LIPIDS PROFILE AND FATTY ACIDS PATTERN IN THE PREGNANT RABBITS

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Received at 25 - 2 - 2003

ABSTRACT

Twenty mature pregnant New Zealand rabbit were used to clarify the blood biochemical changes in lipids and fatty acid profile during pregnancy. Blood samples were obtained 0, 10, 20 and 30 days following pregnancy. Sera were collected and stored frozen at −20°C until determination of total lipid, triacylglycerol, cholesterol, phospholipids, HDL-C, LDL-C and fatty acids pattern. The obtained results revealed that the serum total lipid, triacylglycerol, cholesterol and phospholipids showed a significant decrease till parturition time reaches. Both HDL-C & LDL-C were also significantly decreased along the period of pregnancy and finally there’s some fatty acids patterns as laurate, palmitic, linoleic, linolenic, exhibited a significant increase through the pregnancy period where the myristate showed non-significant change and the arachidonic acid showed a highly significant increase throughout the pregnancy period.

INTRODUCTION

Pregnancy is associated with hypercholesterolemic and hyperlipidemic state. Plasma analysis of pregnant rabbits showed a decrease in total cholesterol and lipoprotein with starting the 10th day, and a significant elevation was noticed at the end of pregnancy. Moreover, triglycerides metabolism was significantly increased after 20 days (Montoudis et al., 1999). In pregnant rats, circulating triglycerides and VLDL levels are high indicating that liver-synthesized triglycerides are rapidly released to the circulation. This increase is coincident with decrease plasma lipoprotein lipase activity (Herrera et al., 1988). In the
same respect, Brian et al., (1986) mentioned that, cattle with severe hepatic lipoidosis had greater concentration of hepatic triglycerides before calving and after parturition. At ante-partum, the serum triglyceride concentration increased approximately two folds in women with preeclampsia relative to uncomplicated pregnancies (Hubel et al., 1996). While, Gratac et al., (1996) recorded that; triglycerides levels were significantly higher than control values, in women with severe gestational hypertension. In heterozygous WHHL and NZW rabbits, the triglycerides were higher during gestation and decreased to the lowest value during lactation when compared to the level at mating (Mortensen and Frandsen (1996). Moreover, Hussein and Azab, (1998) found that plasma concentration of triglycerides increased significantly at 4, 3 and 2 weeks before parturition in goat. Watson et al., (1993) concluded that, the mass phospholipids in VLDL from lactating mare was significantly lower than before and during pregnancy while Bennis et al., (1992) concluded that, the serum phospholipids decreased at the time of parturition when compared with the last two weeks before pregnancy in does and then the level increased again after parturition. Also, there was a significant decrease in the level of phospholipids in the last month of pregnancy.

Meyers and Vohr, (1996) mentioned that, the mean concentration of LDL-C was significantly higher in the gestational diabetic mothers than in control and also they added that HDL-C was inversely correlated with insulin. While Henson et al., (1997) showed that maternal low density lipoproteins is the principle source of cholesterol substrate for progesterone biosynthesis in the primate placental syncy

