***In vivo* kinetic study of the materno-fetal fatty acid transfer in obese and normal weight pregnant women**

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**Abbreviations:** BMI, body mass index;CE, cholesterol esters;DHA, docosahexaenoic acid; FA, fatty acids;FSR,fractional synthesis rate; GC-C-IRMS, gas chromatography combustion isotope ratio mass spectrometry analysis; HDL, high density lipoprotein; HOMA, homeostatic model assessment; LDL, low density lipoprotein; MPE, molar percentage excess; NEFA, non-esterified fatty acids; NW, normo-weight; OA, oleic acid; OB, obese; PA, palmitic acid; PL, phospholipids; SA, stearic acid; TG, triglycerides; VLDL, very low density lipoprotein.

**Key Points**

Placental structure and function can be modified by maternal obesity affecting the materno-fetal fatty acid transport.

We provide for the first time, in humans and *in vivo,* the kinetic of the placental fatty acid transfer in normo-weight and also in normolipemic obese pregnant women using stable isotopes.

Administration of different tracer fatty acids with similar behaviour to the mother at different time points allows the collection of kinetic information on the materno-fetal transfer of fatty acids despite only one sample of placenta and cord can be collected per subject.

Computational modelling showed a good fit to the data when considering all maternal plasma lipid classes but not based only on non-esterified fatty acids.

The novel approach using multiple tracer FA administration combined with computational modelling showed a consistent time course of placental tracer fatty acids and predicted total fatty acid accumulation.

**Abstract**

We analyze for the first time the *in vivo* materno-fetal kinetic transfer of fatty acids (FA) labeled with stable isotopes in control and obese (OB) pregnant women.

Methods: Labelled FA with similar metabolism (stearic acid: 13C-SA, palmitic acid: 13C-PA and oleic acid: 13C-OA) were orally administered at -4h, -8h, and -12h, respectively prior to elective caesarean section to 10 pregnant women with a body mass index (BMI)>30 (OB) and 10 with BMI 25-30 (NW). Placenta, venous and arterial cord blood were collected obtaining a wide range of FA enrichments. A combined experimental and computational modeling analysis was applied.

Results: FA Fractional synthesis rate (FSR) in placenta was 11-12% per hour. No differences were observed between NW and normo-lipidemic OB. It was not possible to estimate FA FSR in cord blood with this oral bolus dose approach. Computational modelling demonstrated a good fit to the data when all maternal plasma lipid classes were included but not with modelling based only on the non-esterified FA fraction (NEFA). The estimated materno-fetal 13C-FA transfer was around 1%.

In conclusion, our approach using multiple 13C-FA tracers allowed us to estimated FSR in placental/maternal plasma but not in fetal/maternal compartments. Computational modelling showed a consistent time course of placental 13C-FA transfer and predicted total fetal FA accumulation during the experiment. We conclude that in addition to NEFA in the maternal circulation, maternal plasma VLDL and other lipoproteins are important contributors to placental FA transfer to the fetus.

**Keywords:** Fatty acids, placenta, kinetic, transfer, fetus.

**Introduction**

Fetal growth and development depends on nutrient transfer across the placenta (Lager & Powell, 2012). The placenta provides the fetus with the required nutrients including fatty acids (FA) that serve to provide energy and are building blocks for membrane structures and precursors of bioactive compounds, thereby modulating tissue growth, differentiation and function (Haggarty, 2002; Wiktorowska-Owczarek *et al.*, 2015). FA can be taken up by the placenta from maternal plasma, in part as non-esterified FA (NEFA) via passive or facilitated diffusion by transport proteins (Duttaroy, 2009). NEFA represent only about 2-3% of circulating FA, whereas the vast majority of FA maternal circulation are present in esterified form packaged into serum lipoproteins (Herrera & Desoye, 2016). FA may be released from maternal lipoproteins by lipases on the surface of the placenta (Gil-Sanchez *et al.*, 2012). Recent studies using perfused placentas and computational modelling highlighted that placental FA metabolism may explain the discrepancy between placental uptake and fetal delivery of 13C-FA (Perazzolo *et al.*, 2017). The placenta may buffer the supply of FA to the fetal circulation, since placental-fetal transport capacity is lower than maternal-placental uptake (Dancis *et al.*, 1973; Perazzolo *et al.*, 2017). Since *ex vivo* placenta perfusion studies only use13C-NEFA or 14C-NEFA but not other lipid classes, they may not fully represent placental lipid transfer (Perazzolo *et al.*, 2017).

Stable isotope labelled-FAs have been used for the *in vivo* assessment of the materno-fetal FA transfer in healthy and gestational diabetes pregnancies (Larque *et al.*, 2003; Gil-Sanchez *et al.*, 2010; Pagan *et al.*, 2013). Stable isotope labelled FAs become distributed through all maternal lipid classes and lipoproteins, which need to be considered when evaluation placental lipid transfer. Previous studies used only a single oral bolus dose of different labelled 13C-FA administrated to pregnant women, which does not yield kinetic *in vivo* information on the FA transfer process since only one sample from placenta and cord blood per subject was available (Gil-Sanchez *et al.*, 2010; Pagan *et al.*, 2013). Administration of different tracer FAs with similar behavior to the mother at different time points (staggered supply) allows the collection of kinetic information on the enrichment of 13C-FA in both placenta and cord blood for an *in vivo* kinetic model of FA transfer between mother and infant (Van Harskamp *et al.*, 2017).

Maternal obesity is a major predictor of offspring obesity (Catalano & deMouzon, 2015). Cinelli and colleagues reported that erythrocyte polyunsaturated FA in cord blood were inversely associated with both maternal body mass index (BMI) and excessive gestational weight gain, suggesting that obesity can be also a risk factor for visual and cognitive development during childhood (Innis, 2007). Better knowledge on the kinetics of materno-fetal transfer of FA in obese pregnancies is needed.

In the present study, we aimed to apply a novel approach to describe the kinetics of placental FA transfer in obese (OB) and normo-weight (NW) pregnant women prior to elective caesarean section using timed administration of different tracer FA.

**Subjects and Methods**

*Ethical approval*

All procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Written informed consent was obtained from all participating women. The protocol of this study was approved by the Ethics Committee of the Virgen de la Arrixaca Clinical Hospital, Murcia, Spain. This study was registered in the ISRCTN database (trial registry number: ISRCTN69794527).

