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Randomised Controlled Trial
Basic science

The unexplored role of sedentary time and physical activity in glucose and lipid metabolism-related placental mRNAs in pregnant women who are obese: the DALI lifestyle randomised controlled trial

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Objective We aimed to explore: (i) the association of sedentary time (ST) and physical activity (PA) during pregnancy with the placental expression of genes related to glucose and lipid metabolism in pregnant women who are obese; (ii) maternal metabolic factors mediating changes in these placental transcripts; and (iii) cord blood markers related to the mRNAs mediating neonatal adiposity.

Design Multicentre randomised controlled trial.

Setting Hospitals in nine European countries.

Population A cohort of 112 pregnant women with placental tissue.

Methods Both ST and moderate-to-vigorous PA (MVPA) levels were measured objectively using accelerometry at three time periods during pregnancy.

Main outcome measures Placental mRNAs (FATP2, FATP3, FABP4, GLUT1 and PPAR-γ) were measured with NanoString technology. Maternal and fetal metabolic markers and neonatal adiposity were assessed.

Results Longer periods of ST, especially in early to middle pregnancy, was associated with lower placental FATP2 and FATP3 expression ($P < 0.05$), whereas MVPA at baseline was inversely associated with GLUT1 mRNA ($P = 0.02$). Although placental FATP2 and FATP3 expression were regulated by the insulin–glucose axis ($P < 0.05$), no maternal metabolic marker mediated the association of ST/MVPA with placental mRNAs ($P > 0.05$).

Clinical trial registration: ISRCTN70595832.

Additionally, placental FATP2 expression was inversely associated with cord blood triglycerides and free fatty acids (FFAs; $P < 0.01$). No cord blood marker mediated neonatal adiposity except for cord blood leptin, which mediated the effects of PPAR-γ on neonatal sum of skinfolds ($P < 0.05$).

Conclusions In early to middle pregnancy, ST is associated with the expression of placental genes linked to lipid transport. PA is hardly related to transporter mRNAs. Strategies aimed at reducing sedentary behaviour during pregnancy could modulate placental gene expression, which may help to prevent unfavourable fetal and maternal pregnancy outcomes.

Keywords Fatty acid, fetal development, gene expression, gestation, gestational diabetes mellitus, nutrient transport, placental development.

Tweetable abstract Reducing sedentary behaviour in pregnancy might modulate placental expression of genes related to lipid metabolism in women who are obese.

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What is already known?

- Obesity during pregnancy is associated with disrupted placental development and function, which can lead to pregnancy complications and future maternal and child diseases (e.g. obesity and type-2 diabetes mellitus).
- Improving lifestyle behaviours (i.e. reducing sedentary time, ST, and increasing physical activity, PA) are powerful stimuli with the capacity to counteract some disruptions during pregnancy.
- Evidence regarding the role of ST and moderate-to-vigorous physical activity (MVPA) during pregnancy on placental transcripts related to glucose and lipid metabolism in pregnant women who are obese is lacking.

What are the key questions?

- Is reducing ST and increasing MVPA effective for modulating placental gene expression related to glucose and lipid metabolism in women who are obese?
- Which maternal metabolic factors related to lifestyle mediate the placental transcript changes involved in glucose and lipid metabolism?
- Which cord blood markers related to specific mRNAs mediate neonatal adiposity?

What are the new findings?

- ST in earlier pregnancy periods is inversely associated with term placenta fatty acid transport protein 2 (FATP 2) and FATP3 mRNA expression.
- MVPA during pregnancy has little if any effect on placental mRNAs.
- Placenta FATP2 and FATP3 expression appear to be regulated by the maternal insulin–glucose axis.
- No maternal metabolic factor mediates the relationship of ST or MVPA with transporter mRNAs.
- Placenta FATP2 expression is inversely associated with cord blood triglycerides and free fatty acids (FFAs, only in female fetuses), but not with neonatal adiposity. Neither triglycerides nor FFA mediate neonatal adiposity.
- No cord blood metabolite examined mediates neonatal adiposity, except for cord blood leptin.
- An increased sum of skinfold in neonates from women characterised by greater placental PPAR-γ expression is partially explained via higher cord blood leptin.

Introduction

The placenta is a multifunctional organ that regulates key aspects of pregnancy maintenance and fetal development.[1–4] Under pathological conditions, such as obesity and gestational diabetes mellitus (GDM), placental metabolism is often dysregulated.[1,2,58] Impaired placental development and function, especially in early pregnancy, is closely related to pregnancy complications and future maternal and child diseases, such as obesity and type-2 diabetes mellitus.[1,2,4–6]

Unfortunately, the mechanisms connecting an obesogenic intrauterine environment to short- and long-term consequences in the offspring have remained elusive.[5,9,10] Previous literature has emphasised that maternal obesity is associated with changes in the expression and activity of placental transporters such as glucose transporter 1 (GLUT1),[9,11,12] fatty acid transport protein 2 (FATP 2),[9,11] FATP3[13] and fatty acid binding protein 4 (FABP4).[9,14,15]

Interestingly, GLUT1, which is the main placental glucose transporter, and FATP2, FATP3 and FABP4, which are relevant proteins for cellular free fatty acid (FFA) uptake and intracellular transport, associate with excessive fat accumulation in offspring born to women who are obese.[9–12,14,15] Peroxisome proliferator-activated receptor gamma (PPAR-γ) is the master regulator of fatty acid-related transcripts, including FATP2, FATP3 and FABP4.[14,16–18] It is associated with maternal obesity,[9,13] and also plays a fundamental role in fatty acid metabolism, adipogenesis and, hence, in fetal development.[9,14,18] Thus, obesity-related changes of these placental transporter isoforms could potentially alter placental uptake and, by inference, the transport of nutrients into the placental–fetal circulation, thereby contributing to suboptimal fetal growth (e.g. fetal overgrowth).

