Changes in the composition of plasma very low density lipoprotein during pregnancy and lactation in genetic lines of pigs

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Abstract

Plasma very low density lipoproteins (VLDL) of gilts were separated into two subfractions according to their affinity for heparin. The proportion of VLDL present as subfraction 2 (higher affinity for heparin) varied, according to the genetic line of the pigs, between 0.21 and 0.78 in virgin gilts. The proportions were related to the variation in piglet survival in the same nine genetic lines by a quadratic equation, which predicted that greater than 90% survival to weaning was to be found in piglets born to gilts having between about 0.3 and 0.7 of their VLDL as subfraction 2. This observation suggests a simple measurement that could be used in the selection of sows for a breeding programme. The proportion of subfraction 2 fell throughout pregnancy in each of three genetic lines measured and only returned to pre-pregnancy values after weaning; these changes did not correlate with the changes in the lipid composition of plasma VLDL measured during pregnancy and lactation. The findings suggest a rôle for the VLDL subfractions in controlling the nutrition of the neonatal pig.

Keywords: lactation, lipoproteins, plasma lipids, pregnancy, sows.

Introduction

Very low density lipoproteins (VLDL) form one of the two classes of plasma lipoprotein responsible for the majority of the transport of triacylglycerol between tissues by the blood system of mammals. In animals given a diet high in carbohydrate and low in fat, the rôle of the diet-derived chylomicrons is reduced and VLDL are the major triacylglycerol-rich lipoproteins. Their rôle is to transport newly synthesized triacylglycerol from the liver to tissues, principally adipose tissue, cardiac muscle, lung and lactating mammary gland, which either store triacylglycerol or use it as a source of energy or of intermediates for further synthesis (Fielding and Fielding, 1991).

The rôle of VLDL in supplying triacylglycerol for milk production presumably has greater significance in a species such as the pig, which is born with very low reserves of body fat (typically only 10 to 20 g/kg of the neonatal carcass is fat, compared with about 160 g/kg in humans (Petigrew, 1981; Gurr, 1988)) but receives milk with a high fat content compared with many other terrestrial mammals (70 to 90 g/kg compared with about 40 g/kg in human milk) (Ribadeau-Dumas, 1983; Gurr, 1988). It can be concluded that the high content of fat in the milk (and hence the correspondingly high rates of precursor uptake and of milk-fat biosynthesis by the mammary gland of the sow) is critical to the survival of the neonatal pig, which has no other reserves of energy. Mortality in neonatal pigs is indeed high (see, for example, English and Wilkinson, 1982) and, while the problem clearly results from a combination of different causes (English and Morrison, 1984), the supply of energy-rich nutrients in the milk may be a contributory factor (Boyd, Moser, Peo and Cunningham, 1978; Pettigrew, 1981; Moser, 1983).

Several laboratories have shown that VLDL from rat and human plasma can be separated into at least two subfractions by chromatography on heparin-agarose (Shelburne and Quarfordt, 1977; Trezzi, Calvi, Roma and Catapano, 1983; Huff and Telford, 1984; Herrera, Gomez-Coronado and Lasuncion, 1987; Evans, Huff and Wolfe, 1989; Fielding, Ishikawa and Fielding,
The separation depends upon the affinity of the apoprotein components of the VLDL for the negatively charged heparin. It has been shown that the binding depends upon the presence of the positively charged apolipoprotein E (apo-E) and not upon apolipoprotein B (apo-B) (Fielding et al., 1989). Different numbers of apo-E molecules per VLDL particle, therefore, result in different affinities for heparin, thus enabling the separation. It has been shown that the least retarded fraction of both rat and human VLDL (having the lowest content of apo-E) also has the highest relative content of triacylglycerol (Shekurne and Quartordt, 1977; Trezzi et al., 1983).

Herrera et al. (1987) have shown that the more retarded subtraction of VLDL is the major subtraction in virgin female rats but that, by the 20th day of pregnancy (the day before parturition), it had almost disappeared in favour of the less retarded subtraction. One interpretation is that the changes are an adaptation necessary as a preparation for lactation. The purpose of the work reported herein was to undertake a similar analysis of VLDL subfractions in pre-pregnant, pregnant and lactating pigs where it is likely that the delivery of VLDL-triacylglycerol to the mammary gland is especially important. The hypothesis was tested further by comparing different genetic lines of pigs which have established different rates of survival for their piglets.

