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Linoleate supplementation in steers modifies lipid composition of plasma lipoproteins but does not alter their fluidity

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The health value for man of lipids in bovine muscles can be improved by the addition of PUFA to the animals' diets, but such treatments can modify fluidity of plasma lipoproteins and therefore their metabolic functions. The aim of the present study was to analyse whether changes in chemical composition of lipoproteins in steers fed sunflower oil-rich diets altered lipoprotein fluidity, measured by fluorescence polarization and electron spin resonance. LDL, light HDL and heavy HDL fractions were isolated by ultracentrifugation from plasma of eighteen crossbred Charolais × Salers steers. For a period of 70 d, animals were given a control diet (C, *n* 6) consisting of hay (540 g/kg) and concentrate mixture (460 g/kg) or the same basal diet supplemented with sunflower oil rich in *n*-6 PUFA (40 g/kg diet DM), given either as crushed seeds (S, *n* 6) or as a free oil infused directly into the duodenum (O, *n* 6), thus avoiding ruminal hydrogenation of PUFA. We have shown that in bovine animals: (1) fluidity measurements by fluorescence polarization must be made at the bovine physiological temperature (38·5°C); (2) heavy HDL always appear as the less fluid lipoparticles; (3) electron spin resonance, which does not depend on lipoparticle size, is more appropriate to compare the fluidity of LDL with that of light HDL. The values for lipoprotein fluidity measured by both methods indicated that linoleate-rich diets did not have any effect when compared with diet C; however, chemical variables support a fluidification of lipoparticles, since in steers given the diet O, *n*-6 PUFA concentrations increased in polar (× 1·8) and neutral (× 1·6) lipids in lipoparticles ($P=0{\cdot}0001$). The phospholipid:protein ratio increased in light (+20 %, $P=0{\cdot}019$) and heavy (+23 %, $P=0{\cdot}06$) HDL and especially in LDL (+46 %, $P=0{\cdot}0001$); the total cholesterol:phospholipid ratio decreased in the three lipoprotein classes (− 15 to − 30 %, NS). Diet S led to similar but less pronounced effects. We concluded that linoleate-rich diets modified the chemical composition of plasma lipoproteins in steers, but did not alter their fluidity; this probably occurred as a result of 'homeoviscous adaptation', which ensured their functional capacity.

Bovine: Fluidity of lipoproteins: Dietary *n*-6 polyunsaturated fatty acids

Diets for ruminant animals usually contain forage and concentrates that are relatively poor in lipids (20–70 g/kg DM; Doreau *et al.* 1997). Phospholipids and digalactosyl diglycerol (from forage) and triacylglycerol (from concentrates) are extensively hydrolysed in the rumen by bacterial lipases favouring an intense biohydrogenation of their PUFA and subsequent production of saturated fatty acids (SFA) and *cis* and *trans* MUFA (Jenkins, 1993). Such ruminal metabolism of lipids explains the low PUFA:SFA ratio generally observed in meat lipids (0·11; Wood *et al.* 1999); this ratio does not correspond with the recommendations for prevention of CVD in man. The recommended value for this nutritional index is far greater, ranging from 0·45 to 0·64 (Wood *et al.* 1999; Legrand *et al.* 2001).

In this context, many studies in ruminant animals have shown it is possible to increase the PUFA:SFA ratio of lipids in meat or in milk by using high-fat rations rich in PUFA (Demeyer & Doreau, 1999; Wood *et al.* 1999). PUFA have been incorporated as triacylglycerol into rations for steers or bulls during the fattening period (Clinquart *et al.* 1995) and for dairy cows during the lactating period (Chilliard *et al.* 2001). The main lipid sources are crushed or extruded seeds (rapeseed, sunflower seed, soyabean, linseed), free oils (Clinquart *et al.* 1995), or seed supplements protected by encapsulation against ruminal lipolysis and hydrogenation (Storry *et al.* 1980; Ashes *et al.* 1982).

In bovine animals, such lipid supplements can modify their lipid and lipoprotein metabolisms. Plasma lipoproteins are dominated by HDL (80 % total lipoproteins), which are mainly involved in cholesterol transport (Bauchart, 1993). Such treatments have led to marked hypercholesterolaemic and hyperphospholipidaemic effects in preruminant calves (Richard *et al.* 1980; Jenkins

Abbreviation: SFA, saturated fatty acid.

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& Kramer, 1986; Leplaix-Charlat *et al.* 1996) and in dairy cows (Ashes *et al.* 1982, 1984). This was associated with an increase in plasma HDL concentrations, especially light and very-light HDL, enriched in cholesteryl esters (Ashes *et al.* 1984; Leplaix-Charlat *et al.* 1996). Moreover, such modifications of distribution and chemical composition of plasma lipoproteins induced by dietary treatments might lead to alteration of lipoprotein fluidity, as observed in human subjects (Berlin *et al.* 1991*a*; Sola *et al.* 1993), rabbits (Berlin *et al.* 1991*b*; Loo *et al.* 1991), rats (Belcher *et al.* 1988), swine (Tall *et al.* 1977; Pownall *et al.* 1980) and chicks (Talavera *et al.* 1997). It has been clearly demonstrated that PUFA-rich diets increase lipoprotein fluidity compared with a diet rich in SFA; conversely, hypercholesterolaemic diets lead to a decrease in their fluidity. Such changes in the physical state of lipoproteins can interfere with their physiological roles by affecting lipoprotein–receptor interactions (Berlin *et al.* 1987, 1991*a*; Dachet *et al.* 1990) and therefore reverse cholesterol transport (Sola *et al.* 1993).

In the present study we investigated the effects of sunflower oil-rich diets (rich in linoleic acid) on the fluidity of the main families of plasma lipoproteins (LDL, light HDL and heavy HDL) in finishing steers. Effects of the degree of PUFA protection against ruminal biohydrogenation have been identified by using sunflower oil given either as seeds incorporated into the diet (PUFA partially protected against ruminal biohydrogenation) or as free oil directly infused into the duodenum (PUFA avoiding ruminal biohydrogenation). Fluidity changes were monitored either by indicators of lipoprotein fluidity derived from the composition of lipids and their fatty acids distributed in the core and the hydrophilic envelope, or directly measured by two spectroscopic methods (fluorescence polarization and electron spin resonance). This analysis should enable us to determine the existence of a potential risk of these PUFA-rich diets on animal health via a deterioration of the metabolic role of the lipoproteins induced by fluidity changes, according to the degree of protection of the PUFA.