Lifestyle behaviours, i.e. sedentary time (ST) and physical activity (PA), can counteract some obesity-related metabolic disruptions during pregnancy,[6,19–22] and can modulate concentrations of relevant maternal and cord serum molecules.[20,23–27] However, there is a paucity of evidence about the influence of lifestyle on placental transporters and the underlying mechanisms. The few studies exploring this issue have shown that PA modulates the expression of placental molecules involved in glucose, fatty acid, amino acid and water transport,[28–30] and in insulin and mTOR signalling.[30] Nonetheless, these studies were limited to the middle period of gestation and by scarce sample size, and did not explore by which mechanisms ST or PA could alter the expression of placental genes or impact fetal health.

Of note, in previous analyses of the DALI (Vitamin D And Lifestyle Intervention for Gestational Diabetes Mellitus Prevention) lifestyle trial we have shown that ST mediates intervention effects on offspring adiposity.[31] Whether placental transport of glucose and placental lipid uptake and metabolism are involved in the negative association of ST with neonatal adiposity in women who are obese remains unknown. As improving lifestyle behaviours may represent a promising strategy to prevent placental dysregulation and inadequate fetal development in pregnant women who are obese, this information is imperative to guide clinical practice.

Therefore, the main aim of the current study was to analyse the associations of ST and PA levels during pregnancy with the placental expression of GLUT1, as well as PPAR-γ and its downstream targets FATP2, FATP3 and FABP4, in women who are obese. Our secondary study aims were to explore which maternal metabolic factors mediate lifestyle-induced changes in these placental transcripts, and which cord blood metabolites related to these placental mRNAs mediate neonatal adiposity.

Methods

Study design and population

The DALI lifestyle study was a multicentre randomised controlled trial (RCT) characterised by a 2 × 2 factorial design and performed in nine European countries (Austria, Belgium, Denmark, Ireland, Italy, Netherlands, Poland, Spain and the UK) between 2012 and 2015. The study was prospectively registered as an RCT in November 2011 (ISRCTN70595832) and was individually approved by local clinical research ethics committees in each country. All pregnant women aged ≥18 years with a pre-pregnancy body mass index (BMI) of ≥29 kg/m^2 (eligible for inclusion) provided signed informed consent. Women diagnosed at baseline with GDM using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria, with pre-existing diabetes, psychiatric diseases or other chronic medical conditions, or requiring complex diets were excluded from the trial. The rationale, along with all procedures and inclusion–exclusion criteria of the DALI lifestyle study have been described elsewhere.[32]

Sample size

The required sample size for the main DALI trial was determined for the primary outcomes (gestational weight gain, fasting glucose and insulin resistance in late pregnancy) but, given the exploratory nature of this work, not for the secondary outcomes analysed in this study.

Randomisation and lifestyle counselling intervention

Pregnant women were randomly (computerised random number generator, pre-stratified for site) allocated to the healthy eating (HE), PA, HE&PA or control groups. Briefly, HE and PA counselling interventions consisted of five face-to-face and four telephone individual booster coaching sessions, between baseline and 35 weeks of gestation, to promote HE and/or PA habits (Figure S1). Additional information about the lifestyle interventions can be found in Appendix S1. The professionals who were involved in assessments were blinded to the intervention.

Procedures

Women were assessed three times by the research midwife or nurse (at baseline, <20 weeks of gestation, at 24–28 weeks of gestation and at 35–37 weeks of gestation). At

baseline, sociodemographic and clinical characteristics, body composition, anthropometry and sleep patterns were assessed by questionnaire, and women underwent a 75-g oral glucose tolerance test, with samples taken at 0, 60 and 120 minutes. Before leaving, women were given an activity log and an accelerometer along with a food diary, to assess ST and PA levels and nutritional intake, respectively. After these baseline measurements, women were randomised, and the lifestyle intervention was started. At 24–28 and at 35–37 weeks of gestation, the same assessments were performed. At delivery, placental tissue and cord blood samples were taken and processed within 30 minutes of the birth, and data related to the delivery were obtained from perinatal obstetric records. After delivery (<48 h), maternal and neonatal measurements were performed. The assessment procedures are detailed further elsewhere.[32]

Exposures, outcomes and confounding factors

Sociodemographic clinical data, obstetric history and other outcomes

Sociodemographic (e.g. age, ethnicity) and clinical (e.g. pre-existing conditions, comorbidities, medications) data, reproductive history, adverse events for mother and for neonate, and tobacco, alcohol and sleep habits were obtained from questionnaires and medical files.

Maternal body weight and height

Pre-pregnancy weight was self-reported. Maternal body weight was measured twice (no shoes, light clothes) at each time point by calibrated electronic scales (SECA 888, SECA 877; SECA, Hamburg, Germany). Maternal weight gain was defined as the weight change from baseline to 35–37 weeks of gestation. Height was measured once at baseline with stadiometers (SECA 206). Body mass index (BMI) was calculated: weight (kg)/height $(m)^2$.

Dietary habits

The frequency and quantity of specified foods were collected with a 12-item list (fish, vegetables, fruit, fruit juice, potatoes/pasta, wholegrain bread, meat/eggs, high-fat milk products, non-diet soft drinks, cakes/muffins, fast food and breakfast) and a 3-day food diary.[32] These data were used to estimate the servings per week of foods rich in fibre, protein, fat and carbohydrates.[32]

Sedentary time and physical activity

The ST and PA levels at <20, 24–28 and 35–37 weeks of gestation were objectively measured with uniaxial/triaxial accelerometers (GT3X+ or GT1M; ActiGraph, Pensacola, FL, USA) using an epoch length of 60 seconds and a sampling frequency of 60–80 Hz. Women wore the devices at their waist for at least 3 days during sleeping and waking hours (except for water-based activities). A minimum register of 3 days (two weekdays and one weekend day, for 8 hours per day) was required to be included in the analyses. Considering the information entered in the activity logs by the participants, 'accelerometer wear time' was estimated by deducting the non-wear and sleeping time from the time registered during the whole day. The time spent in sedentary, light and moderate to vigorous PA (MVPA) behaviours was calculated based on the vertical axis cut points of ≤100, 101–1951, ≥1952 counts/minute, respectively, provided by Freedson et al., and was expressed in minutes/day.[33] Data download, cleaning and analyses were performed using ACTILIFE 6.8.1 (ActiGraph).

