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NUTRITIONAL REGULATION OF MILK FAT SYNTHESIS

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■ **Abstract** Certain diets cause a marked reduction in milk fat production in ruminants. Commonly referred to as milk fat depression (MFD), the mechanism involves an interrelationship between rumen microbial processes and tissue metabolism. Numerous theories to explain this interrelationship have been proposed and investigations offer little support for theories that are based on a limitation in the supply of lipogenic precursors. Rather, the basis involves alterations in rumen biohydrogenation of dietary polyunsaturated fatty acids and a specific inhibition of mammary synthesis of milk fat. The biohydrogenation theory proposes that under certain dietary conditions, typical pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates that inhibit milk fat synthesis. *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) has been identified as one example that is correlated with the reduction in milk fat. Investigations with pure isomers have shown that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis, and similar to diet-induced MFD, the mechanism involves a coordinated reduction in mRNA abundance for key enzymes involved in the biochemical pathways of fat synthesis. A more complete identification of these naturally produced inhibitors of fat synthesis and delineation of cellular mechanisms may offer broader opportunities for application and understanding of the regulation of lipid metabolism.

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INTRODUCTION

Fat is the major energy component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk products. Milk fat consists predominantly of triglycerides (over 95%) in all mammals, but actual fat content of milk varies widely among species. For example, the fat content of milk ranges from over 50% in pinnipeds and whales to less than 1% in the rhinoceros (72). Dairy producers have long been interested in milk fat in ruminants because of its economic value, and research has been directed toward understanding the biosynthesis of milk fat and the factors that influence its quantity and fatty acid composition. These scientific investigations have characterized mammary metabolism, established biochemical pathways of fat synthesis, and identified the regulation of these processes in milk synthesis; results have been of special value because they provided insight into lactation biology in all species. Classical work in the area of milk fat by E.F. Annison, C.L. Davis, S.F. Folley, J.E. Kinsella, J.L. Linzell, and S. Patton serve as a few examples.

Fat content and composition of milk can be markedly affected by diet. This has been most extensively investigated for ruminants, and several excellent reviews summarize dietary effects (49, 65, 76, 97, 115). However, there are specie differences, and Neville & Picciano (91) recently summarized the literature for a range of species, including human. For many species, the fatty acid composition of milk fat strongly reflects the fatty acid composition of the diet. Ruminants are an exception because dietary lipids are extensively altered by bacterial metabolism in the rumen, and one of the major changes is the biohydrogenation of polyunsaturated fatty acids (PUFA). Milk fat from ruminants is estimated to have over 400 different fatty acids, and this relates in large part to the extensive lipid metabolism that occurs in the rumen (76). However, diet can markedly affect the bacterial population and rumen microbial processes, and as a consequence diet and nutrition have major effects on the fat content and fatty acid composition of milk, even in ruminants. Davis & Brown (44) characterized one of the most striking examples of this as the low-fat milk syndrome, more commonly referred to as milk fat depression (MFD). MFD occurs when feeding particular diets markedly reduces the fat content and alters the fatty acid composition of milk. MFD was first recognized in 1885 when Boussingault observed a reduction in milk fat yield when beets were fed to dairy cows [cited by Van Soest (121)], and MFD was reported numerous times through the early twentieth century when dairy producers began to follow "scientific feeding" practices to supply the nutritional requirements of dairy cows. This has resulted in an extensive number of investigations focused on the biology of MFD, especially over the last half century. The dietary conditions that cause MFD can now be predicted with great confidence, but its biological basis remains elusive.

The low-fat milk syndrome represents a challenging biological problem involving interrelationships between digestive processes and tissue metabolism. In the

following sections we will initially provide background on milk fat synthesis and the etiology of MFD. A number of theories have been proposed to explain MFD, and those most widely referenced will be considered. Finally, we will discuss recent developments in the biology of MFD and consider the extent to which they provide the framework for a global basis to explain the low-fat milk syndrome.

BACKGROUND

Origin of Fatty Acids and Milk Fat Synthesis

Mammals differ widely in the fatty acid composition of milk fat (72, 74). The composition of ruminant milk fat (bovine) is contrasted with that of selected species in Table 1. The fatty acids in milk arise from two sources, uptake from circulation and de novo synthesis within the mammary epithelial cells (20, 47, 91). In large part, specie differences in milk fat composition reflect the source of fatty acids used for the synthesis of milk fat. Short-chain fatty acids (4 to 8 carbons) and medium-chain fatty acids (10 to 14 carbons) arise almost exclusively from de novo synthesis. Long-chain fatty acids (>16 carbons) are derived from the uptake of circulating lipids, and fatty acids of 16 carbons in length originate from both sources. Thus, for a given species the importance of the different sources of fatty acids for milk fat synthesis can be reasonably predicted by examining milk fat

TABLE 1 Comparison of milk fatty acid composition (molar percent) of domestic cows with other selected species[a]

Fatty acid	Domestic cow	Human	Laboratory rat	Indian elephant	Northern fur seal
4:0	12				
6:0	5	<1	<1	1	
8:0	2	<1	4	8	
10:0	4	2	12	49	
12:0	4	4	11	21	
14:0	11	6	13	3	7
16:0	24	21	28	7	23
16:1	3	6	2	2	11
18:0	7	3	3	<1	2
18:1	24	45	16	7	33
18:2	3	13	10	2	2
18:3	1	1	1	<1	8
>18:3	<1	<1	<1		15

[a]Pattern for cow adapted from Jensen (76) and data for other species adapted from Jenness (74)

composition. For example, de novo synthesis is clearly the major source of milk fatty acids in the elephant (91 molar percent are ≤16 carbons), whereas milk fat produced by the seal is derived predominantly by uptake of fatty acids from the blood (94 molar percent are ≥16 carbons) (Table 1). These differences become an important consideration when the regulation of milk fat synthesis is considered.

