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Blood Lipids

3. PLASMA LIPIDS OF THE COW DURING PREGNANCY AND LACTATION*

By W. R. H. DUNCAN AND G. A. GARTON

Rowett Research Institute, Bucksburn, Aberdeen

(Received 13 May 1963)

Maynard, Harrison & McCay (1931) found that, after parturition in cows, there was a rapid increase in the plasma concentrations of total fatty acids, phospholipid fatty acids and total cholesterol; this was followed by a gradual decline throughout the remainder of the period of lactation. Schaible (1932) found that average plasma concentrations of total fatty acids and lipid P were considerably greater in lactating cows than in non-lactating cows or in steers, and similar observations for plasma concentrations of total cholesterol were made by Lennon & Mixner (1957). These workers also confirmed the findings of Long, Hibbs & Gilmore (1953) that plasma concentrations of total cholesterol at parturition were significantly lower than those observed before and after calving. Riis (1959) reported observations that corroborated

those of Maynard *et al.* (1931), and also found that the changes in the concentration of total cholesterol were not accompanied by any significant alteration in the relative proportions of free and esterified cholesterol.

Our continuing interest in the role of plasma lipids in lactation led us to study these changes in more detail, with particular reference to the fatty acid composition of the major classes of plasma lipid in blood samples obtained at intervals during pregnancy and lactation.

EXPERIMENTAL

Animals and diet. Five Ayrshire heifers, aged 2-3 years, were used in this study; three (animals A, B and C) were pregnant for the first time and two (animals D and E) had been served but failed to conceive. The pregnant animals were stall-fed to appetite on grass silage from about

* Part 2: Lough & Garton (1957).

3 months before calving and throughout lactation; before this they received a ration of grass silage together with turnips and distiller's grains. The non-pregnant heifers were similarly fed. All animals had free access to water and to salt and mineral 'licks'.

Blood samples and preparation of plasma lipids for analysis. Blood samples, each usually of about 300 ml., were obtained by jugular venepuncture from the pregnant animals at several intervals from 7 weeks before calving to 8 weeks afterwards; samples from the non-pregnant heifers were taken during the same period. In particular it was hoped to obtain samples about a week before calving, but this proved possible with two only of the three pregnant animals. The blood samples were drawn into heparin-treated receivers and centrifuged to yield the plasma. Then 100–150 ml. of plasma was poured slowly, with stirring, into twice its volume of ethanol and the precipitated protein (containing most of the lipid) was removed by centrifuging. The supernatant aqueous ethanol was reduced to about 50 ml. by vacuum-distillation at 40° and extracted three times with chloroform in a separating funnel. Lipids were extracted from the protein precipitate by adding a volume of methanol equal to that of the original plasma and twice that volume of chloroform. The mixture was brought to the boil, cooled and filtered; after two further such extractions the pooled filtrates, together with the chloroform extracts of the concentrated aqueous ethanol, were distilled to dryness under reduced pressure at 40° in an atmosphere of nitrogen. The crude lipid thus obtained was dissolved in warm chloroform and the solution was filtered. The filtrate was made to a known volume (usually 25 ml.) with chloroform containing a trace of ethanol and the solution was stored at 1° in the dark.

Analysis of lipids. A small volume of the lipid solution was used for determination of free and total cholesterol by the method of Schoenheimer & Sperry (1934). Values for total fatty acids were obtained either by saponification of a sample of the total lipid or from the sum of the fatty acids obtained on hydrolysis of the component lipids after their separation by silicic acid chromatography (Duncan & Garton, 1962).

Fatty acid methyl esters, prepared as described by Duncan & Garton (1962), from each lipid class were analysed before and after hydrogenation (Popják & Tietz, 1954) by gas-liquid chromatography by using a Pye Argon Chromatograph (W. G. Pye and Co. Ltd., Cambridge) containing a ⁹⁰Sr detector. The standard glass columns contained Embacel (60–100 mesh acid-washed kieselguhr; May and Baker Ltd., Dagenham) impregnated with 20% by weight of polymerized ethylene glycol succinate [prepared as described by Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) for the corresponding adipate] or Apiezon L grease (Shell Chemicals Ltd., London). Column temperatures were in the range 170–180° and the argon flow rate was usually 50 ml./min. Calculation of the percentage by weight of each component in an ester mixture was made directly from the relative peak areas on the chromatograms as determined by the 'triangulation' procedure. The chromatograms of the hydrogenated esters were used to confirm the chain length of the unsaturated components. Identity of peaks was established from a knowledge of the relative retention times of the esters and of authentic esters on each of the two liquid phases. In the description of fatty acid compositions the terms C₁₈ mono-, di- and tri-unsatur-

ated acids are used since ruminant tissues contain geometrical and positional isomers of oleic acid, linoleic acid and linolenic acid owing to the absorption of the products of microbial hydrogenation of feed lipids in the rumen (Shorland, Weenink, Johns & McDonald, 1957; see also review by Garton, 1961).

