Journal of Dairy Research (2007) **74** 160–166. © *Proprietors of Journal of Dairy Research 2007*
doi:10.1017/S002202990600224X *First published online 12 February 2007 Printed in the United Kingdom*

Conjugated linoleic acid (CLA) effects on pups growth, milk composition and lipogenic enzymes in lactating rats

Amanda Aparecida Hayashi[1], Sérgio Raposo de Medeiros[2], Marina Hojaij Carvalho[1] and Dante Pazzanese Duarte Lanna[1]*

[1] Department of Animal Science, University of São Paulo – ESALQ, Piracicaba, SP – Brazil
[2] EMBRAPA Beef Cattle, Campo Grande, MS – Brazil

Received 4 October 2005 and accepted for publication 20 August 2006

Conjugated linoleic acid (CLA) has a range of biological properties, including effects on lipid metabolism, milk and body composition in animals. This study investigated the effects of dietary CLA on lactating rats and development of the suckling pups. Dams were fed either a control diet or the same diet supplemented with 25 g/kg of a fat supplement containing 540 g CLA/kg (final concentration of 13·5 g CLA/kg diet) from parturition to the 15th day post-partum. The CLA mixture used in this study contained the following isomers (per 100 g): cis-9, trans-11 (24 g); cis-10, trans-12 (35 g); cis-8, trans-10 (15 g); cis-11, trans-13 (17 g) and others (9 g). On d 15 post partum, CLA supplementation reduced milk fat content by 33 % and pup growth by 21 %. The milk fatty acid profile, with decreased content of short and medium chain acids, suggests CLA inhibition was more pronounced for *de novo* lipid synthesis. Consistent with these results, activities of fatty acid synthase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were reduced by CLA treatment in the mammary gland and liver. In contrast, the activity of NADP-malate dehydrogenase was unchanged.

Keywords: Conjugated linoleic acid (CLA), lactating rat, fatty acid profile, enzyme activity, lipogenesis.

Conjugated linoleic acid (CLA) is a term used to describe positional and geometrical derivatives of linoleic acid containing conjugated double bonds. Although there are as many as 56 possible isomers with these characteristics only 20 have been found in milk fat and two of them (cis-9, trans-11 and trans-10, cis-12) have received most attention due to their known biological effects. Investigations on the transfer of CLA to milk fat in dairy cows observed that CLA supplements resulted in a dramatic reduction in milk fat secretion (Baumgard et al. 2000; Moore et al. 2004). CLA supplementation also affects body composition in growing animals, reducing fat content in mice and pigs (Ostrowska et al. 1999; Park et al. 1999).

Mechanisms by which CLA alters lipid metabolism are not well defined. In rodents, the fatty acids of milk triacylglycerol are derived from *de novo* synthesis within the mammary gland and from lipids of dietary origin or lipids mobilized from adipose tissue (Williamson, 1980). Variations in the fatty acid composition of the maternal diet during lactation can strongly alter fatty acid composition of milk lipids (Brandorff, 1980). Ringseis et al. (2004) suggested that dietary CLA reduces triacylglycerol concentrations in milk of lactating rats via reduced *de novo* fatty acid synthesis in the mammary gland and impaired uptake of fatty acids from lipoproteins to the mammary gland. In this study, the observed effect in lipid metabolism resulted in reduced growth and increased mortality of suckling pups.

In this study we evaluated the effect of CLA on milk composition and pups growth. We also analysed milk fatty acid profile and activity of lipogenic enzymes in the main lipogenic tissues during lactation, including the effects of dietary CLA on development of the suckling rats.

*For correspondence; e-mail: dplanna@esalq.usp.br

Materials and Methods

Animals and diet

Sixteen female Wistar pregnant rats (Growth and Nutrition Laboratory – Department of Animal Science ESALQ-USP), on d 7 of gestation and 260±13 g initial weight, were acclimatized to individual plastic shoebox cages until parturition. The control and CLA animals had ad-libitum access to a non-purified diet (Labina E, cat 5002, Purina,

Table 1. Fatty acid composition of diets fed to lactating rats (g total fatty acids/100 g)

	Diet	
Fatty acids	Control	CLA†
14 : 0	0·64	0·51
16 : 0	17·5	14·07
16 : 1 cis-9	0·76	0·61
18 : 0	3·62	2·91
18 : 1 cis-9	23·0	18·49
18 : 2 cis-9, cis-12	49·5	35·6
18 : 2 CLA	<0·10	24·1
20 : 0	0·37	0·30
18 : 3	4·61	3·7

†CLA Calcium salts contained 650 g CLA/kg (Natural Lipids Ltd) and 350 g long chain fatty acids of palm oil/kg.