In ruminants, about one-half of the milk fatty acids (molar percent) are derived from de novo synthesis [see review by Bauman & Davis (20)]. Whereas glucose is used for de novo synthesis by nonruminants, ruminants utilize acetate produced in rumen fermentation of carbohydrates as the major carbon source. In addition, β-hydroxybutyrate, produced by the rumen epithelium from absorbed butyrate, provides about one half of the first four carbons of de novo synthesized fatty acids in the ruminant. There are also differences in the biochemical pathways to provide the needed reducing equivalents [nicotinamide adenine dinucleotide phosphate (NADPH)] to support fatty acid synthesis between ruminants (pentose-phosphate cycle and the isocitrate cycle) and nonruminants (pentose-phosphate cycle and the malate transhydrogenation cycle). However, the overall processes in fatty acid synthesis are remarkably similar among mammals [see reviews by Barber et al. (16), Bauman & Davis (20), and Neville & Picciano (91)].

Preformed fatty acids taken up by the mammary gland and directly used for milk fat synthesis are derived from circulating lipoproteins and nonesterified fatty acids (NEFA) that originate from the absorption of lipids from the digestive tract and from the mobilization of body fat reserves, respectively (16, 20). In ruminants, fatty acids in milk fat that are taken up from circulation are derived predominantly from the intestinal absorption of dietary and microbial fatty acids. Typically, lipolysis and the mobilization of body fat account for <10% of the fatty acids in milk fat. However, when cows are in a negative energy balance, the contribution from mobilized fatty acids increases in direct proportion to the extent of the energy deficit (22). Interestingly, this is also an area where species differ. Whales and pinnipeds do not eat throughout lactation, and as reflected in their milk fat composition (72, 74), mobilization of body fat must be their exclusive source of the fatty acids in milk fat. Body reserves are also of greater importance in humans, where an estimated 60% or more of the fatty acids in milk may be from this source (91).

The Low-Fat Milk Syndrome

The low-fat milk syndrome is of special interest as a natural situation where dietary regulation of milk fat occurs. Historical aspects in identifying dietary relationships with MFD in dairy cows have been reviewed (22, 44, 52, 53, 97). Davis & Brown (44) divided diets causing MFD into two broad groups. One group involved diets that provided large amounts of readily digestible carbohydrates and reduced amounts of fibrous components, the most common being a high-grain/low-roughage diet. However, diets where the fiber content is adequate but the fiber source is ground or pelleted also fall into this group because these processes reduce the ability of fiber to maintain normal rumen function. Corn silage

is used in many areas of the world as the main forage source for lactating dairy cows. Although a corn silage-based diet may appear to contain an adequate level of fiber, its effectiveness to maintain rumen function that supports normal milk fat is less than the fiber in grass silage-based diets. The high proportion of grain in corn silage (typically 40% to 50%) further reduces its effectiveness as a forage source. In ruminants, the effectiveness of dietary fiber is the main determinant of the ability of forages to buffer the rumen, so we will use the term low-fiber (LF) diets as the description for the first group of diets that cause MFD.

The second group of diets that induce MFD, according to Davis & Brown (44), represented dietary supplements containing polyunsaturated oils (e.g., plant and marine oils). MFD occurs when oil supplements are added directly to the diet, but it can also occur when the diet is supplemented with full-fat seeds or meal containing the polyunsaturated fatty acids. In fact, the presence of polyunsaturated fatty acids (PUFAs) is a prerequisite for MFD to occur with LF diets (62). Also, plant oil supplements will not depress milk fat yield if roughage intake is high or the effectiveness of roughage fiber is sufficient to maintain normal rumen function (33, 79). Thus, plant oil supplements are an additional example of MFD caused by LF diets.

Fish oil and oils from marine mammals and marine algae are characterized by the presence of two PUFAs: eicosapentaenoic (C20:5) and docosahexaenoic acid (C22:6). We will refer to these collectively as marine oil (MO) diets. In contrast to plant oils, MO supplements will induce MFD even when diets contain an adequate level of effective fiber (6, 35, 93). Thus, MO diets represent the second major group of diets that induce MFD.

A number of theories have been proposed to explain diet-induced MFD, and dietary alterations in rumen microbial processes form the basis for all of them. Powell (106) first recognized this and concluded "there is apparently a positive correlation between the activities of the rumen and the composition of the milk fat produced." Subsequent work established that the alterations in rumen activity with LF and MO diets involved both the microbial fermentation of dietary carbohydrates and the microbial biohydrogenation of dietary unsaturated fatty acids (44, 49, 114, 120). Rumen alterations generally include changes in the rumen molar ratio of volatile fatty acids, specifically a decreased ratio of acetate:propionate. In addition, these diets cause alterations in rumen biohydrogenation, and a key feature is the accumulation of biohydrogenation intermediates, particularly *trans*18:1fatty acids (Figure 1).

Several of the proposed theories to explain MFD have been disproved, but three theories that are based on diet-induced changes in rumen microbial processes continue to have support in the scientific literature [see reviews by Bauman & Griinari (22) and Doreau et al. (49)]. One theory is that the alterations in rumen fermentation result in an inadequate rumen production of acetate and butyrate to support milk fat synthesis. A second theory is that increased rumen production of propionate and enhanced hepatic rates of gluconeogenesis cause an increase in circulating insulin, thereby resulting in an insulin-induced shortage of precursors

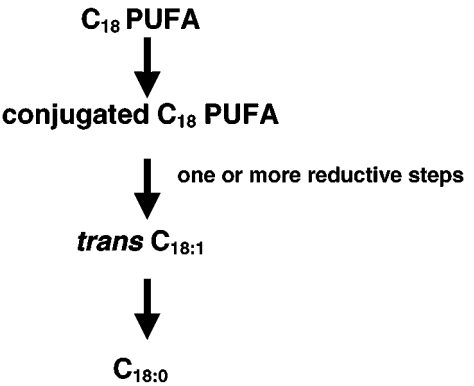

Figure 1 General pathway for rumen biohydrogenation of polyunsaturated 18-carbon fatty acids. PUFA, polyunsaturated fatty acid. Adapted from Harfoot & Hazlewood (67).

for mammary synthesis of milk fat. The third theory that has gained support over the last decade is that mammary synthesis of milk fat is inhibited by unique fatty acids that are produced as a result of the alterations in rumen biohydrogenation. We examine these possibilities in the following section. Of special interest is whether there might be multiple causes for MFD involving several of these mechanisms, or whether a single theory might provide a global basis to account for most, if not all, types of diet-induced MFD.

