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Placental Lipid Droplet Composition; Effect of a Lifestyle Intervention (UPBEAT) in Obese Pregnant Women

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Running title: Placenta Lipid Droplet Composition in Obesity.

Abbreviations

ARA, arachidonic acid; Chol, cholesterol; DGLA, dihomo-γ-linolenic acid; FABP, fatty acid binding protein; GDM, gestational diabetes mellitus; LC-PUFA, long-chain polyunsaturated fatty

acid; LD, lipid droplets; LGA, large for gestational age; LysoPC, lyso-phosphatidylcholine; NEFA, non-esterified fatty acid; PC, phosphatidylcholine; PL, phospholipid; SM, sphingomyelin.

Abstract

Maternal obesity is associated with adverse outcomes. Placental lipid droplets (LD) have been implicated in maternal-fetal lipid transfer but it is not known whether placental LD fat composition is modifiable. We evaluated the effects of a diet and physical activity intervention in obese pregnant women compared to routine antenatal care (UPBEAT study) on placental LD composition. LD were isolated by ultracentrifugation. Total FAs and phospholipids (phosphatidylcholines, PCs; sphingomyelins, SMs and lyso-phosphatidylcholines, Lyso-PCs) were analyzed by LC-MS/MS. Placenta MFSD2a expression was assessed by western blot. Placental LDs from obese women were comprised of predominantly saturated and monounsaturated FAs. TG and Chol composition was similar between intervention (n=20) and control (n=23) groups. PCs containing dihomo-ɣ-linolenic acid in LD were positively associated with gestational weight gain (P<0.007), and lowered by the intervention. In the whole sample, PCs carrying DHA and arachidonic acid were inversely associated with placental weight. Placenta MFSD2a expression was associated with DHA cord blood metabolites and relationships were observed between LD lipids, especially DHA carrying species, and cord blood metabolites. We describe placenta LD composition for the first time and demonstrate modest, potentially beneficial effects of a lifestyle intervention on LD FAs in obese pregnant women.

Keywords: lipid droplets, placenta, omega-3 fatty acids, phospholipids, randomised controlled trial, MFSD2a.

1. Introduction

Lipid droplets (LD) are cytosolic depots of neutral lipids that exist in virtually all cell types and in all forms of life, from bacteria to mammals [1]. Unlike common cell organelles, LD have a unique organization, composed of a neutral lipid core surrounded by a protein-coated phospholipid (PL) monolayer [2]. The LD core contains predominantly TGs and sterol esters, but other endogenous neutral lipids such as retinol esters may also be present [3]. The monolayer surface presents mainly PL, with phosphatidylcholine (PC) being the most abundant, together with a varying amount of free cholesterol (Chol) and a long list of embedded proteins [4-6].

LD size can change with the cell stage or nutritional status [7]. Most cells types contain a set of relatively small LD (<5μm diameter) while supersized LD may be found in white adipose tissue cells (>100μm diameter). The formation and composition of LD strongly depends on the available FA in the local extracellular and intracellular environment [8-9], affecting the function of these particles within specific tissues.

LD regulation and metabolism have been the focus of attention in tissues such as the heart [10], gut [11] or skeletal muscle [12], however, little is known regarding LD composition or function in placenta. Extrapolating from other tissues to the human placenta, variations in maternal plasma FAs during pregnancy could affect the formation and composition of placental LD and hence the FA materno-fetal transfer process.The possible involvement of placental LD in the materno-fetal transfer of FA and in fetal development and growth has not been studied to date despite the fundamental role of the placenta in fetal nutrition and the global escalation in maternal obesity which is strongly associated with increased risk of fetal macrosomia and large for gestational age (LGA) infants. Excessive size at birth leads to problems at delivery and may also be a precipitating factor in development of obesity and metabolic syndrome in later life [13-15]. Whilst a recent study has reported a 17.5% increase in lipids in the placenta of obese pregnant women compared to lean women [16] it is not known whether any of the interventions currently under evaluation in obese pregnant women which aim to improve maternal and neonatal outcomes can

modify the placental lipid fat content of the placental lipid storage depots as measured in the LD, or affect the transfer of fats from the mother to the developing embryo and fetus [17].

The UK Pregnancies Better Eating and Activity Trial (UPBEAT) in 1555 obese pregnant women showed that a complex behavioural intervention comprising diet and physical activity reduced measures of maternal gestational weight gain (GWG), adiposity, and was associated with reduction in infant adiposity at 6 months of age [18], although the primary objectives of reducing maternal gestational diabetes and LGA were not met [19]. We hypothesised that placenta LD composition would be improved by the UPBEAT intervention. In addressing this hypothesis we have described for the first time the lipid composition of LD in placenta and evaluated relationships with maternal and newborn clinical parameters, and have investigated a potential role of the LD lipid species in FA transfer to the fetus.

2. Materials and Methods

2.1. Subjects

Obese pregnant women (BMI ≥30kg/m^2) were recruited from UK antenatal clinics between 15 weeks and 18 weeks' plus 6 days of gestation and randomly assigned to either a behavioural intervention or standard antenatal care. All participants were older than 16 years and had a singleton pregnancy. Exclusion criteria included: unwilling/unable to give informed consent, multiple pregnancy, underlying disorders including GDM, type 1 or type 2 diabetes and hypertension. Subjects in the intervention arm were invited to attend 8 one-to-one weekly sessions with a health trainer to promote a healthier pattern of eating (low glycemic index diet, reduced saturated fat intake and reduced free sugars) and increased physical activity. Standard care women attended routine antenatal care. All women gave informed consent. The study was approved by the NHS Research Ethics Committee (UK Integrated Research Application System; reference 09/H0802/5). Full details of the study have been published previously [19]. Clinical and socio-demographic characteristics of the sub-group of participants in the present study were representative of the women from the main UPBEAT trial participants, with the only significant difference being the average age of the women (2 years older in the sub group).

