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Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes

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Summary. Sixteen Holstein cows in mid-lactation were used to determine whether alterations of mammary fatty acid metabolism are responsible for the milk fat depression associated with consumption of fish oil. Cows were given a total mixed ration with no added fish oil (control), unprotected fish oil (3·7% of dry matter), or glutaraldehyde-protected microcapsules of fish oil (1·5% or 3·0% of dry matter) for 4 weeks. Milk samples were taken once a week and a mammary biopsy was taken from a rear quarter at the end of the treatment period. Milk fat content was lower in cows given unprotected fish oil (26·0 g/kg), 1·5% protected fish oil (24·6 g/kg) and 3% protected fish oil (20·4 g/kg) than in cows fed the control diet (36·0 g/kg). This was mainly due to a decrease in the synthesis of short-chain fatty acids. Consumption of protected fish oil decreased the abundance of lipogenic enzymes mRNA in the mammary gland. Acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase mRNAs for cows given 3% protected fish oil averaged only 30%, 25% and 25% of control values, respectively. Dietary addition of unprotected fish oil slightly decreased mRNA abundance of these enzymes but markedly reduced the amount of lipoprotein lipase mRNA. Milk fat content was significantly correlated with gene expression of acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase but not lipoprotein lipase. These results suggest that fish oil reduces milk fat percentage by inhibiting gene expression of mammary lipogenic enzymes.

Keywords: Milk composition, fish oil, lipogenic enzymes, gene expression.

Of the solids in milk, fat is by far the most responsive to dietary manipulation. Indeed, diets high in concentrate or unsaturated fat, or containing forage of small particle size, can cause substantial milk fat depression (MFD; Sutton, 1989). MFD was long believed to result from a shortage of lipid precursors to the mammary gland. Early studies on the effect of low-roughage diets on milk fat secretion established that these diets decreased the ruminal concentration of acetate relative to propionate,

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and concluded that the fall in milk fat content was the result of a reduction in the amount of acetate available for synthesis of fatty acids (Storry & Rook, 1965). However, variable recoveries in fat content of cows fed low-roughage diets after intraruminal or dietary addition of sodium acetate (Balch & Rowland, 1959) indicated that simple shortage of acetate was an unlikely cause. It has also been proposed that diets that result in increased propionate production induce insulin release, which stimulates use of milk-fat precursors by adipose tissue at the expense of the mammary gland (Van Soest, 1963). However, studies in which insulin concentration was chronically elevated by insulin injection (Schmidt, 1966) or by hyperinsulinaemic-euglycaemic clamp (McGuire *et al.* 1995; Griinari *et al.* 1997; Léonard & Block, 1997) do not offer convincing support to the glucogenic-insulin theory of MFD.

Currently, the most widely accepted hypothesis for the cause of MFD involves direct inhibition of milk fat synthesis in the mammary gland. Davis & Brown (1970) hypothesized that an extrinsic factor produced in rumen fermentation, as a result of feeding a milk-fat-depressing diet, alters body metabolism in such a manner that milk fat synthesis is reduced. Pennington & Davis (1975) further speculated that the extrinsic factor might be trans-octadecenoic acids (*trans* C18:1), arising from partial hydrogenation of unsaturated fatty acids in the rumen. Recently, conjugated linoleic acid (CLA), another intermediate in ruminal biohydrogenation of fatty acids, has also been implicated in MFD (Bauman & Griinari, 2000). The mechanism by which these factors might cause MFD has yet to be elucidated.

Most fish oils are very high in polyunsaturated fatty acids (PUFA) and are known to induce severe MFD (Pennington & Davis, 1975; Chilliard & Doreau, 1997*b*). Infusions of fish oil into the rumen or abomasum also decrease milk fat percentage in lactating cows (Storry *et al.* 1974; Pennington & Davis, 1975; Chilliard & Doreau, 1997*a*). These data support a postruminal effect of PUFA on lipid metabolism. Thus, MFD induced by fish oils may be attributed to a decreased uptake of plasma fatty acids by the mammary gland or to a direct inhibition of one or more steps in the synthesis of milk fat in the mammary gland, or both. Fish oil has been shown to inhibit activity of hepatic lipogenic enzymes in rodents (Iritani *et al.* 1980; Herzberg & Rogerson, 1988) but there is little information on the effect of fish oil on lipogenic enzymes in the mammary gland of ruminants.