Paulínia, SP, 13140-000, Brazil) and water, and were maintained at 22 ± 2 °C with a 12 h light–dark cycle. The experimental protocol was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985) and approved by University of Sao Paulo, ESALQ Ethics Committee. From the day of parturition (considered d 1 of lactation) to d 15 of lactation, dams were assigned to one of the two dietary treatment groups: control diet (n=8) or control diet plus 25 g supplement/kg containing 540 g CLA/kg for a final concentration of 1·35 g CLA/100 g diet as feed basis (n=8). The mixture of CLA isomers used in this study was prepared by Natural Lipids Ltd (Hovdebygda, Norway) and then processed into calcium salts (Church & Dwight Co, Inc, New Jersey, 08543-5297 US). Sixty-five percent of the lipid used for the formation of calcium salts contained a 4 isomer CLA mixture (Natural Lipids Ltd) and the balance of fatty acids were from palm oil. The profile of the CLA isomers was 24 g cis-9, trans-11/100 g; 35 g trans-10, cis-12//100 g; 15 g cis-8, trans-10/100 g; 17 g cis-11, trans-13/100 g and 9 g other/100 g.

Diets were mixed daily before being provided to each rat in order to minimize lipid oxidation. The diet composition provided the nutritional requirements according to Nutrient Requirements of Laboratory Animals (1995). Control diet composition was 253·7 g crude protein/kg, 50·5 g crude fibre/kg, 38·6 g fat/kg, 82·9 g ash/kg, 13 g Ca/kg and 8·3 g P/kg on a DM basis. The CLA treatment diet composition was 240 g crude protein/kg, 48 g crude fibre/kg, 55 g fat/kg, 83 g Ash/kg, 14·8 g Ca/kg and 8·3 g P/kg on a DM basis. Source of fat in the basal diet was mainly corn with minor contribution of ether extract from soybean and rice (<15%). The fatty acid profiles of the diets are presented in Table 1.

Measurements and sampling

Food intake was monitored daily between d 0 and d 15 post-partum. Based on intake from previous days, a sufficient amount was offered to ensure at least 10% refusal. The dams and their litters were weighted before feeding on d 0, 4, 8, 12 and 15 post-partum to evaluate body weight gain. Pups were weighted individually during all experimental period.

Rats were milked once at peak lactation because serial milking affects milk composition (Keen et al. 1980). On d 15 of lactation dams were separated from their litters 4 h before milking to ensure accumulation of milk in the mammary gland. Subsequently, all dams were milked between 12:00 and 14:00 h, to minimize possible diurnal variations in milk composition (Picciano, 1978). Dams were anesthetized with sodium thiopental (48 mg/kg BW) (Abbott Laboratories, Chicago, IL Chicago, 60614, USA). Oxytocin (1.2USP units) (Univet S.A., Sao Paulo, SP, 01523-000, Brazil) was injected intraperitoneally to stimulate milk flow. Milk was drawn by applying intermittent mild suction to each nipple through vacuum and collected in plastic tubes. An average of 6 ml milk could be obtained using this method. Total milk from each dam was lyophilized and frozen (−20 °C) until analysed. Immediately after milking, all dams were killed by decapitation. Blood from dams was collected into heparinized polyethylene tubes (Sarstedt) and plasma was separated by low-speed centrifugation (3000 ***g*** for 15 min at 4 °C) and stored at −20 °C.

Nonesterified fatty acids (NEFA) concentration was determined by the acyl CoA-synthetase and acyl CoA-oxidase enzymatic method, kit NEFA C-test (Wako Chemical GmbH, Neuss, D-41468, Germany). Triglycerides and cholesterol were assayed with a commercial kit (Bio Diagnostica, Pinhais, PR, 813321-090, Brazil). The liver, mammary gland and periovarian fat pad (adipose tissue) were excised immediately after milking, frozen with liquid nitrogen and stored at −80 °C until analysis.

For analysis of dams body weigh and food intake; pups body weight; plasma biochemical variables (NEFA, tryglicerides and cholesterol); milk composition and fatty acid profile (mammary gland, adipose tissue and liver) 8 animals from the CLA group and Control group and the corresponding pups were used. For enzymatic activity in the mammary gland the same number of animals was used. For enzymatic activity in the liver only 5 samples, and for enzymatic activity in adipose tissue only 4 samples, were collected for each treatment.