2.2. Biological samples

Placenta samples were collected at delivery from 43 women (23 control and 20 intervention) delivering at the London Guy's and St. Thomas' NHS Foundation Trust trial site. Fasting blood samples from the mothers were taken after the intervention period, at between 27 weeks and 28 weeks plus 6 days of gestation. Mixed cord blood was obtained immediately after delivery. Triglycerides, total HDL and LDL cholesterol were evaluated in maternal serum by NMR metabolomic profiling, and insulin in maternal plasma by immunoassay [20]. Samples were collected as a pool of four imaginary quadrants, rinsed in cold 0.9% NaCl solution and frozen at -80ºC until analysis.

2.3. Gestational Weight Gain and Maternal and Neonatal Anthropometry

Maternal weight gain during pregnancy was measured and anthropometric measures in mother and newborn infant recorded as reported for the main trial [21].

2.4. Lipid droplets isolation

LD were isolated from placental tissue as previously described [22]. Briefly, 5g of placenta were gently rinsed with cold 0.9% NaCl to eliminate blood. Samples were transferred to 12mL buffer (25mM tricine, 250mM sucrose, 0.2mM phenylmethylsulfonyl fluoride, pH 7.6) and homogenized using a T25 digital Ultra-Turrax® (IKA, Staufen, Germany) disperser for 1min followed by homogenization in a tissue grinder Potter-Elvehjem (25 times). Homogenates were centrifuged (3,000g, 10min, 4ºC). Supernatants were transferred to an Ultra-Clear centrifuge tube (Beckman Coulter, CA, USA) and 2mL buffer (20mM HEPES, 100mM KCl, 2mM $MgCl_2$, pH 7.4) were loaded on the top of the tissue homogenate. The supernatants were again centrifuged (288,000g, 54min, 4ºC) in a Beckman Optima XL100 ultracentrifuge equipped with a swinging bucket SW41Ti rotor (Beckman Coulter, CA, USA). After centrifugation, the floating white band containing LD was collected. Analyses were performed by duplicate; one aliquot was used for TG and Chol measures and the other one for FAs quantification.

2.5. Triglycerides and cholesterol quantification

As the LD band is a colloidal suspension, total lipids were initially extracted with chloroform:methanol (2:1 v/v) according to Folch et al. [23]. The lipid dry residue was dissolved in ultrapure water containing 2% Triton X-100 (Sigma-Aldrich, MO, EEUU). TGs and Chol content of LD were quantified by spectrophotometry using commercial kits of determination for liquid samples (QCA, Barcelona, Spain). Chol determination included both free Chol and Chol from sterol esters quantification. Data were expressed per mg of protein, analyzed by Bradford assay [24] in the placenta homogenate.

2.6. Fatty acids quantification

Total lipids from LD were extracted according to the method of Folch et al. [23]. For LD total FAs profile, an additional step of FA hydrolysis with 40% KOH during 30min 60ºC was

performed before quantification [25]. Lipidome analyses of PCs, SMs and Lyso-PCs from LD, maternal plasma and cord blood were performed by LC-MS/MS as described previously [26]. PCs were described with (XX:Y/XX:Y) as a characterization of the sn-1/sn-2 FAs (XX, number of carbon atoms; Y, number of double bounds). Analyzed PC metabolites were expressed as the sum parameter of both FAs (e.g. PC.34:1) and the two single most common FAs combination based on previous works (e.g. PC.16:0/18:1) [27-28]. Analyst 1.6.2 software (AB Sciex, Darmstadt, Germany) was used for data acquisition.

2.7. Quality Control

Six samples were used as quality control per batch of analyzed samples, 20% intra-batch and 30% inter-batch coefficient of variance were used as threshold. Metabolites exceeded these coefficients of variance in control samples were excluded from the analyses.

2.8. Western blot analysis

30mg of placenta were homogenized in 0.3mL cell lysis buffer (Cell Signaling Technology, MA, EEUU) containing 1mM phenylmethanesulfonyl fluoride [29]. Samples were homogenized in a Tissue Lyser LT device (Qiagen Iberia SL, Madrid, Spain). After homogenate centrifugation (15min, 10,000g, 4ºC), supernatant with protein lysates was collected and protein quantified by Bradford assay [24].

Primary rabbit polyclonal antibody against the orphan transporter “Major Facilitator Superfamily Domain Containing 2A” (MFSD2a) (Abcam, Cambridge, United Kingdom) and primary anti-beta-actin mouse monoclonal antibody (Sigma-Aldrich, MO, EEUU) were used. Secondary antibodies were conjugated with horseradish peroxidase (Santa Cruz Biotechnology, TX, EEUU). 15μg of protein were resolved on 10% polyacrylamide gels, and transferred onto polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany). Membranes were blocked with PBS 0.05% Tween-20 (PBS-T) containing 2% bovine serum albumin (1h room temperature). Then membranes were incubated with primary antibodies (4ºC overnight). The following day, incubation with secondary antibodies conjugated with horseradish peroxidase was

performed (room temperature 1h). Proteins were detected using a chemioluminescence kit (Pierce ECL 2 Western Blotting Substrate; Thermo Fisher Scientific, MA, EEUU)[30]. Band density was determined by densitometry (Image Quant LAS 500 software, GE Healthcare, CA, EEUU). Loading controls were used for normalization of protein expression. Beta-actin loading controls were used for the normalization of protein expression data.

2. 9. Statistical analysis

Anthropometric characteristics, biochemical parameters of mothers and neonates as well as LD composition between intervention (n=20) and control (n=23) groups were analyzed by t-test. Chi-square test was used for caesarean section rate between groups. Pearson correlations were performed in order to assess associations between maternal and newborn/6 month old parameters and LD composition/metabolites. SPSS 23.0 software (SPSS, Inc., IL, EEUU) was used for statistical analysis of data. Significance level was established as $P<0.05$. Data are expressed as means ± SEM.

3. Results

3.1. Anthropometric Characteristics and Biochemical Parameters

In this sub-group of the UPBEAT trial participants (n=43), as reported for the main trial the behavioural intervention significantly reduced the glycaemic index of the maternal diet compared to those that received a routine antenatal program (**Table 1**). Fasting plasma TGs were also reduced in obese women from the intervention group, however plasma Chol levels and placental weight did not differ between groups (**Table 1**). In this small sample of trial participants, birth weight and abdominal circumference were reduced in the intervention group (**Table 1**), although this was not observed in the entire UPBEAT cohort.

