**Ex-vivo Placental Lipid Metabolism of Normal Weight, Overweight and Diabetic Women: A Preliminary Study**


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**INTRODUCTION**

Maternal obesity has become one of the major public health problems during pregnancy. A greater percentage of women nowadays enter pregnancy as overweight or obese [1]. The increase in the rate of obesity among pregnant women goes in parallel with the increasing trend of obesity in the world general population. Therefore the percentage of women becoming obese during pregnancy has increased tremendously to nearly 60% within the past two decades with every 1 in 3 women being involved [2,3].

The increasing rate of maternal obesity hurts women’s and child’s health [3-5]. The effect could be present not only during the pregnancy but also in the later life of both the child and the mother. The major maternal risks during pregnancy include: gestational diabetes mellitus (GDM) and pre-eclampsia, with the fetus having congenital anomalies and stillbirth [6]. In later life, mothers with previous GDM have an increased risk of type 2 diabetes, heart disease, hypertension and cardiovascular diseases (CVD) [6,7] and those women with preeclampsia have the susceptibility to future CVD and metabolic syndrome. Thus the child is at risk of obesity, heart disease and diabetes in later life [5].

The late gestation human placenta is mostly of fetal origin. Fetal growth is reliant on a constant supply of nutrients from the mother through placental transport. This transport mechanism is mainly influenced by factors of maternal, placental and fetal origin [8]. The lipid metabolism in the placenta could be an important predictor of fetal growth and later metabolic disorder in the offspring. Recently, the placental environment of obese women was found to be lipotoxic characterized by increased inflammation, oxidative stress, macrophage accumulation and decreased regulators of angiogenesis [9-13]. Hence, the lipid metabolism of the placenta of obese/overweight and normal-weight women could as well differ due to the change in the lipid environment.

**ABSTRACT**

Obesity and diabetes mellitus are documented major risk factors for maternal and fetal health complications. Maternal lipid metabolism is greatly changed when pregnancy is complicated with obesity and diabetes mellitus. The effects of maternal obesity on placental lipid metabolism are not fully understood and thereby needs to be elucidated. Our objective is to study the lipid metabolism in the placenta of normal weight, overweight/obese and diabetic women, and to assess placental, maternal and cord serum triglycerides (TG). The term placenta, maternal and cord blood were collected from three (3) normal weight, three (3) overweight/obese and two (2) diabetic women during elective caesarean sections. The total lipid content of the placenta was measured using the Folch method. Lipolysis assay was carried out in placental tissue explant using an optimized method. Triglyceride levels of placental tissue, maternal, and cord serum were measured using a commercial reagent kit. The Placental total lipid (mg/500mg placental tissue) deposition was highest in the overweight (35.7±0.5), followed by diabetic (30.7±3.4) women, while both these groups had significantly higher (P<0.05) lipid contents than normal-weight women (19.6±3.9). However, the placental lipolysis among the three groups did not differ. The maternal-to-placental-to-cord TG ratio of the overweight women was 22:2:2, while it was 19:2:4 in diabetic and 16:2:1 in normal-weight women. Our study implies that although maternal obesity and diabetes affect placental lipid deposition, they may not have a notable effect on placental lipolysis. However, cord TG and maternal-to-cord TG ratio were markedly high among diabetic women.

**KEYWORDS**

placental tissue explants
total lipid
lipolysis
triglycerides
maternal obesity
The primary purpose of this research was to study and compare the lipid metabolism in the placenta of obese/overweight, diabetic with the normal weight (control) pregnant women. The secondary purpose was to determine the correlation between the placental triglycerides and total lipid content and to assess the maternal and cord blood triglycerides contents.

MATERIALS AND METHODS

Ethics Statement
Approval of the ethics clearance for the use of human subjects from the Ethics Review Committee of the Faculty of Medicine University of Colombo and permission from the Director, De-soysa Maternity Hospital (DMH) was obtained. Written informed consent was obtained from all participants.