Sample analyses

Total lipids from milk, liver, mammary gland, adipose tissues and diet were extracted with a mixture of hexane isopropanol (3 : 2 v/v; Hara & Radin, 1978). The fatty acid composition of total lipids from the diets, liver, mammary gland, adipose tissue and milk was determined by gas chromatograph as described previously by Hara & Radin (1978), using a hexane: isopropanol (3 : 2 v/v) solution (18 ml/g total lipids) followed by a 67 g sodium sulphate/l

https://doi.org/10.1017/S002202990600224X Published online by Cambridge University Press

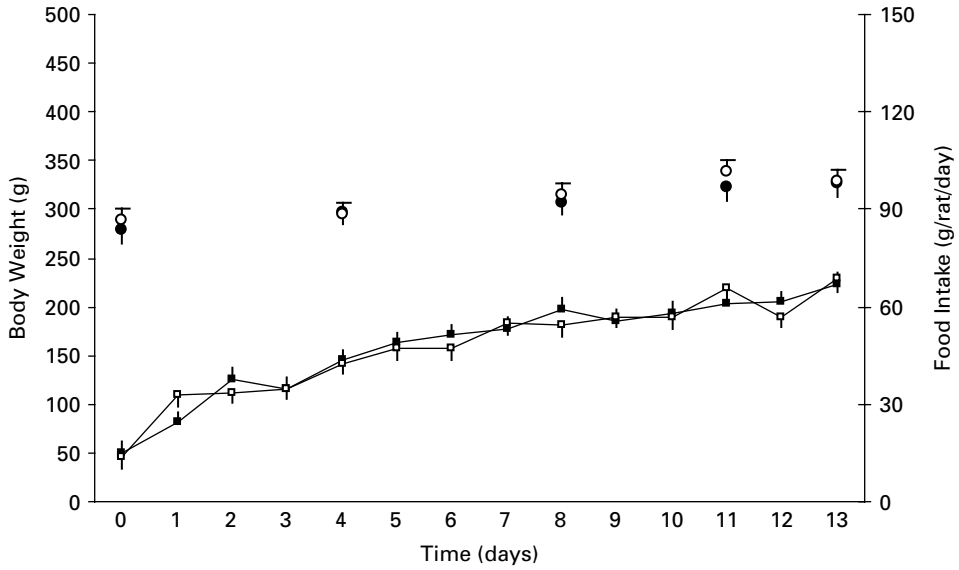

Fig. 1. Body weight (circles) and feed intake (squares) of dams fed control (open symbols) or CLA-supplemented (13·5 g/kg) diets (filled symbols). Body weight and feed intake were subjected to t-test ($P>0·10$).

Values are mean ± SE for $n=8$ per treatment

solution (12 ml/g of lyophilized milk). Fatty acids samples were transesterified by sodium methoxide, prepared in our laboratory according to the method of Christie (1982) with some modifications as described in Medeiros (2002): An aliquot of the hexane, containing fatty acid methyl esters (FAME), was taken and used directly for chromatographic determination. Each peak was identified and quantified by the use of a milk fat standard, CRM-164 (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium).

Total milk fat was determined in the lyophilized sample in a Soxlet apparatus using ether as a solvent.

Enzyme analysis

Tissue samples were homogenized as described by Mellenberger et al. (1973). Enzyme activities were assayed in the cytosolic fraction at 37 °C in the linear range of activity.

Fatty acid synthase (FAS) activity was assayed spectrophotometrically (Ingle et al. 1973) by measuring malonyl-CoA-dependent oxidation of NADPH per min per mg cytosolic protein. Glucose 6-phosphate dehydrogenase (G6PDH) activity and 6-phosphogluconate dehydrogenase (6PGDH) activity were measured spectrophotometrically (Glock & McLean, 1953) by measuring reduction of NADPH/min per mg cytosolic protein. NADP-malate dehydrogenase activity was also performed spectrophotometrically (Mellenberger et al. 1973). Protein concentrations were measured according to the Gornall et al. (1949) procedure using bovine albumin as standard.

Statistical analysis

Data of food intake, dams' and pups' development were analysed by MIXED Procedure in Statistical Analysis System (2000). Litter size (number of pups) was used as a covariate for analysis of pup weight and pup growth rate (CLA group 11 ± 4 pups/litter, control group 10 ± 3 pups/litter). The effect of litter size on dams' feed intake and dams' weight was tested, but no significant effect was observed ($P>0·05$). Data for plasma parameters, milk composition and enzyme activities were analysed using General Linear Models Procedure in Statistical Analysis System (2000). Differences between treatments were tested using the Tukey test with differences considered significant when $P<0·05$.