3.2. Lipid Droplets Composition

TG and Chol content of placental LD was similar between the intervention and control groups (**Fig. 1A and 1B**). In the combined groups, the TG content was 2.5-3 fold higher than Chol. The total FA concentration in placental LD showed a significant correlation with the TG content in LD (**Fig. 1C**), indicating a substantial contribution of TGs to the lipid composition of LD particles.

Total FA in LD, which represent the FA composition of the core plus the envelope of LD did not differ between randomised groups (**Fig. 2**). Total FA in LD predominantly comprised saturated FAs, especially palmitic (16:0) and stearic acid (18:0), while LC-PUFAs such as dihomo-γ-linolenic acid (DGLA, 20:3n-6), arachidonic acid (ARA, 20:4n-6) and DHA (22:6n-3) were less abundant (**Fig. 2**). Saturated FAs represented more than 65% of total FAs of LD while LC-PUFAs less than 15% in both groups.

FAs in PLs represent the envelope or surface of LD and comprise PCs, sphingomyelins (SMs), lysophospatidyl cholines (lyso PCs) and others. PCs represented approximately 2.1-2.4% of the total FA content of LD, SMs 0.8-1% of total FA content, while the contribution of Lyso-PCs was very low (0.03-0.05%). PCs containing DGLA (20:3n-6) were lower in the intervention group than in controls and the DGLA content was positively related to GWG (PC.16:0/20:3 and PC.18:0/20:3, R=0.44 / 0.53 and P=0.007 / <0.001 respectively). In contrast, PCs carrying ARA

were inversely correlated with GWG (PC.16:0/20:4 and PC.18:0/20:4, R= -0.37 / -0.36 and *P*= 0.027 / 0.027 respectively).

SMs mainly comprised saturated and monounsaturated species, with SM.16:0 being the most abundant (**Table 2**). No major differences were observed between intervention and control subjects, with the exception of SM.16:1n-7 and SM.17:0 but their contribution to the SM profile was low. Subjects in the intervention arm had a higher proportion of saturated SMs and lower monounsaturated SMs metabolites compared to controls. SM.24.2n-6 was positively associated with birthweight (R=0.45, *P*=0.003), newborn mid arm circumference (R=0.50, *P*=0.004) and abdominal circumference at birth (R=0.65, *P*<0.001).

The content of LC-PUFAs in PCs of LD, especially those carrying ARA (**Fig. 3A and 3B**) and DHA (**Fig. 3C**) was inversely related to placental weight. These LC-PUFAs were also positively associated with the corresponding PCs in cord blood (**Table 3**). Maternal total DHA serum concentration correlated with PC carrying DHA in LD (PC.16:0/22:6, R=0.35, *P*=0.033).

Since LysoPCs are known to play an important role in DHA placental uptake, the LD LysoPC profile was also analyzed. The LysoPC.18:1 (oleic acid) percentage was lower in the intervention arm when compared to controls but no other LysoPCs were different (**Table 2**). Among all Lyso-PCs, Lyso-DHA alone correlated with its lyso-FA form in cord blood.

As Lyso-DHA transport has been associated with MFSD2a carrier in placenta, we analyzed MFSD2a but placental expression did not differ between groups (**Fig. 4A**). MFSD2a protein was inversely related to the total FAs in LD (**Fig. 4B**), which might indicate a lower expression of this protein when increasing fat stores of LD. Maternal DHA concentration in serum was positively associated with placenta MFSD2a (R=0.37, *P*=0.026) and placental MFSD2a expression was associated with cord blood metabolites carrying DHA such as PC.18:0/22:6 (**Fig. 4C**) and followed the same trend for PC.16:0/22:6 (R=0.293, *P*=0.078) and non-esterified DHA (R=0.328, *P*=0.051), supporting the role of this protein for DHA transfer across the placenta. There was no correlation between placental MFSD2a and cord blood lyso-DHA (R=0.028, *P*=0.868).

4. Discussion

This study explored placental LD composition in a subset of subjects from the UPBEAT trial of obese pregnant women undergoing a complex behavioural intervention comprising nutritional coaching and physical activity advice *vs.* routine antenatal care. Differences observed in LD lipid species following the intervention suggest that this life style intervention has the potential to modify lipid storage in the placenta. Moreover, we have described for the first time the individual FA profile of these intracellular particles in placental tissue of obese women.

There were no differences either in LD TG or Chol content between randomisation arms. As anticipated from LDs in other tissues, TGs were the predominant LD lipid class, being strongly correlated with the total FA content. A recent study has shown higher TG accumulation in placenta in obese women compared to lean women [31]. The authors also reported a positive correlation between maternal pre-pregnancy BMI and the up-regulation of six genes involved in lipid metabolism in placental tissue, notably CGI-58, a co-activator of adipose TG lipase. Moreover, CGI-58 correlated with the maternal fasting insulin concentration, highlighting a potential role of pre-gravid obesity on placenta lipid metabolism, even with glucose and serum TGs levels within normal range [31]. In this sub-study (but not the overall trial), maternal serum TGs were lower in the intervention group, but this reduction was not reflected in the LD TG content. It is possible therefore that in obese pregnant women, placental LD may reflect placental FA synthesis in placenta arising from excess of glucose associated with insulin resistance, rather than maternal TG uptake from serum.

Saturated and monounsaturated FAs were the predominant components in the total lipids of LD. These can be synthesized de novo from glucose, but also from non-esterified FAs (NEFAs); enhanced FA esterification and TG deposition in placenta LD has been reported following incubation with NEFAs [32-34] which show a higher capacity to influence FA deposition than glucose or insulin. Moreover, since placental tissue has low desaturase activity, required for LC-

PUFA biosynthesis [35], this may also contribute to the FAs pattern observed in placental LD, i.e a high content of saturated and monounsaturated FAs.