Materials and methods

Animals and diets

Eighteen crossbred Charolais × Salers steers (454 (SD 20) d old, live weight 528 (SD 36) kg) were divided into three groups according to diet. The animals were given a control diet (diet C, *n* 6) for 70 d, consisting of 540 g natural grass hay and 460 g concentrate mixture/kg DM. The average composition of the concentrate mixture was (g/kg DM): maize seed 575, soyabean meal 240, dehydrated lucerne 120, cane molasses 20, urea 25, vitamin and mineral mixture 20. The two experimental diets consisted of the same basal diet and 40 g sunflower oil/kg DM, offered either as sunflower seeds incorporated into the feed (diet S, *n* 6) or as sunflower oil continuously infused into the proximal duodenum (diet O, *n* 6) through a permanent cannula. The composition of DM and the fatty acid composition of the three diets are shown in Tables 1 and 2 respectively. Crushed sunflower seeds and their corresponding free oil were provided by Vamo Mills Society (Lezoux, France). The three diets were calculated to be isoenergetic and isonitrogenous (Table 1).

Table 1. DM concentration and DM composition of the control diet and the same diet supplemented with sunflower seed or with sunflower oil infused into the duodenum

Diet...	Control (C)	Seed (S)	Oil (O)
DM (g/kg feed)	915	917	918
Composition of DM (g/kg)			
Organic matter	935	936	895
Net energy value*	0·85	0·85	0·86
Crude protein (N × 6·25)	188	181	180
Lipid	36·7	73·6	70·2
Total fatty acids	20·0	54·8	51·5

* Feed units for maintenance and meat production (Vermorel, 1978).

Analytical procedures

Lipoprotein isolation. At the end of the experimental period and just before the morning meal, blood samples (300 ml) were collected from the jugular vein into tubes containing Na_2EDTA, sodium azide and merthiolate (final concentrations 3 mM, 0·1 g/l and 0·01 g/l respectively). Plasma was separated by centrifugation at 4500 rpm for 10 min at 15°C. Main lipoprotein classes, such as the triacylglycerol-rich lipoproteins ($d < 1{\cdot}006$ g/ml), LDL ($1{\cdot}019 < d < 1{\cdot}060$ g/ml, containing about 6% intermediate-density lipoproteins), light HDL ($1{\cdot}060 < d < 1{\cdot}091$ g/ml) and heavy HDL ($1{\cdot}091 < d < 1{\cdot}180$ g/ml), were purified from plasma by sequential ultracentrifugal flotation according to the density limits defined by Bauchart *et al.* (1989). Centrifugation was performed in a

Table 2. Fatty acid composition (g/100 g total methyl esters) and daily total fatty acid intake in steers offered a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into the duodenum*

(Mean values for six steers per group)

Diet...	Control (C)	Seed (S)	Oil (O)
14:0	1·4	0·5	0·6
16:0	20·8	11·0	11·1
16:1	1·4	0·5	0·6
18:0	2·4	3·7	3·4
18:1	12·8	20·5	19·3
18:2*n*-6	30·2	52·2	52·0
18:3*n*-3	21·3	7·6	8·3
20:2*n*-6	2·5	0·8	0·8
20:3*n*-6	2·2	0·7	0·7
20:5*n*-3	0·6	0·3	0·5
22:5*n*-6	2·3	0·7	0·8
22:6*n*-3	0·6	0·2	0·2
Sum of:			
SFA	25·2	16·0	15·9
MUFA	14·9	21·2	20·4
n-6 PUFA	37·7	54·6	54·5
n-3 PUFA	22·5	8·2	9·1
Total Fatty acid intake (g/d)	134	372	347

SFA, saturated fatty acids.
* For details of diets, see Table 1.

Centrikon T-2060 ultracentrifuge equipped with the TFT 38–70 fixed-angle rotor (Kontron Analysis Division, Zurich, Switzerland). Lipoprotein fractions were then dialysed at 4°C for 7 h against the buffer containing 0·02 M-NH_4HCO_3, 1·5 mM-NaN_3 and 1 mM-Na_2EDTA, pH 8·6. Dialysis was performed in the Gibco BRL Microdialysis System using membranes with cut-off of 12–14 kDa (Bethesda Research Laboratories, Rockville, MD, USA).

Chemical composition of lipoproteins. Lipids (non-esterified cholesterol, cholesteryl ester, triacylglycerol, phospholipid) were determined enzymatically. Proteins were determined by the colorimetric method of the bicinchonic acid, as described by Leplaix-Charlat *et al.* (1996).

Total lipids in the three main lipoprotein fractions (LDL, light HDL and heavy HDL) were extracted according to the method of Folch *et al.* (1957), as described by Leplaix-Charlat *et al.* (1996). Their two main components, polar lipids (monoacylglycerol + phospholipid) and neutral lipids (cholesteryl ester + triacylglycerol), were prepared after isolation of the different lipids by semi-preparative TLC using glass plates (200 × 200 mm) covered with lipid-free (Kieselgel G, Merck, Darmstadt, Germany) (0·75 mm). Fatty acids were extracted from polar and neutral lipids and converted into methyl esters according to the method described by Bauchart & Aurousseau (1981). Fatty acid composition of polar and neutral lipids was determined by GLC performed on the Delsi DI 200 GC, equipped with the automatic injector ALS 104 (Périchrom, Saulx-les-Chartreux, France) and using a CP-Sil 88 glass capillary column (length 100 m, diameter 0·25 mm, film depth 0·20 μm; CP 7489, Varian, Middelburg, The Netherlands). GLC conditions were as follows: the oven temperature was programmed at 70°C for 30 s, increased from 70°C to 175°C at 20°C/min, constant at 175°C for 25 min, then increased to 215°C at 10°C/min and finally constant at 215°C for 41 min; injector and detector temperatures were 235 and 250°C respectively; the carrier gas was H_2 (flow 1·1 ml/min) (split injection 1:50). Fatty acids were identified by comparing their retention times with those of fatty acid standards (Sigma Chemical Corp., St Louis, MO, USA). Chromatographic signals were analysed by Winilab software Chromatography Data System (Périchrom, Saulx-les-Chartreux, France).

Fluidity measurements. For the polarization fluorescence studies, lipoproteins were labelled with the lipophilic fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (Sigma Chemical Corp.). Three ml of a fresh dispersion of 1,6-diphenyl-1,3,5-hexatriene in tetrahydrofuran (final concentration 1 μM) were added to solutions of dialysed LDL (100 μl), light HDL (50 μl) and heavy HDL (50 μl) against the NH_4HCO_3 buffer, pH 8·6. Mixtures were gently stirred for 30 min at 20°C in the dark.

Fluorescence polarization intensity was measured with a PTI spectrofluorometer (PTI, Surbiton, Surrey, UK) equipped with three polarizers. The sample was excited with vertically polarized light (360 nm) and emission (460 nm) was measured through polarizers parallel and perpendicular to the excitation polarizer (Shinitzky & Barenholz, 1978). The anisotropy (r) was calculated at 24·0°C (reference temperature given in the literature) and 38·5°C (bovine physiological temperature) from the following equation:

$$r = \frac{I_{//} - I_{\perp}}{I_{//} + 2I_{\perp}},$$

where $I_{//}$ and $I_{\perp}$ refer to the parallel and perpendicular fluorescence intensity respectively. A high value for r refers to low fluidity and *visa versa*.