Study population
Eight (8) pregnant women undergoing elective caesarian section delivery at DMH were enrolled for the study based on the inclusion and exclusion criteria. The inclusion criteria were: pregnant women within the age range of 20-40 years, caesarian section delivery, fetal gestational age of 28 to 42 weeks, diabetes mellitus and singleton pregnancy. The exclusion criteria were: gestational diabetes mellitus, elevated systolic/diastolic blood pressure, hyperlipidemia, vaginal delivery, reported the use of substances such as alcohol/illicit drugs, multiple gestations, complications in pregnancy and preeclampsia. The participants were randomly selected and assigned into three groups (group 1 -normal weight, group 2-preeclampsia. The participants were randomly selected and assigned into three groups (group 1-normal weight, group 2-obese/overweight, diabetic with the normal weight (control)) according to their BMI and diabetes mellitus respectively. The baseline data for the mother and the child were obtained from the medical records.

Sample collection
The blood and the placenta were collected according to the standard protocols as follows.

Blood
The maternal venous blood was drawn just before the elective caesarian section through the cannula. The reason for collecting the blood at that time was to minimize the distress and curtail further venous puncture of the patient. Arterial umbilical cord blood was collected into a plain blood collection tube at the delivery. The blood samples were kept at room temperature for 45 min. The serum was separated after centrifuging at 2200rpm for 10 min.

Placenta
The placenta was positioned on an absorbent pad with the maternal surface facing up. The membrane was gently grasped and elevated in the centre of the placental disc and a small incision was made. The opening in the membrane was carefully increased and the membrane was transferred to the fetal side exposing the full maternal surface of the placenta. Large blood clots were removed. A quadrant of the placenta to be sampled was selected. An area of tissue that is at least 1.5 cm away from the closest edge of the placental disc and at least 1.5 cm away from the centre of the placental disc was gently grasped with forceps. 4 equal-sized vertical deep cuts (each ~1.5 cm in length) were made straight down and at a right angle to each other to form a square-shaped tissue core. A final cut was made horizontally just above the chorionic plate to completely free the tissue core. The tissue core was briefly washed in a clean kidney-shaped bowl containing ice-cold phosphate buffer saline (PBS) solution [14]. The washing steps were repeated for the remaining 3 quadrants of the placenta. After excising the 4 tissue cores, each core was divided into 2 parts, 1 part was placed into a tube containing cold PBS and the other part was transferred into a tube containing pre-warmed Minimum Essential Medium Eagle’s (MEM). The samples were transported to the laboratory within 10 min. From the sample in PBS, a piece of 1 cm² tissue from each core was cut out and transferred to cryovials (contains a total of 4 pieces). The cryovials were snap-frozen in liquid nitrogen and immediately kept at −80°C for gene expression analysis, while a pool of the remaining tissue core in PBS was used for biochemical analysis.

Placental Lipolysis
The placental tissue in MEM was transferred into a pre-warmed MEM medium at 37°C for assay of basal lipolysis. The tissue explant was then pre-incubated at 37°C for 60 min in fresh MEM medium containing 20µM isoproterenol (lipolysis stimulator), 1% Bovine Serum Albumin (BSA) and 0.077% sodium bicarbonate (NaHCO3). The medium was replaced with an identical fresh medium and incubated for up to 120 min at 37°C. Incubation media were taken out at 20, 40, 60, 80,100, and 120 min respectively. Incubation media at baseline and 20-120 min intervals were kept at 70°C to inactivate enzymes. Lipolysis was assayed by measurement of glycerol concentration in the media using a Free Glycerol Reagent kit (Catalogue no. F6428, Sigma USA (Sigma-Aldrich)). After the incubation steps in MEM, the tissue explant was transferred into a lysis solution (NaOH/SDS 0.3N/0.1%) and incubated at 55°C overnight at constant shaking. The protein content of the lysates was analyzed by Hartree-lowry’s method [15]. Lipolysis was expressed as mmolglycerol/mg protein/hour [16].

