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Dietary effects on gene expression of lipogenic enzymes in mammary gland of lactating mice

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ABSTRACT

The effects of a fat-free or clofibrate-supplemented diet on gene expression of several lipogenic enzymes was investigated in mammary gland and liver of lactating mice. In lactating mammary gland, stearoyl CoA desaturase (SCD) mRNA expression was increased 2.1-fold ($P<0.05$) by feeding the fat-free diet, whereas clofibrate had no influence compared to control diet. Fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), thioesterase II and lipoprotein lipase (LPL) mRNA levels in lactating mammary gland were unchanged after feeding the fat-free or clofibrate diets compared to control. In livers from lactating mice, the fat-free diet increased expression of SCD 5-fold ($P<0.05$) and FAS 10-fold ($P<0.001$) compared to control. ACC expression was unchanged by either the fat-free or clofibrate diet compared to control diet. These data show that, in lactating mammary tissue, expression of key lipogenic enzymes may be manipulated by diet, thus offering a potential means to modify milkfat composition.

Keywords: milkfat; lactation; mice; mRNA; lipogenesis; diet.

INTRODUCTION

Bovine milk fat, composed of mainly triacylglycerols, contains a mixture of short and long chain fatty acids which are derived from *de novo* synthesis of the mammary gland or from the diet via the blood. Fatty acid synthesis in the bovine mammary gland produces approximately 50% of total milk fatty acids through the action of fatty acid synthase (FAS), the major product being palmitate (16:0) (Vernon and Flint, 1983; Williamson, 1986). In non-ruminants, there is an additional enzyme, thioesterase II, that catalyses the hydrolysis of fatty acids of medium chain lengths, 8:0 to 12:0 from the FAS complex (Safford *et al*., 1987; Naggert *et al*., 1988). Acetyl CoA carboxylase (ACC), which is the rate-limiting enzyme of fatty acid synthesis, catalyses the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA, the activated donor of two-carbon units for fatty acid chain elongation by FAS. Longer chain fatty acids in milk, such as stearic acid (18:0) are derived from blood lipids via hydrolysis of lipoprotein triacylglycerols by lipoprotein lipase (LPL) (Larson, 1985). Stearoyl CoA desaturase (SCD) is the rate-limiting enzyme in monounsaturated fatty acid biosynthesis. It catalyses the D^9-cis desaturation of palmitoyl-CoA (16:0) and stearoyl-CoA (18:0) converting these to palmitoleoyl-CoA (16:1) and oleoyl-CoA (18:1). Genetic regulation of these different genes has been described for liver of non-lactating rodents. Low fat diets have been shown to increase transcription of liver SCD and FAS (Ntambi, 1992; Paulauskis and Sul, 1989). Clofibrate, an ethyl ester of clofibric acid, is a hypolipidemic drug that acts at a cellular level by causing a proliferation of peroxisomes. It has multiple effects on lipid metabolism including lowering blood lipid by depressing fatty acid synthesis and fatty acid esterification into glycerolipid while increasing fatty acid oxidation (Zakim *et al*., 1970; Reddy and Mannaerts, 1994). Clofibrate represses transcription of lipogenic genes such as S14 and FAS (Jump *et al*., 1995) and induces transcription of SCD in livers of male mice (Miller and Ntambi, 1996).

In bovine milk, palmitic acid (16:0), is the most abundant saturated fatty acid (20-25% of total fatty acids) followed by stearic acid (13-18%). Oleic acid is the major unsaturated fatty acid (25-30%). The ability to alter the 18:1/18:0 ratio by increasing the SCD activity should result in a softer and potentially more healthy milkfat. The aim of the present study was to provide further understanding of the regulation of lipogenic enzymes involved in synthesis and composition of milkfat, using a mouse model.

MATERIALS AND METHODS

Lactating Brown Swiss mice (n=3 per group) were fed a control diet (consisting of lactalbumin 22%, vitamin mix 5%, salt mix 5%, corn oil 8%, cellulose 5%, cornflour 55%) for 2 days prior to fasting for 24 h. The groups were then refed either a control diet, fat-free diet (control diet minus the corn oil) or control diet supplemented with clofibrate (0.5% w/w), for 24 or 48 h. Non-lactating Brown Swiss mice were also fasted for 24 h then fed the control and test diets for 24 h. The pups were separated from the dams 3-4 h before each slaughter. Animals were then sacrificed and aliquots of liver and mammary gland were snap-frozen in liquid nitrogen.

Total RNA was extracted from tissues using TRIzol reagent as described by the manufacturers (Life Technologies Ltd, Auckland). RNA, 2.5 mg for SCD, FAS, thioesterase II, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and 10 mg for ACC and LPL, was separated in 1.2% formaldehyde agarose gels, transferred to Hybond N membrane and stained with methylene blue (Sambrook *et al*., 1989). The cDNA probes for SCD (Cameron *et al*., 1994), FAS (Naggert *et al*., 1988), thioesterase II (Naggert *et al*., 1987), ACC (Lopez-Casillas *et al*., 1991), LPL (Jensen *et al*., 1994) and GAPDH (Fort *et al*., 1985) were random prime labelled using ^{32}P-dCTP and hybridised to the membranes and autoradiographs were prepared. Densitometry was carried out for quantitative analysis. Data were normalised to GAPDH mRNA and values in arbitrary units were analysed by ANOVA.