Material and methods

Material

Kits for the determination of lipoprotein composition were ordered from Sigma Chemical Company Ltd, Poole, Dorset (protein determination, procedure no. P5656), Biomen Ltd, Croydon, Surrey (total cholesterol and triacylglycerol) and the Boehringer Corporation (London), Lewes, East Sussex (free cholesterol and phospholipid). Heparin-agarose (heparin-Sepharose CL-6B) was obtained from Pharmacia Biosystems Ltd, Milton Keynes, Buckinghamshire. Single-draw vacutainer needles (50 mm × 1-1 mm) and vacutainers (7 ml, containing freeze-dried ethylene diamine tetra acetic acid (EDTA)-Na₂) were obtained from Becton Dickinson Vacutainer Systems, Rutherford, New York. All other reagents were of analytical grade.

Animals

The Landrace sows and gilts used were derived from the seventh generation of a selection experiment being undertaken at Wye College, University of London and The Institute of Animal Physiology and Genetics, Edinburgh Research Station, Roslin. The eight genetic lines and the unselected ‘control’ pigs were described by Webb and Curran (1986). Animals were fasted overnight and bled prior to the morning feed. Blood samples were collected from the jugular veins, as described by Muirhead (1981), directly into vacutainers. Piglet survival rate figures were based on piglets born alive and surviving to weaning at about 35 days after birth.

Preparation of VLDL

The collected blood was transferred to centrifuge tubes and plasma prepared by centrifuging the blood at 8500 g for 30 min. The supernatant plasma was transferred to ultracentrifuge tubes where sufficient 0·189 mol/1 NaCl, 1 mmol/1 EDTA-Na₂ to give a total volume of 20 ml was carefully layered over the plasma. The tubes were centrifuged at 100000 g for 18 h at 10°C. VLDL were collected as the cloudy translucent layer, with a density of <1-006 g/cm³, floating at the top of the tube. The collected VLDL were diluted with further 0·189 mol/1 NaCl, 1 mmol/1 EDTA-Na₂ to a total volume of 20 ml and subjected to a repeat of the centrifugation step. The resulting washed VLDL were dialysed, prior to fractionation, against at least 100 volumes of 50 mmol/1 NaCl, 5 mmol/1 Tris buffer (pH 7·4) with four changes of medium over a period of more than 8 h. This procedure had previously been shown to reduce the conductivity of the VLDL solution to less than that of 60 mmol/1 NaCl solution.

Subfractionation of VLDL

VLDL were subfractionated according to their affinity for heparin on a column of heparin-agarose using a modification of the method of Trezzi et al. (1983). A column containing 6 ml heparin-agarose was prepared according to the manufacturer’s instructions and equilibrated in 50 mmol/1 NaCl, 5 mmol/1 Tris, 0·02% (w/v) Na₂CO₃, 25 mmol/1 MnCl₂, pH 7·4. Dialysed VLDL solutions were adjusted to 25 mmol/1 MnCl₂ and samples containing 0·2 mg to 0·5 mg protein were loaded onto the column followed by 5 ml of the loading buffer. The column was eluted with 200 mmol/1 NaCl, 5 mmol/1 Tris, 0·02% (w/v) Na₂CO₃, pH 7·4 until a first protein peak (as judged by the absorption at 280 nm) had emerged. The column was then eluted with a continuous linear gradient made from 15 ml of the above 200 mmol/1 NaCl buffer and 15 ml of 1·5 mol/1 NaCl, 5 mmol/1 Tris, 0·02% (w/v) Na₂CO₃. The relative sizes of subfractions were assessed by integrating the recorder trace obtained by monitoring the absorption of the eluant at 280 nm using a Pharmacia UV-1 absorbance monitor (Pharmacia Biotech Ltd, St Albans, Hertfordshire).

Analysis of VLDL composition

VLDL protein, triacylglycerol, phospholipid, total and free cholesterol were determined using the appropriate kits and essentially following the
Table 1: Details of the survival of piglets born to sows of generation seven, according to genetic line