THEORIES OF MILK FAT DEPRESSION

Rumen Production of Acetate and Butyrate

A clear relationship between changes in the rumen volatile fatty acid (VFA) pattern and the reduction in milk fat yield has been established for a range of diets. This led to suggestions that decreased rumen production of acetate and butyrate will limit milk fat synthesis and contribute to the effect of MFD diets (49, 52, 115, 119). Sutton et al. (116) estimated that up to 80% of the variation in milk fat concentration can be accounted for by variations in molar proportions of VFA in the rumen. Oldham & Emmans (95) also found a close correlation between ratios of milk fat and milk lactose precursors (acetate + butyrate + long-chain fatty acids and glucose + propionate, respectively) and the fat content of milk. They proposed that prediction of milk fat responses to MFD diets might be achieved by relatively simple functions of precursor-product ratios.

The production of VFA in the rumen of lactating cows can be quantified using isotope dilution methods. Unfortunately, there have been only a limited number of isotope dilution measurements involving LF diets, but results are relatively consistent showing that the rate of acetate production is not decreased even under

conditions where the molar proportion of acetate in the rumen fluid is markedly decreased (2, 43, 117). Rather, the decrease in the molar proportion of acetate is due to an increase in the rate of propionate production (21). Only one study has examined the whole body entry of β-hydroxybutyrate, which correlates closely with the rate of butyrate production in the rumen, and it demonstrated that the entry rate was unaffected when a low-fiber diet was fed (98). The effect of an LF diet on these variables is illustrated in Table 2, and overall it is clear that the supply of acetate and butyrate is not appreciably altered when LF diets are fed. Also, in the case of MO-supplemented diets where the shift in molar proportion of VFA is less pronounced (29, 44, 48), it is even less likely that rumen production and the supply of acetate and butyrate limit milk fat synthesis.

In spite of the evidence that LF diets do not appreciably affect rumen production rates of acetate and butyrate, a shortage of these VFAs is frequently considered to contribute to diet-induced reduction in milk fat, even if in a minor way (49). Changes in molar proportions of VFA in the rumen fluid are often cited as support, with the assumption that these correlate with the rates of production. This correlation is relatively good for diets high in fiber, particularly for sheep. However, these

TABLE 2 Rumen volatile fatty acids (VFA) and milk fat depression for cows fed a low-fiber (LF) diet consisting of high grain plus low roughage

	Diet	
Variable	**Control**	**LF**
Milk[a]		
Yield, kg/d	19.1	20.9
Fat content, %	3.6	1.7*
Fat yield, g/d	683	363*
Rumen VFA, molar percent[a]		
Acetate	67	46*
Propionate	21	46*
Butyrate	11	9
Acetate:propionate ratio	3.2	1.0*
Rumen production, moles/d		
Acetate[b]	29.4	28.1
Propionate[c]	13.3	31.0*
Whole body entry rate, mg/min $kg^{.75}$		
β-hydroxybutyrate[d]	3.40	4.43

[a]Averaged from Davis (43) and Bauman et al. (21)

[b]Davis (43)

[c]Bauman et al. (21)

[d]Palmquist et al. (98)

*Significant difference

relationships appear not to apply when LF diets are fed (3, 114). This divergence may be attributable to the fact that LF diets typically reduce rumen pH, and this will affect the relative rates of absorption of individual VFA (46, 81).

The possibility that acetate supply was limiting milk fat synthesis has also been examined by providing exogenous acetate. Davis & Brown (44) summarized six studies that involved dietary supplementation of sodium acetate or infusions of acetate in milk fat-depressed cows fed LF diets. These studies are complicated by the fact that ruminal supplementation of VFA in any form may have an effect on the rumen environment and microbial processes in the rumen. Nevertheless, milk fat responses to acetate supplementation were modest, and Davis & Brown (44) concluded that a simple deficiency in acetate could not adequately explain the reduction in milk fat in diet-induced MFD. Studies using arterio-venous (A-V) difference techniques have demonstrated that blood concentrations of acetate and uptake of acetate by the mammary gland are decreased in MFD cows (summarized in Reference 44). It is also well established that there is a linear relationship between arterial concentration of acetate and A-V difference across the mammary gland (e.g., References 2, 111). This linear relationship is often interpreted to suggest that supply of a nutrient may be limited by a low arterial concentration. However, a reduced acetate uptake would also be the expected consequence of a reduction in milk fat secretion. As Davis & Brown (44) succinctly concluded, "If, in fact, acetate uptake by the mammary gland is reduced in cows secreting low-fat milk, further evidence is needed to determine whether this is a cause or an effect of decreased mammary lipogenesis."

If we accept that the main contributing factor in MFD is inhibition by biohydrogenation intermediates as will be discussed in a later section, then we conclude that a limitation in rumen production of acetate and butyrate can be ruled out. As a result of the initial reduction in rates of milk fat synthesis due to inhibition, the need for milk fat precursors is reduced. Therefore, even if a minor decrease in rumen formation of acetate and butyrate occurred, it would not result in any substantial shortage of precursors. Furthermore, acetate and butyrate are also important sources of energy, and possible small decreases in rumen production of acetate and butyrate would be associated with an equal or larger increase in energy supply from the production of propionate. Thus, we conclude that possible shifts in the rumen production of acetate and butyrate could not be responsible for the reduction in milk fat synthesis during diet-induced MFD.