*Subjects*

10 obese pregnant women with pre-pregnancy BMI >30 kg/m2 (OB) and 10 women with a pre-pregnancy BMI of 20-25 kg/m2 (NW) were recruited at term in the Obstetrics and Gynecology Service of the Virgen de la Arrixaca Clinical Hospital (Murcia, Spain). All participants fulfilled the following inclusion criteria: singleton pregnancy, age 18-40 years, plan to undergo elective caesarean section at term, omnivorous diet, no docosahexaenoic acid (DHA) supplements during the last trimester of pregnancy, non-smoking and normal fetal Doppler scan within the normal reference range (Parra-Cordero *et al.*, 2007) on the day before caesarean section. Women with any health problem or pregnancy complications were excluded. All women had a negative result in the O´Sullivan test in the second trimester, thus patients with gestational diabetes were not included.

*Stable isotope tracer administration*

Three free FAs uniformly labelled with 13C stable isotope (ISOLIFE S.L., The Netherlands) were administered orally spread on a small piece of bread at different time points prior to the time of planned, elective cesarean section: - 12 h for 13C-oleic acid (OA, 18:1n-9, 0.5 mg/kg body weight); - 8 h for 13C-palmitic acid (PA, 16:0, 0.5 mg/kg) and -4 h for 13C-stearic acid (SA, 18:0, 0.5 mg/kg). They were accurately weighted for their addition to the bread according to maternal body weight. The timing of the different 13C-FA administration was scheduled in order to minimize FA interconversions, placing at shorter times from delivery/sampling saturated FAs (13C-PA -8h and 13C-SA -4h) and monounsaturated FA at the beginning (13C-OA -12h) since it should not be converted in PA or SA. Subjects received a breakfast free of DHA and remained fasted until surgery. The caesarean section was scheduled at 21:00 h in order to take the maternal sampling during the day, to avoid disturbing the mother’s night time sleep with the serial administration of the tracers.

*Blood and placenta sampling*

Maternal blood samples were collected before tracer intake (basal time at -12 h prior to caesarean section) and every four hours (-8 h and -4 h), with an additional point at -2 h prior to the caesarean section (0 h), using a venous line placed in the forearm of the subject. We collected 10 mL of maternal blood at each time point except for the sample -2 h before delivery when only 5 mL blood were taken. Directly after birth, 2 mL of both cord venous and arterial blood were collected. All blood samples were collected into EDTA-coated tubes and centrifuged for 3min at 1200 g to separate plasma and red blood cells. Plasma samples were stored at -80ºC until analysis.

We collected 6 pieces of 0.5 x 0.5 x 0.5 cm from different placenta locations: 4 pieces from the four imaginary quadrants of maternal basal plate, that included villi tissue, and also 2 pieces from chorionic plate; they rinsed in cold 0.9% NaCl solution, immediately frozen in liquid nitrogen and stored at -80°C until analysis.

*Isolation of plasma lipoproteins*

 Plasma lipoproteins were isolated from 1 mL of fresh maternal plasma by ultracentrifugation using a discontinuous NaCl/KBr density gradient (Chung *et al.*, 1980) in an Optima L-100 XP ultracentrifuge equipped with 100Ti rotor (Beckman Coulter, CA, EEUU). The purity of isolated lipoproteins was determined by electrophoresis in an agarose gel using a commercial kit (Paragon Lipoprotein Electrophoresis kit, Beckman, UK). The gel was dried and the lipoprotein pattern visualized by using the Sudan Black B Stain-containing Paragon Lipo Stain (0.07% v/v).

*Plasma biochemical analysis*

Maternal plasma biochemical parameters at delivery and venous cord insulin were analyzed by chemioluminescence (DIAsource INSIRMA, Nivelles, Belgium). Glucose, serum total cholesterol, triglycerides (TG), LDL (low density lipoprotein) cholesterol and HDL (high density lipoprotein) cholesterol were quantified by an automatic analyzer (Roche-Hitachi Modular PyD Autoanalyzer, Mannheim, Germany).

The homeostatic model assessment (HOMA) index for insulin resistance was calculated according to the following formula: (glucose mg/dL × insulin µU/mL)/405.

*Analysis of fatty acid concentrations and enrichments*

FA were analyzed in maternal plasma and separated plasma lipoprotein samples from all time points, placental tissue, venous and arterial cord plasma. Total lipids from 250 µL of plasma/lipoproteins and 0.8 g of placental tissue were extracted into 3 mL (plasma and lipoproteins) or 6 mL (placenta) chloroform-methanol (2:1 v/v) applying a modified Folch method (Folch *et al.*, 1957). Free pentadecanoic acid, dipentadecanoyl phosphatidylcholine, tripentadecanoin and cholesterylpentadecaoate (Sigma-Aldrich, Steinheim, Germany) were added as internal standards. The lipid extract was dried under nitrogen flow, resuspended in 400 µL chloroform-methanol (1:1 v/v) and applied to silica gel plates (Merck, Darmstadt, Germany) for the isolation of lipid classes. Phospholipids (PL), TG, cholesterol esters (CE) and NEFA were isolated by development of the plates in N-heptane/diisopropylether/glacial acetic acid (60:40:3, by vol). Synthesis of FA methyl esters was performed with 3 N methanolic HCl (Supelco, PA, USA) at 90°C for 1 h (Carnielli *et al.*, 1996). A direct isolation and transesterification method was applied for NEFA quantification in plasma samples (Husek *et al.*, 2002). After drying under a gentle stream of nitrogen the samples were dissolved in hexane containing butylated hydroxytoluene (2 g/L) and stored at -20°C until gas chromatography combustion isotope ratio mass spectrometry analysis (GC-C-IRMS).

13C enrichment of FA was measured by GC-C-IRMS (Delta XP, Thermo Electron, Bremen, Germany) equipped with a BPX70 (30 m x 0.25 mm x 0.25 µm) GC column (SGE, Milton Keynes, United Kingdom). The injection volume varied amongst the samples, to achieve areas under the curve for all methylated FA within the linear dynamic range of the instrument. The procedure was slightly adapted from the one previously described (Demmelmair *et al.*, 1998; Reijngoud & Verkade, 2017).

*Calculations and expression of results*

From the 13C/12C ratio of the samples measured by GC-C-IRMS, the δ13C relative to the international Pee Dee Belemnite standard and the 13C-FA molar percentage excess (MPE) were calculated (Brossard *et al.*, 1994), which represented the FA 13C-enrichment.

