS, CV-ANOVA $P < 0.0001$). **c)** Matsuda index calculations from intraperitoneal glucose tolerance tests in SC and HFD fed mice (*$P < 0.05$). **d)** HFD-induced changes in diacylglycerols and ceramides from mice soleus **(d1)** and vastus lateralis muscle **(d2)**. *# indicate a significant (P<0.05) OPLS correlation with insulin sensitivity (Matsuda). c: Data are expressed as the mean ± SD.*

Sketelal muscle lipid profiles differed significantly between the oxidative soleus (high in type 1 muscle fiber) [24] and glycolytic vastus lateralis muscle (low in type 1 muscle fiber) [24], as well as their response to a high fat diet (HFD) compared to standard chow (SC) (OPLS, CV-ANOVA $P < 0.0001$, Fig. 3a). Total lipid content did not differ between muscle types in SC-fed mice (Fig. 3b). As expected, the oxidative soleus muscle exhibited significantly higher total cardiolipin content, a marker of mitochondrial content [25], along with elevated levels of specific cardiolipin lipid species, compared to the vastus lateralis muscle (Fig. 3b). While there were no significant differences in total ceramide or DAG content between muscle types in SC-fed mice, the soleus muscle had higher levels of DAG(38:6), DAG(40:6) and C23–C24-ceramides, and lower levels of C18:1-ceramide than the vastus lateralis muscle (Fig. 3b). The HFD resulted in significantly lower insulin sensitivity, as indicated by the Matsuda index (Fig. 3c).

In both muscle types, HFD led to a notable increase in C18-ceramides, specifically Cer(d18:1/18:0) and Cer(d18:1/18:1), along with an accumulation of specific DAGs in the soleus muscle (rich in type 1 muscle fibers) (Fig. 3d1 and 3d2, OPLS, CV-ANOVA $P < 0.0001$). However, there were no significant differences in DAGs between HFD and SC mice in the glycolytic vastus lateralis muscle (low in type 1 muscle fiber, Fig. 3d2). Furthermore, soleus muscle DAGs, including total DAG content, and C18-ceramides were inversely associated with insulin sensitivity (Fig. 3d1, indicated by #), whereas no such association was found in the vastus lateralis muscle (Fig. 3d2).

4. Discussion

Diacylglycerols (DAG) have been established as signaling molecules with the potential to impact insulin signaling pathways [8]. In this study, we provide evidence of a novel link between increased DAG content in skeletal muscle and lower peripheral insulin sensitivity (pIS) in individuals of European ancestry (EA), but not in individuals of African ancestry (AA). We propose that the absence of this link in AA individuals may be partly attributed to the lower content of oxidative type I muscle fibers compared to EA individuals. Furthermore, we posit that the contribution of lipids to altered pIS may differ between individuals with high and low type I muscle fiber content, and that this warrants further investigation.

It is well known that type I muscle fibers have a greater capacity (2-3-fold) to buffer increases in fatty acids by partitioning into triacylglycerol (TAG) in lipid droplets, compared to glycolytic type II fibers [26]. Instead, during fatty acid overflow and obesity, skeletal muscle low in type I muscle fibers may be prone to accumulate lipid precursors of TAGs, e.g., DAGs [11]. Of major interst is that TAG hydrolysis does not generate the 1,2-DAG isomer that can activate PKCθ in the muscle and interfere with insulin signaling [9]. Thus, in obesity, skeletal muscle DAGs in muscle low in type I muscle fiber content may instead reflect increases in 1,3- and 2,3-DAG isomers that result from incomplete incorporation of intramyocellular triglycerides [9]. Notably, the 1,3- and 2,3-DAG isomers are not able to activate PKCθ and interfere with insulin signaling. Consistently, our findings showed higher DAG, i.e., DAG(34:2) in the AA (lower in typ I muscle fiber) compared with EA (higher in type I muscle fiber), despite no association with pIS. Similarly, we also show that DAGs were uniquely associated with a high fat diet (HFD)-induced decrease in insulin sensitivity in mouse soleus muscle, which mainly consist of oxidative type I muscle fibers. In contrast, we found no association between insulin sensitivity and DAG in the glycolytic vastus lateralis muscle (low in type I fiber). Taken together, these observations support the hypothesis that the impact of lipids on alterations in insulin sensitivity varies according to myofiber composition.