Arrhenius plots (log η *v.* 1/T) were used to indicate phase transitions between 20 and 40°C for each lipoprotein fraction. Arrhenius plots were also used to calculate the flow activation energy (ΔE, expressed in kcal/mol) from the equation:

$$\eta = A \times e^{\Delta E/RT},$$

where η is the microviscosity derived from the anisotropy value by the empirical relation (Shinitzky & Barenholz, 1978):

$$\eta\,(\text{poises}) = \left(\frac{2{\cdot}4r}{0{\cdot}362 - r}\right),$$

and A is a constant, R is the perfect gas constant and T is the absolute temperature of measurement.

Electron spin resonance allows mobility measurements of the probe embedded in the membranes. The probe was a spin label containing a nitroxide group that was positioned on C_{16} of a stearic acid (16-doxyl-stearic acid; Sigma Chemical Corp.). Lipoprotein fractions (200 μl) were spin-labelled with 2 μl probe (10 mM), in conditions where the ratio between probe and phospholipids was less than 1:500 in order to prevent any artifactual modification of the membrane structure. Electron spin resonance spectra were recorded at room temperature with the Bruker ECS 106 spectrometer (Bruker, Wissenbourg, France). Time of correlation–relaxation (Tc) was calculated from the relation of Keith *et al.* (1973):

$$Tc(ns) = 0{\cdot}65 \times W_0\left(\sqrt{(h_0/h_{-1})} + \sqrt{(h_0/h_{+1}) - 2}\right),$$

where W_0 refers to the main line width (Gauss) and h_0, h_{-1} and h_{+1} to the height of the main line and the two secondary lines of the spectrum respectively. Tc describes the degree of freedom of the spin label embedded in the lipid moiety. A high value of Tc corresponds to a low fluidity and *vice versa* (Hubbell & McConnell, 1969).

Statistical analyses

All data were subjected to ANOVA using the general linear models procedure of SAS (1987; Statistical Analysis Systems Inc., Cary, NC, USA) according to 3 × 3 factorial designs (three diets × three lipoprotein classes). Both the main effects and the interaction of diet × lipoprotein were included in the model. When the main factors were statistically significant, differences between diets or between lipoprotein classes were compared by Student's *t* test (Snedecor & Cochran, 1979). When interactions were

statistically significant, the significances of the main factors were not taken into account and treatment mean values were compared between lipoprotein classes by Student's *t* test.

Results

Fatty acid composition of plasma lipoproteins

The fatty acid compositions of polar and neutral lipids in the three lipoprotein classes are shown in Tables 3 and 4 respectively. The fatty acid composition of polar lipids was similar for the three lipoprotein classes considered whatever the diet, but heavy HDL contained more *trans* MUFA than LDL and light HDL (Table 3). However, differences between lipoprotein classes were observed in neutral lipids (Table 4). LDL has the highest content of SFA (27·5 g/100 g total fatty acids) and the lowest content of *n*-6 PUFA (54·9 g/100 g total fatty acids) compared with HDL particles. The heavy HDL were 1·3 times richer in SFA than light HDL ($P=0·041$), mainly to the detriment of *n*-6 PUFA.

Moreover, large modifications of fatty acid composition in lipoproteins were induced by the diets (Tables 3 and 4). Sunflower oil infusion (diet O) increased the content of *n*-6 PUFA 1·8 times in polar lipids ($P=0·0001$) and 1·5 times in neutral lipids ($P=0·0001$) of the three main lipoprotein classes, but to the detriment of *cis* MUFA, SFA and *n*-3 PUFA. In steers fed sunflower seeds (diet S), enrichment of the three lipoprotein classes with *n*-6 PUFA was also observed, but at a lower intensity than with diet O ($\times 1·2$ *v.* $\times 1·6$, $P=0·0001$). Furthermore, this enrichment in *n*-6 PUFA was not to the detriment of MUFA as was shown for diet O, but to the detriment of *n*-3 PUFA and of SFA (Tables 3 and 4).

Chemical variables as indicators of lipoprotein fluidity

Values of chemical variables (lipid:protein, phospholipid:protein, total cholesterol:phospholipid, cholesteryl ester:total triacylglycerol, unsaturated fatty acid:SFA, PUFA:SFA ratios) as indicators of lipoprotein fluidity are shown for the main lipoprotein classes in Table 5.

Among fluidity variables specific to the entire lipoprotein particles, the lipid:protein ratios, known to be positively related to higher fluidity, were significantly higher for the three lipoprotein classes in steers given diet O than those given diet C (+14 %, $P=0·0001$) and diet S (+17 %, $P=0·0004$). Among fluidity variables in relation to the hydrophilic envelope of lipoparticles, the phospholipid:protein ratios increased significantly in LDL (+46 %, $P=0·0001$) and to a lesser extent in light and heavy HDL (+20 % ($P=0·019$) and + 23 % ($P=0·06$) respectively) in steers fed diet O, indicating a higher fluidity of their envelope compared with diet C. The effect of diet S on these variables was less pronounced (+18 % ($P=0·0019$) and + 13 % ($P=0·0423$) in LDL and in light HDL respectively). The total cholesterol:phospholipid ratio (Table 5), a variable inversely correlated with fluidity of the envelope of lipoparticles, as well as non-esterified cholesterol:phospholipid and cholesteryl ester:phospholipid ratios (results not shown) tended to be lower in lipid-rich

Table 3. Fatty acid composition (g/100 g total fatty acid methyl esters) of polar lipids of the major lipoprotein classes in steers offered a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into the duodenum*

(Mean values for six steers per group)

	SFA	*cis* MUFA	*trans* MUFA	*n*-3 PUFA	*n*-6 PUFA
LDL (1·018<d<1·060 g/ml)					
C	43·9	17·3	1·4	11·7	24·6
S	45·0	17·2	1·6	4·9	30·9
O	42·7	6·7	1·3	2·1	47·1
SEM	0·2	0·1	0·1	0·3	0·4
Light HDL (1·060<d<1·091 g/ml)					
C	50·2	17·0	1·0	7·4	24·0
S	44·8	17·4	1·5	4·5	31·3
O	42·7	8·1	1·4	1·4	46·1
SEM	0·7	0·2	0·1	0·2	0·1
Heavy HDL (1·091<d<1·180 g/ml)					
C	49·3	15·7	1·8	6·3	26·7
S	43·7	17·8	2·1	7·0	29·3
O	41·1	8·0	1·9	3·1	45·9
SEM	0·3	0·2	0·1	0·4	0·3
Statistical significance of effects: *P*					
Diet	0·0120	0·0001	NS	0·0007	0·0007
C *v.* S	NS	NS		0·0249	0·0103
C *v.* O	0·0037	0·0001		0·0002	0·0001
S *v.* O	NS	0·0001		0·0171	0·0001
Lipoprotein	NS	NS	0·0449	NS	NS
LDL *v.* light HDL			NS		
LDL *v.* heavy HDL			0·0425		
Light HDL *v.* heavy HDL			0·0220		
Diet × Lipoprotein	NS	NS	NS	NS	NS

SFA, saturated fatty acids; C, control diet; S, sunflower oil-supplemented diet; O, sunflower oil infused into duodenum.
* For details of diets and procedures, see Tables 1 and 2 and p. 576.