RESULTS

Fig. 1 shows the changes in the plasma concentrations of total lipids; in this Figure (and also in Fig. 2) values are plotted relative to the respective calving dates of animals A, B and C, and, for the non-pregnant heifers (animals D and E), in relation to the calving date of animal A. The calving dates were 9, 16 and 25 February for animals C, A and B respectively.

Though the plasma concentrations of lipid of the non-pregnant animals showed a fairly steady decline from the beginning of the sampling period, those of the pregnant animals fell to reach minimal values at the time of calving, to be followed by increasing values when the animals were lactating. During the week immediately preceding parturition a rise in concentrations was observed in animals A and B, followed by a sharp decline at calving. It may well be that this took place in animal C also, but unfortunately no blood sample was obtained a week before it calved.

The content of free and total cholesterol in the plasma reflected closely the overall changes in total lipid; for animals A, B and C this is illustrated in Fig. 2 which, for clarity of presentation, does not include the values for animals D and E. The values for the ratio of free cholesterol to total cholesterol for animals A, B and C were within the ranges

Fig. 1. Plasma concentrations of total lipids in the cow. Pregnant animals (solid lines): ○, animal A; ●, animal B; △, animal C. Non-pregnant animals (broken lines): ▽, animal D; □, animal E.

0.186–0.224, 0.192–0.218 and 0.183–0.228 respectively; the corresponding ranges for animals D and E were 0.172–0.210 and 0.206–0.226. The plasma concentrations of total fatty acids (Table 1) showed a similar trend to those for free and total cholesterol, although a more limited number of determinations was made.

No major change was observed in the relative contribution of the fatty acids of each class of plasma lipid to the plasma total fatty acids, though the small proportion contributed by the triglycerides declined progressively during pregnancy to parturition and then further during

lactation. These observations are illustrated in Table 2, which gives the values for three samples (one *pre-partum*, one at parturition and one during lactation) from each of the animals A, B and C.

The component fatty acids of the lipid classes from the plasma of each of these animals are shown in Table 3, and those from the plasma lipids of the non-pregnant non-lactating heifers are given in Table 4. To simplify presentation minor components with less than 18 carbon atoms are grouped together and, with one exception, the acids having more than 18 carbon atoms are similarly recorded as a group; the exception is the C₂₀ branched-chain saturated acid. This acid, which contributed significantly to the fatty acids in glyceride and phospholipid combination, has been identified in this Laboratory as 3,7,11,15-tetramethylhexadecanoic acid (Lough, 1963).

DISCUSSION

The results presented above agree with those of previous workers (Maynard *et al.* 1931; Lennon & Mixner, 1957; Riis, 1959) in showing that the concentrations of total lipids, free and esterified cholesterol and total fatty acids of ox plasma increase with the onset of lactation and that the quantitative relationships between the individual lipid classes are maintained. These relationships resemble those observed in non-lactating non-pregnant heifers by Duncan & Garton (1962). It seems likely that the changes observed reflect changes in the amounts of lipoproteins normally

Fig. 2. Plasma concentrations of free and total cholesterol in the cow in late pregnancy and in lactation. ○, Animal A; ●, animal B; △, animal C. Solid lines show total cholesterol and broken lines show free cholesterol.

Table 1. Plasma concentrations of total fatty acids in the cow

Experimental details are given in the text. The results are expressed as mg./100 ml. The numbers in parentheses show days before (–) or after (+) calving, except for animals D and E where they refer to days relative to the calving date of animal A.

Animals during pregnancy and lactation			Non-pregnant animals	
Animal A	Animal B	Animal C	Animal D	Animal E
200 (–38)	173 (–29)	179 (–23)	275 (–31)	263 (–31)
155 (–3)	155 (–2)	89 (+0.5)	178 (–10)	240 (–10)
133 (+1)	164 (+0.5)	221 (+22)	168 (+18)	179 (+54)
206 (+22)	176 (+7)	186 (+50)		
	223 (+21)			

Table 2. Percentage contribution of the fatty acids of each lipid class to the plasma total fatty acids in the cow

Experimental details are given in the text.