PLs comprise about 2% of total FAs in placental LD, similar to the composition observed in LD from other cell types [36-37]. PC has been described as the major PL class in LD structures, followed by phosphatidylethanolamine and phosphatidylinositol species [5, 38]. In the placenta LDs from the obese women, a relatively high proportion of SMs was found compared to the PC content (~30%) while additional PL types were not quantified. Other authors have also reported a high content of SMs in LD from mouse adipocytes [36]. PL metabolism in LD is, however, not completely understood. The presence in the monolayer surface of key enzymes involved in PC synthesis e.g. phosphocholine cytidylyltransferase [39] responsible for CDP-choline synthesis, a rate-limiting step in the Kennedy pathway of PC synthesis, implies a role of LD in PL cell homeostasis and LD maintenance [38-39].

The percentage of DGLA (20:3n-6) in LD PCs was lower in the intervention group compared to controls. DGLA has previously been related to insulin resistance and the promotion of adiposity in adults and children [40-42]. Two cohort studies have found that the maternal DGLA plasma concentration throughout pregnancy is associated with a higher childhood BMI and other adiposity measures at age 6-7 [43-44]. Thus, the intervention lowering effect of DGLA content in placental LD could have long-term beneficial effects on childhood adiposity and obesity-related disorders. It is relevant that a recent report from the UPBEAT trial has reported that the intervention was associated with a reduction in subscapular skinfold thickness, a measure of adiposity [18]. Moreover, DGLA PC species were positively associated with GWG. Excessive GWG has been associated with increased risk of obesity, type II diabetes and metabolic syndrome in the mother and in offspring obesity, and might induce early programming of obesity in the fetus [45-46]. GWG was reduced in the main UPBEAT study [21], although it was similar between groups in the small set of subjects considered in the present study.

Despite no major differences in PCs and SMs pattern by randomisation arm, SM.24.2 was positively associated with birthweight and some skinfolds at birth. The relevance of this observation is not clear, although it is of interest that a recent study reported an association between maternal plasma 24:2n-6 concentration during pregnancy and the child's diastolic blood pressure [47].

We carried out further analyses in the combined (intervention and control arm) placenta samples to gain further insight into FA handling by the placenta. The inverse association between placental weight and LC-PUFA containing PCs (such as those carrying DHA and ARA), indicates a lower LC-PUFA content in LD of larger placentas. Higher placental thickness and size are associated with maternal obesity, GDM and birthweight [48-49] and it would appear increased size may alter the capacity for placenta transfer of LC-PUFA to the fetus. This is supported by a recent cohort study of 435 mothers and offspring, in which the pre-pregnancy BMI was inversely associated with decreased PUFAs in the offspring at birth, both n-6 and DHA [50]. Maternal obesity is reported to modify some placental genes related to lipid transfer and intracellular lipid metabolism including adipose triglyceride lipase, FA transporter proteins (FATPs) 1 and 3, perlipin 2, peroxisome proliferator-activated receptor G (PPARG) and FA binding protein 4 (FABP 4) [31, 34], but none of these is involved in the selective transfer of DHA in placenta. More studies are needed to better understand the extent of maternal obesity on essential FA transfer and its influence on the long-term health of the newborns.

MFSD2a protein was recently described as one of the major carriers of Lyso-DHA transport across the blood brain barrier [51]. MFSD2a expression in placenta has also been related to DHA-containing metabolites in cord blood from women with GDM [30]. Our finding that MFSD2a was inversely associated with total LD FA content is indicative of lower expression with increasing fat stores in placenta. Surprisingly, Lyso-DHA in placental LD and cord blood showed a positive association, while no relationship was evident for any other Lyso-PCs analyzed. This may indicate that lyso-phospholipids can cross the placenta without further hydrolisis. Lyso-DHA has been proposed as a preferred carrier of DHA for red blood cells and the brain, and appears to act as a

DHA carrier also in placenta [52-54]. An n involvement of LD in the transfer of FAs, especially DHA, seems possible in placenta. However, more studies are needed to confirm this hypothesis.

Strengths of the study include the rigour of sample collection in the setting of a large randomized control trial in which a rich data set of maternal variables and newborn anthropometric parameters were available. The lipidome of placental LD has been described for the first time. A limitation is the small sub-set of subjects who provided placenta samples (n=43) which limits the statistical power.

5. Conclusions

In summary we have described for the first time the FAs composition of placental LD. We conclude that the placental LD composition may be modestly modified by maternal life style. The observation of a lower proportion of DGLA LD PCs in the intervention arm, a FA associated with obesity and insulin resistance, could have repercussions for obesity in later life. We also propose that DHA transport may be influenced by placental size and that placenta LD may play a role in the transfer to the fetus of some specific FAs such as DGLA, ARA and DHA.

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Table 1. Characteristics of the mothers and neonates.

	Intervention (n = 20)	**Control (n = 23)**	***P***
Maternal			
Maternal pregestational BMI (Kg/m^2)	34.50 ± 5.08	33.78 ± 3.49	0.599
Gestational weight gain (Kg)	6.86 ± 1.15	8.59 ± 0.98	0.261
Dietary glycaemic index	52.95 ± 1.05	57.86 ± 0.88	**0.001**
Maternal TG (mmol/L)	1.86 ± 0.13	2.25 ± 0.13	**0.044**
Maternal total cholesterol (mmol/L)	6.15 ± 0.23	6.26 ± 0.22	0.732
Maternal LDL cholesterol (mmol/L)	3.54 ± 0.21	3.78 ± 0.19	0.397
Maternal HDL cholesterol (mmol/L)	2.18 ± 0.14	1.97 ± 0.09	0.188
Placental weight (g)	620.75 ± 149.03	650.43 ± 107.09	0.453
Caesarean section rate (%)	55.00	56.52	0.920
Infant			
Birth weight (g)	3289.75 ± 83.75	3590.43 ± 77.39	**0.012**
Birth length (cm)	49.99 ± 0.59	50.25 ± 0.90	0.826
Birth abdominal circumference (cm)	31.18 ± 0.46	32.47 ± 0.38	**0.036**
Birth mid arm circumference (cm)	11.20 ± 0.26	11.69 ± 0.23	0.172

Results are expressed as means ± SEM. 6mo, 6 months old follow up. Significantly different at $P < 0.05$.

Table 2. Phosphatidylcholines (PCs), sphingomyelins (SMs) [a] and lysophosphatidylcholines (LysoPCs) in placental lipid droplets from obese pregnant women randomized to a complex behavioural intervention, comprising nutrition and physical activity, during pregnancy or to routine antenatal care (control). Intervention: n=20, control: n=23.