<table>
<thead>
<tr>
<th>Genetic line</th>
<th>No. of litters</th>
<th>Piglets born alive</th>
<th>Piglets surviving to weaning</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGAH</td>
<td>23</td>
<td>203</td>
<td>146</td>
<td>96.6</td>
</tr>
<tr>
<td>LGA L</td>
<td>19</td>
<td>146</td>
<td>138</td>
<td>94.5</td>
</tr>
<tr>
<td>VFHL</td>
<td>24</td>
<td>225</td>
<td>184</td>
<td>81.8</td>
</tr>
<tr>
<td>VFH L</td>
<td>21</td>
<td>219</td>
<td>195</td>
<td>91.3</td>
</tr>
<tr>
<td>LGSH</td>
<td>20</td>
<td>123</td>
<td>111</td>
<td>90.2</td>
</tr>
<tr>
<td>LGSL</td>
<td>23</td>
<td>219</td>
<td>187</td>
<td>85.4</td>
</tr>
<tr>
<td>LFAH</td>
<td>18</td>
<td>147</td>
<td>133</td>
<td>90.5</td>
</tr>
<tr>
<td>LFA L</td>
<td>21</td>
<td>196</td>
<td>183</td>
<td>93.4</td>
</tr>
<tr>
<td>Control</td>
<td>27</td>
<td>249</td>
<td>226</td>
<td>90.8</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>1717</td>
<td>1553</td>
<td>90.4</td>
</tr>
</tbody>
</table>

Results

The data presented in Table 1 summarize the survival of piglets produced by the different genetic lines in generation 7 of the selection experiment. Clear differences between the proportions of piglets surviving to weaning were recorded, with the highest survival rate being 97% for the LGAH line and the lowest survival rate being 82% for the VFHL line (results of a χ²-test using a 9 x 2 contingency table, χ² = 41.56; P < 0.001). Similar differences were observed for the cumulative data (generations 1 to 7) with LFAL showing the highest survival at 93% and VFHL being the lowest at 84% (contingency table, χ² = 88.39; P < 0.001). Analysis of variance of the survival data for generation 7 on a litter by litter basis also showed significant differences between the lines (P < 0.001).

Analysis of the composition of VLDL obtained from sows throughout their first pregnancy and lactation revealed an increase in all the lipid components measured (expressed per ml of plasma) in the 5 weeks prior to farrowing, reducing to the values obtained prior to pregnancy by 1 week after farrowing (Figure 1). In contrast VLDL-protein varied little during the period measured except for a large increase 1 week after farrowing.

The loadings of VLDL used for chromatography on heparin-agarose were shown, in preliminary experiments, not to cause any elution of protein in the loading buffer. Those preliminary experiments used a shallow gradient from 50 mmol/l NaCl to 750 mmol/l NaCl to elute the VLDL from the column. Under such conditions major peaks eluted with maxima at about 130 mmol/l NaCl (subfraction 1) and over 250 mmol/l NaCl (subfraction 2). In order to obtain better resolved peaks, the much steeper elution gradient described in Material and Methods was employed. A typical recorder trace obtained by chromatography under the standard conditions is shown in Figure 2. Subfraction 1 eluted in the initial buffer containing 200 mmol/l NaCl and subfraction 2 eluted at between 300 mmol/l and 450 mmol/l NaCl under these conditions. It should be noted that occasionally one or both of the subfractions showed 'shoulders' indicating the potential for further fractionation and that on a few occasions a third distinct subfraction was seen at about 600 mmol/l NaCl.

Table 2 summarizes the results of the heparin-agarose chromatography carried out on VLDL obtained from nulliparous gilts belonging to the nine genetic lines. The recorder trace, obtained by monitoring the absorbance of the eluant at 280 nm, was integrated and the area under the peak corresponding to subfraction 2 expressed as a proportion of the total area under the peaks corresponding to subfractions 1 and 2. Analysis of variance showed clear differences between the nine lines (P < 0.001). The VLDL from the VFHL line contained a significantly higher proportion of subfraction 2 than any of the other genetic lines except LFAL (P < 0.05). The lines VFIL and LGSL contained lower proportions of subfraction 2 than all the other lines with the exception of LGSH (P < 0.05). When these data from the genetic lines on piglet survival and on the composition (by subfractions) of the VLDL were analysed by non-linear regression, a clear relationship described by a quadratic equation was observed (Figure 3). The quadratic correlation was significant (r² = 0.865; P < 0.01). The equation predicts maximum survival for piglets born to gilts having roughly 0.5 of their VLDL as subfraction 2 before the onset of pregnancy and greater than 90% survival for those between 0.3 and 0.7. The survival data shown were derived from the cumulative experience with generations 1 to 7 inclusive and were considered more valid because a total of 9978 piglets were included in the calculations. A single generation such as that shown in Table 1 included fewer litters and was thus more susceptible to the occurrence of atypical results. Nevertheless, the relationship was still observed when the survival data were derived from a single generation (piglets born to generation 7) (r = 0.710; P < 0.05).
Figure 1 The variation in the concentration of VLDL components in the plasma of pregnant and lactating sows. The time scale is expressed in weeks prior to (-) or following (+) parturition (zero weeks). Service was at -16.5 weeks. Data were obtained from one animal belonging to each of six different genetic lines each sampled at intervals of 3 weeks. Points on the time axis are grouped to the nearest 3 week interval. Panel (a) shows VLDL triacylglycerol; (b), VLDL protein; (c), VLDL total cholesterol; (d), VLDL free cholesterol; (e), VLDL phospholipid. Error bars are ±1 standard error of the mean.
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Figure 2. A sample chromatogram of a VLDL preparation on a heparin-agarose column. The absorbance of the eluate at 280 nm is shown as the unbroken line. The concentration of NaCl is shown as the dotted line. The graduation marks on the volume-axis represent intervals of 5 ml of eluate.