RESULTS

Effects of diet on mammary gland mRNA levels of lactating mice

No significant differences were detected in the mRNA levels for SCD, FAS, ACC, thioesterase II or LPL between the 24 and 48 h samples for any of the treatments, allowing the values for these time points to be combined for further analysis.

SCD mRNA levels were increased significantly with respect to the control values by 2.1-fold ($P<0.05$) by feeding the fat-free diet, whereas the clofibrate diet resulted in an apparent 1.6-fold increase that was not statistically significant (Table 1a). The levels for the other mammary gland mRNAs were not significantly altered by either dietary treatment (Table 1a).

TABLE 1: Effects of the fat-free and clofibrate diets on mRNA levels of genes encoding lipogenic enzymes in tissues of lactating mice

A: Mammary gland

	Control	Fat-free	Clofibrate	SED
Stearoyl CoA desaturase	1.70[+]	3.52*	2.82	0.68
Fatty acid synthase	3.29	3.05	2.38	0.80
Acetyl CoA carboxylase	5.33	3.46	5.40	1.37
Thioesterase II	1.52	0.89	1.70	0.54
Lipoprotein lipase	5.90	5.10	17.53	6.63

* $P<0.05$ vs control diet

B: Liver

	Control	Fat-free	Clofibrate	SED
Stearoyl CoA desaturase	0.26	1.38*	0.65	0.21
Fatty acid synthase	0.03	0.30**	0.08	0.06
Acetyl CoA carboxylase	0.006	0.182	0.056	0.09

* $P<0.05$ vs control and clofibrate; ** $P<0.001$ vs control and clofibrate
[+] Values are expressed as means (n=6 per treatment) and are in arbitrary units.

Effects of diet on liver mRNA levels in lactating and non-lactating mice

The levels of the liver SCD mRNA from the lactating mice were significantly increased by feeding the fat-free diet (5-fold, $P<0.05$, Table 1b). Feeding the clofibrate diet did not result in significant differences at 24 h, however, at 48 h the SCD mRNA levels were significantly higher than those for the mice fed the control diet (0.94 vs 0.30, SED 0.3, $P<0.05$). Liver FAS mRNA levels were also increased by feeding the fat-free diet (10-fold, $P<0.001$) but no differences of FAS were detectable following feeding the clofibrate diet (Table 1b). Similarly, levels of liver ACC mRNA were not altered by either treatment (Table 1b). No signals were detected for either thioesterase II or LPL with the liver RNA indicating that these genes are expressed specifically in the mammary gland.

No statistically significant differences could be detected in the levels of SCD or FAS mRNA in the livers of non-lactating (0.021, sed 0.25 and 0.018, sed 0.06, respectively) and lactating mice (0.212 and 0.021, respectively). In addition, in contrast to the livers from the lactating mice, no differences relating to feeding either diet could be detected in the non-lactating mice.

DISCUSSION

This study has examined the effects of a fat-free diet and a clofibrate supplemented diet on the expression of several mammary gland and liver genes encoding proteins involved in lipogenesis in the lactating mouse. The results have shown that the expression of mammary gland and liver SCD in lactating mice can be enhanced by feeding a fat-free diet, suggesting regulation at the transcriptional level. In contrast, feeding clofibrate, a peroxisome proliferator, failed to modify these mRNA levels significantly in mammary tissue and only a small increase in SCD mRNA was detected at one time point in liver tissue of lactating mice. In the livers of non-lactating mice, SCD expression was not affected by the dietary manipulations. This was unexpected as previous studies in mice have shown up to 40-fold increases by feeding a fat-free high carbohydrate diet after a period of fasting (Ntambi, 1992) and up to 20-fold increases by dietary supplied clofibrate (Miller and Ntambi, 1996). There is no obvious explanation for this apart from the observation that these previous studies were carried out in male mice in contrast to the present study. The expression of SCD mRNA has been reported to be dependent on gender, with levels in livers of female mice 5-fold those in male mice and it was hypothesised that this gender difference may be due to differences in levels of hormones such as oestrogen and testosterone (Lee *et al*., 1996). Oestrogen has been shown to induce SCD enzyme in avian livers (Lippiello *et al*., 1979) and peroxisome proliferators induce SCD activity differently in male and female rats in which this difference has been suggested to be due to higher levels of testosterone in males (Kawashima *et al*., 1989).

FAS mRNA was also enhanced by feeding a fat-free diet, although in contrast to SCD, only in the liver of lactating mice and not in the mammary gland. As with SCD, no dietary effects were detected in livers of non-lactating mice. This again is in contrast to previous results demonstrating a dramatic increase in FAS mRNA of livers of mice after refeeding animals that had been fasted (Paulauskis and Sul, 1988; 1989).

The fact that SCD mRNA levels can be manipulated by dietary methods suggests that these treatments may be useful in terms of controlling the level of unsaturation of the fatty acids found in milk fats. This is significant in ruminants in which dietary fatty acids reaching the mammary gland are saturated as a result of the action of rumen microorganisms. Further studies are being carried out using the bovine model to characterise associated effects on milkfat composition.

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