In each subject, the enrichment ratio (MPE values) of OA, PA and SA in placenta relative to maternal plasma (product/precursor enrichment ratio) was plotted in relation to the time of tracer administration for the calculation of the fractional synthesis rate (FSR) of FA by placental tissue. From that, we calculated the slope of the MPE in total lipids of placenta relative to MPE in total lipids of maternal plasma and the correlation coefficient of the linear trend line. The FSR was derived by using the following equation (van den Akker *et al.*, 2008):

*FSR (% per day) = slope of the trend line x -1 x 100* $FSR \left(\% per day\right)=slope of the trend line × -1 ×24 h ×100$ (Eq. 1)

The distribution ratio (%) of the tracer FA enrichment between mother and fetus was estimated by the calculation of the ratio between tracer MPE enrichment in total lipids of venous cord blood relative to maternal plasma MPE enrichment (average of all time point values).

*Computational modelling of fatty acid transfer kinetics*

The main aim of the model was to rationalise the time course of the results observed for OA, PA and SA at delivery to predict the total amount of FA transferred to the fetus during the experiment. Since fluxes and volumes between compartments need to be used for the modelling, concentration values of 13C-FA instead of 13C-FA enrichment values (MPE) were used. Concentrations of labelled FA (µmol 13C/L or nmol 13C/g) were calculated by multiplying absolute concentrations of unlabelled FA obtained by gas chromatography by their tracer enrichment (MPE values) obtained by GC-IRMS. Since these 13C-FA were administrated orally at different time points, a compartmental modelling approach of 13C-FA concentrations was adopted to describe the transfer of FA to the fetus as a function of time, based on the measured maternal plasma lipid profile, the plasma umbilical cord concentrations and placental tissue values at delivery. The model schematic is shown in **Figure 1**. From the maternal plasma, the uptake flux *Juptake* reflects the transfer of lipids to the placenta, modelled as a single compartment. From the placenta, the flux *Jdelivery* represents the delivery to the fetal plasma compartment. Finally, from the fetal plasma, the flux *Jfetaltissue* represents the rate of lipid deposition into the fetal tissue.

The fluxes between compartments were modelled using linear kinetics, *i.e.* the flux leaving the compartment was proportional to the compartmental concentration (Eq. 2-4). The reason for this is that the tracer concentrations modelled were much lower than those for endogenous FA and unlikely to be saturating. To keep the model as simple as possible in light of the *in vivo* data available, only uni-directional fluxes were considered to represent the net fluxes based on the overall concentration gradients from mother to fetus. The equations for the fluxes *J* (µmol/min) are then given by:

$J\_{uptake}= k\_{uptake} C\_{M}$, (Eq. 2)

$J\_{delivery}= k\_{delivery} C\_{P}$, (Eq. 3)

$J\_{fetaltissue}= k\_{fetaltissue} C\_{F}$, (Eq. 4)

where *CM* (µmol/L) was the maternal plasma tracer concentration; *CP* and *CF* (µmol/L) were the placental and fetal plasma compartmental tracer concentrations and *k* (L/min) were the flux rate constants. The resulting compartment concentrations were governed by:

$\frac{dC\_{P}}{dt}= \frac{1}{V\_{P}}\left( J\_{uptake} -J\_{delivery} \right),$ (Eq. 5)

$\frac{dC\_{F}}{dt}= \frac{1}{V\_{F}}\left( J\_{delivery} -J\_{fetaltissue}\right),$ (Eq. 6)

with $V\_{P}$ and $V\_{F}$ the placental tissue and fetal plasma volume (L), respectively. The placental delivery to the fetus $J\_{delivery}$ was related to the umbilical flow rate $Q\_{umbilical}$ (l/min) and venous-arterial tracer concentration difference $\left(C\_{V}-C\_{F}\right)$, as follows:

$J\_{delivery}=Q\_{umbilical}\left(C\_{V}-C\_{F}\right)$ (Eq. 7)

in which the fetal umbilical artery tracer plasma concentrations were considered to correspond to those in the fetal plasma compartment $C\_{F}$. Note that the concentration in the fetal tissue was not modelled, as only the total output based on *Jfetaltissue* was of interest. The model input consisted of the measured maternal plasma concentrations *CM*, prescribing the concentrations in the maternal plasma compartment as a function of time. Different scenarios were evaluated in which the uptake into the placenta was assumed to be a function of NEFA alone, NEFA+TG, or the total sum of the maternal plasma lipid classes, NEFA + TG + PL + CE.

In line with the main assumption underlying this study, it was assumed that OA, PA and SA have similar kinetics (Duttaroy, 2009; Haggarty, 2010) therefore 13C-OA, 13C-PA and 13C-SA were fitted together. In addition, it was reported that labelled saturated FA (represented by 13C-PA) and monounsaturated FA (represented by 13C-OA) were higher in maternal plasma TG, while polyunsaturated FA followed a different distribution and were found mainly in PL and TG (Gil-Sanchez *et al.*, 2010; Larque *et al.*, 2011). Model equations Eq. 2-7 were implemented in Matlab version R2016a (The Mathworks Inc, MA, USA) for each FA and time integration was performed using the ode45 function (Runge-Kutta 4, 5 method). A least square criterion normalised by concentration was used to match the measured umbilical artery and umbilical vein concentrations and placental tissue concentrations for OA, PA and SA. The only parameters estimated were the 3 values of *k* that determine the lipid fluxes in Eq. 2-4. The remaining parameters used in the model are given in **Table 1**. Plasma volumes and flow rates were based on measured clinical parameters using established relationships from literature (Yao & Lind, 1972; Sutton *et al.*, 1990; El Behery *et al.*, 2011; Abduljalil *et al.*, 2012). The data for each placenta was averaged and the model fit to this average data (N = 10). The complete analysis was repeated for the obese subjects.

*Statistics*

The results are expressed as means ± SEM. Obese and control groups were compared using unpaired two-sided t-test. The effect of time and obesity on MPE values was analyzed with two ways ANOVA followed by *post hoc* of Bonferroni. The significance level was set at *P*<0.05. Statistical analysis was performed with SPSS software version 23.0 (SPSS, IL, USA).