Reynaert et al., (1976) reported that, during the days before and after parturition, no change in the serum free fatty acids could be determined in heifer and cow. Moreover, Ogburn et al., (1980) found that there is a general rise in total fatty acids as the pregnancy progressed, followed by a marked elevation in total fatty acids during labour. In the same respect (Kashyap et al., 1976) observed that, there is a dramatic two-fold increase in maternal plasma free fatty acids during labour in women. Furthermore the level of FFA and fatty acids in blood
of pregnant women significantly increased in the second and third trimesters of pregnancy. Sanjurjo et al. (1993) reported that in the first trimesters of pregnant women there was a significant increase in palmitic, palmitoleic, stearic and docoahexonic acids where as linolenic and eicosapentaenoic decreased. Between the first and the second trimester a significant increase in the proportion of palmitic acid and a significant decrease in arachidonic acid was detected in the second trimester and delivery. In the same aspect, (Holman et al., 1991) reported that the changes seen in phospholipid profiles suggest a significant transfer of omega 3 and omega 6 polyunsaturated fatty acids from the mother to the fetus. These fatty acids are essential for normal fetal growth. Wang et al, (1992) reported that, the total polyunsaturated fatty acid levels were not significantly different between non-pregnant and normal pregnant women. Normal pregnant patient has significantly higher levels of eicosapentaenoic and decosahexanoic acid. This may reflect normal physiological changes in pregnancy and the decreased level of eicosapentaenoic acid seen in preedomptic patients may play a significant role in the nearly identical in the two groups suggesting that in intrauterine growth rate the essential fatty acids will be transported to the fetus at the expense of the placenta. Furthermore, Lorentzen et al., (1995) reported that, the free fatty acids significantly increased in woman with preeclampsia the level and composition of the esterified fatty acids in phospholipids, triglycrides and cholesterol esters did not increase. The present work aimed to clarify the blood biochemical changes in lipids and fatty acids profile during pregnancy in order to throw light about the feed requirements during this period.

**MATERIALS & METHODS**

The present study was carried out on twenty female mature and virgin New Zealand rabbits obtained from private farm in Kafr El-Sheikh governorate. The age ranges from 4 - 4.5 months and the individual body weight averaged 2.9±0.4 kg. Animals were examined for external and internal parasites and kept under good hygienic conditions throughout the experimental period. Rabbits were fed on concentrated diet obtained from Cairo Company for poultry, feed and water were ad libtum. Blood
samples were collected from all females at zero time. Then each 2-non pregnant mature females were put in a special wire cage for one day in contact with adult mature male rabbit (5-6 month age). Ten days later the pregnancy was diagnosed by abdominal palpation. Thereafter, all pregnant females were separated in special cages for completion of the pregnancy period. From each pregnant female blood was collected from the ear vein at 10, 20 and 30 days of pregnancy and left to coagulate. The serum samples were separated and stored frozen until used for determination of serum total lipids, triacylglycerols, total cholesterol phospholipids, HDL-C and LDL–C according to Frings and Dunn (1970); Fossati (1982); Svenson, (1982); Zilversmit and Davis (1950); Grove (1979) and Friedewald et al. (1972) respectively. The serum fatty acids were measured using Shimadzu CR 3A gas liquid chromatography according to the method described by Frakas et al., (1980). Data were statistically analyzed according to Snedecor and Cochran, (1969).

RESULTS & DISCUSSION

Data presented in table (1) and fig. (1) showed that, there was a significant decrease (P < 0.05) in the total lipids in the serum at 20 and 30 days of pregnancy compared to 0 and 10 days of pregnancy. The obtained results came in accordance with that reported by Hussein et al., (1995) in cattle and Hussein and Azab, (1998) in goats, which attributed the decline in total lipids to the gradual exchange of fatty acids containing lipids from the blood to the mammary glands. Also they observed that serum total lipid concentrations in Holstein fresian were lower at late stage of non-lactating pregnant females due to onset of lactogenesis (production of precolostrum). Based on the biochemical findings of this study the decrement in the total lipids could be attributed theoretically to the increases of fetal demand for its normal intrauterine growth which require oxidation of fatty acids to yield high amounts of ATP as a source of energy.

The obtained data revealed that, triacylglycerols of pregnant rabbits were non-significantly changed throughout the experimental period. In the same respect La Borde et al., (1999) found a three-fold elevation of serum triacylglycerol levels in rats near term with a subsequent decrease
at birth. In contrast, Bennis et al., (1992) attributed the increase of triacylglycerol level at late pregnancy in goat to the increased hepatic triacylglycerol synthesis. Schulz et al., (2001) reported that, when lipoproteins decreased, the triglycerides content progressively increased. He added that the fractions of higher density may represent the vehicles for triglycerides transport, while the composition of lipoprotein of lower density reflects the extent to which the vehicle is loaded with triglycerides.