No. of days before (–) or after (+) calving	Animal A			Animal B			Animal C		
	–38	+1	+22	–29	+0.5	+21	–23	+0.5	+22
Source of fatty acids									
Cholesterol esters	50.0	70.6	62.3	56.4	55.4	68.1	39.8	47.4	49.8
Triglycerides	11.3	4.7	1.8	8.8	4.8	2.3	11.0	8.1	5.1
Free fatty acids	15.9	3.0	6.2	1.5	7.0	4.4	4.5	6.6	4.1
Phospholipids	22.8	21.7	29.7	33.3	32.8	25.2	44.7	37.9	41.0

Table 3. *Component fatty acids of plasma lipids in the cow expressed as percentage by weight of the total fatty acids of each class of lipid*

Experimental details are given in the text. The designations of the fatty acids in col. 1 are according to the shorthand nomenclature of Dole, James, Webb, Rizack & Sturman (1959), indicating the number of carbon atoms and, where applicable, the number of double bonds; Br, branched-chain. N.D., Not detected.

No. of days before (-) or after (+) calving ... Fatty acid	Animal A			Animal B			Animal C		
	-38	+1	+22	-29	+0.5	+21	-23	+0.5	+22
Fatty acids of cholesterol esters									
< 18*	4.7	3.3	5.5	6.1	4.3	1.1	7.4	8.3	4.4
16:0	8.4	8.1	9.3	8.2	9.5	9.9	7.4	10.0	9.1
18:0	8.6	13.4	10.9	5.0	5.8	8.6	5.2	6.4	6.0
18:1	9.7	15.0	9.7	9.2	11.1	12.9	9.8	12.6	11.4
18:2	49.2	22.5	28.7	40.1	35.9	34.7	47.3	29.2	31.0
18:3	13.4	26.1	26.3	25.1	26.9	22.3	15.9	25.4	29.3
Br 20:0	0.8	0.7	1.1	Trace	Trace	2.0	Trace	Trace	0.5
> 18†	5.2	10.9	8.5	6.3	6.5	8.5	7.0	8.1	8.2
Fatty acids of phospholipids									
< 18*	2.6	3.1	3.4	3.8	5.0	1.4	5.2	4.7	4.1
16:0	17.9	18.1	20.5	23.7	20.6	17.4	23.8	22.9	20.5
18:0	25.5	27.8	19.7	18.6	18.4	23.4	26.6	18.4	17.9
18:1	14.9	15.7	17.9	19.4	19.9	14.5	16.2	19.3	17.3
18:2	19.5	7.7	9.0	8.0	8.7	9.0	13.8	7.4	8.1
18:3	2.6	4.5	4.4	3.6	3.0	4.1	2.9	3.0	3.9
Br 20:0	4.6	7.0	8.2	6.2	6.5	14.2	Trace	9.8	12.5
> 18†	12.7	16.1	16.9	16.7	17.9	16.0	11.5	14.5	15.7
Fatty acids of triglycerides									
< 18*	11.6	9.7	8.1	7.8	13.9	8.7	5.6	9.7	8.0
16:0	28.0	29.1	18.9	21.6	27.8	27.0	25.1	26.4	25.9
18:0	39.1	22.6	37.8	30.7	19.0	30.4	39.5	33.3	19.6
18:1	13.8	14.5	19.0	15.0	16.5	17.5	14.9	19.4	17.9
18:2	4.0	3.2	2.7	5.9	6.3	5.3	3.1	2.8	2.7
18:3	1.7	3.2	2.7	4.6	3.8	2.6	2.6	1.4	3.6
Br 20:0	1.8	16.1	10.8	6.5	10.1	8.5	5.1	4.2	12.5
> 18†	—	1.6	N.D.	7.9	2.5	N.D.	4.1	2.8	9.8
Free fatty acids									
< 18*	2.8	9.0	2.4	7.7	1.7	13.5	3.8	3.4	6.5
16:0	19.5	13.6	10.2	19.2	14.8	16.7	13.9	13.6	15.2
18:0	31.5	12.8	15.7	23.1	13.0	10.4	21.5	15.2	18.5
18:1	18.2	23.6	26.8	26.9	34.9	21.8	40.5	40.6	26.1
18:2	16.7	8.3	12.6	11.6	11.3	11.5	16.5	10.2	7.6
18:3	2.8	6.5	8.7	3.8	5.2	4.2	1.3	5.1	4.3
Br 20:0	1.9	10.0	5.5	3.9	7.8	6.3	2.5	11.9	12.0
> 18†	6.6	16.2	18.1	3.8	11.3	15.6	N.D.	N.D.	9.8

* Includes 14:0 and 16:1, together with traces of other acids.