Glucogenic-Insulin Theory

Insulin is involved in the coordination of nutrient partitioning through its central role in the regulation of glucose and energy homeostasis. In the case of the ruminant mammary gland, insulin is required for the maintenance of normal mammary cell function. However, this requirement is met by a relatively low blood concentration, and the daily fluctuations in circulating insulin that occur with meal pattern have no apparent effect on glucose utilization by the mammary gland. Both in vitro (12, 23) and in vivo studies (68, 69, 84, 85) have demonstrated that insulin has no acute

effect on glucose uptake, which is consistent with ruminant mammary epithelial cells having only glucose transporter 1 (125, 126). However, insulin does acutely regulate the metabolism of other tissues in the ruminant, including rates of lipogenesis (stimulatory) and lipolysis (inhibitory) in adipose tissue (17, 123), and this may indirectly affect the supply and pattern of nutrients available to the mammary gland.

The glucogenic-insulin theory is based on a competition for nutrients between the mammary glands and nonmammary tissues, and tissue differences in response to insulin. The theory was first proposed by McClymont & Vallance (88) to explain the MFD that occurs with LF diets, and it was subsequently elaborated by Jenny et al. (75), Annison (1), and others (10, 49, 52, 89, 115). Propionate and glucose are secretagogues for pancreatic release of insulin, and LF diets result in increased rumen production of propionate (114) and hepatic rates of gluconeogenesis (2). In addition, LF diets generally result in a substantial increase in net energy balance because of the greater energy intake and a reduction in milk fat secretion. As a consequence of this combination of factors, blood concentrations of insulin are elevated. According to the glucogenic-insulin theory, the increase in circulating insulin diverts nutrients from the mammary gland because of insulin-induced increases in adipose tissue utilization of acetate, β-hydroxybutyrate and diet-derived long-chain fatty acids, and the insulin-induced reduction in mobilization of long-chain fatty acids from body fat reserves. Overall, these changes in rates of lipid synthesis and lipolysis are proposed to preferentially channel nutrients to adipose tissue, thereby causing a shortage of lipogenic precursors for mammary synthesis of milk fat.

The glucogenic-insulin theory has been tested with exogenous infusions of propionate and glucose. Davis & Brown (44) summarized 13 experimental treatments involving propionate infusion and observed that effects on milk fat were highly variable, ranging from 0 to 14% reduction in yield. Many of the studies infused propionate at a rate of 1000 g/d or more, an amount that approximates the daily propionate production in cows fed control diets. More recent investigations of propionate infusion verify both the variability and range in the response of milk fat yield (55, 70, 71, 90, 118). When recent data are included, reductions in milk fat yield appear to be independent of the dose of ruminally infused propionate. Likewise, Bauman & Griinari (22) recently summarized 24 investigations involving infusion of glucose and found a similar variability and range for effects on milk fat yield ($+4$ to -16%).

Infusion of insulin secretagogues generally results in hypoglycemia and counter-regulatory changes because of insulin's central role in glucose homeostasis, and this complicates interpretation. Use of the hyperinsulinemic-euglycemic clamp allows an examination of the role of insulin without the complication of hypoglycemic and counter-regulatory changes (45). Bauman & Griinari (22) reviewed data from five experimental treatments by the Cornell group involving this approach in well-fed cows in positive net energy balance. Circulating insulin was elevated approximately fourfold over basal levels and clamps were maintained for four days, thereby allowing for an evaluation of both acute and chronic effects. There was no evidence of insulin resistance based on the constant rates of glucose

infusion required to maintain euglycemia and the steadfast antilipolytic effect of insulin as indicated by the reduction in plasma concentrations of NEFAs. Relative to the glucogenic-insulin theory, effects on milk fat were minimal with the reduction in milk fat yield averaging 5% during the hyperinsulinemic-euglycemic clamps. Similar observations were reported by Bequette et al. (32) in a recent hyperinsulinemic-euglycemic clamp study with lactating goats.

The energy status of the animal is an important consideration in evaluating the regulation of milk fat yield. In cows in positive energy balance, an estimated 4% to 8% of milk fatty acids originate from mobilized body fat reserves (99, 108). Circulating concentrations of NEFAs are directly proportional to their turnover rate and the extent of the energy balance deficit. Furthermore, mammary uptake of NEFAs is directly related to their circulating concentration (4, 24, 34, 50, 51). Based on this, we proposed that the variability in the response in milk fat yield observed with propionate and glucose infusions was due to differences in the energy status of cows among the different studies (22). Fatty acids derived from body fat stores make a minimal contribution to milk fat for cows in positive energy balance, but their importance increases in direct relation to the extent of a cow's nutrient deficit. Accordingly, the magnitude of the insulin-induced reduction in lipolysis observed with propionate and glucose infusions or during a hyperinsulinemic-euglycemic clamp would reflect this relation.

The relationship between net energy balance and the effect of insulin on milk fat yield is shown in Table 3. In early lactation when voluntary intake was inadequate,

TABLE 3 Effects of a hyperinsulinemic-euglycemic clamp on milk fat synthesis in dairy cows

	Early lactation study[a]		Mid lactation study[b]	
Variable	**Control**	**Insulin clamp**	**Control**	**Insulin clamp**
Milk yield, kg/d	19.7	19.1	27.5	28.8
Milk fat				
Percent	4.93	3.31*	3.54	3.15*
Yield, g/d	980	637*	956	893
Milk fatty acids, g/d				
<C16	113	121	283	286
C16	209	161*	253	281
>C16	550	285*	315	228*
Plasma				
Glucose, mg/dL	39.2	40.0	49.4	48.0
Nonesterified fatty acids, μM	1021	330*	113	79*

[a]Cows were 10 d postpartum at the start of the 4-d clamp, and net energy balance averaged −7.5 Mcal/d during the hyperinsulinemic-euglycemic clamp. Adapted from S.T. Butler, W.R. Butler, B.A. Corl, & D.E. Bauman (unpublished).