**Results**

Obese mothers did not show hyperlipidaemia, and maternal serum TG and cholesterol levels were similar in OB and NW. Insulin and HOMA index tended to be higher in OB women compared to NW, reflecting an early state of insulin resistance. Placental weight also tended to be higher in the OB mothers (*P*=0.051) (**Table 2**).

13C-FA enrichment in the maternal-placental-fetal compartments was time dependent, with lower 13C-FA MPE at delivery in maternal plasma of 13C-OA that was administered first at -12 h prior to caesarean section compared to those that were administered at -8 h and -4 h, 13C-PA and 13C-SA. This appears to reflect both metabolic utilisation and dilution of tracers in the maternal lipid pool over time (**Table 3**). In placenta and cord blood, tracer enrichment increased with time suggesting that the FA transfer process towards these compartments takes more than 12 h (**Table 3**).

We estimated FA kinetics within 12 h prior to delivery, assuming a similar behaviour of the three 13C-FA administered at different time points. Peak of tracer enrichment in plasma VLDL (very low density lipoprotein) and HDL plasma occurred 4 h after administration (13C-SA value), followed by a fast drop in MPE values during the last 8 h of the study (13C-PA and 13C-OA values) (**Figure 2A and 2B**). MPE values in VLDL and total lipids of placental tissue 4 h after administration (13C-SA value) were correlated (R = 0.583, *P* = 0.011), following the correlations with placental PL, NEFA and TG the same trend. At later time points after administration (8 and 12 h), all these associations tended to disappear (data not shown). In contrast to VLDL and HDL, MPE in LDL increased continuously over time **(Figure 2C**), indicating a slower FA turnover in LDL respect to VLDL and HDL in these fasted subjects. 13C-FA MPE were not different between OB and NW subjects in any lipoprotein.

The ratio between 13C-FAs MPE in total lipids of placenta and maternal plasma showed a linear-trend relationship over time (**Figure 3A**), which provides a basis for estimating the FSR based on the slope of the product (placenta)/precursor (maternal plasma) ratio of 13C-FA appearance. The average linear regression coefficients were 0.993 (0.986-0.999) for NW and 0.982 (0.951-0.998) for the OB group. The calculated FSR of 13C-FA from MPE values of total lipids in maternal plasma and placenta indicated a placental FA uptake per hour of 10.72 ± 1.48 % for OB *vs.* 11.88 ± 1.66 % for NW (*P* = 0.565). In contrast, the ratio of MPE in venous cord blood total lipids versus maternal plasma total lipids did not show a linear trend relation **(Figure 3B)**, and hence, FSR could not be estimated in the fetal compartment. This finding may reflect the contribution of placental FA metabolism to materno-fetal transfer.

13C-FA enrichment in placenta total lipids (**Table 3**), PL (**Figure 4A**) and NEFA (**Figure 4B**) increased over time both in NW and OB women. However, placental TG behaved different and surprisingly, OB had lower 13C-FA enrichment in placental TG than NW (**Figure 4C**). Venous and arterial cord blood did not differ between groups (**Table 3**).

Computational modelling was applied to represent the kinetics of placental transfer and predict fetal lipid accumulation. Modelling placental uptake using only NEFA concentrations of 13C-FA from maternal plasma did not fit the data (results not shown), either NEFA+TG or total lipids (NEFA+TG+PL+CE) of maternal plasma had to be considered and both approaches fit the model. The time course of FA transfer was modelled by combining the data for 13C-OA, 13C-PA and 13C-SA in total lipids, showing initial high rates of uptake following administration leading to a peak of concentration at 8h (13C-PA) with a decrease over time (**Figure 5**). This peak of 13C-PA concentration in maternal plasma was due to the higher unlabelled PA concentration respect to OA or SA in maternal plasma (Table 3). Since unlabelled FA data are used to calculate 13C-FA concentrations from enrichment values, that explained the discrepancies between 13C-FA concentrations (Figure 5) and 13C-FA enrichment values (MPE) in the different compartments (Table 3). Fitting the 13C-OA, 13C-PA and 13C-SA data with a single set of parameters *k* resulted in an overall satisfactory fit (Figure 5).

Parameters used for the modelling such as estimated uterine artery and umbilical vein plasma flow rates tended to be higher in OB subjects since they were theoretically calculated using the subjects’ weight data (Table 1). These parameters were used for the calculation of estimated 13C-FA amounts shown in **Table 4**. The amount of each tracer entering the placenta via the maternal uterine plasma over the course of the experiment was higher than the dose given to the mother due to recirculation (i.e. the FAs can pass through the maternal intervillous space multiple times if they are not taken up) (Table 4).

The set of estimated model parameters were similar between the NW and OB “average subject” groups (Table 4). The FA amount transferred related back to the initial oral dose administered to the mothers showed a maximum transfer of 1.5-2% of the oral dose over the 12 h before delivery.

To evaluate placental function, the materno-fetal transfer was also calculated as a percentage based on the predicted fetal/metabolism accumulation (µmol) using the umbilical arterious-venous difference and multiplied by the umbilical flow rate and divided by the total amount of FA tracer entering the placenta via the maternal uterine plasma (Table 4). The percentage of FA transfer expressed in this manner worked out lower than using the initial dose administered to the mother, around 1%, which is due to recirculation of FAs in the maternal blood. Again, results were similar for both OB and NW pregnant women (Table 4). Statistical assessment between the OB and controls must be done as part of future work.

**Discussion**

This is the first study addressing the *in vivo* placental transfer of FA using a kinetic modelling approach applying labelled FA with similar chemical structure and metabolism (13C-SA, 13C-PA and 13C-OA) at different time points prior to elective caesarean section to OB and NW pregnant women. Saturated and monounsaturated FA (13C-PA and 13C-OA) behave similarly and after oral intake they were mainly found in maternal plasma TG, while polyunsaturated FA followed a different distribution and were found mainly in PL and TG (Gil-Sanchez *et al.*, 2010; Larque *et al.*, 2011). Such modelling provided flux estimates for FA uptake, delivery and fetal deposition during the 12 hours of the experiment. Each flux can be calculated by multiplying the corresponding estimated parameter *k* with the concentration at given time points. The incorporation of the three tracers FAs into total plasma lipids and plasma lipoproteins was similar in NW and OB women who showed similar plasma lipid concentrations. Some but not all other studies have also reported similar serum TG levels in normal, overweight and obese pregnant women (Martino *et al.*, 2016).