%		**Intervention (n=20)**	**Control (n=23)**	***P***
PC				
PC.32:0	(16:0/16:0)	12.53 ± 0.61	11.44 ± 0.39	0.262
PC.32:1	(16:0/16:1)	1.64 ± 0.06	1.69 ± 0.08	0.647
PC.34:1	(16:0/18:1n-9)	9.02 ± 0.33	9.74 ± 0.30	0.107
PC.34:2	(16:0/18:2n-6)	11.39 ± 0.34	11.43 ± 0.40	0.947
PC.36:1	(18:0/18:1n-9)	1.34 ± 0.07	1.41 ± 0.05	0.417
PC.36:2	(18:0/18:2n-6)	4.92 ± 0.13	5.07 ± 0.17	0.492
PC.36:3	(16:0/20:3n-3)	6.34 ± 0.20	6.97 ± 0.21	**0.037**
PC.36:4	(16:0/20:4n-6)	17.28 ± 0.47	16.97 ± 0.47	0.638
PC.38:3	(18:0/20:3n-3)	1.33 ± 0.07	1.56 ± 0.08	**0.037**
PC.38:4	(18:0/20:4n-6)	6.55 ± 0.23	6.67 ± 0.11	0.617
PC.38:5	(18:0/20:5n-3) [§]	4.43 ± 0.13	4.58 ± 0.14	0.433
PC.38:6	(18:2/20:4n-6)	4.02 ± 0.14	3.93 ± 0.14	0.652
PC.40:6	(18:0/22:6n-3)	0.89 ± 0.04	0.94 ± 0.04	0.446
PC.32:0*	(16:0/16:0*)	1.02 ± 0.07	0.92 ± 0.04	0.235
PC.32:1*	(16:0/16:1n-9*)	0.93 ± 0.06	0.82 ± 0.03	0.117
PC.34:1*	(16:0/18:1n-9*)	1.21 ± 0.04	1.13 ± 0.03	0.136
PC.36:5*	(16:0/20:5n-3*) [†]	1.02 ± 0.03	0.96 ± 0.03	0.141
PC.38:5*	(18:0/20:5n-3*) [§]	0.98 ± 0.04	1.02 ± 0.03	0.417

PC.40:0* (16:0/24:0*)	1.84 ± 0.09	1.95 ± 0.07	0.352
PC.40:1* (16:0/24:1n-9*)	1.05 ± 0.05	0.96 ± 0.05	0.229
SM			
15:0	1.25 ± 0.08	1.21 ± 0.06	0.644
16:0	37.96 ± 0.76	37.06 ± 0.54	0.310
16:1n-7	3.58 ± 0.11	3.87 ± 0.09	**0.043**
17:0	0.94 ± 0.05	0.78 ± 0.04	**0.014**
18:0	4.71 ± 0.22	4.80 ± 0.13	0.753
18:1n-9	1.63 ± 0.09	1.75 ± 0.07	0.280
20:0	4.03 ± 0.28	4.19 ± 0.22	0.637
20:1n-9	2.14 ± 0.13	2.42 ± 0.11	0.115
22:0	12.61 ± 0.42	11.61 ± 0.38	0.080
22:1n-9	3.65 ± 0.18	4.10 ± 0.16	0.065
23:0	2.11 ± 0.09	2.20 ± 0.09	0.458
23:1n-9	0.96 ± 0.08	0.95 ± 0.05	0.916
24:0	9.61 ± 0.30	9.22 ± 0.29	0.354
24:1n-9	8.96 ± 0.23	9.49 ± 0.20	0.081
24:2n-6	4.04 ± 0.19	4.45 ± 0.17	0.111
SFA SMs	74.21 ± 0.58	72.11 ± 0.39	**0.003**
MUFA SMs	21.23 ± 0.45	22.89 ± 0.33	**0.003**
PUFA SMs	4.56 ± 0.20	5.00 ± 0.19	0.123
LysoPC			
16:0	29.83 ± 3.01	30.97 ± 2.69	0.775
18:0	11.25 ± 1.04	9.78 ± 0.72	0.226
18:1n-9	3.97 ± 0.72	6.83 ± 0.77	**0.011**

18:2n-6	14.59	±	1.75	13.25	±	1.59	0.567
20:3n-6	6.44	±	1.00	5.85	±	1.10	0.695
20:4n-6	24.73	±	3.12	24.52	±	2.31	0.954
20:5n-3	3.98	±	0.90	3.90	±	0.85	0.951
22:6n-3	5.20	±	0.71	4.91	±	0.68	0.760
SFA LysoPCs	41.08	±	3.58	40.75	±	3.08	0.944
MUFA LysoPCs	3.97	±	0.72	6.83	±	0.77	**0.011**
PUFA LysoPCs	54.95	±	3.67	52.42	±	3.10	0.591

[a]Only metabolites >0.9% content are listed. 20 PCs ester-linked, 25 PCs ether-linked and 5 SMs <0.9% are not listed. SFA, saturated fatty acids. Results are expressed as means ± SEM. *ether-linked PCs. [§]This metabolite can be also composed by 16:0/22:5 and [†]by 18:1/20:4. Significantly different at $P < 0.05$.

Table 3. Correlations between the same metabolites measured in placental lipid droplets (LD) and in cord blood.

Metabolite		**R**	***P***
PC.32:0	(16:0/16:0)	0.309	0.076
PC.32:1	(16:0/16:1n-9)	0.022	0.189
PC.34:1	(16:0/18:1n-9)	0.395	**0.021**
PC.34:2	(16:0/18:2n-6)	0.443	**0.009**
PC.36:1	(18:0/18:1n-9)	-0.005	0.978
PC.36:2	(18:0/18:2n-6)	0.336	0.052
PC.36:3	(16:0/20:3n-6)	0.598	**<0.001**
PC.36:4	(16:0/20:4n-6)	0.636	**<0.001**
PC.38:3	(18:0/20:3n-6)	0.348	**0.044**
PC.38:4	(18:0/20:4n-6)	0.695	**<0.001**
PC.38:6	(18:2/20:4n-6)	0.340	**0.049**
PC.40:6	(18:0/22:6n-3)	0.491	**0.014**
	LysoPC.16:0	0.125	0.480
	LysoPC.18:0	0.270	0.123
	LysoPC.18:1n-9	0.004	0.903
	LysoPC.18:2n-6	0.118	0.701
	LysoPC.20:3n-6	0.036	0.841
	LysoPC.20:4n-6	0.050	0.779
	LysoPC.22:6n-3	0.476	**0.004**

LysoPC, lyso-phosphatidylcholine; PC, phophatidylcholine. N=43. Statistically significant at $P < 0.05$.