[b]Cows were 184 d postpartum at the start of the 4-d clamp, and net energy balance averaged +7.9 Mcal/d during the clamp. Adapted from Griinari et al. (63).

*Significant difference

cows were in a substantial negative energy balance, and circulating NEFAs were elevated. In this situation mobilized fat reserves represented a major source of fatty acids for milk fat, and insulin infusion markedly reduced rates of lipolysis as indicated by changes in plasma NEFA and resulted in a 35% reduction in milk fat yield. In contrast, cows in midlactation were in positive energy balance and circulating NEFAs were very low. Lipolysis was also inhibited during the insulin clamp as indicated by the reduction in plasma NEFAs, but in this case fatty acids derived from body fat reserves represented a minor source of milk fatty acids so that milk fat yield was reduced by only 6%. The reduction in milk fat yield was exclusively confined to long-chain fatty acids during the insulin clamp in both early lactation and midlactation (Table 3). This is consistent with the antilipolytic effects of insulin and contrasts with the lack of any effect of the insulin clamp on milk fat yield of short or medium-chain fatty acids. Thus, the fourfold elevation in circulating insulin during the hyperinsulinemic-euglycemic clamp did not result in a shortage in the supply of precursors for de novo synthesis of milk fat. As a consequence of the changes in yields of specific fatty acids during the insulin clamp studies, there was a shift in milk fat composition reflecting a reduction in the proportion of longer chain fatty acids and an increase in the proportion of de novo synthesized fatty acids. A similar shift in milk fat composition was also observed in studies involving glucose infusions [reviewed by Bauman & Griinari (22)].

Circulating insulin concentrations are closely related to energy balance, and several studies report dietary situations where insulin is elevated but milk fat is unaltered. These have been discussed in an earlier review (22), and as outlined above, represent situations where cows were in positive energy balance, thereby minimizing the importance of body fat reserves as a source of milk fatty acids. Activities of adipose tissue enzymes related to lipid synthesis are also increased in milk fat depressed cows consuming LF diets (11, 31, 96), and again these changes appear to be a response to the energy balance characteristic of cows fed LF diets rather than a cause of MFD.

Overall, results from studies involving propionate and glucose infusions and hyperinsulinemic-euglycemic clamps offer little support for the glucogenic-insulin theory as the basis for diet-induced MFD in terms of the magnitude of response or the pattern of changes in milk fatty acid composition. Clearly, milk fat response to an increase in blood insulin is a consequence of the antilipolytic effects of insulin, and the magnitude of the insulin-induced reduction in milk fat yield is related to the importance of body fat reserves as a source of fatty acids for milk fat. Elevations in circulating insulin correspond to dietary intakes where animals are in positive energy balance, and under these situations mobilized fatty acids represent only a minor source of fatty acids for milk fat synthesis in dairy cows. Thus, we conclude that increased supplies of propionate and glucose, and the related response in insulin that occurs with LF diets, are not the basis of diet-induced MFD. Furthermore, changes in glucogenic nutrients and insulin would contribute minimally to the decrease in milk fat yield that occurs with diets causing MFD.

Trans Fatty Acid Theory

Davis & Brown (44) were the first to suggest a possible relationship between *trans* octadecenoic acid and MFD. In postulating a possible role for *trans* fatty acids, they pointed out that the increase in *trans* octadecenoic acids in milk fat during MFD indicated that rumen biohydrogenation was incomplete. Investigators subsequently elaborated the *trans* fatty acid theory and demonstrated that increases in milk fat content of *trans* 18:1 occurred for a wide range of diets related to MFD (8, 13, 14, 54, 56, 57, 66, 100, 112, 124). However, a summary of published literature revealed that inconsistencies were often observed, although milk fat content of *trans* 18:1 was generally correlated with the depression in milk fat percent [see review by Bauman & Griinari (22)]. Studies by Selner & Schultz (112) and Kalscheur et al. (79) provide examples where dietary manipulations resulted in substantial increases in milk fat content of *trans* 18:1, but corresponding reductions in milk fat yield were not observed.

Trans-11 18:1 is an intermediate in the biohydrogenation of linoleic acid (Figure 2) and linolenic acid. *Trans*-11 18:1 is also the major *trans* octadecenoic acid present in milk fat, and it was generally assumed that the increase occurring with MFD was this isomer. However, a range of *trans* 18:1 positional isomers are produced in the rumen and subsequently absorbed from the small intestine and incorporated into milk fat (39, 40, 80, 105, 107). The only studies to examine the effect of pure isomers of *trans*18:1 on rates of milk fat synthesis observed no effects with abomasal infusion of 25 g/d of *trans*-9 18:1 (109) or 25 g/d of an equal mixture of *trans*-11 and *trans*-12 18:1 (61).

An important development was the discovery by Griinari et al. (62) that MFD was associated with a specific increase in *trans*-10 18:1, rather than *trans* 18:1 isomers in general. The relationship between *trans*-10 18:1 content of milk fat and milk fat percentage is presented in Figure 3. Subsequent studies have extended

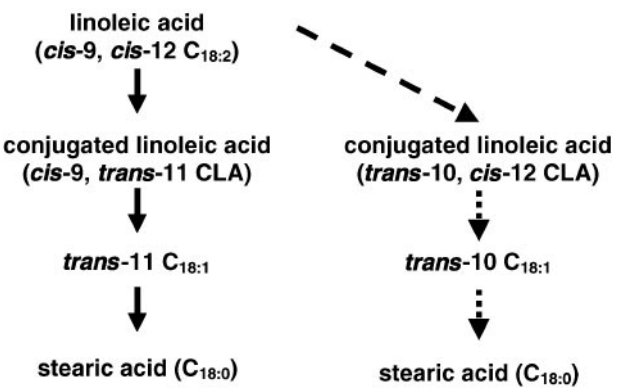

Figure 2 Pathways of rumen biohydrogenation of linoleic acid. Adapted from Griinari & Bauman (58).