† Comprising mainly 20:4, together with 20:0 and traces of 19:0.

present in ox plasma rather than changes in the structural nature of the lipoproteins. It is, however, reasonable to suppose that in lactation the increased plasma concentrations of lipid are associated with the demands of the udder for fatty acids for milk glyceride synthesis. Riis, Luick & Kleiber (1960) concluded from isotope experiments that about 50% of the fatty acids of cow butter-fat were derived from plasma lipids though, apart from the uptake of triglycerides by the udder, it is not yet clear what part other plasma lipids play in the metabolism of secreting mammary tissue in the living cow. Experiments *in vivo* (Riis, 1959; Riis *et al.* 1960) and studies involving perfusion of the isolated udder (Lough, Duncan, Garton & Peeters,

1960) indicated that phospholipids and cholesterol esters may have been involved, and, in addition, udder perfusion studies (Laurysens *et al.* 1960; Laurysens, Verbeke & Peeters, 1961) have shown that plasma free fatty acids can be absorbed by the udder tissue and incorporated into triglycerides. In addition to the post-parturient rise in the plasma concentrations of lipid which takes place at the onset of lactation, our experiment indicates that a temporary pre-parturient increase in the plasma concentrations of lipid occurs, the significance of which is not readily apparent.

Lactation is normally accompanied by increased feed consumption and hence increased lipid intake which might affect plasma concentrations of lipid.

Table 4. *Component fatty acids of plasma lipids in the non-pregnant cow expressed as percentage by weight of the total fatty acids of each class of lipid*

Experimental details are given in the text. The designations of fatty acids (col. 1) are as indicated in Table 3.

No. of days before (-) or after (+) calving date of animal A ...	Animal D		Animal E		Animal D		Animal E	
	-31	+18	-10	+54	-31	+18	-10	+54
Fatty acid	Fatty acids of cholesterol esters				Fatty acids of phospholipids			
< 18*	8.3	7.9	8.8	6.8	1.7	1.9	4.0	4.7
16:0	9.1	8.9	8.0	10.3	14.7	12.0	14.7	17.0
18:0	6.2	9.2	6.4	12.8	20.4	18.7	22.0	19.6
18:1	9.2	9.6	11.7	17.4	10.2	11.8	16.6	21.3
18:2	23.9	21.7	25.6	21.5	2.0	6.9	6.0	5.5
18:3	35.4	27.9	27.5	18.7	1.7	4.2	3.6	3.4
Br 20:0	1.6	1.2	1.5	1.1	43.0	17.3	12.3	7.3
> 18†	6.3	13.6	10.5	11.4	6.3	27.2	20.8	21.2
	Fatty acids of triglycerides				Free fatty acids			
< 18*	4.2	8.5	10.8	9.1	10.1	6.1	4.6	2.3
16:0	24.5	23.6	22.0	24.7	23.9	18.3	14.9	10.3
18:0	30.2	35.2	37.7	22.1	26.6	29.5	22.1	11.5
18:1	8.3	13.9	13.0	18.2	18.4	18.2	25.6	32.3
18:2	1.6	3.7	2.2	5.2	4.6	6.8	8.7	9.2
18:3	1.5	1.8	1.8	2.6	4.5	4.5	4.1	5.7
Br 20:0	22.4	9.1	9.0	14.3	10.1	4.5	2.6	8.0
> 18†	7.3	4.2	3.5	3.8	1.8	12.1	17.4	20.7

* Includes 14:0 and 16:1, together with traces of other acids.

† Comprising mainly 20:4, together with 20:0 and traces of 19:0.

However, Maynard *et al.* (1931) and Schaible (1932) showed that lactation alone is accompanied by increased plasma concentrations of lipid which are independent of any effect that may result from increased feed consumption. It thus seems that a hormonal influence is involved, possibly thyroid activity (cf. Long *et al.* 1953) which is well known for its effect on all plasma lipids (Deuel, 1955): hyperthyroidism is accompanied by low concentrations and, when gland activity is low, the concentrations tend to be increased above normal.