Neonatal adiposity

Triceps, subscapular, supra-iliac and quadriceps skinfold thicknesses were measured (within 48 hours after birth) and the values were summed.[31] All skinfold measurements were performed twice.

Laboratory methods

Placental tissues collection

Placental tissue was sampled following recommendations from previous research,[34,35] with some modifications. At delivery, placental biopsies were collected from each of the four quadrants from the central part in relation to the cord insertion (avoiding areas of necrosis and calcification). Each of these placental biopsies was equally divided into maternal and fetal parts by cutting them into two pieces in the middle of the villous tissue (similarly to Roberts et al.).[36] Tissue from the decidua basalis and chorionic plate were avoided. The two pieces of remaining villous tissue were stored in cryotubes filled with RNA-later (Sigma-Aldrich, St. Louis, MO, USA) and kept at 4°C for at least 24 hours to allow the RNA-later to fully penetrate the tissue. Thereafter, these cryotubes were stored in a freezer held at −20°C until being shipped to the central lab in Graz for analyses.

RNA isolation

After removing RNA-later at the central lab, two pieces, one from the maternal and one from the fetal side (approx. 20 mg per piece), were pooled. Subsequently, 700 μL of Quiazol was added to each pooled sample, and then the tissue was homogenised using the MagNa Lyser Instrument (two or three runs, 6500 rpm, 20 seconds; Roche, Basel, Switzerland). Standard procedures were performed with the miRNeasy Mini Kit (#217004; Qiagen, Hilden, Germany) for RNA isolation and DNAse digestion in the lysates. RNA concentration and quality were determined using the QIAexpert System (Qiagen) and Agilent 2100 Bioanalyser System (Agilent, Santa Clara, CA, USA), respectively. An RNA integrity number of ≥4 for lysates was required to be included in the analyses.

Gene expression analysis by the nCounter system

Overall, the quantification of the different placental mRNAs (FATP2, FATP3, FABP4, GLUT1 and PPAR-γ) was performed with molecular counting using the NanoString nCounter Analysis Technology (NanoString Technologies, Seattle, WA, USA). The probes for the genes investigated were part of a customised CodeSet with 24 probes in total (nCounterTM PlexSetTM), including probes for three validated reference genes, *ornithine decarboxylase antizyme 1* (*OAZ1*), *WD repeat-containing protein 45-like* (*WDR45L*) and *tata-box-binding protein* (*TBP*), which was used for hybridisation according to the manufacturer's protocol (Appendix S2). A total of 490 ng of RNA per sample was applied for hybridisation. Quality control and normalisation was performed using the NanoString analysis software NSOLVER 4.0 (NanoString Technologies).

Blood samples – laboratory analyses

Maternal blood was collected at baseline, 24–28 and 35–37 weeks of gestation. In maternal fasting blood samples, plasma glucose, insulin, glycated haemoglobin (HbA1c), insulin, lipids (triglycerides, FFA, total cholesterol and high- and low-density lipoprotein cholesterol, HDL-C and LDL-C) and leptin concentrations were measured. Cord blood (from placental chorionic vessels) was processed within 30 minutes of birth. From cord venous blood samples, plasma glucose, C-peptide, the aforementioned lipids and leptin were determined. All of the analytes were quantified by conventional clinical chemistry methods, except for insulin and leptin (quantified by enzyme-linked immunosorbent assay, ELISA).

Insulin resistance and beta-cell function

As proxy measures of insulin resistance and beta-cell function, the homeostasis model assessment (HOMA)-IR and HOMA-B were calculated, respectively, according to standard formulas.[37]

Statistical analysis

Descriptive statistics were performed to show the characteristics (Table 1) and the ST and PA levels of participants during pregnancy (Table 2).

The few influential outliers found for some outcome variables were adjusted (Appendix S3). Subsequently, Box-Cox transformations were used for models characterised by asymmetry in the placental mRNAs. Interaction between offspring sex and the independent/predictor variables (intervention and ST/MVPA) was assessed in linear regression analyses. Afterwards, multilevel analyses were used to account for the clustering effect of the different countries. All multilevel analyses were based on a two-level hierarchy (country and individual), with random intercept and slope.

Table 1. Sociodemographic and clinical characteristics of pregnant women (n = 112)

Maternal age (years)	32.7	5.3
Gestational age (weeks)		
At baseline	14.7	2.3
At delivery	39.7	1.3
Ethnicity, *n* (%)		
Maternal European descent	85	(75.9)
Living with a partner, *n* (%)	108	(96.4)
High educational level, *n* (%)	62	(55.4)
Working, *n* (%)	91	(81.3)
Body composition		
BMI pre-pregnancy (kg/m^2)	33.6	3.9
GWG, baseline to 35–37 weeks of gestation (kg)	8.1	4.6
Dietary behaviour (baseline) (n = 107)		
Fibre (number consumed per week)	29.5	(20.3, 42.8)
Protein (number consumed per week)	7	(5, 12)
Fat (number consumed per week)	4	(2, 8)
Carbohydrates (number consumed per week)	39	(26, 58)
Multiparous, *n* (%)	56	(50)
Female offspring sex, *n* (%)	55	(49.1)
Active smoking at baseline, *n* (%)	15	(13.4)
Developed GDM during pregnancy, *n* (%)	40	(37.7)
Placental weight (g) (n = 103)	634.4	151.4
Weight of the neonate (g)	3540.9	500.4
Neonatal sum of skinfolds (mm) (n = 103)	20.4	4.4
Cord blood parameters (n = 89)		
C-peptide (μg/L)	0.7	(0.5, 0.9)
Glucose (mmol/L)	4.6	(3.6, 5.4)
Triglycerides (mmol/L)	0.4	(0.3, 0.7)
Free fatty acids (mmol/L)	0.3	(0.2, 0.4)
Leptin (μg/L)	8.5	(4.2, 12.4)

BMI, body mass index; GDM, gestational diabetes mellitus; GWG, gestational weight gain. Continuous variables are presented as means with standard deviations or medians (interquartile ranges), unless otherwise indicated.