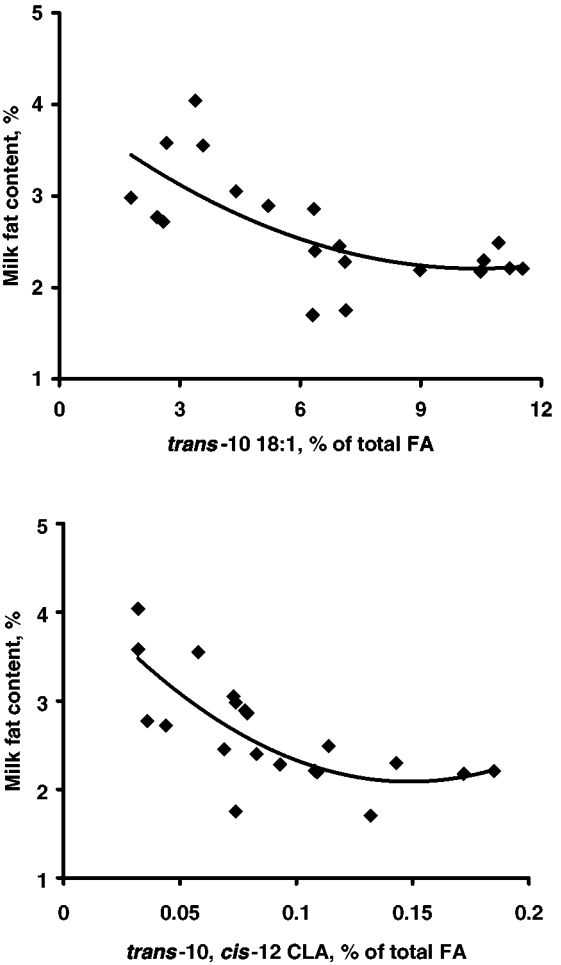

Figure 3 Relationship between the change in the fat content of milk and the *trans*-10 18:1 and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) content of milk fat [expressed as percent of total fatty acids (FA)] in cows fed a low-fiber diet supplemented with sunflower oil. Adapted from Griinari et al. (64) and Bauman & Griinari (22).

these results and established that an increased milk fat content of *trans*-10 18:1 is typical for diets that cause MFD (5, 6, 9, 42, 60, 64, 87, 92–94, 104). Griinari & Bauman (58) presented a putative pathway where *trans*-10 octadecenoic acid was formed from the reduction of *trans*-10, cis-12 CLA in a minor pathway for rumen biohydrogenation of linoleic acid (Figure 2). Just as for *trans*-10 18:1, cows fed a low-fiber diet have a curvilinear relationship between the increase in milk fat content of *trans*-10, *cis*-12 CLA and milk fat percentage (Figure 3). Kepler et al. (82)

demonstrated that *Butyrivibrio fibrisolvens* was able to hydrogenate *trans*-10, *cis*-12 CLA to *trans*-10 18:1, and another rumen bacterium strain, *Megasphaera elsdenii* YJ-4, recently has been shown to produce *trans*-10, *cis*-12 CLA (83). An anaerobic bacteria of the genus *Propionibacterium* isolated from mouse cecum also produced *trans*-10, *cis*-12 CLA when cultured in the presence of linoleic acid; more than 50% of the linoleic acid was converted to *trans*-10, *cis*-12 CLA, and 10% was converted to *trans*-10 18:1 (122).

Consistent with a product/precursor relationship, milk fat concentrations of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA are linearly related ($y = 0.013 \times +0.011$; $R^2 = 0.70$) for cows fed a low-fiber diet (64). The low value for "slope" suggests that *trans*-10, *cis*-12 CLA is a transient intermediate and *trans*-10 18:1 accumulates in the rumen consistent with the pattern established for *cis*-9, *trans*-11 CLA and *trans*-11 18:1 [see discussion in Griinari & Bauman (58)]. Also, the ratio between *trans*-10 18:1 and *trans*-10, *cis*-12 CLA observed in milk fat is similar with the ratio in rumen and duodenal fluid ($\sim$0.01) for lactating dairy cows fed LF diets (40, 105). Further evidence regarding the precursor/product relationship was provided by a study where *trans*-10, *cis*-12 CLA was infused to the rumen and an increased concentration of *trans*-10 18:1 was detected in plasma lipids (86).

The *trans* theory of MFD is based on total *trans*18:1 octadecenoic acids as inhibitors of milk fat synthesis (44, 53, 54, 100), but based on the preceding considerations this is clearly not the case. Recent investigations to identify strategies to enhance the concentration of CLA in milk fat unexpectedly provided insight related to diet-induced MFD. In examining the transfer of CLA to milk fat, it was discovered that a preparation containing a mixture of CLA isomers markedly inhibited milk fat synthesis in dairy cows (36, 37). Given that *trans*-10, *cis*-12 CLA was one of the isomers in the infusion mixture and an intermediate in the putative pathways of biohydrogenation occurring under conditions of MFD as discussed previously, the possible connection was obvious. Baumgard et al. (26) were the first to directly examine this by utilizing postruminal infusions of pure isomers of CLA. They demonstrated that *trans*-10, *cis*-12 CLA inhibited milk fat synthesis, whereas the *cis*-9, *trans*-11 CLA isomer had no effect.