Placental Total Lipsids Extraction
Total lipid was extracted from placental tissue using the method [17] developed by Folch in 1957. The tissue was homogenized with Chloroform: Methanol (2:1, v/v) to a final volume 20 times the volume of the tissue sample (0.5 g in 10ml of solvent mixture). The mixture was agitated for 15 min in an orbital shaker at room temperature. The solution was centrifuged at 14,000 rpm for 10 min to obtain the clarified supernatant. The volume of the supernatant was measured and transferred to a fresh tube. 0.2 volume of 0.9% NaCl was added to break the phases of the organic solvents. The tube was vortexed hard for 1 min. After centrifuging at 2000 rpm for 10 min the two phases were separated. The lower organic phase containing the lipid was collected into a separate fresh tube and evaporated under a vacuum.

Placental, Maternal and Cord Serum Triglycerides Determination
The triglycerides content of the placenta, maternal and cord serum was analyzed using the triglycerides reagent kit (triglycerides liquid colour Catalogue no. 10724, Human Germany) following the manufacturer’s instruction manual.

Statistical Analysis
Data were normally distributed and expressed as mean values (experimental replicates) ± SD. Statistical differences in means of two groups were determined using a two-tailed paired student’s t-test. ANOVA test was done to describe the relationship among the three groups. Values of p < 0.05 were considered statistically significant in all analyses. All the data were analyzed using IBM SPSS 20.0 statistical package software for Windows (SPSS Inc., Chicago, IL, USA) and Microsoft excel.

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RESULTS AND DISCUSSION

Placental lipolysis

As shown in Table 1 we found no significant difference in the mean concentrations of basal and stimulated placental lipolysis among the normal weight, overweight/obese and diabetic women groups.

Table 1: Comparison of mean placental lipolysis (nmol glycerol/mg protein/hour) at a different time interval of the three groups.

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>basal</td>
<td>34.4 ± 12.2</td>
<td>44.4 ± 18.2</td>
<td>24.6 ± 5.5</td>
</tr>
<tr>
<td>20</td>
<td>55.7 ± 54.9</td>
<td>67.7 ± 18.7</td>
<td>52.4 ± 11.6</td>
</tr>
<tr>
<td>40</td>
<td>85.5 ± 67.2</td>
<td>88.0 ± 30.6</td>
<td>67.3 ± 39.5</td>
</tr>
<tr>
<td>60</td>
<td>118.5 ± 88.6</td>
<td>110.8 ± 39.5</td>
<td>41.1 ± 32.6</td>
</tr>
<tr>
<td>80</td>
<td>70.9 ± 59.4</td>
<td>114.1 ± 61.3</td>
<td>73.2 ± 11.1</td>
</tr>
<tr>
<td>100</td>
<td>75.8 ± 56.2</td>
<td>97.6 ± 25.9</td>
<td>75.0 ± 42.9</td>
</tr>
<tr>
<td>120</td>
<td>112.6 ± 64.2</td>
<td>132.8 ± 19.7</td>
<td>78.6 ± 46.5</td>
</tr>
</tbody>
</table>

Note: Group 1, normal-weight women; group 2, obese women; group 3 diabetic women; NS, no significance; S significant, *p-value between groups 1 and 2, †p-value between groups 1 and 3, ‡p-value between groups 2 and 3.

Fig 1. Association between the placental rates of lipolysis of the three groups.

Placental total lipids

The mean placental total lipids content was significantly higher in both groups 2 and 3 when compared with group 1 (P = 0.001 and P = 0.009) (Table 2) respectively. No significant difference in the placental total lipids content between groups 2 and 3 was observed.