Maternal plasma FA tracer enrichment decreased during the 12 h after tracer administration in maternal plasma while increasing in placenta. Previous studies also revealed that labelled FA appearance into maternal plasma lipid after a single bolus administration required at least 12 h for full incorporation in all lipid fractions (Larque *et al.*, 2003; Gil-Sanchez *et al.*, 2010). Human placenta expresses VLDL/apoE receptor, HDL and LDL receptors and the LDL receptor-related protein (Herrera, 2002), but the role of maternal lipoproteins uptake for placental FA transfer is poorly understood. VLDL was the lipoprotein with highest 13C-FA enrichment. VLDL is mainly composed of TG, and TG and NEFA from maternal VLDL may serve as rapidly available source of FA to the placenta. Late pregnancy is characterized by a catabolic state with lipolysis of maternal adipose tissue and enhanced hepatic production of VLDL particles, both contributing to maternal hypertriglyceridemia (Wasfi *et al.*, 1980). HDL and LDL also become enriched in TG at the end of gestation (Alvarez *et al.*, 1996). LDL showed a slower FA turnover than HDL and VLDL, with a constant increase of MPE during the 12 h of the study.

This study suggests that VLDL and other lipoproteins are important contributors to placental FA transfer to the fetus. The model was unable to fit the data based on only maternal plasma NEFA and instead all four lipid fractions (total lipids) allowed kinetic modelling (Table 4). In addition, we detected underprediction of placental tissue values mainly for labelled polyunsaturated FA in pilot calculations in which only TG+NEFA were considered. While OA, PA and SA could be represented reasonably well by the same kinetics, linoleic and DHA could not (data not shown). This is not surprising since polyunsaturated FA are mainly transported in maternal plasma PL, which therefore need to be considered for explaining placental FA transfer (Gil-Sanchez *et al.*, 2010). Thus, all lipid fractions should be considered for the studies of kinetic modelling.

To date, all stable isotope turnover studies in which the kinetics of lipoproteins have been investigated rely on endogenous labelling procedures, with most using the FSR formula in which a the link between the precursor and the product is required as a prerequisite (Foster *et al.*, 1993). FSR has been also applied in pregnant women to estimate human albumin fetal synthesis (van den Akker *et al.*, 2008). We tried a kinetic approach based on the FSR considering either 13C-FA enrichment in total lipids of maternal plasma/placenta or maternal plasma/cord venous to estimate placental uptake or materno-fetal FA transfer (Figure 2). We found a good linear trend relationship between the MPE of 13C-FA in total lipids of placenta and maternal plasma (Figure 2A), with the FSR calculation indicating a relatively high uptake rate of about 11-12% per hour from an oral bolus dose. *In vitro* perfusion studies using 13C-NEFA showed a placental uptake over 3 h of about 60% while about 6% was delivered to the fetus (Dancis *et al.*, 1973; Perazzolo *et al.*, 2017).

Surprisingly, we could not estimate the slope of 13C-FA enrichments in total lipids of cord blood respect to maternal plasma and hence FSR in cord blood could not be estimated. The delay in appearance and metabolism of placental lipid fractions would require different time-frame design for cord FSR. Perazzolo *et al.* reported that placental FA metabolism was necessary for explaining the discrepancy between placental uptake and delivery of 13C-FA using computational modelling with data from *ex vivo* placental perfusion study (2017). Without considering placental metabolism, the model overestimated fetal delivery of 13C-FA 15 fold (Perazzolo *et al.*, 2017). With respect to the current study, it is important to bear in mind that the estimated rates of placental uptake from the maternal circulation represent the minimum fluxes necessary to match the measured data but the uptake rate from the maternal circulation may be higher if the loss due to placental oxidation is included. Direct measurements of the maternal arterial-venous difference *in vivo* are required to address this question.

In a similar way to that observed for total 13C-FA enrichment in placental total lipids, placental PL MPE also increased over time. PL are by far the largest lipid fraction in placental tissue (Klingler *et al.*, 2003). However, significantly lower MPE values were observed in placental TG fraction at all time points and lower in the OB group than NW group. It is possible that placental TG in the OB group mainly derive from lipogenesis which require longer times. Other authors reported higher fat content in placenta of overweight and obese compared to normal weight women (Saben *et al.*, 2014; Calabuig-Navarro *et al.*, 2017), while one other study did not (Segura *et al.*, 2017). In placentas from pregnancies complicated by gestational diabetes mellitus, the FA oxidation capacity was reduced along with higher glucose uptake, which could explain the higher placenta lipid content in diabetes (Visiedo *et al.*, 2015). These adaptations may serve to limit the materno-fetal transfer of fat and to protect the fetus from excessive adiposity in this population of glucose-tolerant OB pregnant women.

In the present study, a computational kinetic model for the *in vivo* transfer of FA was applied using a compartmental modelling approach in order to discern the effects of maternal obesity on the materno-fetal transfer of FA. The applied model accounted for differences in placental weight and flow rates, which tended to be increased in OB subjects. The estimated materno-fetal transfer was around 1% for 13C-FA (Table 4), which seems low although similar low transfer rates for PA have been observed in *ex vivo* placental perfusion studies (Perazzolo *et al.*, 2017). This was also similar to the ratio between fetal and maternal plasma area under the concentration curve after one oral dose of 13C-FA 12 h before elective caesarean section in healthy pregnant women and gestational diabetes mothers (estimated around 1%) (Pagan *et al.*, 2013).

Application of the model to the experimental data demonstrated that the placental tissue and umbilical artery and vein concentrations following administration of OA, PA and SA at different time points could be described by the same kinetics, based on the maternal plasma profiles. In particular, the model predicted a coherent time course following FA administration with an increase in fetal plasma concentrations followed by a slow decay, which has important implications for the interpretation of the experimental data in this type of experiments (Figure 5). The discrepancy between experimental and predicted model values in cord blood 13C-PA (8h) is related to the higher unlabelled PA pool concentration in cord respect to OA and SA. This limitation of using different FAs with different size pools is compensated by the advantage of an in vivo model in humans that provides data at 3 different time points available in both placenta and cord compartments. In addition, we also tested three separate models for each independent 13C-FA using their specific maternal plasma time profiles but with only one data point available in placenta and cord at delivery. Fitting the 13C-OA, 13C-PA and 13C-SA data with a single set of parameters *k* resulted also in an overall satisfactory fit and the predicted data of 13C-FA transfer (Annex 1 and 2) were quite similar to those included the present manuscript, which confirmed in part our results.