Table 4. Fatty acid composition (g/100 g total fatty acid methyl esters) of neutral lipids of the major lipoprotein classes in steers offered a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into the duodenum*
(Mean values for six steers per group)

	SFA	*cis* MUFA	*trans* MUFA	*n*-3 PUFA	*n*-6 PUFA
LDL (1·018<d<1·060 g/ml)					
C	33·3	12·0	3·7	5·7	44·9
S	26·0	12·6	3·7	4·5	52·7
O	23·1	6·0	1·6	1·9	67·2
SEM	0·2	0·5	0·3	0·2	0·4
Light HDL (1·060<d<1·091 g/ml)					
C	17·1	16·7	3·1	10·1	52·5
S	13·5	14·4	1·2	6·6	64·2
O	11·5	4·6	1·1	1·4	81·4
SEM	0·2	0·2	0·1	0·2	0·4
Heavy HDL (1·091<d<1·180 g/ml)					
C	22·6	15·4	3·0	10·1	51·3
S	17·7	14·0	3·3	9·7	58·1
O	14·2	5·7	1·4	2·7	76·0
SEM	0·8	0·4	0·1	0·6	0·9
Statistical significance of effects: *P*					
Diet	0·0025	0·0003	NS	0·0007	0·0001
C *v.* S	0·0126	NS		NS	0·0020
C *v.* O	0·0008	0·0002		0·0003	0·0001
S *v.* O	NS	0·0005		0·0026	0·0001
Lipoprotein	0·0001	NS	NS	NS	0·0011
LDL *v.* light HDL	0·0001				0·0003
LDL *v.* heavy HDL	0·0002				0·0094
Light HDL *v.* heavy HDL	0·0410				NS
Diet × Lipoprotein	NS	NS	NS	NS	NS

SFA, saturated fatty acids; C, control diet; S, sunflower oil-supplemented diet; O, sunflower oil infused into duodenum.
* For details of diets and procedures, see Tables 1 and 2 and p. 576.

diets (−15 to −30 % according to the diet and the lipoprotein class considered) compared with that with diet C. Among fluidity variables characteristic of the inner core of lipoproteins, the cholesteryl ester:triacylglycerol ratio did not differ significantly between diets (Table 5), even if it increased greatly in LDL (×6·1 compared with the control) and tended to be raised in light (×1·3 compared with the control) and heavy HDL (×1·6 compared with the control) of steers given sunflower oil by duodenal infusion (Table 5).

The degree of unsaturation of lipids represented an additional variable that can be correlated with the fluidity of lipoproteins (Table 5). In the three lipoprotein classes, the unsaturated fatty acids : SFA and PUFA:SFA ratios increased in steers fed diet O in polar lipids (+23 % (P=0·0021) and +61 % (P=0·0001) respectively), but more especially in neutral lipids (+71 % (P=0·0010) and +110 % (P=0·0003) respectively) compared with the control steers. In diet S, the PUFA:SFA ratio was not significantly different from that in diet C, but was lower than in diet O in polar (−29 %, P=0·0003) as well in neutral lipids (−31 %, P=0·0127).

Physical measurements of lipoprotein fluidity

Analyses of fluidity were performed by fluorescence polarization and by electron spin resonance; these are highly sensitive and reproducible spectroscopic methods.

Anisotropy of lipoproteins (inversely related to lipoprotein fluidity) was determined by fluorescence polarization at 24·0 and 38·5°C (Table 6). When measured at 38·5°C (i.e. the physiological temperature of bovines), anisotropy was significantly higher in heavy HDL than in LDL (+3 %, P=0·0161) and in light HDL (+5 %, P=0·0001). Similarly, correlation-relaxation time (inversely related to lipoprotein fluidity), measured by electron spin resonance, was higher in heavy HDL than in other lipoproteins (Table 6). However, conversely to the other variables of fluidity, correlation-relaxation time in LDL was lower than in light HDL (×1·2, P=0·0002).

Whatever the lipoprotein class considered, anisotropy and correlation-relaxation time did not differ between the three diets (Table 6). Thermodynamic behaviour of lipoproteins analysed by fluorescence polarization with the 1,6-diphenyl-1,3,5-hexatriene probe between 20 and 40°C showed that the three lipoprotein fractions of steers were invariant phases for the three diets (results not shown). In steers fed diet C, flow activation energy (ΔE), which determined the intensity of lipid–protein interactions in particles, averaged 46·9 kJ (11·2 kcal) mol whatever the lipoprotein class; the value was 1·2-fold higher with diet S than with diet O (P=0·01; Table 7).

Discussion

The purpose of the present study was to investigate the impact of sunflower oil-rich diets on fluidity variables of the main lipoparticle classes related to their chemical composition in steers. PUFA from sunflower oil were totally protected against ruminal degradation, a process that occurs when sunflower seeds are added to the diet, by means of infusion into the duodenum through a chronic cannula. Using this approach, for the first time we have

Table 5. Fluidity variables derived from the chemical composition of the major lipoprotein classes in steers offered a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into duodenum*

(Mean values for six steers per group)

		Polar lipids				Neutral lipids		
	Lipid:protein	PL:protein	TC:PL	UFA:SFA	PUFA:SFA	CE:TG	UFA:SFA	PUFA:SFA
LDL (1·018<d<1·060)								
C	2·31	0·67[a]	2·54	1·28	0·85	7·14	2·00	1·53
S	2·26	0·79[b]	1·75	1·23	0·81	10·46	2·85	2·22
O	2·66	0·98[c]	1·71	1·34	1·16	43·44	3·35	3·02
SEM	0·01	0·01	0·02	0·01	0·01	0·27	0·03	0·03
Light HDL (1·060<d<1·091)								
C	1·63	0·60[a]	1·79	1·03	0·67	143·97	4·91	3·74
S	1·67	0·68[b]	1·34	1·23	0·81	137·05	6·45	5·28
O	1·88	0·72[b]	1·51	1·36	1·13	186·28	7·80	7·31
SEM	0·01	0·01	0·01	0·03	0·03	2·19	0·12	0·11
Heavy HDL (1·091<d<1·180)								
C	0·84	0·30[a]	1·90	1·03	0·68	39·84	3·57	2·84
S	0·86	0·34[a]	1·36	1·29	0·83	46·87	5·11	4·27
O	0·95	0·37[a]	1·43	1·43	1·19	63·14	7·27	6·77
SEM	0·01	0·01	0·01	0·01	0·01	0·71	0·29	0·29
Statistical significance of effects: *P*								
Diet	0·0001		NS	0·0071	0·0001	NS	0·0037	0·0012
C *v.* S	NS			NS	NS		0·0540	NS
C *v.* O	0·0001			0·0021	0·0001		0·0010	0·0003
S *v.* O	0·0004			NS	0·0003		0·0506	0·0127
Lipoprotein	0·0001		0·0247	NS	NS	0·0001	0·0002	0·0005
LDL *v.* light HDL	0·0001		0·0165			0·0001	0·0001	0·0002
LDL *v.* heavy HDL	0·0001		0·0191			0·0462	0·0013	0·0019
Light HDL *v.* heavy HDL	0·0001		NS			0·0001	NS	NS
Diet × Lipoprotein	NS	0·0001	NS	NS	NS	NS	NS	NS

PL, phospholipid; TC, total cholesterol; UFA, unsaturated fatty acids; SFA, saturated fatty acid; CE, cholesteryl ester; TG, triacylglycerol; C, control diet; S, sunflower oil-supplemented diet; O, sunflower oil infused into duodenum.