Table 2: Comparison of mean of placental total lipids contents between the groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
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<tr>
<td>Maternal TG</td>
<td>75.8 ± 42.9</td>
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<td>75.0 ± 42.9</td>
</tr>
<tr>
<td>Cord TG</td>
<td>25.9 ± 4.9</td>
<td>22.5 ± 7.4</td>
<td>28.3 ± 5.6</td>
</tr>
<tr>
<td>Placental TG</td>
<td>13.1 ± 3.2</td>
<td>24.1 ± 5.3</td>
<td>54.6 ± 17.1</td>
</tr>
</tbody>
</table>

Note: Group 1, lean women; group 2, obese women; group 3 lean diabetic women, NS, no significance; S significant, *p-value between groups 1 and 2, †p-value between groups 1 and 3, ‡p-value between groups 2 and 3.

DISCUSSION

We studied the placental lipid metabolism to gain more understanding of the rate and flow of lipids within and through the placenta and relate that to maternal and cord serum triglycerides (TG) levels. It has become progressively clear that the maternal intrauterine milieu in pregnancy can have profound effects on their offspring later in life, a phenomenon referred to as “fetal programming”. Therefore, due to this phenomenon, three (3) different sets of women with different conditions (i.e. normal weight, overweight/obese and diabetic) were selected and studied for their placental lipid metabolism which was compared and related to TG contents of the mother and the fetus.

Maternal serum, cord serum and placental tissue triglycerides

The triglycerides concentration (mg/dL) and the difference between the mean concentrations of maternal, cord and placental triglycerides of the study groups are presented in Table 3. No significant difference was found in the mean maternal triglycerides between the three groups. The mean concentration of the cord serum triglycerides was significantly higher (0.012 and 0.003) in group 3 when compared to groups 1 and 2. And no significant difference was seen between groups 1 and 2. The mean placental triglycerides concentrations show no significant difference among the three groups.

Table 3. Comparison of the mean of cord, placental and maternal triglycerides levels.

<table>
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Note: Group 1, lean women; group 2, obese women; group 3 lean diabetic women, NS, no significance; S significant, *p-value between groups 1 and 2, †p-value between groups 1 and 3, ‡p-value between groups 2 and 3.

Maternal TG: placental TG: cord TG

<table>
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<tbody>
<tr>
<td>Normal weight women (n = 3)</td>
</tr>
<tr>
<td>Overweight/obese women (n = 3)</td>
</tr>
<tr>
<td>Diabetic normal weight women (n = 2)</td>
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</table>

For the ex vivo placental lipolysis, we were able to observe both basal and stimulated lipolysis of the placental tissue explants. Our findings found no significant difference in the mean concentrations of basal and stimulated placental lipolysis among the normal weight, overweight/obese and diabetic women groups. Although we obtained higher values from the overweight/obese woman’s placenta, the normal weight women placenta had the middle values and the diabetic placenta had the lowest lipolysis rate. From Fig. 1 a similar pattern of the rate of lipolysis was observed in the three groups from the basal level up to 40 min of stimulated level. A change of pattern was observed from 60 min up to 120 min. So far, according to a literature search, there are limited available data on the placental lipolysis rate. Some of the few findings, we came across discovered that the human placental lactogen have the inducing effect on adipose tissue and adipocytes lipolysis in pregnancy [18,19].
Relating our findings with the maternal adipose tissue lipolysis, we, therefore, suggest that the reason for observing similar lipolysis in the placenta among the normal weight and overweight/obese women could be due to the findings that normal-weight women experience a higher rate of lipogenesis in early pregnancy and increased lipolysis in late pregnancy, on the other hand, the overweight/obese women usually experience lipogenesis during the pre-gravid period and lipolysis predominate both in early and late pregnancy \cite{8,20,21}. Therefore, this is in context with our study that found a higher lipid deposition in the overweight women because of early transfer of fat to the placenta and hence the higher placental lipolysis rate. Surprisingly, our study observed a lower rate of lipolysis in the placenta of diabetic women. Therefore the reason for this kind of occurrence is not clearly understood.