Results and Discussion

Food intake; plasma triglycerides, cholesterol and glucose concentration in dams; milk composition and body weight of dams and pups

CLA supplementation during lactation did not affect dam's food intake ($P>0·05$) or body weight ($P>0·05$; Fig. 1). There is evidence suggesting that CLA has effects on body weight changes (Delany et al. 1999) and feed intake (Park et al. 1999). However, these studies were conducted with different animal species and in different physiological conditions. In humans, results concluding that CLA could attenuate obesity are conflicting and inconsistent. This discrepancy is probably due to

https://doi.org/10.1017/S002202990600224X Published online by Cambridge University Press

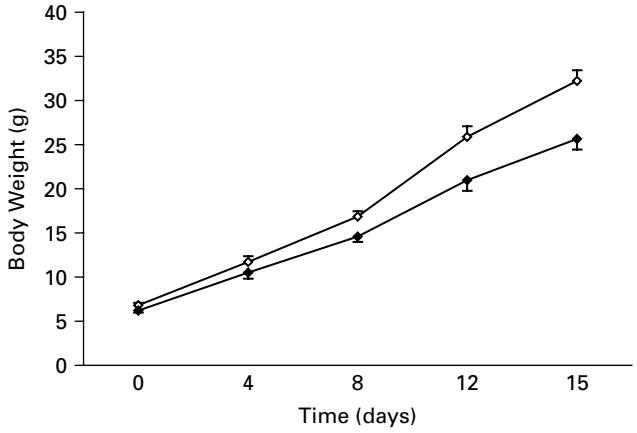

Fig. 2. Body weight of pups from dams fed control (open symbols) or CLA supplemented diets (filled symbols) from day 0 to 15 post-partum.

Values are mean ± SE, mean litter size = 10 pups/dam; 8 litters/treatment

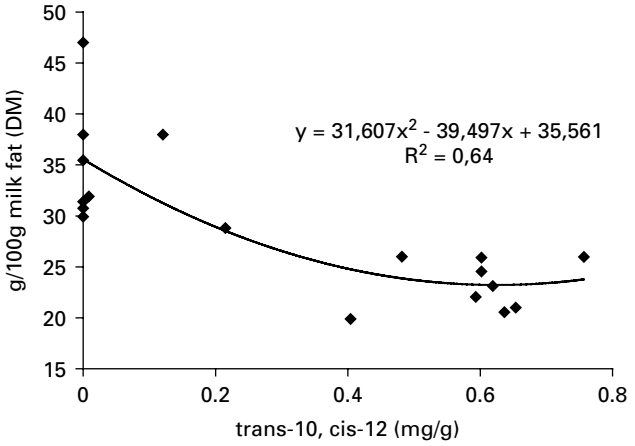

Fig. 3. Relationship between milk fat content (g/100 g of dry matter) and content of trans-10, cis-12 isomer in milk fat (mg/g). The regression analysis included values (n = 16) for milk from both control and CLA groups. The predicted quadratic equation followed data well and was selected using regression analysis SAS (2000).

isomer-specific mechanisms and animal/diet model (Brown & McIntosh, 2003). In rodents, Park et al. (1999) concluded that the 18 : 2 trans-10, cis-12 isomer was responsible for changes in body composition during growth, with significant reductions in body weight gain, food intake and carcass fat percentage. The authors concluded that CLA effects were mediated through alteration on lipid biosynthesis pathways. However, in adipose tissue during lactation peak in rats, lipids are mobilized and rates of lipid synthesis are extremely low. Thus, in the present experiment an inhibition of lipid synthesis should not result in significant differences in either body weight or food intake.

On d 1 of lactation, control and CLA pups weight averages were not statistically different (P<0·05). For the dams fed the diet containing CLA, pups' body weight was significantly lower by d 8 (P=0·02) when compared with control pups and remained lower (P<0·05) throughout the study. At the end of the study, the litters nursing from the dams fed CLA weighed 21% less than litters from the control group (25·6 × 32·2 g; P<0·02; Fig. 2). This is contrary to the response observed by Chin et al. (1994), who reported moderately enhanced body weight gain for the pups. However, recent results from Ringseis et al. (2004) confirm our observations, they observed reduced pups weight after d 8 post partum accompanied by increased death rate. In addition, Herbein's group (Loor et al. 2003) confirmed a decrease in body weight gain of the pups suckling milk from dams fed pure trans-10, cis-12 isomer compared with the control group. No differences in litter body weight were observed between the cis-9 trans-11 group and the control or 18 : 0 enriched diet. From the results presented in Fig. 2, it is possible to suggest that the lower fat content (energy) presented in milk of CLA-dams may have been responsible for the lower weight gain of the CLA pups.