The model allowed us to predict total fetal accumulation during the experiment, however it has to be noted that these predictions will ultimately depend on the accuracy of the measured umbilical cord venous-arterial differences at birth. As a first assumption linear kinetics were adopted to minimise the number of parameters, however effects of saturation may need to be taken into account depending on the concentrations and transport mechanisms involved. Further investigation into the time course of transfer could be performed by administering the same type of FA at different time points before delivery in different subjects. This would increase the time resolution and confirm the ability of the model to represent the kinetics of placental uptake and materno-fetal transfer of specific FA as a function of time.

Strengths of the study include the novel approach applied to describe the kinetics of placental FA transfer, combining labelled FA *in vivo* administration at different time points and computational modelling for the first time. A limitation was the application of a single oral bolus of FA tracer instead of a continuous tracer infusion, which led to decreasing values of enrichment in the maternal compartment while increasing in placenta and fetus over time. The different turnover of lipid fractions and plasma lipoproteins should be considered, which limits the definition of FA precursor, although new information on their metabolism is provided. It is important to bear in mind that tracer FA were not identical, each FA has its own processing even while having similar metabolism. Some of the subsequent variations may be related to those differences, it is possible that the fetus modified the tracer FA administered differently and that may change the basic assumptions. The limited sample size was due to the very demanding study protocol with caesarean sections at night. Concerning differences between OB and NW pregnant women, no statistical assessment was performed because of high inter-individual variation within the data permitted only modelling of overall trends.

*In vivo* kinetic modelling is challenging, but our approach using multiple 13C-FA tracers has allowed us to gain additional insight into the kinetics of materno-fetal lipid transfer. No clear differences in FA transfer process between NW and normolipemic OB pregnant women were observed. After oral bolus administration 11-12%/h of synthesis rate of FA by placental tissue was estimated which should be confirmed in further studies. The administration of similar 13C-FA at different time points before delivery in combination with computational modelling is a valuable approach to obtain a more comprehensive overview of the materno-fetal transfer of FA considering that only one placenta and cord sample can be collected per subject.

**Competing interests**

None declared.

**Author contributions**

AG, HD, HS, JBvG, BK and EL designed research; MTPS and JEBC performed research; AG, DvH, JEO and HS performed samples and data analysis. SP, BGS and RML analyzed data. AG performed statistical analysis. AG, SP, BGS and EL wrote the manuscript and had primary responsibility for final content. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspect of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designate as authors qualify for authorship and all those who qualify for authorship are listed.

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Table 1. Characteristics of normal weight and obese subjects used for modeling.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Control (n=10)** | **Obese (n=10)** |
| Maternal weight | 74.55 ± 6.3 kg | 93.55 ± 14 kg |
| Maternal haematocrit | 0.331 ± 0.3 | 0.345 ± 4.3 |
| Uterine artery blood flow (Abduljalil *et al.*, 2012) | 541 mL/min | 598 mL/min |
| Uterine artery plasma flow | 362 mL/min | 392 mL/min |
| Fetal haematocrit | 0.48 ± 0.03 | 0.45 ± 0.03  |
| Umbilical vein blood flow (El Behery *et al.*, 2011) | 347 | 376 |
| Umbilical vein plasma flow | 180 mL/min | 208 mL/min |
| Fetal blood volume | 0.34 L | 0.37 L |
| Fetal plasma volume (Yao & Lind, 1972) | 0.18 L | 0.21 L |

Experimental values are mean ± SD, other values were derived using established relationships from literature based on the average clinical parameters from Table 2. Note the placental tissue volume was simply equated to the weight in kg. The umbilical vein blood flow was based on the average value per kg of fetal weight (El Behery *et al.*, 2011) and the observation that umbilical vein blood flow increased linearly with fetal weight during gestation (Sutton *et al.*, 1990).

Table 2. Characteristics of the mothers and neonates at delivery.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Control (n=10)** | **Obese (n=10)** | ***P*** |
| Maternal Age (years) | 33.8 ± 1.9 | 34.5 ± 2.3 | 0.807 |
| Gestational Age (weeks) | 38.5 ± 0.3 | 38.4 ± 0.4 | 0.838 |
| Maternal Pregestational BMI (kg/m2) | 22.5 ± 0.5 | 32.2 ± 0.9 | **<0.001** |
| Maternal BMI at delivery (kg/m2) | 28.2 ± 0.6 | 35.6 ± 1.2 | **<0.001** |
| Placental Weight (g) | 613 ± 58 | 755 ± 42 | 0.051 |
| Maternal Glucose (mg/dL) | 59.80 ± 2.48 | 62.10 ± 6.271 | 0.377 |
| Maternal Insulin (μU/mL) | 9.47 ± 1.50 | 14.73 ± 2.68 | 0.090 |
| HOMA index | 1.39 ± 0.24 | 2.40 ± 0.55 | 0.093 |
| Maternal TG (mmol/L) | 1.94 ± 0.25 | 1.95 ± 0.17 | 0.973 |
| Maternal NEFA (mmol/L) | 0.90 ± 0.11 | 0.91 ± 0.07 | 0.938 |
| Maternal total cholesterol (mmol/L) | 6.77 ± 0.40 | 6.31 ± 0.39 | 0.399 |
| Maternal LDL cholesterol (mmol/L) | 3.81 ± 0.31 | 3.37 ± 0.42 | 0.385 |
| Maternal HDL cholesterol (mmol/L) | 2.06 ± 0.24 | 2.00 ± 0.18 | 0.834 |
| Maternal total FA (mmol/L) | 14.54 ± 1.02 | 14.52 ± 0.98 | 0.986 |
| Placental total FA (nmol/g) | 32.57 ± 2.35 | 37.21 ± 1.99 | 0.127 |
| Cord venous total FA (mmol/L) | 3.27 ± 0.38 | 3.13 ± 0.36 | 0.776 |
| Cord artery total FA (mmol/L) | 4.83 ± 0.29 | 4.41 ± 0.30 | 0.303 |
| Birth Weight (g) | 3241 ± 134 | 3512 ± 169 | 0.203 |
| Birth Length (cm) | 49.80 ± 0.54 | 50.50 ± 0.71 | 0.418 |
| Cord Glucose (mg/dL) | 32.80 ± 5.19 | 46.63 ± 5.85 | 0.105 |
| Cord Insulin (μU/mL) | 6.16 ± 0.57 | 5.86 ± 0.42 | 0.676 |

Results are expressed as means ± SEM. Significant differences when *P* <0.05. BMI, body mass index; FA, fatty acids; HDL, high density lipoprotein; HOMA, homeostatic model assessment; NEFA, non-esterified fatty acids; LDL, low density lipoprotein; TG, triglycerides.