Our study showed that the mean concentration of the total lipid contents in the placenta was significantly higher among the overweight/obese and diabetic women when compared to the normal-weight women. There was no significant difference in the placental lipid content between the overweight/obese women and the diabetic women, although the overweight/obese women have higher values.

When comparing the total lipid levels of the placenta of the overweight/obese and that of the normal-weight women, the findings of \cite{12} is by our results, in which they found almost 50\% more lipid levels within the placenta of overweight/obese than the normal-weight women. Moreover, the study of \cite{22} and \cite{23} on animal models showed that overweight/obese rat placenta had higher lipid accumulation in all junctional zones upon oil red o staining than those of the non-obese rats. It, therefore, appears that the accumulation of lipid depositions observed in the placenta of the overweight/obese women is a result of the mothers having an excess pre gravid adipose tissue deports together with an augmented lipid metabolism during pregnancy.

Consequently, the higher amounts of total lipids in the placenta women, which exceeded that of their normal-weight women counterparts, could be due to increased insulin resistance allied with hypertriglyceridaemia and hyperglycemia, as well as changes in the gene expression and activity of metabolic enzymes and proteins associated with a diabetic condition. The result of the present study showed no statistically significant difference in the mean values of maternal serum TG among the three study groups, but higher values were noted in the overweight/obese women, and the lowest among the normal-weight women.

Partially, this result is in agreement with the findings of \cite{6,24} and \cite{25}, which showed a significantly higher maternal TG level of overweight/obese pregnant mothers than their normal-weight women's counterpart. Also, in the animal model \cite{26}, there was a higher maternal TG levels among overweight/obese ewe. In contrast with our findings, the study of \cite{27} revealed significantly lower maternal TG levels among the overweight/obese women than the normal-weight women throughout the gestational period. The high maternal TG observed in the overweight/obese women may be due to a reduced ability to clear circulating TG even after several hours of fasting before the caesarean section delivery.

When considering the diabetic normal-weight women, their maternal serum TG level was much higher than their normal-weight match and closer to the overweight women. As diabetes in pregnancy is associated with an exaggerated altered lipid metabolism with more susceptibility towards hypertriglyceridaemia, thus our study participants have pre-existing type 2 diabetes. The Study of \cite{28} Goblet et al., 2010, has revealed that the changes in lipid metabolism in pregnancy among type 2 diabetic mothers is less pronounced when compared to mothers with type 1 diabetes, this might be the reason why their TG levels do not exceed those of the healthy overweight/obese mothers. However, \cite{29} Scifres, 2008 and \cite{30} Ciamm et al., 2005, found higher maternal TG levels among diabetic obese women when compared with healthy obese women. This implies that maternal metabolic conditions in pregnancies complicated by both obesity and diabetes could result in more adverse effects than when having a single pregnancy complication as it is with our study participants (diabetic normal-weight women).

The TG content of the placenta was examined to determine whether an increase in maternal circulating TG levels corresponds to their increase within the placenta. Our findings showed no significant difference in the mean values of placental TG levels among the groups. Although the placental TG levels of the diabetic women were slightly higher than those of the overweight/obese women and the normal-weight women have the lowest placental TG content. Similar to our finding, the study of Hirschmugl et al. \cite{9} found increased TG content in the placenta of overweight/obese women when compared to lean women. Likewise Heerwagen et al. \cite{23} and Strakovsky and Pan \cite{22} found in their study on an animal model that the placenta TG content of the overweight/obese rat was significantly higher when compared to those of the non-obese rats. As revealed by the study of Lindegaard et al. \cite{6} the TG level of the placenta in diabetic conditions was found to be augmented when compared to that of a healthy pregnancy. Even though the maternal TG levels of the diabetic women were lower than that of the overweight/obese, but the placental TG levels in the diabetic pregnancy exceeded those of the overweight women, this implies that the enzymes and the proteins involved in the influx of lipid into the placenta and those involved in the metabolic pathways within the placenta are extensively changed in diabetic condition during pregnancy.