To address the first aim, linear regression analyses (multilevel models) were employed to assess the effects of a PA counselling intervention on placental mRNAs (per-protocol basis; Table S1). Multilevel linear regression analyses were also used to examine the association of ST and PA levels at the different time points (baseline, 24–28 and 35–37 weeks of gestation), and changes in ST and PA levels (from baseline to 24–28 and 35–37 weeks of gestation), with the levels of placental mRNAs (Table 3).

To achieve our secondary aims, linear regression, moderation and mediation analyses (Appendix S4) were used to explore maternal lifestyle-related metabolic factors mediating placental transcript changes and measured mRNA-related cord blood markers mediating neonatal adiposity (Figures S3–S6; Tables 4, 5, S2–S11).

Table 2. Sedentary time and physical activity levels during pregnancy (n = 112)

Sedentary time and PA levels		
Baseline (<20 weeks of gestation), n = 112		
Sedentary time (minutes/day)	577.7	(102.5)
MVPA (minutes/day)	40.0	(24.3, 56.0)
Relative percentage of daily sedentary time (%)	71.2	(64.8, 79.4)
Relative percentage of daily MVPA (%)	4.6	(3.0, 6.8)
24–28 weeks of gestation, n = 88		
Sedentary time (min/day)	596.8	(100.9)
MVPA (min/day)	39.0	(24.2, 56.9)
Relative percentage of daily sedentary time (%)	72.8	8.4
Relative percentage of daily MVPA (%)	4.6	(2.9, 7.0)
35–37 weeks of gestation, n = 79		
Sedentary time (min/day)	593.2	(102.1)
MVPA (min/day)	31.2	(16.8, 46.8)
Relative percentage of daily sedentary time (%)	74.0	(7.1)
Relative percentage of daily MVPA (%)	4.3	(2.8)
Changes in sedentary time and PA levels from baseline		
24–28 weeks minus baseline, n = 72		
Sedentary time (min/day)	12.4	(88.5)
MVPA (min/day)	−2.4	(25.4)
35–37 weeks minus baseline, n = 64		
Sedentary time (min/day)	−12.6	(−65.0, 67.2)
MVPA (min/day)	−13.9	(−27.9, 4.6)

PA, physical activity; MVPA, moderate-to-vigorous physical activity. Data are means (standard deviations) or medians (interquartile ranges: Q1, Q3).

Potential confounding factors identified from previous literature that modified the relationship between the predictors and outcomes (change in the regression coefficient >15%) were included in the models. Specifically, the main cofounding factors included in the analyses (specified in each table) besides site were: the intervention group; gestational week at delivery; smoking at baseline; the relative percentage of daily ST, i.e. (ST/accelerometer wearing time) * 100, when analysing MVPA, or MVPA (when exploring ST); and the use of prostaglandins at delivery (induction of labour).[38] All the assumptions related to the generalisation of the results were met. The statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). The statistical level of significance was set at $P \leq 0.05$. False discovery rate corrections were made using the Benjamini–Hochberg step-up procedure.[39]

Patient and public involvement

The counselling interventions were designed considering women's needs from previous projects. Women were involved in their development. Once all data were processed, women were delivered 'assessment informs'. The dissemination of the results was performed online.

Results

Placental mRNAs were analysed in a subsample of the DALI cohort (n = 112 for 'ST and PA' analyses, n = 183 for 'intervention and secondary' analyses; see flow chart in Figure S1). Sociodemographic and clinical characteristics, and ST and MVPA levels of the participants during pregnancy, are shown in Tables 1 and 2. The baseline characteristics of the women included in this study and in the entire DALI lifestyle cohort were similar. The expression of placental genes and their bivariate Pearson correlations are shown in Figure S2. Gene expression was similar between women who developed GDM and those who did not ($P > 0.05$; data not shown).

Associations of sedentary time and physical activity with placental mRNAs

No differences in placental mRNAs were found between the HE&PA, HE or PA groups, compared with the control group (Table S1; all $P > 0.05$). Given that there were no differences between groups, all intervention groups were combined into one cohort to assess the associations between ST and MVPA with placental mRNAs. This provides the opportunity to explore greater variation in placental mRNAs and increases the statistical power.

The associations of ST and MVPA levels at each time point, and the absolute changes in ST and MVPA levels from baseline to either 24–28 or 35–37 weeks of gestation with placental mRNAs, are shown in Table 3. Notably, most of the significant associations were found with ST, and not with MVPA. After adjusting for confounding factors (model 2), ST at baseline was inversely associated with FATP2 and FATP3 mRNA levels (P = 0.03 and P = 0.05). At 24–28 or 35–37 weeks of gestation, no statistically significant associations were found, except for PPAR-γ mRNA, which showed evidence of statistical significance with ST at 24–28 weeks of gestation (P = 0.06). The change in ST from baseline to 24–28 weeks of gestation was inversely associated with FABP4 mRNA (model 2, P = 0.05). From baseline to 35–37 weeks of gestation, the change in ST showed an inverse association with GLUT1 mRNA (model 1, P = 0.01), which was not significant after adjusting for additional confounding factors (model 2).

The MVPA level at baseline was inversely associated with GLUT1 mRNA (model 1, P = 0.01), but not when additionally adjusted for ST (model 2). No further associations with (changes in) MVPA were found.