The total serum cholesterol of pregnant rabbits in table (1) and fig.(1) showed a significant decreased (P<0.05) at 20 days and highly significant decrease (P<0.01) at 30 days of pregnancy compared to 0 and 10 days. This results come in accordance with that of Mortensen and Frandsen (1996) who attributed the decrease in the level of serum cholesterol during pregnancy to the increase biosynthesis of sex hormones. Moreover, Girgis et al., (1961) found that, the concentration of progesterone increased gradually to reach the peak at the mid pregnancy period and then followed by gradual decline in the 2nd half of pregnancy, in rabbits.

It is clearly observed from the present results that, there was a highly significant decrease in the mean values of serum phospholipids during pregnancy period. The observed decrement in serum phospholipids during this period might be attributed to the great exchange of phospholipids into mammary glands to be utilized in milk synthesis (Yang et al., 1978).

The present study revealed that, there was a significant decrease (P<0.05) in HDL-C and LDL-C at 10 days and highly significant decrease (p<0.01) at 20 and 30 days of pregnancy. In the same respect, Hussein and Azab. (1998) reported a significant decrease in plasma LDL-C concentration during late stage of pregnancy. In contrast, Bennis et al., (1992) observed that HDL-C fraction is predominant during late stage of pregnancy and parturition.

The mammary alveolar cellssynthesize the major portion of the short chain fatty acids (C4 to C16) for milk triacylglycerols. However, long chains fatty acids (C16-C18) appear to be peculiar to a significant
extent from the lipolysis of bovine lipoproteins as they circulate through capillaries in the mammary gland (Patton and Jenson, 1976). The present study (table 2, fig. 2 and 3) declared that, there was a significant increase in lauric acid throughout the pregnancy period. The increment in lauric acid might be attributed to increased lipolysis in this period to meet the requirements of foeti for energy during their growth Hussein and Azab, (1998). The mean values of palmitic acid were significantly higher throughout the pregnancy period. This increase agreed with the results of Sanjurjo et al., (1993) in women. This result was previously explained by Fowden et al., (1984), who attributed the increment of long chain fatty acids to the peripheral insensitivity to the action of insulin hormone in goats. The present data agree with that of Naylor et al., (1980) who reported that the excessive mobilization of all fatty acids from adipose tissue as a response to negative balance, the long chain fatty acids increased in the serum of pregnant ewes. The present study demonstrated that there was a significant increase in stearic acid in the sera of does during pregnancy. This result disagreed to that reported by Sanjurjo et al., (1993) who found that, the mean values of stearic acid were higher at the first trimester of pregnancy in women and decreased between the second trimester and delivery. This increment could be attributed to the excessive mobilization of fatty acids from adipose tissue as affected by hormone-sensitive lipase secreted during such period (Robert et al., 2000). The linoleic acid exhibited significant changes throughout the experimental period. The previously mentioned results were confirmed by the work of Chen et al., (1992) who suggested that, the increment of linoleic acid in the sera of rats during pregnancy especially in the last trimester could be attributed to the transfer of linoleic acid to the neonates in the period prior to lactation.

It could be concluded that, lipoproteins and fatty acids showed a noticeable changes during pregnancy so, it seems necessary to increase their concentrations specially unsaturated fatty acids in the diet of rabbits to meet the increased physiological requirements during such period.
**Table 1:** Effect of pregnancy on the level of different serum lipid profile (mg/dl) in rabbits.

<table>
<thead>
<tr>
<th>Criteria Time</th>
<th>Total lipids</th>
<th>Triacylglycerol</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>О day</td>
<td>320.58±5.9</td>
<td>125.78±2.1</td>
<td>67.25±1.2</td>
<td>88.25±1.9</td>
<td>6.40±0.5</td>
<td>37.03±1.0</td>
</tr>
<tr>
<td>10 days</td>
<td>314.44±4.3</td>
<td>126.39±2.0</td>
<td>63.23±1.2</td>
<td>82.74±1.8**</td>
<td>4.33±0.5*</td>
<td>33.67±1.0*</td>
</tr>
<tr>
<td>20 days</td>
<td>305.22±3.9*</td>
<td>124.43±1.7</td>
<td>59.39±1.6*</td>
<td>79.08±1.7**</td>
<td>4.30±0.4**</td>
<td>30.78±0.9**</td>
</tr>
<tr>
<td>30 days</td>
<td>294.01±3.4*</td>
<td>126.16±1.4</td>
<td>54.18±1.2**</td>
<td>74.87±1.7**</td>
<td>4.01±0.3**</td>
<td>26.42±1.5**</td>
</tr>
</tbody>
</table>