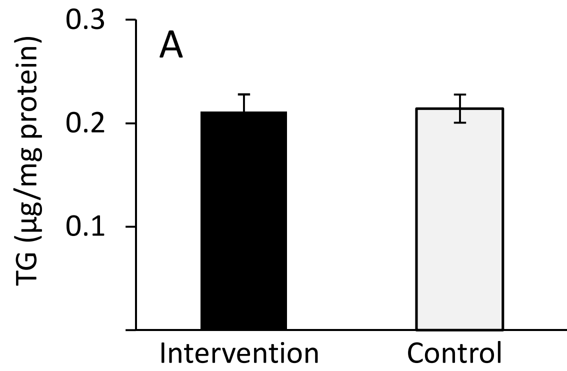
Fig. 1. A) TG and **B)** cholesterol content (Chol) of placental lipid droplets from obese pregnant women randomized to a complex behavioural intervention, comprising nutrition and physical activity, during pregnancy or to routine antenatal care (control). **C)** Correlation between total FAs and TG content in lipid droplets (Intervention: crosses, R= 0.418, *P*= 0.107; control: dots, R= 0.736, *P*< 0.001). Differences statistically significant when *P*<0.05. Mean ± SEM. Intervention: n=20, control: n=23. Total correlation analysis: n=43.

Fig. 2. Total FAs composition of placental lipid droplets (LD) from obese pregnant women randomized to a complex behavioural intervention, comprising nutrition and physical activity, during pregnancy or to routine antenatal care (control). Significant differences between groups, *P*<0.05. Means ± SEM. Only >1% FA are plotted, other 12 FAs were quantified (<1%): 18:3, 20:1, 20:2, 20:5, 22:3, 22:4, 22:5, 24:3, 24:4, 24:5, 26:1 and 26:3. Intervention: n=20, control: n=23.

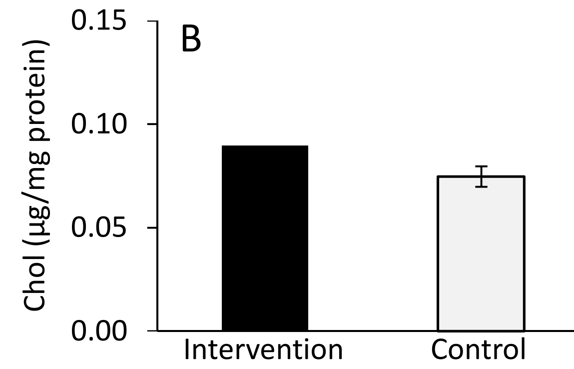
Fig. 3. Correlation between placental weight and lipid droplets metabolites: **A)** PC composed of stearic acid (SA, 18:0) and arachidonic acid (ARA, 20:4n-6) (Intervention: R= -0.409, *P*= 0.103; control: R= -0.326, *P*= 0.129), **B)** PC composed of linoleic acid (LA, 18:2n-6) and ARA (Intervention: R= -0.513, *P*= 0.035; control: R= -0.589, *P*= 0.003), **C)** PC composed of SA and docosahexaenoic acid (DHA, 22:6n-3) (Intervention: R= -0.396, *P*= 0.116; control: R= -0.444, *P*= 0.038). Intervention: crosses (n=20), control: dots (n=23). Significance, *P*<0.05. Total correlation analysis: n=43.

Fig. 4. A) MFSD2a expression (arbitrary units) in placenta of obese pregnant women participating in a randomized controlled trial of a complex behavioural intervention, **B**) correlation between MFSD2a expression and total FAs content of placental lipid droplets (LD) (Intervention: R= -0.451, *P*= 0.091; control: R= -0.363, *P*= 0.089), and **C**) cord blood phosphatidylcholine (PC) carrying stearic (SA, 18:0) and DHA (22:6n-3) (Intervention: R= 0.358, *P*= 0.173; control: R= 0.299, *P*=

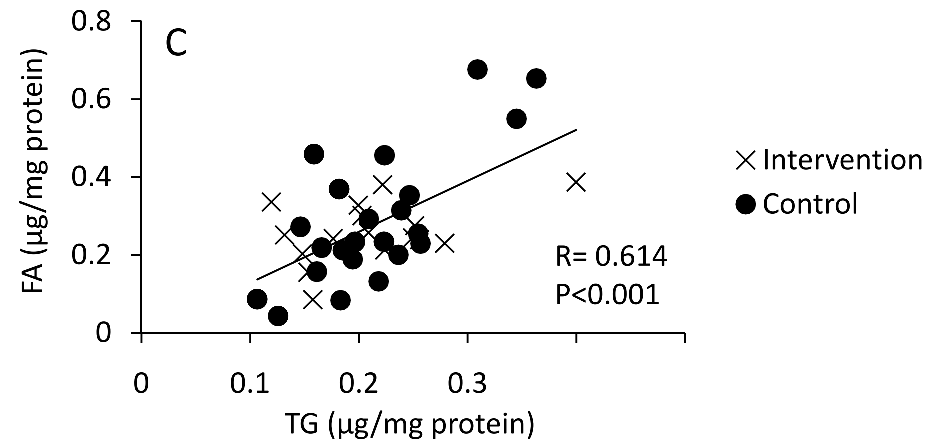
0.201). Intervention (n=20): crosses, control (n=23): dots. Total correlation analysis: n=43. Significance, $P<0.05$. N=43.