BIOHYDROGENATION THEORY

Conceptual Basis

Bauman & Griinari (22) proposed the "biohydrogenation theory" of MFD to accommodate limitations in the *trans* theory. They suggested the name "biohydrogenation theory" based on the concept that under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates, some of which are potent inhibitors of milk fat synthesis (22). The study by Griinari et al. (62) was critical in the development of the concept as it established that two conditions were required to observe diet-induced MFD—a dietary supply of unsaturated fatty acids and a change in the microbial processes

in the rumen. The change in microbial processes involves an alteration in the pathways of biohydrogenation that results in an increase in the formation of *trans*-10 18:1 and related intermediates. In the case of LF diets, the change in microbial processes is also characterized by a decline in rumen pH and a shift in the rumen pattern of VFA (52, 115, 120). In the case of MO diets, pH and VFA proportions in the rumen are minimally affected (48, 49), and instead components of the MO diet apparently alter microbial processes by directly affecting critical steps in the biohydrogenation processes (18, 58).

Rumen production of *trans*-10 18:1 is evidence of the altered pathways of biohydrogenation with diet-induced MFD. Although an increase in the milk fat content of *trans*-10 18:1 has been observed for all types of diets that cause MFD, the role of *trans*-10 octadecenoic acid as an inhibitor of milk fat synthesis has not been directly examined due to the lack of pure material. Supplements of partially hydrogenated vegetable oils (PHVOs) offer a less definitive method to investigate the role of *trans*18:1 isomers; PHVOs typically contain 40 to 50% *trans* octadecenoic acids and about 10% *trans*-10 octadecenoic acid. Several studies have demonstrated that dietary supplements or postruminal infusions of PHVOs cause a reduction in milk fat (8, 56, 110, 112), and based on the characteristic fatty acid composition of PHVO, these studies would have supplied about 50 to 60 g/d of *trans*-10 18:1. These results are often cited as evidence that *trans* octadecenoic acids cause a decrease in milk fat, but caution is needed for such an interpretation. The chemical hydrogenation process is typically optimized to produce a range of *trans* 18:1 isomers, with *trans*-9 or *trans*-10 as the major isomer, but *trans* 18:2, *trans* 18:3, and conjugated PUFA are also present (15, 41, 78, 107). Thus, fatty acids other than *trans*-10 18:1 and the *trans* octadecenoic fraction could play a role in the reduction in milk fat observed with PHVO.

In contrast to the lack of direct evidence for *trans*-10 18:1, studies have clearly established that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis in the dairy cow; a 25% reduction in milk fat yield is observed with as little as 3.5 g/d of *trans*-10, *cis*-12 CLA (25–28, 101). Investigations involving abomasal infusion of pure isomer demonstrated a curvilinear relationship between the dose of *trans*-10, *cis*-12 CLA and the reduction in milk fat yield (Figure 4) (28, 101). Most investigations have been short term (<7d) and involved abomasal infusion of the CLA as a convenient experimental means to avoid alterations by rumen bacteria. Results have been similar to those obtained with diet-induced MFD where milk fat output was decreased up to 50% and effects of the *trans*-10, *cis*-12 CLA were generally specific for milk fat with other milk components being unaltered [see review by Bauman et al. (18)]. Technologies exist to formulate dietary supplements that protect the CLA from rumen fermentation, and longer term studies (6- to 20-week duration) feeding rumen-protected CLA have demonstrated that the reduction in milk fat persisted throughout the treatment period and returned to normal when treatment was terminated (see References 19, 59). CLA has also been shown to reduce milk fat content in other lactating mammals including humans, and to reduce body fat accretion during the growth phase in several species (reviewed in References 19, 30, 73).

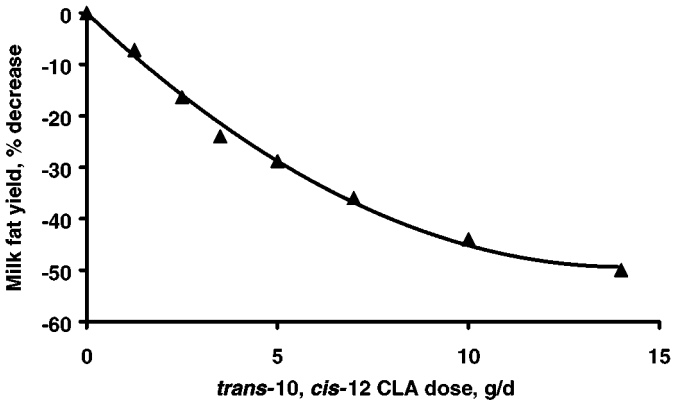

Figure 4 Relationship between abomasally infused *trans*-10, *cis*-12 conjugated linoleic acid (CLA) and the percent decrease in milk fat yield of lactating dairy cows. Adapted from Peterson et al. (101) using data points from Baumgard et al. (27, 28) and Peterson et al. (101).

The *trans*-10, *cis*-12 CLA isomer is also incorporated into milk fat, and studies involving abomasal infusion of the pure isomer established a curvilinear relationship between the increase in milk fat content of *trans*-10, *cis*-12 CLA and the reduction in milk fat secretion (101). Just as with *trans*-10 octadecenoic acid, milk fat content of *trans*-10, *cis*-12 CLA was also increased when LF diets were fed (58, 102, 104). However, at comparable reductions in milk fat yield, the concentration range of *trans*-10, *cis*-12 CLA in milk fat for cows fed LF diets was less than one-half of the concentration of *trans*-10, *cis*-12 CLA in milk fat observed in the studies where MFD was caused by supplements of pure isomer (5, 102). Furthermore, little or no *trans*-10, *cis*-12 CLA was detected in milk fat from cows fed MO diets, although the MFD is accompanied by an increase in milk fat content of *trans*-10 18:1 (6, 35, 60, 94). Thus, for MFD induced by both LF diets and MO diets, results indicate that there must be biohydrogenation intermediates in addition to *trans*-10, *cis*-12 CLA that inhibit milk fat synthesis. In proposing the biohydrogenation theory, Bauman & Griinari (22) suggested that the rumen environment that results in the formation of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA would likely result in other unique biohydrogenation intermediates that would inhibit milk fat synthesis.