The comparison of the mean values of TG concentration in the cord serum of the study groups showed significantly higher levels among the diabetic normal weight group when compared to the normal weight and overweight group. The cord TG of the overweight/obese women was significantly higher than that of the normal-weight women.

Our study is in parallel with the study of \cite{31} Murthy et al., 2014, in which they found higher cord TG levels among the newborns of diabetic mothers when compared to those of healthy women. Similarly, the cord TG of infants of diabetic obese women was higher than the cord TG of obese infants as indicated by \cite{29} Scifres et al., 2008. Likewise, in the animal model \cite{26} Zhu et al., 2010 observed a higher cord TG level of an obese ewe when compared with normal weight ewe.

In contrast to our results, \cite{6} Lindegaard et al. found no significant difference in the cord TG level of diabetic women when compared with the TG levels found in healthy women infants. Similarly, \cite{24} and \cite{32} observed no significant difference between the cord TG levels of the overweight/obese women and normal-weight women. From our outcomes, we can therefore obviously say that the infants born to diabetic mothers are at higher risk for future health complications. The result of the current study presents a higher maternal to placental TG ratio among the overweight/obese women when compared to the normal weight and the diabetic women’s ratios. But, the TG levels in the diabetic pregnancy exceeded those of the overweight women, this implies that the enzymes and the proteins involved in the influx of lipid into the placenta and those involved in the metabolic pathways within the placenta are extensively changed in diabetic condition during pregnancy.
concentrations both in the placenta and fetal cord serum were higher in the diabetic women's group. However, the values obtained from the overweight/obese women were also higher than the normal-weight women. The higher cord to maternal TG ratios found in neonates of diabetic mothers denotes that the metabolic disturbances resulting from diabetes complications in pregnant women may reflect the increased lipid uptake by the placenta and perhaps delivery to the fetus which predominantly affect the development of fetal adipose tissue. Thus the neonates of the overweight/obese women are also at risk of developing more fat mass as indicated in their cord to maternal TG ratio even though their birth weight was lower among the groups.

As suggested by earlier studies that fetal lipid accumulation is caused by increased maternal-to-fetal lipid concentration gradients [33-37] and believed widely that membrane transport, not metabolism is the rate-determining step in nutrient transport to the fetus [38-42]. However, the study of [43] Perazzolo et al., thoroughly investigated the determining factors and the influence of the placenta in the transfer of fatty acids to the fetus using a combined experimental and computational modelling approach on a perfused placenta with radiolabelled fatty acids. Consequently, they found that lipid metabolism and not membrane transport was the determining factor of lipid transfer from the mother to the placenta. However, from the placenta to the fetus both metabolism and membrane transport affect lipid supply to the fetus. And this was due to their observation of a continual steady rate of fetal vein fatty acid concentration regardless of variation in the maternal lipid concentration.

Finally, as observed from our study groups, it appears that in healthy pregnancy (normal weight and overweight/obese women) maternal-to-placental lipid transport capacity is higher than the placental-to-fetal transport capacity. Unlike in the diabetic condition where the augmented TG level was observed in the infant's cord blood, which indicates somewhat an alteration in both their metabolism and membrane transport of lipids.

CONCLUSION

Our study implies that although maternal obesity and diabetes affect placental lipid deposition, they may not have a notable effect on placental lipolysis. However, cord TG and maternal-to-cord TG ratio were markedly high among diabetic women.

ACKNOWLEDGEMENT

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REFERENCES

2. Valsamakis, G. et al., 2015. Effect of maternal obesity on pregnancy outcomes and long-term metabolic consequences. Hormones, 14 (3);345-357
7. Jin, W., et al., 2016. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China. BMC Pregnancy Child. 1(60); 1-9
43. Perazzolo, S. et al., 2016. The influence of placental Metab on fatty acid transfer to the fetus. J Lipid Res. 58(2);443–