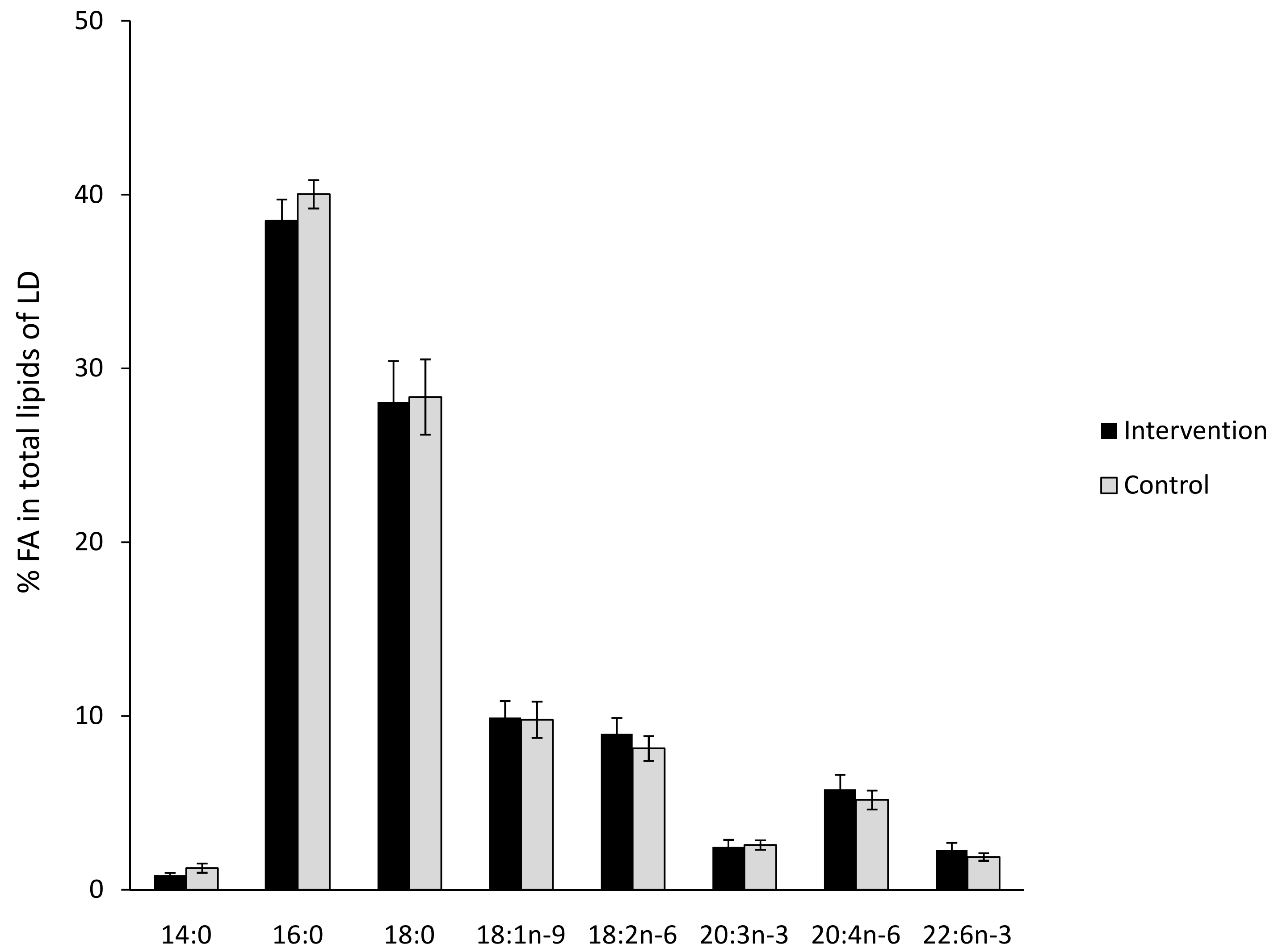
A
TG (µg/mg protein)
0.3
0.2
0.1
Intervention
Control

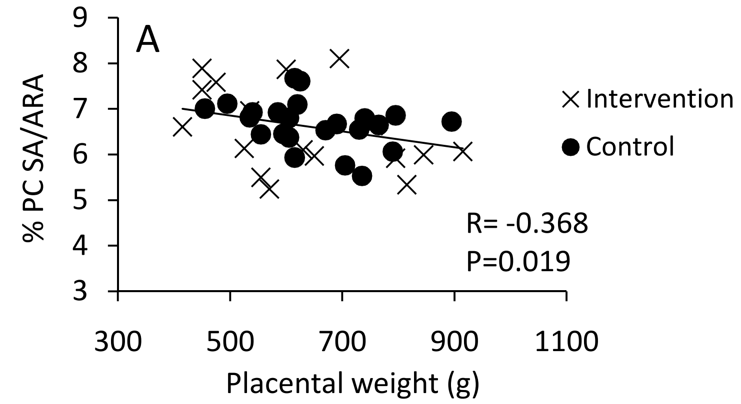


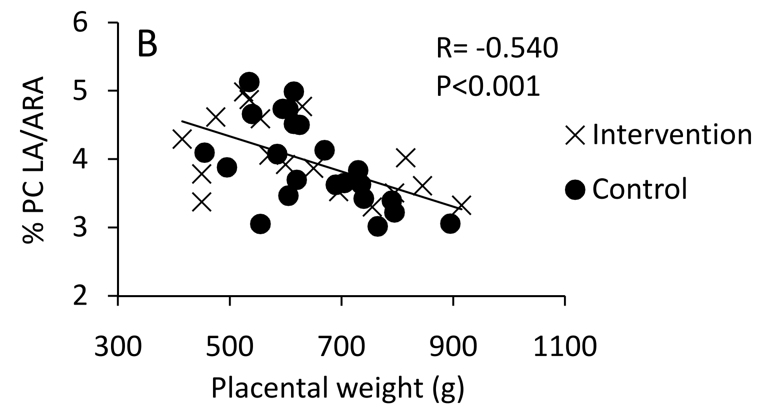
B
Chol (µg/mg protein)
0.15
0.10
0.05
0.00
Intervention
Control