The objective was to determine the mechanism by which fish oil affects lipid metabolism in the mammary gland in lactating cows. Two forms of fish oil were used, glutaraldehyde-protected and unprotected fish oil, and their effects on lipogenic enzyme expression determined.

Materials and methods

Animals and diets

Sixteen Holstein cows ($162 \pm 4{\cdot}7$ d in lactation) were given a total mixed ration (TMR) based on grass silage, maize silage and rolled barley once a day at 11.00. Four cows were allocated to each treatment according to their days in lactation and milk yield. After a preliminary period of 1 week, rations were supplemented with nothing (control), 3·7% of dry matter (DM) as unprotected fish oil (UFO; 74% lipids; Vaculift, Newark, CA, USA) or 1·5% (L) or 3% (H) glutaraldehyde-protected fish oil (PFO; 58% lipids; Ocean Nutrition Ltd, Bedford, NS, Canada) for 4 weeks. The composition of the diet and the fatty acid profiles of the basal diet and experimental supplements are presented in the companion paper (Lacasse *et al.* 2002).

Table 1. *Effects of dietary addition of fish oil on feed intake and milk production*

(Values are least squares means ± SEM for samples ($n = 4$) taken during the last week of the experiment)

	Control	L-PFO	H-PFO	UFO	SEM
DMI, kg/d	21·4	21·9	20·2	16·9	1·75
Milk, kg/d	27·5	33·9	30·3	22·2*	2·63
Fat, g/kg	36·0	24·6*	20·4**	26·0*	0·32
Fat, kg/d	0·98	0·83	0·63*	0·55*	0·10
Protein, g/kg	35·0	32·3	30·4*	32·6	0·12
Protein, kg/d	0·97	1·09	0·91	0·72	0·08

Values differ from those of the Control: *$P < 0·05$; **$P < 0·01$.

Sampling and analyses

Feed intake was measured daily. Feed samples were collected once a week and analysed for DM, crude protein, neutral-detergent fibre, acid-detergent fibre, minerals and ether extract. Milk production was recorded daily. Milk samples were collected once a week (a.m. and p.m. milking) and were analysed for fat (Roese-Gottlieb method, AOAC, 1990) and protein (nitrogen analysis based on the Dumas method; LECO FP-428, Leco Corp., St-Joseph, MI, USA). A portion of each milk sample was extracted, esterified, and analysed by gas chromatography to determine fatty acid profiles (Lacasse *et al*. 2002). Blood samples were taken from the tail vein at 0, 2, and 4 h after feeding on the last day of the experiment. Blood concentrations of non-esterified fatty acids (NEFA; WACO Chemicals Co., Richmond, VA, USA), total cholesterol and triglycerides (Sigma Chemical Co., St Louis, MO, USA) were measured with commercial kits. Biopsy samples were taken at the end of the experiment, from the mammary gland of all cows. Cows were lightly sedated (Atravet, 75 mg i.m.), and biopsies were performed as described by Farr *et al*. (1996). Tissue samples were stored in liquid nitrogen until assays were performed. The procedure was undertaken with approval of the local animal care committee.

Isolation of RNA and Northern blot analyses

Total RNA from mammary gland tissues was extracted by the guanidinium isothiocyanate, phenol/chloroform method (Chomczynski & Sacchi, 1987) using Trizol reagent (Gibco BRL, Life Technologies, Rockville, MD, USA). Quantity and quality of RNA were determined by absorbance at 260 and 280 nm. Twenty micrograms of RNA in ethidium bromide (1 μg/ml) were loaded onto a 1% agarose/6·6% formaldehyde gel. After electrophoresis, the gel was photographed under u.v. light using high-speed Polaroid films. Then the samples were transferred to a nylon membrane (Micron Separation Inc., Westborough, MA, USA). For each blot, equal loading of lanes and integrity of RNA was confirmed by ethidium bromide fluorescence of 18S and 28S ribosomal RNA. A 2·0-kb cDNA fragment of ovine acetyl-CoA carboxylase (ACC; EC 6.4.1.2) cloned in pGEM7zf+ was obtained from M. Barber and M. Travers (Hannah Research Institute, Ayr, Scotland, UK). A 2·5-kb cDNA fragment of rat fatty acid synthase (FAS; EC 2.3.1.85) was obtained from S. Smith (Childrens Hospital Oakland Research Institute, Oakland, CA, USA). A 2·4-kb cDNA fragment of human lipoprotein lipase (LPL; EC 3.1.1.34) cloned in pUC19 was obtained from T. Clandinin (University of Alberta, Edmonton, AL, Canada). A 2·6-kb cDNA fragment of rat stearoyl-CoA desaturase-1 (SCD; EC 1.14.99.5) cloned in the vector p91023-B was obtained from E. Moore (University

College, Dublin, Ireland). A 0·3-kb fragment from the exon 7 of bovine β-casein gene was PCR-amplified and used as a probe.