Means±S.E. * (p<0.05) ** (p<0.01)

**Table 2:** Effect of pregnancy on the level of different serum fatty acids patterns (mg/dl) in rabbits.

<table>
<thead>
<tr>
<th>Criteria Time</th>
<th>Laureate</th>
<th>Myristate</th>
<th>Palmitoleate</th>
<th>Palmitate</th>
<th>Stearate</th>
<th>Linolate</th>
<th>Lenoleic</th>
<th>Arachidonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0.072±0.028</td>
<td>0.401±0.089</td>
<td>0.224±0.048</td>
<td>0.115±0.030</td>
<td>0.438±0.083</td>
<td>0.184±0.045</td>
<td>0.252±0.084</td>
<td>0.040±0.006</td>
</tr>
<tr>
<td>10 day</td>
<td>0.156±0.041</td>
<td>0.464±0.093</td>
<td>0.284±0.055</td>
<td>0.184±0.026</td>
<td>0.480±0.075</td>
<td>0.217±0.039</td>
<td>0.187±0.033</td>
<td>0.094±0.028</td>
</tr>
<tr>
<td>20 days</td>
<td>0.243±0.037*</td>
<td>0.521±0.108</td>
<td>0.320±0.058</td>
<td>0.240±0.027*</td>
<td>0.502±0.046</td>
<td>0.301±0.034</td>
<td>0.230±0.032</td>
<td>0.175±0.038*</td>
</tr>
<tr>
<td>30 day</td>
<td>0.336±0.053*</td>
<td>0.573±0.108*</td>
<td>0.327±0.052</td>
<td>0.345±0.046*</td>
<td>0.638±0.067</td>
<td>0.372±0.018</td>
<td>0.277±0.028</td>
<td>0.228±0.032**</td>
</tr>
</tbody>
</table>

Means±S.E. * (p<0.05) ** (p<0.01)
Figure (1) Effect of pregnancy on the level of different lipid parameters (mg/DL)

Figure (2) Effect of pregnancy on the level of different fatty acids (mg/DL)
Standard curve of fatty acids

Fig. 3
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فصل مصل الدم وحفظه عند درجة حرارة -20°م لتحليل كلاً من الدهون الكلية و الدهون الثلاثية والكولسترول والفوسفوليبيدات والدهون البروتينية عالية الكثافة والمنخفضة الكثافة كما تم فصل الأحماض الدهنية. وقد أظهرت التجارب انخفاض معنوي في نمط الدهون الكلية والدهون الثلاثية والكولسترول والفوسفوليبيدات بطول فترة الحمل، كما وجد أن هناك نقص معنوي في مستوي الدهون البروتينية عالية الكثافة والدهون البروتينية منخفضة الكثافة أثناء فترة الحمل. أما بالنسبة للأحماض الدهنية المشبعة وجد أن هناك زيادة معنوية في مستوي حمض اللوريك وحمض البالانتيك وحمض الأستيارك كما لوحظ وجود زيادة غير معنوية في مستوي حمض المريستيك. وبالنسبة للأحماض الدهنية غير المشبعة وجد أن هناك زيادة معنوية في مستوي حمض الأولينيك وحمض اللينولينك وحمض الأراشيدونيك. من النتائج السابقة لوحظ أنه يجب أن ننتمي ونركز جيداً على مكونات العلائق المعطاة للحيوانات أثناء فترة الحمل مع النصح والوضع في الاعتبار بزيادة نسبة الدهون الكلية في العلبة وخصوصاً الأحماض الدهنية غير مشبعة في العلبة أثناء فترة الحمل.