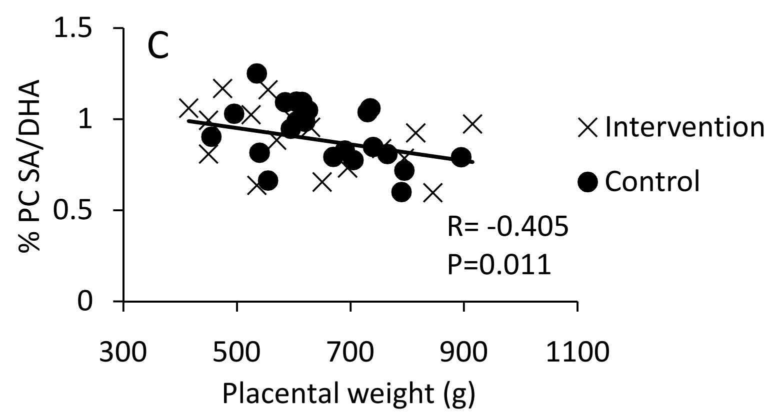


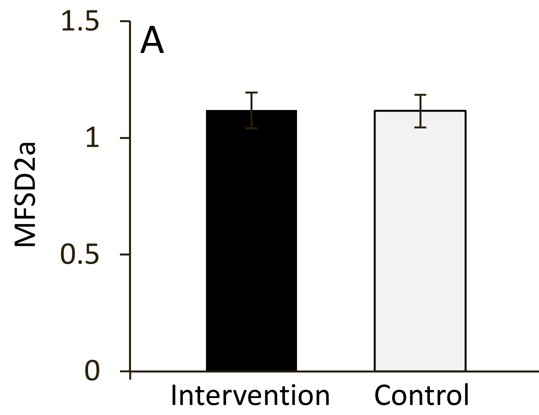
C
FA (µg/mg protein)
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
TG (µg/mg protein)
R= 0.614
P<0.001
Intervention
Control

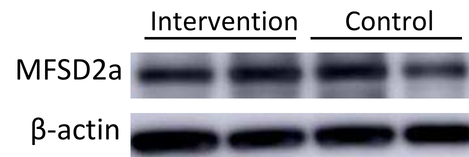

% FA in total lipids of LD
50
40
30
20
10
0
14:0
16:0
18:0
18:1n-9
18:2n-6
20:3n-3
20:4n-6
22:6n-3
Intervention
Control

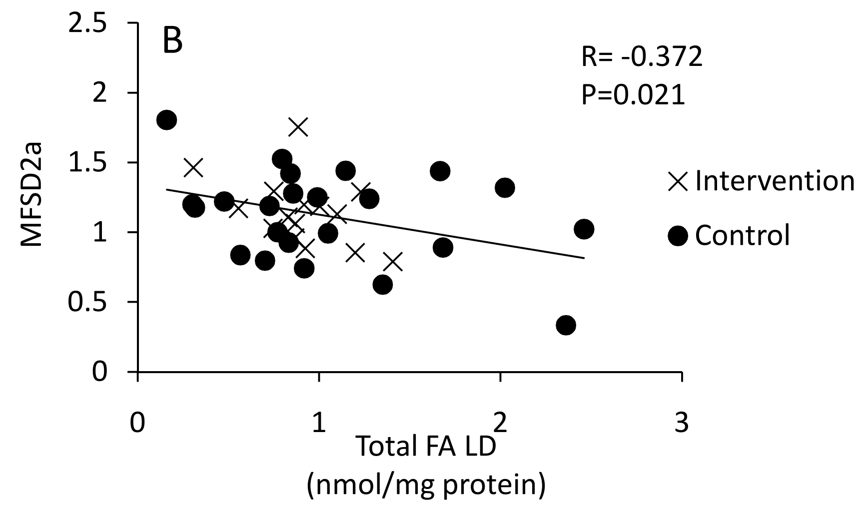

A
% PC SA/ARA
Placental weight (g)
Intervention
Control
R= -0.368
P=0.019
9
8
7
6
5
4
3
300
500
700
900
1100

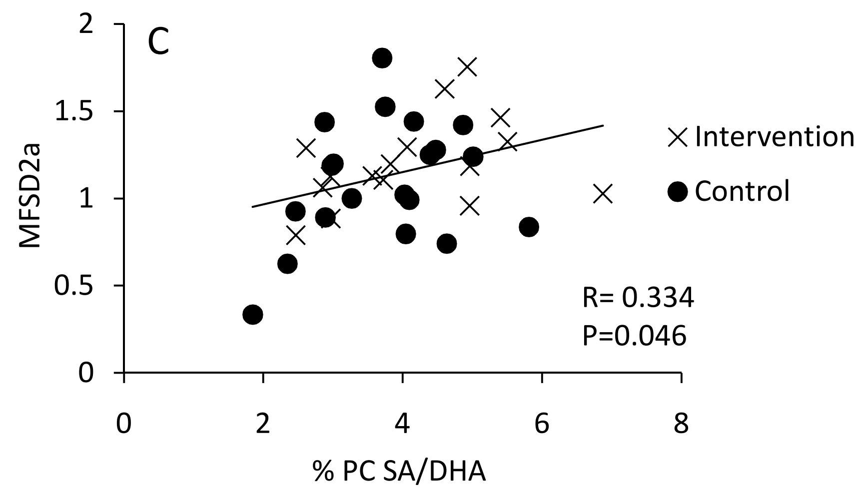

B
% PC LA/ARA
Placental weight (g)
R= -0.540
P<0.001
Intervention
Control
6
5
4
3
2
300
500
700
900
1100


C
% PC SA/DHA
Placental weight (g)
Intervention
Control
R= -0.405
P=0.011
1.5
1
0.5
0
300
500
700
900
1100


A
MFSD2a
1.5
1
0.5
0
Intervention
Control


Intervention
Control
MFSD2a
β-actin


B
R= -0.372
P=0.021
MFSD2a
2.5
2
1.5
1
0.5
0
0
1
2
3
Total FA LD
(nmol/mg protein)
Intervention
Control


C
MFSD2a
2
1.5
1
0.5
0
0
2
4
6
8
% PC SA/DHA
R= 0.334
P=0.046
Intervention
Control