Similarly, recent experimental data from Jani et al. showed an increase in 1,2-DAG-mediated pathways, i.e., increased PKCθ translocation/activation, which disrupts insulin signaling in skeletal muscle with higher oxidative type I fiber content in HFD fed rodents [11]. Jani et al. suggest that altered insulin sensitivity in muscles low in type I muscle fibers is more driven by inflammatory parameters rather than DAG levels. However, a limitation of our and previous studies is the lack of DAG isomerization data in human studies, i.e., the ability to differentiate between 1,2-DAG and 1,3- and 2,3-DAG, which should be a priority for future research. There are a few studies in human [6] and rodent [27] that highlight DAG isomerization and subcellular localization as key determinants of their ability to interact with the insulin signaling machinery, where 1,2-DAG at the plasma membrane induces insulin resistance. However, data in humans are scarce.

We recently showed that improved mitochondrial biogenesis and increased lipid utilization in women of African ancestry were associated with increased skeletal muscle 1,2-DAG production in the endoplasmic reticulum/Golgi, with no effect on the 1,2-DAG production at the plasma membrane, which is more prone to interact with insulin signaling and glucose uptake [28], suggesting that the subcellular location of lipid production is key and should be considered. Notably, previous research has indicated that neither mitochondrial function nor muscle fiber composition alone can fully explain the lower insulin sensitivity observed in individuals of African ancestry [29]. However, other aspects of skeletal muscle composition may affect lipid metabolism and contribute to the development of prediabetes and diabetes [6,30]. Our findings emphasize the importance of bioactive lipid species and their cellular location in enhancing our understanding of the underlying mechanisms by which muscle fiber composition influences the pathophysiology of type 2 diabetes.

Post-glucose-challenge insulin has been shown to be higher in individuals with African ancestry compared to European Americans (EA), irrespective of obesity and insulin sensitivity, and across various states of glucose tolerance [31]. The exaggerated insulin response to glucose in AA individuals may increase the risk for beta-cell exhaustion, dysfunction and finally type 2 diabetes compared to EA [32]. Experimental studies in rodents have shown that hyperinsulinemia can alter muscle fiber type composition, resulting in a reduction in type I muscle fiber content [33]. This finding is also supported by human studies of acute hyperinsulinemia [34]. Indeed, chronic hyperinsulinemia has shown to increase the production of reactive oxygen species [35], leading to cell damage, impaired function of blood vessels, and reduced nutrient delivery to skeletal muscle [36]. Collectively, this can contribute to a switch in muscle fiber composition, resulting in decreased oxidative type I muscle fiber content and lower insulin sensitivity [33,34].

Consistent with existing literature [37], our study demonstrates that total skeletal muscle ceramide content provides misleading

information. We show that C18-ceramides, i.e., a C18 fatty acid in their sn-2 position, such as Cer(d18:1/18:0) and Cer(d18:1/18:1), were inversely associated with peripheral insulin sensitivity (pIS) in both individuals of African and European ancestry. In contrast, ceramides containing C25-26 fatty acids showed a non significant or positive association with pIS. In skeletal muscle, C18-ceramides account for the majority of ceramide content, and deletion of skeletal muscle C18-ceramide synthesis has been shown to improve insulin sensitivity in mice with HFD induced insulin resistance, whereas deletion of C25-26-ceramide production does not affect glucose metabolism in obesity [38]. These findings highlight the differential roles of specific ceramide species in muscle, rather than total ceramide content. Furthermore, we found that C18-ceramides were higher in HFD mouse muscle types with both high (soleus) and low (vastus lateralis) type I muscle fiber content. Our results support existing literature and highlight C18-ceramides as an interesting target for improving skeletal muscle glucose homeostasis [39], regardless of genetic ancestry. The overall ceramide signature was similarly associated with insulin sensitivity in individuals of AA and EA, with the exception of Cer(d18:1/16:0). Experimental studies have shown similar results when inhibiting C16-ceramide synthesis, as for C18-ceramide, i.e., protection from HFD induced obesity and glucose intolerance [40]. However, the synthetic routes of C18- and C16-ceramides differ [41], and further investigation is needed to understand their relation to muscle fiber content and genetic ancestry.