Overall, no potential effect modification by fetal sex was found. None of these results changed when multilevel analyses were adjusted in a stepwise manner for the relative

Table 3. Linear regressions assessing the association of objectively measured sedentary time and physical activity levels at each time point with targeted placental transporters mRNAs (n = 112)

Placental mRNAs	Sedentary time and PA levels (min/day)	Baseline <20 weeks (n = 112)				24–28 weeks (n = 88)				35–37 weeks (n = 79)			
		Model 1			Model 2 (n = 97)	Model 1			Model 2 (n = 80)	Model 1			Model 2 (n = 70)
		B	SE	*P*	*P*	B	SE	*P*	*P*	B	SE	*P*	*P*
PPAR-γ (au)[c]	Sedentary time	0.000	0.000	0.63	0.27	−0.001	0.000	**0.05**	**0.06**	0.000	0.000	0.56	0.45
	MVPA	−0.001	0.002	0.62	0.19	0.000	0.002	0.96	0.62	0.000	0.002	0.96	0.94
FATP2 (au)	Sedentary time	−0.001	0.001	**0.05**	**0.03**	−0.002	0.001	0.06	0.31	−0.001	0.001	0.29	0.48
	MVPA	0.002	0.003	0.48	0.48	−0.001	0.003	0.82	0.35	0.003	0.004	0.54	0.78
FATP3 (au)	Sedentary time	−0.002	0.001	0.09	**0.05**	−0.002	0.002	0.30	0.13	0.000	0.002	0.92	0.55
	MVPA	0.002	0.006	0.78	0.43	−0.006	0.006	0.33	0.28	−0.003	0.007	0.67	0.36
FABP4 (au)[abc]	Sedentary time	0.001	0.001	0.56	0.41	−0.001	0.001	0.43	0.89	0.000	0.001	0.78	0.34
	MVPA	0.000	0.004	0.94	0.40	0.001	0.004	0.91	0.97	−0.009	0.005	0.06	0.08
GLUT1 (au)[ac]	Sedentary time	0.001	0.000	0.10	0.67	0.000	0.000	0.73	0.35	0.000	0.000	0.77	0.37
	MVPA	−0.005	0.002	**0.01**	0.14	−0.002	0.002	0.23	0.43	−0.003	0.002	0.20	0.21

Placental mRNAs	Changes in sedentary time and PA levels (min/day)	Baseline to 24–28 weeks (n = 72)				Baseline to 35–37 weeks (n = 64)			
		Model 1			Model 2	Model 1			Model 2
		B	SE	*P*	*P*	B	SE	*P*-value	*P*-value
PPAR-γ (au)[de]	Sedentary time	−0.001	0.001	0.10	**0.10**	0.000	0.001	0.81	0.69
	MVPA	0.000	0.002	0.85	0.89	0.000	0.002	0.86	0.82
FATP2 (au)	Sedentary time	0.000	0.001	0.88	0.70	0.000	0.001	0.77	0.40
	MVPA	0.001	0.003	0.68	0.45	−0.001	0.004	0.89	0.52
FATP3 (au)	Sedentary time	0.000	0.002	0.79	0.58	0.001	0.002	0.60	0.97
	MVPA	−0.011	0.006	0.09	0.27	−0.011	0.007	0.15	0.11
FABP4 (au)[de]	Sedentary time	−0.002	0.001	0.08	**0.05**	−0.001	0.001	0.47	0.39
	MVPA	−0.001	0.005	0.78	0.76	−0.004	0.005	0.45	0.61
GLUT1 (au)[de]	Sedentary time	−0.001	0.001	0.15	0.25	−0.001	0.001	**0.01**	0.10
	MVPA	0.000	0.002	0.88	0.76	0.002	0.002	0.45	0.49

Abbreviations: B, unstandardised regression coefficient; SE, standard error. Winsorizing of extreme outliers and/or optimum Box-Cox transformations were performed on mRNAs at different time-point analyses (baseline[a], 24–28 weeks of gestation[b], 35–38 weeks of gestation[c], changes between baseline and 24–28 weeks of gestation[d], and changes between baseline and 35–37 weeks of gestation[e]). Model 1 was adjusted for the intervention group, smoking at baseline and gestational week at delivery. In the single time-point analyses, model 2 was additionally adjusted for the relative percentage of daily ST or MVPA at the respective time point, and the requirement of prostaglandins at delivery. In the analyses of changes from baseline, model 2 was adjusted for the intervention group, gestational age at delivery and the relative percentage of ST (35–37 weeks of gestation) or MVPA (baseline). Numbers in bold indicate statistical significance *$P < 0.05$.

Table 4. Simple mediation analyses assessing the potential role of glycaemic and lipid parameters (at 24–28 and 35–37 weeks of gestation) as mediators of the relationship between sedentary time and physical activity levels with placental mRNAs (n = 112)

Outcome (Y)	Predictor (X)	Mediator (M)	Effect of the predictor on the mediator (a-path)			Effect of mediator on the outcome (b-path)			Direct effect of the predictor on the outcome (c′-path)			Indirect effect (path a*b)		
			B	95% CI		B	95% CI		B	95% CI		B	95% CI	
FATP2	ST (baseline)	Insulin (24–28 weeks)	0.013	−0.004	0.030	−0.009	−0.030	0.001	−0.001	−0.003	0.000	−0.000	−0.000	0.000
		HOMA-IR (24–28 weeks)	0.003	−0.001	0.007	−0.049	−0.127	0.030	−0.001	−0.003	0.000	−0.000	−0.001	0.000
FATP3	ST (baseline)	Glucose (24–28 weeks)	0.000	−0.000	0.001	**−0.860**	**−1.620**	**−0.091**	−0.002	−0.004	0.001	−0.000	−0.001	0.000
		HbA1c (24–28 weeks)	−0.000	−0.001	0.000	0.503	−0.292	1.298	−0.003	−0.005	0.000	−0.000	−0.001	0.000
		HOMA-B (24–28 weeks)	0.397	−0.361	1.156	**0.001**	**0.000**	**0.001**	−0.002	−0.004	0.001	0.000	−0.000	0.001
		Triglycerides (35–37 weeks)	−0.000	−0.002	0.001	0.257	−0.132	0.647	−0.002	−0.005	0.001	−0.000	−0.001	0.001
GLUT1[a]	MVPA (baseline)	Glucose (24–28 weeks)	0.002	−0.001	0.004	0.139	−0.104	0.383	**−0.005**	**−0.008**	**−0.001**	0.000	−0.000	0.001
		Insulin (24–28 weeks)	**−0.083**	**−0.156**	**−0.001**	0.002	−0.008	0.012	**−0.004**	**−0.008**	**−0.000**	−0.000	−0.001	0.001
		HDL-C (24–28 weeks)	0.002	−0.001	0.004	**0.350**	**0.068**	**0.634**	**−0.005**	**−0.008**	**−0.001**	0.001	−0.000	0.002
		HDL-C (35–37 weeks)	0.001	−0.002	0.004	**0.349**	**0.091**	**0.606**	**−0.005**	**−0.009**	**−0.002**	0.000	−0.001	0.001
	Change ST (baseline to 35–37 weeks)[b]	HDL-C (35–37 weeks)	**−0.002**	**−0.003**	**−0.001**	0.168	−0.183	0.519	−0.001	−0.002	0.001	−0.000	−0.001	0.000