[a,b,c]Mean values between diets for a lipoprotein class were significantly different ($P<0.05$).

* For details of diets and procedures, see Tables 1 and 2 and p. 576.

determined the characteristics of the fluidity of lipoproteins in bovine fed lipid–rich diets protected or not against rumen bacteria.

Chemical composition of lipoproteins

As previously described, HDL represent the major lipoprotein class (>80 %) in the plasma of preruminant and ruminant animals compared with human subjects where LDL predominate (Bauchart, 1993). In our laboratory (Durand *et al.* 2000) we have shown that linoleic-rich diets increased plasma cholesterol and phospholipids in steers due to a higher concentration of HDL, essentially the light HDL, when dietary PUFA were protected against ruminal degradation (diet O). These results confirmed previous research on preruminant calves fed a diet rich in soyabean oil (Leplaix-Charlat *et al.* 1996) and on dairy cows fed protected soyabean (Storry *et al.* 1980) or protected sunflower seed supplements (Ashes *et al.* 1984).

In the present study we have shown that sunflower-rich diets led to the accumulation of *n*-6 PUFA in polar and neutral lipids in the three major lipoprotein classes, in particular with oil infusion, because the capacity for hydrogenation of C_{18} PUFA from sunflower seeds in the rumen is high: about 80 % for linoleic acid (Doreau & Ferlay, 1994). Protection of dietary PUFA against ruminal degradation led to greater enrichment in *n*-6 PUFA in polar than in neutral lipids of the three lipoprotein classes, as previously observed in preruminant calves fed soyabean-rich milk (Leplaix-Charlat, 1995). However, this enrichment in *n*-6 PUFA observed in steers fed linoleic-rich diets was to the detriment of SFA, MUFA and *n*-3 PUFA according to the lipid and lipoprotein classes, whereas in cows it was mainly to the detriment of MUFA (Leplaix-Charlat, 1995). Such modifications of the fatty acid composition of lipoparticles induced by dietary treatments have been previously related to perturbation of the lipoprotein fluidity in human subjects given a daily supplement of 30 g linoleic acid (Berlin *et al.* 1987), foods with PUFA:SFA ratio 1 (Berlin *et al.* 1991*a*) or 4 (Pownall *et al.* 1980), or diets rich in olive, soyabean or maize oils (Sola *et al.* 1990). Similar variations of the lipoprotein fluidity by treatments have been shown in animals such as rabbits given 50 g cocoa butter, milk fat, coconut oil or maize oil/kg (Berlin & Young, 1980), in rats given 200 g fish oil/kg (Belcher *et al.* 1988) and in chicks given 200 g coconut oil/kg (Talavera *et al.* 1997). It has been clearly demonstrated that the higher the unsaturated fatty acid content of the diet, the higher the lipoprotein fluidity (Shinitzky, 1984; Berlin *et al.* 1987; Sola *et al.* 1990; Loo *et al.* 1991).

Fluidity of lipoproteins

Characteristics of fluidity of the main lipoprotein classes (LDL, light and heavy HDL) have not been reported in

Table 6. Fluidity variables of the three major lipoprotein classes determined by fluorescence polarization (anisotropy) at 24.0°C (reference temperature given in the literature) and 38.5°C (bovine physiological temperature) and by electron spin resonance (correlation-relaxation time) in steers fed a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into duodenum

(Mean values for six steers per group)

Lipoprotein class	Diet	Anisotropy 24°C	Anisotropy 38·5°C	Correlation-relaxation time (ns)
LDL†	C	0·287	0·222	2·229
	S	0·288	0·233	2·131
	O	0·288	0·230	2·388
	SEM	0·001	0·001	0·022
Light HDL‡	C	0·289	0·223	2·859
	S	0·276	0·223	2·637
	O	0·279	0·221	2·647
	SEM	0·001	0·001	0·016
Heavy HDL§	C	0·284	0·236	3·246
	S	0·281	0·237	3·002
	O	0·282	0·230	2·793
	SEM	0·001	0·001	0·028
Statistical significance of effect: *P*				
Diet		NS	NS	NS
Lipoprotein		NS	0·0002	0·0001
LDL *v.* light HDL		–	0·0301	0·0002
LDL *v.* heavy HDL		–	0·0161	0·0001
Light HDL *v.* heavy		–	0·0001	NS
Diet × Lipoprotein		NS	NS	NS

C, control diet; S, sunflower oil-supplemented diet; O, sunflower oil infused into duodenum.
* For details of diets and procedures, see Tables 1 and 2 and p. 576.
† 1.018 < d < 1.060 g/ml.
‡ 1.060 < d < 1.091 g/ml.
§ 1.091 < d < 1.180 g/ml.

bovine species. Analyses of fluidity were performed by fluorescence polarization and by electron spin resonance, which are known to be highly sensitive and reproducible methods (Shinitzky & Barenholz, 1978).