In this study, individuals of EA and AA did not differ in total skeletal muscle lipids, which is consistent with earlier research conducted in black West African men [42]. However, we observed a difference in specific lipid subtypes that are associated with mitochondrial biogenesis and membrane fluidbility, i.e., saturation degree and amount of cardiolipins, phosphatidylcholines, and TAGs [43], indicating lower mitochondrial content in AA muscle. This finding is consistent with our mouse data where lower levels of mitochondria-related lipids were observed in the vastus lateralis muscle that is low in type I muscle fiber, compared to the soleus muscle high in type I fibers. These results suggest that the distinct lipid pattern observed between AA and EA is indeed partly related to differences in type I muscle fiber content.

Previous studies have established that muscle types with higher type I fiber content have higher glucose-handling capacity [44] and lipid utilization capacity [45]. Although some studies report lower type I fiber content in individuals of African ancestry compared to European Americans [46], other studies suggest no difference in muscle fiber composition when accounting for obesity [13]. Muscles that are low in type I muscle fiber content tend to rely heavily on glycogen for energy, which may render them more susceptible to alterations in mitochondrial biogenesis and lower metabolic flexibility [47]. Therefore, to comprehensively elucidate the underlying mechanisms of skeletal muscle insulin resistance and the potential role of lipids, it is crucial to expand our focus beyond lipotoxic intermediates and also consider glycolytic intermediates. This study involved individuals with different obesity levels, which will enhance the external validity by reflecting the diversity seen in the general population. However, this diversity presents a challenge owing to its heterogeneity and potential confounding factors. Furthermore, the relatively small number of participants may have contributed to conflicting results in the obtained data due to interindividual variation. To address this, the findings in humans were further validated using a mouse model in which the levels of obesity, lifestyle, and ancestry were controlled.

In conclusion, our findings suggest that the association between skeletal muscle DAG content and peripheral insulin sensitivity differs between individuals of AA and EA. We propose that this discrepancy may be attributed to muscle fiber composition and its inherent differences in fat oxidation capacity. In a state of fatty acid overflow, muscles with high type I muscle fiber content may be more prone to accumulate 1,2-DAG, which can interact with novel PKCs as a feedback mechanism, potentially interfering with insulin signaling. In contrast, muscles low in type I muscle fibers have lower ability to utilize and store lipids. Insulin resistance in these muscles may instead be due to non-lipid feedback mechanisms. Further research is warranted to investigate these mechanisms in more detail. More specifically, we suggest that fiber type measures and organelle-specific regulators of bioactive lipids should be considered in future studies.

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Ethics statement

All human skeletal muscle materials included in this study was collected at the University of Alabama at Birmingham (UAB), US at the Center for Clinical and Translational Science. All participants provided written consent and the study was approved by the UAB Institutional Review Board.

Animal experiments were performed in accordance with Guidelines for the Care and Use of Laboratory animals and approved by the Animal Review Board at the Court of Appeal of Northern Norrland in Umeå 190219 (approval numbers Dnr: A73–15 and A11-17).

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available due to data privacy laws. However, they are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Tova Eurén: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Barbara Gower:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Pär Steneberg:** Writing – review & editing, Resources, Methodology, Data curation. **Andréa Wilson:** Investigation, Data curation. **Helena Edlund:** Writing – review & editing, Resources, Funding acquisition, Data curation. **Elin Chorell:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32456.

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Abbreviations

AIM: (ancestry information markers)
AA: (African ancestry)
CL: (cardiolipins)
DAG: (diacylglycerol)
EA: (European ancestry)
HFD: (high fat diet)
IR: (insulin resistance)
OPLS: (orthogonal partial least squares)
PC: (phosphatidylcholines)
PCA: (Principal component analysis)
PE: (phosphatidylethanolamine)
pIS: (peripheral insulin sensitivity)
PKC: (protein kinase C)
PS: (phosphatidylserine)
SC: (standard chow)
TAG: (triacylglycerol)