Probes were labelled with ^{32}P using the Random Primer DNA Labelling System (Gibco BRL, Life Technologies, Rockville, MD, USA). After prehybridization for 1 h at 65 °C, membranes were hybridized for 16–18 h in hybridization solution (0·1 % Ficoll, 0·1 % polyvinylpyrrolidone, 0·1 % BSA, 0·5 % SDS, 100 μg/ml salmon sperm DNA, in 6 × SSPE) containing the denatured labelled probe (5–10 × 10^6 cpm). Membranes were washed three times for 20 min at room temperature in 2 × SSPE, 0·1 % SDS, followed by a high stringency wash (0·1 × SSC, 0·1 % SDS for 15 min at 65 °C) when the background required it. Thereafter, the membranes were exposed to Kodak X-OMAT AR films in a cassette with intensifying screens at −70 °C. Intensity of mRNA bands on blots was measured using an imaging densitometer (BioRad Laboratories, Mississauga, ON, Canada).

To control for loading differences, densitometry was performed on Polaroid photographs of ethidium bromide-stained gels. A ratio between the arbitrary units, obtained by linear densitometry, of autoradiograms of exposed blots and those obtained from the 28S RNA band in ethidium bromide-stained gels was used to express the data and to perform statistical analyses.

Statistical analyses

Data were analysed by analysis of variance with the GLM procedure of SAS (1985). For observations repeated in time, namely, milk production, milk composition, DM intake (DMI) and blood metabolites, only analyses of data from the last week of the experimental period are reported. Contrasts were used to compare each fish oil treatment with the control. Pearson correlation coefficients were obtained with the CORR procedure of SAS (1985).

RESULTS

Feed intake and BW

During the last week of the experimental period, DMI tended ($P = 0{\cdot}07$; Table 1) to be lower in cows given UFO than in those given the basal diet. DMI was not affected by PFO ($P > 0{\cdot}25$). Cows given UFO lost BW ($P < 0{\cdot}05$), and average daily gain (ADG) for the experimental period averaged 0·74, 0·69, 0·95 and 0·50 ± 0·23 kg/d for control, L-PFO, H-PFO, and UFO, respectively.

Milk production and composition

Cows produced less milk ($P < 0{\cdot}05$) on the UFO treatment (Table 1). Milk fat percentage and yield were depressed ($P < 0{\cdot}05$) by consumption of PFO and UFO. Milk protein percentage was depressed by the highest level of PFO ($P < 0{\cdot}05$). As a result of lower milk production, milk protein yield for cows given UFO tended to be lower ($P = 0{\cdot}06$) than the yield of control cows (Table 1).

Both supplements changed the fatty acid profile of milk fat (Table 2). The proportion of short-chain fatty acids (< C14) was reduced by 15, 35, and 28 % by L-PFO, H-PFO, and UFO, respectively. Secretion of these fatty acids was significantly reduced ($P < 0{\cdot}05$) by both H-PFO and UFO supplements and averaged 120, 89, 50, and 55 ± 18 g/d during the last week of the experiment for control, L-PFO, H-PFO, and UFO, respectively. Similarly, secretion of C18 fatty acids was reduced ($P < 0{\cdot}05$) by the highest level of PFO (32 %) and by UFO (43 %), owing to a reduction of total fat secretion rather than a decreased proportion of these fatty acids.