B, unstandardised regression coefficient. Confidence intervals are shown as 95% bias-corrected and accelerated CIs, and are based on 5000 bootstrap samples. [a]A subtle variation of winsorizing was performed on extreme outliers of placental mRNAs. [b]n = 64. All models were adjusted for lifestyle intervention, smoking at baseline and gestational week at delivery. When additionally adjusting for the relative percentage of ST or MVPA at the respective time point, the results remained similar. Conditional analyses (Figure S4) showed a significant inverse association of insulin at 24–28 weeks of gestation with GLUT1 in male fetuses. Schematic diagram presented in Figure S3. Numbers in bold indicate statistical significance *$P < 0.05$.

Table 5. Simple mediation analyses assessing the potential role of cord glycaemic and lipid parameters as mediators of the relationship between placental mRNAs and neonatal adiposity (n = 112)

Outcome (Y)	Predictor (X)	Mediator (M)	Effect of the predictor on the mediator (a-path)			Effect of mediator on the outcome (b-path)			Direct effect of the predictor on the outcome (c′-path)			Indirect effect (path a*b)		
			B	95% CI		B	95% CI		B	95% CI		B	95% CI	
Sum of skinfolds	PPAR-γ	Glucose[ab]	0.300	−0.311	0.912	0.444	−0.481	1.369	−1.148	−3.606	1.310	0.133	−0.198	0.742
	FATP2	Cholesterol[ab]	−0.295	−0.602	0.012	0.296	−0.700	2.248	0.869	−0.509	2.248	−0.087	−0.466	0.188
		Triglycerides[a]	**−0.172**	**−0.261**	**−0.083**	−2.976	−6.339	0.386	0.269	−1.175	1.714	0.513	−0.031	1.253
		Free fatty acids[a]	**−0.073**	**−0.126**	**−0.021**	−1.991	−7.763	3.780	0.636	−0.775	2.047	0.146	−0.335	0.705
	FATP3	Free fatty acids[a]	0.006	−0.023	0.036	−2.764	−8.304	2.777	−0.025	−0.747	0.697	−0.017	−0.171	0.101
	GLUT1	Glucose[ab]	0.546	−0.041	1.133	0.488	−0.452	1.428	−1.143	−3.582	1.296	0.266	−0.182	0.969
		Free fatty acids[a]	0.081	−0.012	0.174	−2.198	−7.798	3.405	−1.233	−3.567	1.100	−0.178	−0.997	0.210
Cord blood leptin[a]	PPAR-γ	Glucose[ab]	0.214	−0.368	0.797	**1.469**	**0.291**	**2.647**	2.365	−0.568	5.299	0.314	−0.498	1.538
		Triglycerides[a]	0.074	−0.101	0.250	1.114	−3.362	5.590	2.862	−0.486	6.209	0.083	−0.326	0.717
		Free fatty acids[a]	0.569	−0.046	0.160	−3.943	−11.543	3.657	3.169	−0.171	6.509	−0.225	−1.199	0.405
	FATP2	Cholesterol[ab]	−0.287	−0.611	0.035	0.417	−0.940	1.774	0.312	−1.581	2.206	−0.120	−0.817	0.294
		Triglycerides[a]	**−0.165**	**−0.253**	**−0.076**	2.012	−2.947	6.971	−0.331	−1.387	0.440	−0.331	−1.387	0.440
		Free fatty acids[a]	**−0.068**	**−1.122**	**−0.014**	−3.025	−11.083	5.033	−0.014	−1.944	1.917	0.206	−0.366	0.974
	FATP3	Free fatty acids[a]	0.035	−0.027	0.034	−3.096	−10.788	4.597	0.399	−0.581	1.380	−0.011	−0.199	0.166
	GLUT1	Glucose[ab]	0.433	−0.142	1.009	**1.508**	**0.297**	**2.719**	0.604	−2.411	3.619	0.654	−0.280	1.958
		Free fatty acids[a]	0.079	−0.018	0.176	−3.565	−11.404	4.273	1.222	−2.053	4.498	−0.284	−1.334	0.333

The results remained similar when all women (n = 183) were included in the analyses, except for the direct effect of PPAR-γ on cord blood leptin, which was statistically significant. B, unstandardised regression coefficient. Confidence intervals are shown as 95% bias-corrected and accelerated CIs, and are based on 5000 bootstrap samples. [a]A subtle variation of winsorizing was performed on extreme outlier cord blood markers. [b]Optimum Box-Cox transformations were conducted on cord blood parameters. All models were adjusted for the intervention group, smoking at baseline and gestational week at delivery. When additionally adjusting for the delivery route, the results remained similar. Conditional analyses (Figure S6) showed a significant direct effect of PPAR-γ on sum of skinfolds (inverse association when controlling for cord glucose) in female fetuses and a positive significant association of cord cholesterol with cord leptin only in male fetuses. Schematic diagram in Figure S5. Numbers in bold indicate statistical significance *P < 0.05.

percentage of daily ST, light PA or MVPA at baseline, maternal age, BMI, sleep duration, fat percentage, gestational weight gain, development of GDM, protein, fat and carbohydrate consumption, maternal/paternal ethnicity, parity or mode of delivery (data not shown). Only when additionally adjusting for development of GDM, ST at 24–28 weeks of gestation was inversely associated with PPAR-γ mRNA ($P = 0.03$).

Maternal metabolic parameters mediating changes in placental mRNAs

As maternal ST was highly related to insulin and insulin resistance in this cohort,[22] the hypothesis that maternal metabolic parameters drive the associations between ST and placental mRNAs was tested (Table 4). Mediation analyses were only conducted for the lifestyle behaviours (Table 3), and maternal metabolic (Table S2) and adiposity (Table S3) parameters associated with placental mRNAs.