Unlike human lipoproteins (Dachet *et al.* 1990; Pownall *et al.* 1980; Sola *et al.* 1990), analysis of bovine lipoprotein fluidity by fluorescence polarization showed no differences between the three main lipoprotein classes at the reference temperature of 24°C. By contrast, we have shown that at the physiological temperature (38·5°C), which improves the sensitivity of this method, fluidity of bovine lipoproteins varied as follows: light HDL > LDL > heavy HDL. Comparison with available data on lipoproteins of other mammals, such as human subjects (Jonas, 1977; Dachet *et al.* 1990) or rabbits (Berlin & Young, 1980; Berlin *et al.* 1991*b*), was difficult since the two main classes of HDL were not analysed specifically in these species. From these results, HDL were more rigid than LDL in rabbits due to their higher protein content, known to have a rigidifying effect (Berlin & Young, 1980; Berlin *et al.* 1991*b*); in humans subjects, however, LDL were always more rigid than HDL because of their higher content of cholesterol, another rigidifying substance (Jonas, 1977; Dachet *et al.* 1990). Our present results determined by electron spin resonance have confirmed that bovine heavy HDL were the most rigid lipoparticles because of their higher protein content. Comparison of the relative degree of LDL and light HDL fluidity by fluorescence polarization and electron spin resonance led to apparent contradictory results, which could be explained by differences in the location of 1,6-diphenyl-1,3,5-hexatriene and 16-doxyl-stearic acid probes in the envelope and the core of lipoparticles. By electron spin resonance, the 16-doxyl-stearic acid probe intercalates between acyl chains of phospholipids, giving information specific to the fluidity of the lipoprotein envelope. Consequently, LDL appeared to be more fluid than light HDL because of the higher phospholipid:protein ratio in their envelope, since the relationship between phospholipid:protein and fluidity was established by Shinitzky (1984). By fluorescence polarization, the smaller size of light HDL (diameter 12–15 nm) compared with LDL (diameter 19–25 nm) reported in bovine animals by Bauchart (1993) would favour 1,6-diphenyl-1,3,5-hexatriene incorporation into the core of light HDL, as demonstrated in human subjects by Dachet *et al.* (1990) and Ben-Yashar & Barenholz (1991). Consequently, the high content of PUFA in the cholesteryl ester of the core of light HDL would increase the fluidity of these particles compared with that of LDL. From these results, comparison of the fluidity of LDL with HDL particles appeared more appropriate by electron spin resonance than fluorescence polarization, since incorporation of the corresponding probe (16-doxyl-stearic acid) does not depend on the size of lipoparticles.

Effects of linoleate on lipoprotein fluidity

The lack of effect of dietary linoleate on fluidity values for the three lipoprotein classes in the bovine animal runs contrary to the common concept of a higher fluidity of lipoproteins in mammals given PUFA-rich diets (Pownall *et al.* 1980; Berlin *et al.* 1987; Sola *et al.* 1990; Loo *et al.* 1991; Talavera *et al.* 1997). The same discrepancies have been already reported in rabbits (Berlin & Young, 1980; Berlin *et al.* 1991*b*; Loo *et al.* 1991), in human subjects (Berlin *et al.* 1987, 1991*a*; Sola *et al.* 1990) and in rats (Belcher *et al.* 1988), where the authors showed that lipoprotein fluidity does not necessarily reflect the degree of unsaturation of dietary fatty acids. However, in all these cases, the fluidity values of lipoproteins have been related to their phospholipid, triacylglycerol, cholesterol, protein or MUFA content (Pownall *et al.* 1980; Dachet *et al.* 1990; Sola *et al.* 1990; Berlin *et al.* 1987, 1991*a*).

Lipid:protein, phospholipid:protein, total cholesterol:phospholipid and cholesteryl ester:triacylglycerol ratios are known to be fluidity indicators of the whole lipoprotein, of their envelope and of their core respectively (Shinitzky & Barenholz, 1978; Shinitzky, 1984). The higher value of the lipid:protein ratio in lipoparticles of our steers receiving linoleate infusion suggested a marked fluidifying effect, since restriction of the movement of lipids by the protein moiety has been described by Jonas (1977). For similar reasons, the higher phospholipid:protein ratio of lipoparticles, especially in LDL, in steers fed diet O indicates a higher fluidity at the level of their envelope. The lower total cholesterol:phospholipid ratio should also enhance the fluidity of the envelope of the three lipoprotein classes in steers given linoleate-rich diets, since cholesterol acts as the main lipid rigidifier in lipoproteins (Jonas, 1979; Shinitzky, 1984). At the lipoparticle core

Table 7. Flow activation energy of the three major lipoprotein classes determined by fluorescence polarization in steers fed a control diet or the same diet supplemented with sunflower seed or with sunflower oil infused into duodenum

(Mean values for six steers per group)

Lipoprotein class	Diet	Flow activation energy kJ/mol	Flow activation energy kcal/mol
LDL (1·018 < d < 1·060 g/ml)			
	C	51·752	12·369
	S	51·057	12·203
	O	44·827	10·714
	SEM	0·628	0·150
Light HDL (1·060 < d < 1·091 g/ml)			
	C	46·518	11·118
	S	56·179	13·427
	O	45·727	10·929
	SEM	1·088	0·260
Heavy HDL (1·091 < d < 1·180 g/ml)			
	C	42·656	10·195
	S	46·501	11·114
	O	42·045	10·049
	SEM	0·736	0·176
Statistical significance of effect: *P*			
Diet			0·0328
C *v.* S			NS
C *v.* O			NS
S *v.* O			0·0100
Lipoprotein			0·0550
LDL *v.* light HDL			NS
LDL *v.* heavy HDL			0·0408
Light HDL *v.* heavy HDL			0·0326
Diet × Lipoprotein			NS

C, control diet; S, sunflower oil-supplemented diet; O, sunflower oil-supplemented diet; O, sunflower oil infused into duodenum.
* For details of diets and procedures, see Tables 1 and 2 and p. 576.

level, the rigidifying effect of the higher cholesteryl ester: triacylglycerol ratio in steers fed diet O suggested in human (Berlin *et al.* 1987; Dachet *et al.* 1990) and in animal lipoproteins (Pownall *et al.* 1980; Loo *et al.* 1991; Talavera *et al.* 1997) would be improbable, because the triacylglycerol level was too low to act as a potent fluidizer. Thus, fluidity will be probably more affected by the degree of unsaturation of fatty acids in cholesteryl ester (Dachet *et al.* 1990; Talavera *et al.* 1997).

To predict variations of lipoprotein fluidity induced by dietary linoleate, the degree of unsaturation of fatty acid esterified in polar and neutral lipids must be taken into account (Shinitzky, 1984). In the three lipoprotein classes, sunflower oil infusion increased the PUFA:SFA ratio, especially in neutral lipids because lecithin:cholesterol acyl transferase preferentially transferred *n*-6 PUFA from lecithin to the hydroxyl group of cholesterol, leading to the formation of cholesteryl ester (Frohlich *et al.* 1982). This accumulation of PUFA should increase the lipoprotein fluidity as previously demonstrated in human subjects and animals (Pownall *et al.* 1980; Berlin *et al.* 1987, 1991*a*; Sola *et al.* 1990; Loo *et al.* 1991).

In summary, except for the cholesteryl ester:triacylglycerol ratio, all the predictive factors taken into account in our present study would indicate a higher fluidification of lipoprotein particles in steers fed PUFA-rich diets, particularly with protected PUFA. Conversely, biophysical measurements of fluidity determined by fluorescence polarization and electron spin resonance, two highly sensitive methods, indicated no effects of PUFA-rich diets. We clearly demonstrated that predictive factors usually related to fluidity were insufficient to predict the thermotropic behaviour of lipoparticles in the bovine animal. Only the flow activation energy (ΔE) indicated, according to the definition of ΔE given by Jonas (1977), that intermolecular interactions in lipoparticles were lower in steers receiving oil infusion than in animals given the sunflower seeds, favouring rapid exchange of non-esterified cholesterol between the envelope and core of bovine lipoparticles, as previously described in human subjects (Lund-Katz & Philips 1984).