Table 2. *Effects of dietary addition of protected (PFO) or unprotected (UFO) fish oil on milk fatty acid composition (g/kg of total fatty acids)*

(Values are least squares means for samples ($n = 4$) taken during the last week of the experiment)

Fatty acids	Control	L-PFO	H-PFO	UFO	SEM
C4:0	21·5	23·2	15·8	20·2	2·8
C6:0	25·9	19·4	15·4*	17·5	3·1
C8:0	10·4	8·9	6·2	7·0	1·4
C10:0	27·7	22·2	16·8*	18·2	3·2
C12:0	35·2	28·6	23·8*	24·0*	2·9
C14:0	110	104	96	93	6·1
C16:0	311	285	314	316	9·3
C16:1	16·5	17·3	38·0*	38·8*	6·2
C18:0	74·7	73·2	51·4*	32·5***	6·3
trans C18:1	17	33	78**	84**	13·5
cis-9 C18:1	176	182	141	125*	12·3
C18:2	19·4	23·6	28·6	36·7**	3·2
CLA†	5·2	9·5	12·6*	15·9**	1·8
C18:3	2·9	3·9	3·6	3·7	0·4
C20:5	0·3	1·0*	2·1***	3·0***	0·2
C22:6	1·13	1·56	1·34	1·28	0·3

† *trans* 9, *cis* 11 isomer of C18:2.
Values differ from those of the Control: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

Table 3. *Effects of dietary addition of fish oil on concentrations of blood metabolites*

(Values are least squares means for samples ($n = 4$) taken during the last week of the experiment)

Metabolites	Control	L-PFO	H-PFO	UFO	SEM
Cholesterol, mM	4·18	4·37	5·27	4·18	0·45
Triglycerides, mM	0·101	0·132	0·124	0·111	0·013
NEFA, mEq/l	123·4	132·9	140·8	179·0***	8·2
Glucose, mM	3·63	3·57	3·76	3·61	0·08

Value differs from that of the Control: $***P < 0{\cdot}001$.

Blood metabolites

UFO increased ($P < 0{\cdot}001$) concentrations of NEFA in blood serum (Table 3). Concentrations of triglycerides, cholesterol, and glucose were not affected ($P > 0{\cdot}10$) by fish oil supplements.

Mammary gene expression

H-PFO decreased ($P < 0{\cdot}001$) mRNA abundance of lipogenic enzymes ACC (Fig. 1), FAS (Fig. 2*a*) and SCD (Fig. 2*b*) in the mammary gland. In the H-PFO group, ACC, FAS, and SCD mRNAs averaged only 30%, 25%, and 25% of control values, respectively. However, PFO had no effect on LPL gene expression (Fig. 2*c*). UFO decreased slightly mRNA abundance of ACC ($P < 0{\cdot}05$), FAS ($P < 0{\cdot}01$), and SCD ($P < 0{\cdot}10$) but markedly reduced the amount of LPL mRNA ($P < 0{\cdot}001$). Gene expression of β-casein was reduced by both fish oil supplements (Fig. 2*d*). Milk fat content was significantly correlated with gene expression of ACC ($r = 0{\cdot}72, P < 0{\cdot}002$; Fig. 3), FAS ($r = 0{\cdot}60$; $P < 0{\cdot}05$), and SCD ($r = 0{\cdot}68$; $P < 0{\cdot}01$) but not LPL. Correlations between expression of ACC, FAS, and SCD ranged from $r = 0{\cdot}54$ to $r = 0{\cdot}86$. Gene expression of LPL was negatively correlated with serum NEFA ($r = -0{\cdot}72$; $P < 0{\cdot}01$). Expression of β-casein gene was correlated with milk protein content ($r = 0{\cdot}78$; $P < 0{\cdot}001$) and gene expression of ACC ($r = 0{\cdot}84$; $P < 0{\cdot}001$), FAS ($r = 0{\cdot}68$; $P < 0{\cdot}05$), and SCD ($r = 0{\cdot}82$; $P < 0{\cdot}001$) but not LPL.

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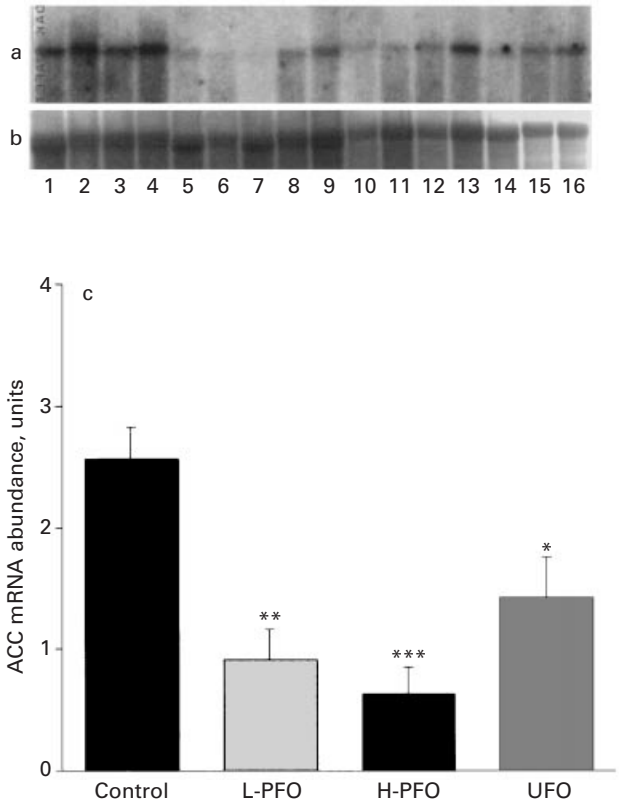