Fat concentration in milk from dams fed the control diet (34 ± 6% on a DM basis) were within normal range, but values from dams fed the CLA diet (23 ± 3% on a DM basis) were 33% lower (P<0·001). This result is similar to a previously reported value in rats fed a diet with 20 g synthetic CLA mixture/kg (Yang et al. 2002) and to a reduction of 31% reported in lactating rats fed CLA mixture (Ringseis et al. 2004).

In a recent study by this group (Peterson et al. 2004) 75 μmol 18 : 2 trans-10, cis-12/l of medium 199 (Gibco) reduced mRNA abundance of critical genes in the lipid synthesis pathway (acetyl CoA carboxylase, fatty acid synthase and stearoyl CoA desaturase), whereas 18 : 2 cis-9, trans-11 treatment had no effect in the mammary epithelial cell line studied (MAC-T cells). Figure 3 shows a negative and curvilinear relationship between the concentrations of trans-10, cis-12 isomer in milk fat and milk fat percentage (R^2 = 0·64). A similar quadratic relationship was observed in cows (Baumgard et al. 2000; Medeiros, 2002). Consistently with Loor et al. (2003), it is likely that, in this experiment, this isomer was responsible for milk fat depression in dams supplemented with CLA and consequently, for the reduction of pups body weight gain observed in Fig. 2.

The percentage of medium fatty acids (C10 : 0 to C14 : 0) was decreased in milk fat consistently with a decrease in *de novo* fatty acid synthesis due to lower activities of key lipogenic enzymes (Table 3). Fatty acids with 8–14 carbon atoms are the main products of *de novo* fatty acid synthesis in the mammary gland. In the present study, we observed reductions in the percentage of these medium-chain fatty acids and an increase in long-chain fatty acids (Table 2). Consistent with these data, Ringseis et al. (2004) reported decrease in mRNA levels and FAS

https://doi.org/10.1017/S002202990600224X Published online by Cambridge University Press

Table 2. Fatty acid concentrations in milk fat from dams collected at d 15 of lactation (g fatty acids/100 g milk)

	Treatment†		
Fatty acid	Control	CLA§	*P*‡
4 : 0	0·08 ± 0·01	0·04 ± 0·01	**
6 : 0	0·23 ± 0·01	0·28 ± 0·01	**
8 : 0	4·9 ± 0·33	5·2 ± 0·26	NS
10 : 0	13·4 ± 0·53	12·2 ± 0·5	*
12 : 0	10·6 ± 0·25	8·8 ± 0·34	***
12 : 1	0·21 ± 0·03	0·15 ± 0·02	NS
14 : 0	10·2 ± 0·41	8·18 ± 0·32	***
14 : 1 cis-9	0·07 ± 0	0·07 ± 0	NS
15 : 0	0·64 ± 0·08	0·41 ± 0·1	*
16 : 0	21·2 ± 0·64	19·6 ± 0·31	**
16 : 1 cis-9	1·12 ± 0·08	1·24 ± 0·07	NS
18 : 0	3·43 ± 0·08	3·64 ± 0·06	**
18 : 1 trans-11	0·21 ± 0·01	0·30 ± 0·02	***
18 : 1 cis-9	12·9 ± 0·43	14·9 ± 0·67	**
18 : 2 trans-11. cis-15	0·04 ± 0	0·08 ± 0	***
18 : 2 cis-9. cis-12	12·3 ± 0·35	12·3 ± 0·34	NS
18 : 3	0·92 ± 0·07	1·06 ± 0·03	*
18 : 2 cis-9. trans-11	0·12 ± 0·03	0·73 ± 0·04	***
18 : 2 trans-10. cis-12	0·04 ± 0·02	0·6 ± 0·03	***
18 : 2 trans-9. trans-11	0·02 ± 0	0·09 ± 0	***