In our opinion, the lack of effect of diet on the fluidity of bovine lipoproteins is the result of animal adaptation to the changes in lipid and fatty acid composition. This adaptation, called 'homeoviscous adaptation' (Shinitzky, 1984), probably involves three possible mechanisms.

A first possible mechanism implicates the degree of unsaturation of fatty acids esterified in polar and neutral lipids of different lipoparticles. In our present experiment, the high fluidifying effect of dietary linoleate was moderated by the fact that they took the place of *n*-3 PUFA and *cis* MUFA in lipoparticles, known also to be fluidifying components (Shinitzky, 1984; Sola *et al.* 1990). These authors emphasized the major role of the unique double bond of MUFA (compared with two double bonds in PUFA) on the fluidity of lipoparticles. Indeed, Sola *et al.* (1990) showed that HDL in women given olive oil (rich in MUFA) were more fluid than those of women given a soyabean or maize oil (rich in *n*-6 PUFA). Moreover, the fluidifying effect of MUFA is specific to the *cis* unsaturated fatty acids known to disorder the membrane, since their melting points are lower than their *trans* counterparts (Klausner *et al.* 1980).

A second possible mechanism concerns the interactions of α-tocopherol with membrane phospholipids. In addition to its major function as an antioxidant, α-tocopherol can play a structural role in membranes, comparable with that of cholesterol, a membrane stabilizing agent (Maggio *et al.* 1977; Urano *et al.* 1988). In our laboratory, we have recently shown that dietary supplementations with sunflower seeds or oil led to a significant increase (× 2·4, $P < 0·0001$) in plasma vitamin E compared with the control group (2·00 μg/ml; D Durand, D Gruffat-Mouty, V Scislowski and D Bauchart, unpublished results). Separate domains of saturated and polyunsaturated phospholipids, were probably present in the lipoprotein envelope and α-tocopherol may intercalate preferentially into polyunsaturated regions to stabilize them physically (Maggio *et al.* 1977; Urano *et al.* 1988).

The last hypothesis implicates the size heterogeneity of lipoparticles. We have shown that the profile of plasma lipoprotein was modified by PUFA-rich diets, especially with infused oil, resulting in an accumulation of large and very-light HDL in LDL density limits (1·018–1·060 g/ml; Durand *et al.* 2000). This heterogeneity of lipoparticle size may in turn regulate their fluidity, as previously described in VLDL from rabbits given saturated fat and cholesterol (Berlin *et al.* 1991*b*). The marked curvature of the envelope of the smallest lipoparticles leads to strong lipid–protein

interactions, which can in turn reduce the fluidity of particles (Dachet *et al.* 1990).

These three mechanisms contribute to 'homeoviscous adaptation'. This process has been already observed in rats fed diets rich in fish oil where VLDL fluidity decreased, consistent with a 'hook' conformation for dietary long-chain *n*-3 PUFA (20 : 5*n*-3 and the 22 : 6*n*-6; Belcher *et al.* 1988). Homeoviscous adaptation has been also identified during stress conditions in micro-organisms, plants, poikilotherms and hibernating animals (Shinitzky, 1984).

In conclusion, our present results clearly show alterations of the chemical composition of lipoproteins without any changes in their fluidity in bovine animals given linoleate-rich diets; this suggests a homeoviscous adaptation phenomenon. From a physiological point of view, it would indicate that the functional capacity of LDL, light HDL and heavy HDL should be optimal following PUFA supplementation. According to the hypothesis of optimal fluidity proposed by Shinitzky (1984), fluidity HDL from steers fed PUFA-rich diets would be optimal in order to ensure their role in the reverse cholesterol transport.

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References

Ashes JR, Burley RW, Davenport JB & Sidhu GS (1982) Effects of dietary supplements of protected lipids on the concentration and transport of ß-carotene and cholesterol in bovine blood and milk: unusual chromatographic behaviour of the high-density lipoprotein with high levels of β-carotene. *J Dairy Res* **49**, 39–49.

Ashes JR, Burley RW, Sidhu GS & Sleigh RW (1984) Effect of particle size and lipid composition of bovine blood high density lipoprotein on its function as a carrier of β-carotene. *Biochim Biophys Acta* **797**, 171–177.

Bauchart D (1993) Lipid absorption and transport in ruminants. *J Dairy Sci* **76**, 3864–3881.

Bauchart D & Aurousseau B (1981) Postprandial lipids in blood plasma of preruminant calves. *J Dairy Sci* **64**, 2033–2042.

Bauchart D, Durand D, Laplaud PM, Forgez P, Goulinet S & Chapman MJ (1989) Plasma lipoproteins and apolipoproteins in the preruminant calf, Bos spp: density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis. *J Lipid Res* **30**, 1499–1514.

Belcher D, Stillwell W, Langsford CA & Wassall SR (1988) Effect of fish oils on rat plasma lipoproteins. *Biochem Biophys Res Commun* **150**, 1063–1068.

Ben-Yashar V & Barenholz Y (1991) Characterization of the core and surface of human plasma lipoproteins. A study based on the use of five fluorophores. *Chem Phys Lipids* **60**, 1–14.

Berlin E, Judd JT, Marshall MW & Kliman PG (1987) Dietary linoleate increases fluidity and influences chemical composition of plasma low density lipoprotein in adult men. *Atherosclerosis* **66**, 215–225.

Berlin E, Judd JT, Nair P, Jones DY & Taylor R (1991*a*) Dietary fat and hormonal influences on lipoprotein fluidity and composition in premenauposal women. *Atherosclerosis* **86**, 95–110.

Berlin E, Shapiro SG & Young C (1991*b*) Relative effects of feeding saturated fats and cholesterol on fluidity of rabbit lipoproteins. *Comp Biochem Physiol* **98**A, 343–346.

Berlin E & Young C (1980) Influence of dietary fats on the fluidity of the lipid domains of rabbit plasma lipoproteins. *Atherosclerosis* **35**, 229–241.

Chilliard Y, Ferlay A & Doreau M (2001) Contrôle de la qualité nutritionnelle des matières grasses du lait par l'alimentation des vaches laitières: acide gras trans, polyinsaturés, acide linoléique conjugué (Dietary control of milk fat nutritional quality in the dairy cow: trans and polyunsaturated fatty acids, and conjugated linoleic acid). *INRA Prod Anim* **14**, 323–335.

Clinquart A, Micol D, Brundseaux C, Dufrasne I & Istasse L (1995) Utilisation des matières grasses chez les bovins à l'engraissement (Use of fat in fattening diets for cattle). *INRA Prod Anim* **8**, 29–42.

Dachet C, Motta C, Neufcour D & Jacotot B (1990) Fluidity changes and chemical composition of lipoproteins in type IIa hyperlipoproteinemia. *Biochim Biophys Acta* **1046**, 64–72.