Fig. 1. Effects of dietary addition of protected (PFO) or unprotected (UFO) fish oil on mammary acetyl-CoA carboxylase (ACC) gene expression. (*a*) Northern blot of ACC; (*b*) ribosomal 28S RNA of the same gel; (*c*) ACC mRNA levels corrected for 28S. Lanes 1–4, control; lanes 5–7 and 16, H-PFO; lanes 8–11, L-PFO; lanes 12–15, UFO. Values are means ± SEM for $n = 4$. Differences from control diet: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

DISCUSSION

In our study, adding fish oil to the diet of lactating cows caused severe MFD, in agreement with other studies (Brumby *et al.* 1972; Wonsil *et al.* 1994; Chilliard & Doreau, 1997*b*). Secretion of short-chain fatty acids was reduced by up to 60% (H-PFO) indicating strong inhibition of *de novo* synthesis of fatty acids. This was also reported by others when fish oil was added to the diet (Chilliard *et al.* 1997) or infused into the rumen (Chilliard & Doreau, 1997*a*). We observed a significant reduction in yield of C18 fatty acids. Again, similar reductions have been observed in most fish oil studies (Brumby *et al.* 1972; Storry *et al.* 1974; Chilliard *et al.* 1997). These fatty acids are obtained from the circulation, not synthesized by the mammary gland, but Storry *et al.* (1969) suggested that the decrease in mammary uptake of fatty acids is due to an inhibition of LPL by C20 and C22 fatty acids. We found a reduction in LPL gene expression only in cows given UFO. Furthermore, correlation of LPL expression with yield of C18 fatty acids was weak ($r = 0{\cdot}43$, $P = 0{\cdot}12$). Blood triglyceride concentration was not affected by treatment, but blood fatty acid profile was modified (Lacasse *et al.* 2002). Except for *trans* C18:1 the proportion of all C18 fatty acids in blood plasma was reduced. Therefore, part of the effect of fish oil on C18 uptake may simply be due to changes in substrate availability.

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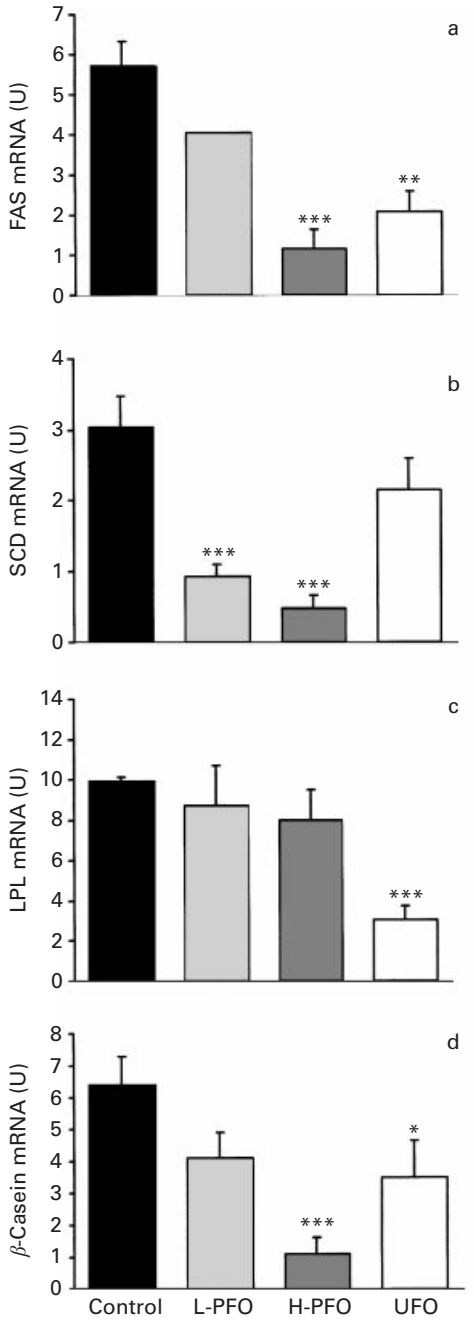