At 24–28 weeks of gestation, higher fasting insulin and HOMA-IR were associated with lower FATP2 mRNA (Table S2; only model 1, $P = 0.04$ and $P = 0.03$, respectively). Higher glucose levels ($P = 0.003$) and lower HbA1c (model 1, $P = 0.03$) and HOMA-B ($P = 0.008$) were related to lower FATP3 mRNA. Higher HDL-C was associated with higher GLUT1 mRNA ($P = 0.004$). At 35–37 weeks of gestation, higher triglycerides were related to higher FATP3 mRNA ($P = 0.03$), and higher HDL-C was associated with higher GLUT1 mRNA ($P = 0.02$). After controlling for false discovery rates, only the association between glucose and FATP3 (24–28 weeks of gestation, $P = 0.003$) remained significant. Maternal adiposity was not related to placental mRNAs (Table S3, $P > 0.05$).

Mediation analyses showed that maternal metabolic parameters did not mediate the relationship of ST or MVPA with placental mRNAs (Table 4, $P > 0.05$).

Cord blood markers mediating neonatal adiposity

Subsequently, the hypothesis that fetal metabolites drive the associations between placental mRNAs and proxy measures of neonatal adiposity (sum of skin folds, cord blood leptin) was tested (Table 5). Mediation analyses were only conducted for the placental mRNAs associated with glucose and lipid transport-related fetal markers (Table S4).

FATP2 mRNA was inversely associated with cord triglycerides and FFA (Table S4, $P < 0.001$). After separating the analyses by fetal sex, the association of FATP2 mRNA with cord FFA was only observed in female fetuses (Table S5, $P = 0.01$). GLUT1 mRNA was positively associated with cord glucose (Table S4, model 1, $P = 0.02$). Greater FABP4 and PPAR-γ mRNAs were associated with higher cord leptin ($P = 0.01$ and $P = 0.03$, respectively); these associations were not observed with the sum of skinfolds. When controlling for false discovery rates, only the association of FATP2 with cord triglycerides and FFA remained significant.

Mediation analyses showed that PPAR-γ mRNA exerted an indirect effect on neonatal sum of skinfolds via cord blood leptin (indirect effect = 0.98, 95% CI 0.07–2.17, data not shown). No other cord metabolic parameter mediated the association of placental mRNAs with neonatal adiposity (Table 5, $P > 0.05$).

Sensitivity analyses

Additional sensitivity analyses of groups with high/low ST or MVPA and obese class I–III are shown in Tables S6–S11. The association between FATP2 mRNA and cord triglycerides was more noticeable in women with higher ST levels at early pregnancy ($P = 0.01$). Of note, PPAR-γ mRNA was positively associated with cord blood leptin only in more sedentary women at 24–28 weeks of gestation (Tables S7, S8, $P < 0.001$).

Discussion

Main findings

This is the first large-scale study examining the influence of objectively measured ST and MVPA at different time periods in pregnancy on targeted placental mRNAs in pregnant women who are obese. The overall result was that MVPA had little if any effect on placental mRNAs. Strikingly, more ST, especially in early to middle pregnancy, was associated with lower FATP2 and FATP3 mRNA in term placenta samples. Although placental FATP2 and FATP3 expression were regulated by the insulin–glucose axis, no maternal metabolic marker mediated the association of ST/MVPA with placental mRNAs. Moreover, the placental FATP2 expression was inversely associated with cord blood triglycerides and FFA. However, no cord blood marker mediated neonatal adiposity, except for cord blood leptin, which mediated the effects of PPAR-γ on neonatal sum of skinfolds.

Strengths and limitations

The main strength of the current study is that it included objective device-based measurements of ST and MVPA at three time points in pregnancy. The use of objective (instead of self-reported) measures of PA is especially relevant in our study of women who are obese, as individuals with greater body fat self-report PA with lower accuracy than individuals who are lean.[40] Furthermore, the cohort is very well phenotyped, with important information on maternal and cord blood metabolic parameters available. In addition, the development of GDM, mode of delivery, sleep duration and maternal diet were considered for the analyses, as they could be important confounding factors of the association between ST/MVPA and placental mRNA. The sample size

was large enough to assess sex differences in associations of placental mRNAs with both maternal and fetal metabolites. Some limitations also should be acknowledged. First, the representativeness of the sample might be compromised because we only analysed placental samples from a subgroup of women (Figure S1). Hence, some selection biases might be present. We preferentially selected women for mRNA analyses from the intervention groups with PA counselling, as we expected the most relevant changes in ST and MVPA levels in these groups. Thus, women from the HE groups are under-represented in this study. Another limitation of the current study is that only gene expression, which does not necessarily represent gene function or protein levels (as a result of post-translational, epigenetic modifications, etc.),[41,42] was analysed in pooled placental tissues. Furthermore, statistical power might have been too limited for mediation analyses.

Interpretation

Although higher levels of maternal insulin and insulin resistance (24–28 weeks of gestation) were associated with lower FATP2, and higher glucose, poorer beta-cell function (24–28 weeks of gestation) and lower triglycerides (35–37 weeks of gestation) were associated with lower FATP3 expression, none of the metabolic parameters mediated the relationship of ST or MVPA with transporter mRNAs. This might be because of a lack of power in our mediation analyses. Contrary to our expectations,[10,14] FATP2 mRNA was inversely associated with cord blood triglycerides and FFA (mainly in female fetuses) and was not associated with neonatal adiposity. In addition to transplacental transport, FFA uptake into fetal tissues contributes to the steady-state levels in cord blood, which might account for the inverse association of FATP2 mRNA with cord blood triglycerides and FFA. Moreover, other placental transporters and transcripts/proteins as well as placental lipid metabolism could play a role in determining the cord blood levels of triglycerides and FFA. It is worth noting that none of the cord metabolic parameters (triglycerides and FFA inclusive) mediated the relationship of placental mRNAs with neonatal adiposity, except for cord blood leptin, which drove the indirect effects of PPAR on neonatal sum of skinfolds.