Demeyer D & Doreau M (1999) Targets and procedures for altering ruminant meat and milk lipids. *Proc Nutr Soc* **58**, 593–607.

Doreau M, Demeyer DI & Van Nevel CJ (1997) Transformations and effects of unsaturated fatty acids in the rumen. Consequences on milk fat secretion. In *Milk Composition, Production and Biotechnology*, pp. 73–92 [RAS Welch, DJW Burns, SR Davis, AI Popay and CG Prosser, editors]. Oxford: CAB International.

Doreau M & Ferlay A (1994) Digestion and utilisation of fatty acids by ruminants. *Anim Feed Sci Technol* **45**, 379–396.

Durand D, Scislowski V, Gruffat-Mouty D & Bauchart D (2000) Effet de rations enrichies en acide linoléique sur les lipoprotéines plasmatiques chez le bouvillon (Effect of linoleic-rich diets on plasma lipoproteins in steers). *Nutr Clin Metab* **14**, Suppl. 2, 151.

Folch J, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.

Frohlich J, McLeod R & Hon K (1982) Lecithin:cholesterol acyl transferase (LCAT). *Clin Biochem* **15**, 269–278.

Hubbell WL & McConnell HM (1969) Orientation and motion of amphiphilic spin label in membranes. *Proc Natl Acad Sci* **64**, 20–27.

Jenkins KJ & Kramer JK (1986) Influence of low linoleic and linolenic in milk replacer on calf performance and lipids in blood plasma, heart and liver. *J Dairy Sci* **69**, 1374–1386.

Jenkins TC (1993) Lipid metabolism in the rumen. *J Dairy Sci* **71**, 3851–3863.

Jonas A (1977) Microviscosity of lipid domains in human serum lipoproteins. *Biochim Biophys Acta* **486**, 10–22.

Jonas A (1979) Interaction of bovine serum high density lipoprotein with mixed vesicles of phosphatidylcholine and cholesterol. *J Lipid Res* **20**, 817–824.

Keith AD, Sharnoff M & Cohn GE (1973) A summary and evaluation of spin labels used as probes for biological membrane structure. *Biochim Biophys Acta* **300**, 379–419.

Klausner RD, Kleinfeld AM, Hoover RL & Karnovsky MJ (1980) Lipid domains in membranes. *J Biol Chem* **255**, 1286–1295.

Legrand P, Bourre JM, Descomps B, Durand G & Renaud S (2001) : *Lipides in Apports Nutritionnels Conseillés pour la population Française (Lipids in Recommended Dietary*

Advances for the French Population), pp. 63–82, Editions TEC & DOC (Paris).
Leplaix-Charlat L (1995) Effets des acides gras et du cholestérol alimentaires sur le métabolisme des lipides et des lipoprotéines aux niveaux plasmatiques et hépatique chez le veau préruminant: conséquences sur la composition lipidique des tissues (Effects of dietary fatty acids and cholesterol on plasma and hepatic metabolisms of lipids and lipoproteins in preruminantscalf: consequence on the lipid composition in tissues), PhD Thesis, University of Aix-Marseille III.
Leplaix-Charlat L, Bauchart D, Durand D, Laplaud PM & Chapman MJ (1996) Plasma lipoproteins in preruminant calves fed diets containing tallow or soybean oil with and without cholesterol. *J Dairy Sci* **79**, 1267–1277.
Loo G, Berlin E, Peters RC, Kliman PG & Wong HYC (1991) Effect of dietary corn, coconut, and menhaden oils on lipoprotein, liver, and heart membrane composition in the hypercholesterolemic rabbit. *J Nutr Biochem* **2**, 594–603.
Lund-Katz S & Phillips MC (1984) Packing of cholesterol molecules in human high density lipoproteins. *Biochemistry* **23**, 1130–1138.
Maggio B, Diplock AT & Lucy JA (1977) Interactions of tocopherols and ubiquinones with monolayers of phospholipids. *Biochem J* **161**, 111–121.
Pownall HJ, Jackson RL, Roth RI, Gotto AM, Patsch JR & Kummerow FA (1980) Influence of an atherogenic diet on the structure of swine low density lipoproteins. *J Lipid Res* **21**, 1108–1115.
Richard MJ, Stewart JW, Heeg TR, Wiggers KD & Jacobson NL (1980) Blood plasma lipoprotein and tissue cholesterol of calves fed soybean oil, corn oil, vegetable shortening or tallow. *Atherosclerosis* **37**, 513–520.
Shinitzky M (1984) Membrane fluidity and cellular functions. In *Physiology of Membrane Fluidity*, pp. 1–51 [M Shinitzky, editor]. Boca Raton, FL: CRC Press, Inc.
Shinitzky M & Barenholz Y (1978) Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim Biophys Acta* **515**, 367–394.
Snedecor GW & Cochran WG (1979) The comparison of two samples. In *Statistical Methods*, 6th ed., Ames, IA: Iowa State University.
Sola R, Baudet MF, Motta C, Maillé M, Boisnier C & Jacotot B (1990) Effects of dietary fats on the fluidity of human high-density lipoprotein: influence of the overall composition and phospholipid fatty acids. *Biochim Biophys Acta* **1043**, 43–51.
Sola R, Motta C, Maille M, Bargallo MT, Boisnier C, Richard JL & Jacotot B (1993) Dietary monounsaturated fatty acids enhance cholesterol efflux from human fibroblasts. *Arterioscler Thromb* **13**, 958–966.
Storry JE, Brumby PE, Tuckley B, Welch VA, Stead D & Fulford RJ (1980) Effect of feeding protected lipid to dairy cows in early lactation on the composition of blood lipoproteins and secretion of fatty acids in milk. *J Agric Sci Camb* **94**, 503–516.
Talavera EM, Zafra MF, Gil-Villarino A, Pérez MI, Alvarez-Pez JM & Garcia-Peregrin E (1997) Changes in chemical composition and physico-chemical properties of chick low- and high-density lipoproteins induced by supplementation of coconut oil to the diet. *Biochimie* **79**, 333–340.
Tall AR, Atkinson D, Small DM & Mahley RW (1977) Characterization of the lipoproteins of atherosclerotic swine. *J Biol Chem* **252**, 7288–7293.
Urano S, Yano K & Matsuo M (1988) Membrane-stabilizing effect of vitamin E: effect of α-tocopherol and its model compounds on fluidity of lecithin liposomes. *Biochem Biophys Res Commun* **150**, 469–475.
Vermorel M (1978) Feed evaluation for ruminants II. The new energy systems proposed in France. *Livest Prod Sci* **5**, 347–365.
Wood JD, Enser M, Fisher AV, Nute GR, Richardson RI & Sheard PR (1999) Animal nutrition and metabolism group symposium on 'improving meat production for future needs': manipulating meat quality and composition. *Proc Nutr Soc* **58**, 363–370.