The detailed study of the component fatty acids of the plasma lipids of animals A, B and C (Table 3) showed that, although the absolute concentrations of plasma lipids fell at parturition and then increased in lactation (Fig. 1), no major change took place in the composition of the phospholipids, triglycerides or free fatty acids. However, the fatty acids esterified to cholesterol (which account for about half the plasma total acids) showed a change in the relative proportions of C₁₈ di- and tri-unsaturated acids. In late pregnancy the proportion of C₁₈ di-unsaturated acid was considerably greater than that of C₁₈ tri-unsaturated acid whereas, at parturition and in lactation, the relative proportions of the two species of acid were similar. Samples of plasma from heifers D and E, taken at a time when animals A, B and C were in late pregnancy, did not contain a high proportion of C₁₈ di-unsaturated acid relative to that of C₁₈ tri-unsaturated acid in the cholesterol esters; the proportions were of a similar order, as they were also in samples taken during the time when animals A, B

and C were lactating (Table 4). The possibility that the relative proportions of C₁₈ di- and tri-unsaturated fatty acids esterified to cholesterol may have some physiological significance in pregnancy would appear to merit further investigation.

The C₂₀ branched-chain acid (3,7,11,15-tetramethylhexadecanoic acid) was present as a significant proportion of the fatty acids of all lipid fractions except the cholesterol esters. Particularly noteworthy is its occurrence as 43% of the total phospholipid acids in a plasma sample from animal D (Table 4). The origin and possible significance of this acid are not known; it was not present as such in the lipids of the silage fed to the animals; it may possibly be derived from phytol in the chlorophyll of the feed. A re-examination of the phospholipid fatty acids of the ox plasma lipids used by Duncan & Garton (1962) has shown that the acid then reported as *n*-heptadecanoic acid included some 3,7,11,15-tetramethylhexadecanoic acid (A. K. Lough & W. R. H. Duncan, unpublished work).

SUMMARY

1. A study was made of plasma lipids of venous blood obtained from three cows during pregnancy and lactation and, concurrently, from two non-pregnant non-lactating heifers. All the animals were stall-fed, to appetite, on grass silage throughout the experiment.

2. Plasma concentrations of lipid fell as parturition approached and then rose when the animals were lactating; values for the non-lactating non-

pregnant animals declined during the same period. In all the animals no major changes took place in the quantitative relationships of the different classes of plasma lipid (cholesterol esters, triglycerides, phospholipids and free fatty acids), though the small proportion of fatty acids contributed by the triglycerides to the plasma total fatty acids declined progressively during pregnancy to parturition and then further during lactation.

3. Though pregnancy, parturition and lactation were not accompanied by any significant changes in the fatty acid composition of the plasma glycerides, phospholipids and free fatty acids, the plasma cholesterol esters during pregnancy contained a much greater proportion of C_{18} di-unsaturated acid relative to that of C_{18} tri-unsaturated acid than was present at parturition and during lactation.

4. 3,7,11,15-Tetramethylhexadecanoic acid was found in the plasma lipids (particularly triglycerides and phospholipids) of all the animals.

We thank Mr J. Gunn for his assistance in the housing and feeding of the animals and Mr W. Thomson for obtaining the blood samples.

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Studies on the Mode of Action of Excess of Vitamin A

8. MITOCHONDRIAL SWELLING*

By J. A. LUCY,† MOLLIE LUSCOMBE‡ AND J. T. DINGLE†

Strangeways Research Laboratory, Cambridge

(Received 4 April 1963)

Many of the changes seen in hypervitaminosis A are explicable on the hypothesis that vitamin A penetrates lipoprotein membranes and that membranes containing an excess of the vitamin exhibit increased permeability or decreased stability. Model experiments with monolayers of lecithin and cholesterol on a Langmuir trough have shown that vitamin A alcohol readily penetrates lipid films and produces an increase of surface area (Bangham,

Dingle & Lucy, 1964). The instability that results from penetration by an excess of vitamin A is exemplified by the rapid haemolysis of erythrocytes that occurs on treatment with the vitamin (Dingle & Lucy, 1962). Phase-contrast-microscopic and electron-microscopic studies have shown that this haemolysis is preceded by drastic changes in the structure of erythrocytes which include buckling of the cells and the formation of vacuoles by a process that resembles micropinocytosis (Glauert, Daniel, Lucy & Dingle, 1963). In experiments on the effect of excess of vitamin A on fibroblasts, disruption of the cell membranes and nuclear membranes and swelling

* Part 7: Glauert, Daniel, Lucy & Dingle (1963).

† Medical Research Council External Staff.

‡ Medical Research Council Rheumatism Research Unit, Bath.