Fig. 2. Effects of dietary addition of protected (PFO) or unprotected (UFO) fish oil on mammary (*a*) fatty acid synthase (FAS); (*b*) stearoyl-CoA desaturase (SCD); (*c*) lipoprotein lipase (LPL); (*d*) β-casein gene expression. Values are means ± SEM for $n = 4$. Differences from control diet: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

Whether fish oil has direct or indirect effects on mammary gland metabolism is not clear. The presence of PUFA is essential for fish oil to induce MFD since hydrogenated fish oil has no effect on milk fat (Brumby *et al.* 1972). Studies in rodents show C20:5 and C22:6 to be strong inhibitors of lipid synthesis (Raclot &

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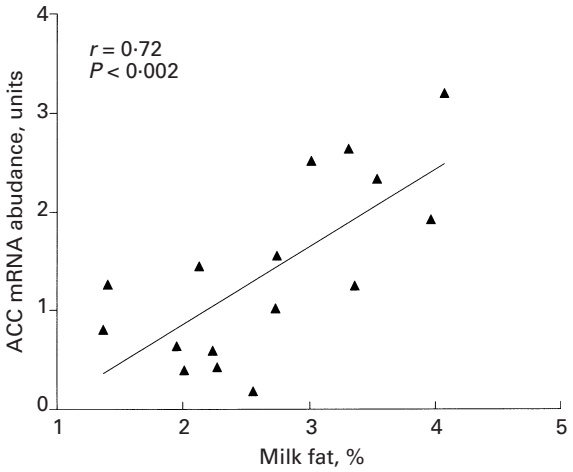

Fig. 3. Relationship between mRNA levels of acetyl-CoA carboxylase (ACC) and milk fat content for the 16 animals in the experiment.

Oudart, 1999). Intravascular (Storry *et al.* 1969) and post-ruminal (Pennington & Davis, 1975; Chilliard & Doreau, 1997*a*) infusions of fish oil induced mild MFD. However, much stronger inhibitions were seen when fish oil was infused into the rumen (Pennington & Davis, 1975; Chilliard & Doreau, 1997*a*). Dietary addition of fish oil increased milk fat content of *trans* C18:1. Pennington & Davis (1975) proposed that *trans* C18:1, arising from partial hydrogenation of unsaturated fatty acids in the rumen, directly inhibits lipid synthesis in the mammary gland. Wonsil *et al.* (1994) reported that dietary and ruminally derived *trans* C18:1 were associated with reductions of milk fat content. Abomasal infusion of a fat mixture containing 43% *trans* C18:1 and 21% *cis* C18:1 induced MFD while an infusion of a fat mixture containing no *trans* C18:1 and 65% of *cis* C18:1 did not (Gaynor *et al.* 1994). Griinari *et al.* (1998) summarized data from 17 studies and found a relationship ($r = 0{\cdot}54$) between change in milk fat percentage and change in *trans* C18:1 content in milk fat. Chilliard *et al.* (1999) reported that fish oil caused a sharp increase in milk fat content of both *trans* C18:1 and CLA. The latter is particularly interesting because its effect on milk fat content is spectacular. Indeed, abomasal infusion of CLA for as short as 12 h reduced milk fat percentage by 25% (Loor & Herbein, 1998). Similarly, Chouinard *et al.* (1999) reduced milk fat content by 50% by infusing into the abomasum a mixture of four CLA isomers for 5 d. Recently, Baumgard *et al.* (2000) have shown that as little as 10 g/d of *trans*-10, *cis*-12 CLA is enough to cause severe MFD. We observed an increase in the concentration of *cis*-9, *trans*-11 CLA in milk of cows given fish oil. Although we were not able to detect the presence of other CLA isomers, it is possible that part of the effect of fish oil on mammary gland metabolism is through *trans* C18:1 or CLA.