Table 3. Enrichments of 13C-fatty acids in maternal plasma, placenta and fetal plasma and their corresponding unlabelled fatty acid concentration at delivery.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Obese** |  | **Control** | **Po** | **Pt** | **P\*** |
| **Tracer enrichment values (MPE)** |
| **Maternal plasma** |  |  |  |  |  |  |  |  |  |  |
| -4h | (13C-SA) | 0.071**a** | ± | 0.010 |  | 0.072**a** | ± | 0.014 | 0.753 | **<0.001** | 0.995 |
| -8h | (13C-PA) | 0.031**b** | ± | 0.003 |  | 0.033**b** | ± | 0.004 |  |  |  |
| -12h | (13C-OA) | 0.028**b** | ± | 0.003 |  | 0.030**b** | ± | 0.003 |  |  |  |
| **Placenta** |  |  |  |  |  |  |  |  |  |  |
| -4h | (13C-SA) | 0.018**a** | ± | 0.001 |  | 0.018**a** | ± | 0.001 | 0.056 | **<0.001** | 0.444 |
| -8h | (13C-PA) | 0.021**b** | ± | 0.001 |  | 0.022**b** | ± | 0.001 |  |  |  |
| -12h | (13C-OA) | 0.031**c** | ± | 0.001 |  | 0.034**c** | ± | 0.002 |  |  |  |
| **Cord venous** |  |  |  |  |  |  |  |  |  |  |
| -4h | (13C-SA) | 0.003**a** | ± | 0.001 |  | 0.003**a** | ± | 0.001 | 0.384 | **0.008** | 0.784 |
| -8h | (13C-PA) | 0.005**b** | ± | 0.001 |  | 0.006**b** | ± | 0.001 |  |  |  |
| -12h | (13C-OA) | 0.004**b** | ± | 0.001 |  | 0.005**b** | ± | 0.001 |  |  |  |
| **Cord artery** |  |  |  |  |  |  |  |  |  |  |
| -4h | (13C-SA) | 0.002**a** | ± | 0.001 |  | 0.003**a** | ± | 0.001 | 0.753 | **0.008** | 0.789 |
| -8h | (13C-PA) | 0.005**b** | ± | 0.001 |  | 0.005**b** | ± | 0.001 |  |  |  |
| -12h | (13C-OA) | 0.004**b** | ± | 0.001 |  | 0.005**b** | ± | 0.001 |  |  |  |
| **Concentration values of unlabelled fatty acids (umol/L or nmol/g)** |
| **Maternal plasma** (µmol/L) |
| -4h | (SA) | 1106**a** | ± | 58 |  | 1180**a** | ± | 58 | 0.185 | **<0.001** | 0.679 |
| -8h | (PA) | 5394**b** | ± | 314 |  | 5614**b** | ± | 326 |  |  |  |
| -12h | (OA) | 3894**c** | ± | 236 |  | 4383**c** | ± | 273 |  |  |  |
| **Placenta (**nmol/g) |
| -4h | (SA) | 3426**a** | ± | 439 |  | 3344**a** | ± | 424 | 0.768 | **<0.001** | 0.985 |
| -8h | (PA) | 6643**b** | ± | 974 |  | 6624**b** | ± | 805 |  |  |  |
| -12h | (OA) | 2225**c** | ± | 315 |  | 2188**c** | ± | 253 |  |  |  |
| **Cord venous (**µmol/L) |
| -4h | (SA) | 556**a** | ± | 27 |  | 634**a** | ± | 41 | 0.167 | **<0.001** | 0.857 |
| -8h | (PA) | 1497**b** | ± | 100 |  | 1618**b** | ± | 88 |  |  |  |
| -12h | (OA) | 846**c** | ± | 67 |  | 888**c** | ± | 67 |  |  |  |
| **Cord artery (**µmol/L) |
| -4h | (SA) | 520**a** | ± | 28 |  | 545**a** | ± | 27 | 0.418 | **<0.001** | 0.952 |
| -8h | (PA) | 1360**b** | ± | 82 |  | 1424**b** | ± | 102 |  |  |  |
| -12h | (OA) | 715**c** | ± | 54 |  | 754**c** | ± | 53 |  |  |  |

MPE, molar percent excess. Values are means ± SEM. N=10/group. Po, obese *vs.* control group; Pt, effect of different times; P\*, interaction obesity\*time. Significantly different at P < 0.05. Values with different superscript letters indicate statistically significant differences between hours/fatty acids (P<0.05).

Table 4. Comparison of estimated model parameters and results for obese and control pregnant women using 13C-FA in total lipids data.

|  |  |  |
| --- | --- | --- |
| **Model parameters** | **13C-FA Control** | **13C-FA Obese** |
| kuptake (mL/min) | 4.0 | 4.6 |
| kdelivery (mL/min) | 7.0 | 7.8 |
| kfetaltissue (mL/min) | 160 | 182 |
| **Estimated amounts** | **13C-FA Control** | **13C-FA Obese** |
| Dose given to mother (µmol)\* | 132 | 165 |
| Tracer influx via maternal uterine plasma (µmol)∞ | 281 | 268 |
| In placental tissue at birth (µmol) | 0.52 | 0.56 |
| In fetal plasma at birth (µmol) | 0.0065 | 0.0065 |
| Predicted fetal tissue at birth (µmol)§ | 2.60 | 2.56 |
| % of maternal dose transferred† | 1.97% | 1.55% |
| % of materno-fetal FA transfer$ | **0.92%** | **0.96%** |

\*Based on experimental data. ∞Equivalent to the area under the curve of the maternal plasma concentration time profile multiplied by the uterine artery plasma flow rate. §Fetal tissue at birth as a model prediction that would be equivalent to the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical vein flow rate, minus the small amount in fetal plasma at birth. †Equivalent to the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical vein flow rate, divided by the maternal oral dose. $Materno-fetal transfer as the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical flow rate, divided by the tracer FA in maternal uterine plasma. OA, oleic acid; PA, palmitic acid; SA, stearic acid. All values represent total lipids (TG+NEFA+PL+CE).