Mechanism

Effects of diet-induced MFD on the pattern of fatty acids in milk fat provide insight about the mechanism for the decrease in milk fat. Across all examples of diet-induced MFD, the reduction in milk fat yield involved decreases in fatty acids of all chain lengths (see reviews in References 22, 97, 115). However, the decrease

in yield of de novo synthesized fatty acids tended to be greater, especially when the reduction in milk fat was more pronounced. This resulted in a shift in the milk fatty acid composition so that longer chain and unsaturated fatty acids increased in proportion while short- and medium-chain fatty acids represented a smaller percent of the milk fatty acid composition. A similar shift in the pattern of milk fatty acids was observed when milk fat yield was reduced by *trans*-10, *cis*-12 supplements. Initial studies used higher doses, and results demonstrated that secretion of all chain lengths of fatty acids were decreased, but effects were most pronounced for those synthesized de novo. As investigations expanded it became evident that at lower doses of *trans*-10, *cis*-12 CLA the reduction in milk fatty acids was more uniformly distributed among short-, medium-, and long-chain fatty acids (28, 101). Likewise the inhibition of Δ^9-desaturase that resulted in a marked shift in the fatty acid composition of milk was observed only at higher doses. For example, when cows received 5 g/d or less of *trans*-10, *cis*-12 CLA, milk fat yield was reduced by up to 30%, but the decrease was relatively uniform across all chain lengths of fatty acids and the ratio of fatty acids representing product/substrate for Δ^9-desaturase was unaltered (28, 101).

The biohydrogenation theory is based on the concept that unique fatty acid intermediates produced in rumen biohydrogenation inhibit the mammary gland's ability to synthesize milk fat. Furthermore, the fact that secretion of all chain lengths of fatty acids was reduced with diet-induced MFD and CLA supplements suggests the mechanism(s) may involve multiple steps in the synthesis of milk fat. However, early studies offered little support for this possibility. Askew et al. (7) examined the effects of feeding a high concentrate-restricted roughage diet on mammary tissue activities of lipoprotein lipase and glyceride synthetase and observed no differences from cows fed a diet high in fiber. However, the study is limited as a test for enzyme changes with diet-induced MFD because there was also no dietary effect on milk fat yield. Early studies by Baldwin's group also examined lipogenic-related enzymes in the mammary gland of cows fed a low-fiber diet and observed minimal effects on enzyme activity (11, 96). In this case the LF diet decreased milk fat percent, but subsequent work has shown that except for fatty acid synthase (FAS), which was numerically reduced in activity, the particular enzymes that were examined do not play critical roles in the regulation of lipid synthesis in ruminants.

Piperova et al. (104) provided a critical examination of the mechanism in an investigation that determined the activity of acetyl CoA carboxylase (ACC) and FAS and ACC mRNA abundance in mammary tissue under conditions of LF-induced MFD. They observed reductions of approximately 40 to 60% for these enzyme activities and ACC mRNA abundance, and this was comparable to the 43% reduction in milk fat yield. Baumgard et al. (27) utilized mammary tissue biopsies obtained on day 5 of treatment with *trans*-10, *cis*-12 CLA and observed that the 48% reduction in milk fat yield corresponded to reductions of similar magnitude in mRNA abundance for genes that encoded for enzymes involved in the uptake and transport of fatty acids (lipoprotein lipase and fatty acid binding protein), de novo fatty acid

synthesis (ACC and FAS), desaturation of fatty acids (Δ^9-desaturase), and triglyceride synthesis (glycerol phosphate acyltransferase and acylglycerol phosphate acyltransferase). A similar approach was used by Peterson et al. (103) to examine mRNA abundance in cows that had LF-induced MFD, and results indicated the same coordinate reduction in mRNA abundance for key mammary enzymes associated with lipid synthesis. The biochemical response to diet-induced MFD and supplements of *trans*-10, *cis*-12 CLA suggests a mechanism involving coordinate regulation of key lipogenic enzymes in the mammary gland. Two candidates for such control that have received recent attention relating to lipid metabolism are peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs), and both are regulated by polyunsaturated fatty acids (38, 77, 113). To date, investigations of these signaling molecules have involved rodents or rodent-derived cell culture models.

CONCLUDING REMARKS

Diet-induced milk fat depression has perplexed dairy producers and scientists for over a century. Following the recognition that the basis involved an interaction between microbial fermentation in the rumen and post-absorptive effects on tissue metabolism, many theories were proposed. A portion were based on the concept that the reduction in milk fat synthesis was due to a shortage in the supply of lipogenic precursors, but research over the last decade has offered little support for these theories. The demonstration that all types of diet-induced milk fat depression resulted in alterations in rumen biohydrogenation and the identification of *trans*-10, *cis*-12 CLA as a potent inhibitor of milk fat synthesis were particularly important developments. These results led to the "biohydrogenation theory," which proposes that diet-induced MFD is the result of a direct inhibition of milk fat synthesis at the mammary gland by unique fatty acid intermediates formed during rumen biohydrogenation of PUFA. *Trans*-10, *cis*-12 CLA is the first such intermediate identified, but it is apparent that others must be formed in the rumen under conditions of diet-induced MFD. Thus, further research is needed to identify additional inhibitors of fat synthesis that are produced during rumen biohydrogenation and delineate their cellular mechanisms; we anticipate this will have a broader application in understanding the regulation of lipid metabolism.

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