SCD is an important enzyme that catalyses Δ9 desaturation of C16:0 and C18:0 to C16:1 and C18:1 in various organs, including the ruminant mammary gland. Mahfouz *et al.* (1980) demonstrated that SCD can produce *cis*-9, *trans*-11 C18:2 (a major isomer of CLA) from *trans*-11 C18:1 in microsomal preparations from rat liver. Chilliard *et al.* (2000) noted a linear relationship between contents of *trans* C18:1 and CLA in milk. Abomasal infusion of a mixture of *trans* C18:1 fatty acids induced a gradual increase in milk concentrations of CLA (Griinari *et al.* 2000). Furthermore,

inhibition of SCD by sterculic acid reduced CLA content in milk fat by 45%. Therefore, it is possible that the fat-depressing effect of *trans* C18:1 is mediated by CLA.

Several mechanisms have been proposed to explain how specific fatty acids might affect mammary lipogenesis. Davis & Brown (1970) first speculated that *trans* fatty acids in blood triglycerides might be less acceptable substrates for LPL. However, no differences in metabolism of *cis*- and *trans*-9 C18:1 were observed when they were infused intravenously into lactating goats (Bickerstaffe *et al*. 1972). Gaynor *et al*. (1994) suggested that the nearly straight-chain configuration of *trans* C18:1 might impair mammary SCD activity or esterification to glycerol. In the present experiment, mammary biopsies revealed that gene expression of several lipogenic enzymes was strongly inhibited by fish oil. Contents of ACC, SCD and FAS mRNA, but not of LPL mRNA, were correlated with milk fat content. Accordingly, Piperova *et al*. (2000) observed a reduction in ACC mRNA abundance and activity in mammary tissue of cows given a diet that induced MFD. In rodents, there is well-documented suppression of hepatic *de novo* fatty acid biosynthesis by PUFA (Clarke & Jump, 1996). Such suppression of enzymic activities does not represent a fatty acid-mediated impairment of enzyme catalytic efficiency but rather reflects a decrease in hepatic enzyme content by a suppression of lipogenic enzyme synthesis (Toussant *et al*. 1981). Subsequently, Clarke *et al*. (1990) demonstrated that this effect is due to an inhibition of gene transcription. Recently, it has been reported that a CLA isomer (*trans*-10, *cis*-12) that causes MFD, also inhibits SCD expression in rat liver (Choi *et al*. 2000) and in 3T3-L1 adipocytes (Park *et al*. 2000). We also observed an inhibition of SCD gene expression in the mammary gland of cows when milk fat was depressed by infusion of a mixture of CLA isomers (Simard *et al*. 2001).

Expression of LPL was reduced only in cows given UFO. These cows were in negative energy balance resulting from severe depression of feed intake. Underfeeding has been shown to depress LPL activity and expression in adipose tissue (Bonnet *et al*. 1998) and mammary gland (Jensen *et al*. 1994). Accordingly, there was a negative correlation between blood NEFA and LPL expression.

Milk protein content was reduced by feeding fish oil, as also reported by Chilliard *et al*. (1997*a*) and Cant *et al*. (1997). The correlation between β-casein gene expression and milk protein content suggests that this effect, at least in part, was due to an inhibition of gene expression of milk proteins.

Coordinated inhibition of the expression of several genes involved in milk synthesis suggests that these genes may share a common regulatory mechanism. Clarke *et al*. (1990) found that PUFA-regulation of gene transcription occurs within a matter of minutes. Such a time frame is too short to be explained by changes in membrane composition, altered hormone release, or altered hormone signalling (Clarke, 2000). However, a ligand-mediated event, such as fatty acid binding to a transcription factor, is compatible with a rapid response. Sterol-regulatory element-binding protein-1 (SREBP-1) is a membrane-anchored precursor protein which, upon proteolysis, releases a peptide that induces gene expression of lipogenic enzymes (Shimomura *et al*. 1998). Transgenic mice over-expressing this protein show several-fold increases in hepatic synthesis of fatty acid (Shimomura *et al*. 1998). Xu *et al*. (1999) reported that ingestion of safflower oil or fish oil, but not saturated or monounsaturated fat, reduced the stability of SREBP-1 mRNA and the membrane content of SREBP-1. Whether SREBP-1 plays a role in bovine mammary gland is not known.

Our observations suggest that dietary fish oil can affect milk fat synthesis by

regulating the expression of several genes involved in lipid synthesis and that these genes may share a common regulatory mechanism.

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