After preliminary studies had shown that the proportion of subfraction 2 observed in virgin VFIL gilts declined during pregnancy and early lactation (data not shown), three lines were chosen for further study. The lines were chosen on the basis of piglet survival rates, VFIL being low, LGAH high and LGAL intermediate. Blood samples for chromatographic analysis of the VLDL were taken from sows of these lines at regular intervals during their first pregnancy and lactation and, in some cases, the subsequent pregnancy. The results presented in Figure 4 show that, in the majority of sows, the proportion of subfraction 2 had already fallen to values below the 'virgin' values by the first 1 or 2 weeks of pregnancy. The fall continued during the first few weeks of pregnancy, the proportion of subfraction 2 becoming relatively stable at a new low value from week 6 until after parturition. There was some evidence of an increase in the proportion of subfraction 2 as weaning approached. Similar trends were observed in the second pregnancy. The relative fall in the content of subfraction 2 was seen most clearly in the VFIL line where the pre-pregnant value of 0.78 fell to between 0.05 and 0.2 in mid pregnancy. A similar consistent, albeit less dramatic, fall from 0.38 to between 0.04 and 0.18 was observed with the LGAL line; data from the LGAH line were less consistent but it can be seen that the proportion of subfraction 2 did not fall to such low values as in the other two lines.

Discussion
An important finding of the work is that porcine VLDL, like that from humans and rats (Shelburne and Quarfordt, 1977; Trezzi et al., 1983; Huff and Telford, 1984; Herrera et al., 1987; Evans et al., 1989; Fielding et al., 1989), can be separated into subfractions according to their affinity for heparin. Previous experiments with rat (Herrera et al., 1987) or human VLDL (Gómez-Coronado, Sáez, Lasunci6n and Herrera, 1993) have shown that four

Table 2. The proportion of plasma VLDL present as subfraction 2 in nulliparous gilts of generation 7

<table>
<thead>
<tr>
<th>Genetic line</th>
<th>No. of pigs</th>
<th>Proportion of total VLDL present as subfraction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGAH</td>
<td>6</td>
<td>0.42 ± 0.017</td>
</tr>
<tr>
<td>LGAL</td>
<td>6</td>
<td>0.38 ± 0.019</td>
</tr>
<tr>
<td>VFIL</td>
<td>4</td>
<td>0.78 ± 0.009</td>
</tr>
<tr>
<td>VFIH</td>
<td>4</td>
<td>0.21 ± 0.009</td>
</tr>
<tr>
<td>LGSH</td>
<td>2</td>
<td>0.35 ± 0.008</td>
</tr>
<tr>
<td>LGSL</td>
<td>2</td>
<td>0.26 ± 0.025</td>
</tr>
<tr>
<td>LFAH†</td>
<td>2</td>
<td>0.71 ± 0.055</td>
</tr>
<tr>
<td>LFAH‡</td>
<td>2</td>
<td>0.58 ± 0.018</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>0.41 ± 0.023</td>
</tr>
</tbody>
</table>

† The error is the standard deviation for four or six pigs and is half the range for two pigs.
‡ Sows of generation 8 were used for the line LFAH. 92.5% of piglets born alive to LFAH sows of generation 8 survived to weaning.
subfractions (named A to D) could be obtained after eluting the heparin-agarose column with a discontinuous gradient. Using the continuous gradient, we found no evidence for the peak A unless the column was overloaded; furthermore, there was no clear evidence for a peak C. Subfractions 1 and 2 appear to correspond to the peaks B and D, respectively. Although the previous papers reported differences in the proportions of the subfractions obtained from human VLDL, compared with those from rat VLDL, the evidence presented herein suggests that within species variation may be as great as that occurring between species.