Nonetheless, PPAR-γ was not associated with ST or MVPA. This is surprising as it is a transcriptional regulator of FATPs and FABPs acting upstream of FATP2, FATP3 and FABP4,[14,16–18] and these transporters were related to ST. A possible explanation is that we measured mRNAs only, and not proteins. However, PPAR-γ expression was positively correlated with FATP2, FATP3 and FABP4 mRNA (see Figure S2). The higher levels of these placental transporters with lower ST levels, although at various time periods, prompted the hypothesis that PPAR-γ upregulation could indirectly explain lifestyle-induced changes on FATP2, FATP3 and FABP4 mRNA. However, we did not find PPAR-γ mediating the association between ST and FATP2, FATP3 or FABP4 (data not shown), which might through a lack of statistical power.

In agreement with an earlier study,[30] our findings also suggested that higher MVPA during early pregnancy was related to downregulated placental GLUT1 expression, which in turn was related to lower cord blood glucose. Noteworthily, this association was dependent on ST levels. By contrast, another study did not observe any association.[29] Methodological differences (e.g. measurements/devices employed, statistical power or maternal phenotype) could explain discrepancies in these findings.

Given that most of the associations were reported with ST during early to middle gestation, a potential explanation is that reducing ST during this period might have induced diverse structural, metabolic and molecular changes in placental cells that remain throughout pregnancy until parturition,[28–30,43] and might dictate placental phenotype.[5,20,28–30] However, we cannot fully exclude that there might also be an acute influence of currently unknown drivers in later pregnancy that account for placental alterations. Neither can we dismiss a potential contribution from obesity, because of the difficulty in separating the biological effects of obesity from ST/MVPA by statistical analysis. If future studies confirm our findings, strategies aimed at reducing ST during this vulnerable tipping point, when pregnancy complications arise,[1,4,44] might be of high relevance for targeting the placental regulation of these transcripts. At this early stage of research, no conclusions can be drawn about the potential for changing placental function by implementing the DALI PA counselling intervention in the clinical context.

Conclusions

The present study shows that ST at specific periods of pregnancy, and changes in ST from baseline, are associated with the expression of different placental genes linked to intracellular lipid transport. However, PA during pregnancy is hardly related to transporter mRNAs. Therefore, the role of PA on these placental mRNAs of women who are obese is debatable. Strategies aimed at lowering ST behaviours are more likely to regulate neonatal growth and adiposity by modulating the expression of relevant placental molecules, which is of clinical interest for the prevention of future maternal and offspring diseases in adulthood. To better understand the role of the placenta in linking maternal lifestyle with neonatal outcomes, future studies are necessary: (i) earlier in pregnancy;[5] (ii) considering the crosstalk among muscle, placenta and other organs;[45] (iii) considering epigenetic changes;[46] and (iv) distinguishing clearly between normal and pathological conditions during pregnancy.

Disclosure of interests
None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship
Conceptualisation: PAM and MNMvP. Methodology: PAM, MNMvP, GD and BLP. Formal analysis, visualisation and writing of original draft: PAM, MNMvP and GD. Supervision: MNMvP. Investigation and resources: all authors except PAM. Writing, reviewing and editing: all authors.

Details of ethics approval
The study was prospectively registered as an RCT in November 2011 (ISRCTN70595832) and was individually approved by local clinical research ethic committees in each country: National Research Ethics Service (NRES), East of England – Norfolk, 11/EE/0221; Medical University Poznan, 1165/12; UZ KU Leuven, ML7625; VUmc Amsterdam, 2012/400; Hospital De La Santa Creu i Sant Pau Barcelona 13/006 (OBS); Medical University Vienna, 2022/2012 – 1369/2013; Region Hovedstaden Copenhagen, H-4-2013-005; Province of Padua, 4201 × 11; Galway University Hospitals, 7/12).

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Data availability statement
The datasets used and/or analysed during the current study are available from the corresponding author, upon reasonable request.

Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. CONSORT flow chart diagram for the DALI lifestyle trial.

Figure S2. Graphical representation and Pearson correlations of placental mRNA levels in pregnant women.

Figure S3. Schematic diagram. The mediator role of maternal glycaemic and lipid markers on the association between sedentary time and moderate-to-vigorous PA (at baseline and at 24–28 weeks of gestation), and changes in sedentary time (between 35–37 weeks of gestation and baseline), with placental mRNAs.

Figure S4. Schematic diagram. The moderator role of fetal sex in all pathways of the mediation models in Figure S3.

Figure S5. Schematic diagram. The mediator role of cord glycaemic and lipid markers on the association between placental mRNAs and neonatal adiposity (<48 hours after delivery).

Figure S6. Schematic diagram. The moderator role of fetal sex in all pathways of the mediation models from Figure S5.

Appendix S1. Lifestyle interventions.

Appendix S2. Target sequence of placental genes.

Appendix S3. Outlier detection and management (main aims).

Appendix S4. Statistical analyses (secondary aims).

Table S1. Effects of the counselling interventions on placental mRNAs.

Table S2. Associations of maternal metabolic parameters (at 24–28 and 35–37 weeks of gestation) with placental mRNAs.

Table S3. Associations of maternal adiposity markers (35–37 weeks of gestation) with placental mRNAs.

Table S4. Associations of placental mRNAs with cord blood glycaemic and lipid parameters, and neonatal adiposity.

Table S5. Associations (grouped by fetal sex) of placental mRNA concentrations with neonatal and maternal metabolic parameters.

Table S6. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women with low or high ST levels (baseline).

Table S7. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women with low or high ST levels (24–28 weeks of gestation).

Table S8. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women who increased less or more ST (from baseline to 24–28 weeks of gestation).

Table S9. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women with low or high MVPA levels (baseline).

Table S10. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women with low or high MVPA levels (35–37 weeks of gestation).

Table S11. Associations of placental mRNAs with neonatal and maternal metabolism markers, and neonatal adiposity, in women in obesity class I and women in obesity class II and III. ■

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