**ANNEX 1**. Comparison of estimated model parameters and results for obese and control pregnant women using 13C-FA in total lipids data and maternal plasma concentration time profiles for each FA separately.

|  |  |  |  |
| --- | --- | --- | --- |
| **Model parameters** | **13C-OA** | **13C-PA** | **13C-SA** |
|  | **Control** | **Obese** |
| kuptake (mL/min) | 4.2 | 5.1 |
| kdelivery (mL/min) | 7.5 | 9.4 |
| kfetaltissue (mL/min) | 165 | 214 |
| **Estimated amounts** | **13C-OA** | **13C-PA** | **13C-SA** |
|  | **Control** | **Obese** | **Control** | **Obese** | **Control** | **Obese** |
| Dose given to mother (µmol)\* | 132 | 165 | 145 | 182 | 131 | 164 |
| Tracer influx via maternal uterine plasma (µmol)∞ | 473 | 465 | 253 | 210 | 25 | 22 |
| In placental tissue at birth (µmol) | 0.50 | 0.53 | 0.63 | 0.64 | 0.15 | 0.16 |
| In fetal plasma at birth (µmol) | 0.0066 | 0.0063 | 0.0083 | 0.0077 | 0.0020 | 0.0019 |
| Predicted fetal tissue at birth (µmol)§ | 5.00 | 5.54 | 2.30 | 2.08 | 0.13 | 0.13 |
| % of maternal dose transferred† | 3.78% | 3.36% | 1.59% | 1.14% | 0.10% | 0.08% |
| % of materno-fetal FA transfer$ | **1.06%** | **1.19%** | **0.91%** | **0.99%** | **0.53%** | **0.58%** |

\*Based on experimental data. ∞Equivalent to the area under the curve of the maternal plasma concentration time profile multiplied by the uterine artery plasma flow rate. §Fetal tissue at birth as a model prediction that would be equivalent to the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical vein flow rate, minus the small amount in fetal plasma at birth. †Equivalent to the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical vein flow rate, divided by the maternal oral dose. $Materno-fetal transfer as the area under the curve of the cord arterial-venous difference over time, multiplied by the umbilical flow rate, divided by the tracer FA in maternal uterine plasma. OA, oleic acid; PA, palmitic acid; SA, stearic acid. All values represent total lipids (TG+NEFA+PL+CE).**Legend of figures**

**Figure 1.** Compartmental modelling schematic. From left to right the model distinguished the fatty acids (FA) tracer concentration for OA, PA and SA in the maternal blood plasma; the tracers in the placental tissue compartment; the tracer concentrations in the fetal plasma compartment (assumed equivalent to those in the umbilical artery); and the accumulation of tracers in the fetal tissue. Compartments are connected by unidirectional fluxes, representing the net transfer occurring from mother to the fetus. Juptake is the placental uptake flux, Jdelivery is the flux of FA from the placenta delivered to the fetal plasma via the umbilical cord, and Jfetaltissue is the flux of FA taken up by the fetal tissue.

**Figure 2.** 13C-fatty acids enrichments in maternal plasma lipoproteins of obese and normal weight pregnant women (control) at delivery. A) VLDL. B) HDL. C) LDL. 13C-Stearic acid (13C-SA) represents enrichment value after 4h of tracer administration; 13C-Palmitic acid (13C-PA) represents enrichment value after 8h of tracer administration and 13C-Oleic acid (13C-OA) represents enrichment value after 12h of tracer administration. Obese group (black squares), N = 10 and control group (open squares), N = 10. Results are expressed as means ± SEM. \*Indicates statistically significant differences (P<0.05) between normal weight and obese group. Common superscript letters indicate similar values between different time points.

**Figure 3.** A)Ratio between placenta and maternal plasma molar percent excess values (MPE). B) Ratio between cord venous and maternal plasma MPE values. 13C-Stearic acid (13C-SA) represents enrichment value after 4h of tracer administration; 13C-Palmitic acid (13C-PA) represents enrichment value after 8h of tracer administration and 13C-Oleic acid (13C-OA) represents enrichment value after 12h of tracer administration. Obese group (black points), n=10 and control group (crosses), n=10. Results are expressed as means ± SEM. Different superscript letters indicate statistically significant differences (*P*<0.05).

**Figure 4.** 13C-fatty acid enrichments in different lipid fractions of placental tissue of obese and normal weight pregnant women (control). A) Phospholipids. B) Non-esterified fatty acids. C) Triglycerides. D) Cholesterol esters. 13C-Stearic acid (13C-SA) represents enrichment value after 4h of tracer administration; 13C-Palmitic acid (13C-PA) represents enrichment value after 8h of tracer administration and 13C-Oleic acid (13C-OA) represents enrichment value after 12h of tracer administration. Obese group (black squares), n=10 and control group (open squares), n=10. Results are expressed as means ± SEM. \*Indicates statistically significant differences (P<0.05) between control and obese group. Common superscript letters indicate similar values between different time points.

**Figure 5.** Computational model predictions of fatty acids (FA) transfer. Model prediction (solid lines) vs. experimental data (symbols) in normoweight (A) and obese subjects (B) from data at delivery of 13C-OA, 13C-PA and 13C-SA, administered respectively at -12 h, -8 h and -4 h before delivery. Close up of model predictions for the umbilical artery and vein in normo-weight (C) and obese subjects (D). Data represent the sum of all lipid classes (TG+NEFA+PL+CE), reported as mean ± SEM.

**ANNEX 2 Figure.** Computational model predictions of fatty acids (FA) transfer. The experimental data for the different FAs 13C-Oleic acid (13C-OA), 13C-Palmitic acid (13C-PA) and 13C-Stearic acid (13C-SA) could be fitted reasonably well based on a single set of parameters. Overall a coherent picture of the time course of FA transfer emerged, with an initial sharp rise after administration, leading to a peak followed by a slow decline. A) Model prediction (solid lines) vs. experimental data (symbols). From left to right, results for 13C-OA, 13C-PA and 13C-SA, administered respectively at -12 h, -8 h and -4 h before delivery. B) Close up of model predictions for the umbilical artery and vein. Data represent the sum of all lipid classes (TG+NEFA+PL+CE), reported as mean ± SEM. Results shown are those for normal weight subjects, obese subjects provide a similar picture.