The finding of the two subfractions immediately begs the question of the possible physiological significance of the phenomenon. The only clues in the literature relate to changes occurring during pregnancy (Herrera et al., 1987). Accordingly, samples of plasma were taken throughout pregnancy and lactation and the composition of the whole VLDL fraction analysed. The results show similar trends to those reported elsewhere for pigs (Reese, Peo, Lewis and Hogg, 1984) and for other species (Knopp, Warth, Charles, Childs, Li, Mabuchi and Van Allen, 1986; Montelongo, Lasunción, Pallardo and Herrera, 1992). The concentration in plasma of all the lipid components of VLDL increased markedly during the 5 weeks prior to parturition, whereas the concentration of VLDL-protein remained roughly constant. Each VLDL particle contains one molecule of apo-B which is the largest of the apoproteins and is the major protein constituent of the lipoprotein (Davis, 1991). This being the case, the concentration of VLDL-protein in plasma gives a measure of the number of VLDL particles in the medium; conversely, phospholipid and free cholesterol both increase with the surface area of the VLDL and triacylglycerol and cholesteryl ester (being total cholesterol minus free cholesterol) both reflect changes in the volume of the VLDL. The results are therefore an indication that plasma contains larger, more lipid-rich, VLDL in the weeks leading up to parturition. In humans, there is a corresponding hyperlipaemia in late pregnancy which results from mobilization of adipocyte triacylglycerols and subsequent incorporation of the liberated fatty acids by the liver into VLDL (Herrera, Lasunción, Martín and Zorzano, 1992). The onset of lactation, on the other hand, coincides with a fall in the concentrations of the lipid components and a rise in the plasma concentration of VLDL-protein. Hence, following parturition, the VLDL fraction of plasma was represented by more, smaller particles. That the VLDL are smaller may be a result of their more rapid catabolism in response to the increased requirement by the mammary gland for the substrates of milk fat synthesis and the consequent presence of more partially digested VLDL in the sample of plasma. Some care must be exercised in the analysis of these results as the six gilts chosen for study came from six different genetic lines, a fact which may be reflected in the relatively high standard errors recorded in some cases.

The proportion of VLDL present as subfraction 2 fell continually throughout pregnancy, agreeing with a
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previous finding in rats (Herrera et al., 1987). Decreased proportions of subfraction 2 were present in the 11 weeks prior to parturition, only returning to pre-pregnancy values after weaning. Cautions necessary in comparing the changes in the subfraction proportions during pregnancy and lactation with the changes in lipid and protein compositions of whole VLDL over the same period because a mixture of genetic lines was used to obtain the data for Figure 1, whereas each panel of Figure 4 represents a single genetic line. Nevertheless, the sharp changes revealed in Figure 1 are not seen in Figure 4; furthermore, the timing of the recoveries post-partum are not coincident. These observations lead to the conclusion that the changes in the proportions of subfractions do not result simply from a change in the size of the VLDL in late pregnancy and early lactation. It seems more likely that the changes in the proportions of the subfractions in VLDL are a longer-term adaptation to the needs of pregnancy or of lactation and may be related to the induction of the enzyme lipoprotein lipase in mammary gland in late pregnancy (Ramirez, Llobera and Herrera, 1983).

Clear differences were observed between the VLDL subfractions of nulliparous gilts. These differences were reflected in the changes observed in the VLDL subfractions of the three lines chosen for study throughout pregnancy and lactation. It may be more than a coincidence that the survival rates of piglets born to the nine genetic lines also varies in a manner which correlates with the variation in the proportions of the VLDL subfractions present. The relationship between the two variables, however, is complex (see Figure 3) and complicated by the fact that the proportions of each subfraction were measured some time before lactation started. It would be of interest to determine what proportions of the subfractions were predicted in late pregnant and lactating gilts by the prepregnant figure. If a causal relationship can be established, the implication is that the synthesis and secretion of each subfraction were predicted in late pregnant and lactating gilts by the prepregnant figure. If a relationship can ultimately only be substantiated when the total and relative amounts of the subfractions can be shown to correlate with the output of triacylglycerol in the milk.

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References


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