† Treatment means ± SE on day 15 of lactation of 8 Control and 8 CLA dams
‡ Probability of difference between treatment means
§ CLA = diet contained 13·5 g/kg of calcium CLA
NS, $P > 0·10$; *, $P < 0·10$; **, $P < 0·05$; ***, $P < 0·01$

activity in the mammary gland of the CLA lactating rats. Yang et al. (2002), using HPLC analysis, showed a reduction in medium-chain fatty acids (12 : 0–14 : 0) and an increase in the concentration of long-chain fatty acids (18 : 0). These authors observed a close relationship between the amount of CLA isomers consumed and the amount of CLA found in milk. There are several studies demonstrating an increase in milk concentrations of CLA in CLA supplemented ruminants. We have demonstrated a dramatic increase in trans-10, cis-12 and cis-9, trans-11 18 : 2 concentrations in milk from rats. This not only suggests an opportunity to increase cis-9, trans-11 in human milk to improve certain milk healthy effects, but also demonstrates that the trans-10, cis-12 isomer could be transferred in significant amounts to nursing infants. In the latter case the milk from the mother could have an altered nutrient composition, which may compromise energy uptake of the child (McGuire et al. 2000).

In contrast to the results of this experiment and those of Ringseis et al. (2004), Yang et al. (2002) observed no changes in litter body weight gain. The absence of effect on litter body weight could be due to different diet composition or isomer profile of the CLA supplement. Thus, regarding the apparently conflicting results from Yang et al. (2002) and Chin et al. (1994), we could speculate that there was no energy deficit for the pups, due either to small litters or to a relatively large milk production (i.e. pups could compensate the lower energy per unity of milk by suckling more milk). Under these conditions, it is possible that the effects of the cis-9, trans-11 isomer were more pronounced than the effects of trans-10, cis-12 isomer, leading to the increased body weight gain of pups observed by Chin et al. (1994).

Plasma NEFA concentrations of dams varied from 0·48 ± 0·05 (CLA group) to 0·5 ± 0·04 mmol/l (control group), cholesterol 1·30 ± 0·13 (CLA group) to 1·35 ± 0·08 mmol/l (control group) and tryglicerides 3·05 ± 0·83 (CLA) to 2·64 ± 0·41 mmol/l (control) and differences were not significant ($P > 0·05$) between the treatment groups. Similarly, studies using lactating Holstein cows abomasally infused with trans-10, cis-12 isomer (Baumgard et al. 2000) showed no effects on plasma levels of NEFA, despite a strong reduction in milk fat production (25 to 50%). In relation to growing animals, it is shown that plasma concentrations of NEFA were slightly higher in pigs fed a diet supplemented with CLA compared with the control group (Ostrowska et al. 1999; Azain et al. 2000). The absence of an effect on this parameter suggests that CLA supplementation has small effects on lipid mobilization, during peak lactation. However CLA supplement have an effect on expression of lipoprotein lipase (LPL), the key factor in the uptake of circulating lipids by the lactating mammary gland (Ringseis et al. 2004). Despite the fact that CLA strongly decreased activities of enzymes involved in of lipid synthesis in the liver, there were no changes in trygliceride concentrations in plasma. This may be explained by the high turnover of triglycerides during peak lactation.

The mechanisms by which CLA alters lipid metabolism are not well defined. CLA could mediate its effects on lipoprotein metabolism via activation of PPAR alpha (peroxisome proliferator activated receptor). In the liver PPAR alpha has been reported (Roche et al. 2001) to have an effect on the regulation of the expression of the genes involved in hepatic lipid metabolism, including those that regulate lipid synthesis and oxidation. Another possible effect of CLA is linked to reductions in cell uptake of preformed fatty acids. Reduction in LPL activity might be important to this effect. Park et al. (1997) observed a reduction in the activity of LPL in cell cultures of 3T3 pre adipocites incubated with CLA. The activity of this enzyme and long chain fatty acid yield were not measured in this experiment. However, the pronounced reduction in fatty acids formed through the *de novo* pathway was enough to explain the reduction in milk fat content.

Fatty acid profiles in mammary gland, liver, adipose tissue and milk of lactating dams

The individual milk fatty acid profiles are presented on Table 2. The sum of the percentages of medium chain fatty acids (10 : 0 to 16 : 0) in milk fat was 13% lower in response to feeding CLA compared with the control treatment ($P < 0·05$). However, dietary treatment did not affect

https://doi.org/10.1017/S002202990600224X Published online by Cambridge University Press

Table 3. Activities of fatty acid synthase (FAS), glucose 6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and NADP-malate dehydrogenase in the mammary gland, liver and adipose tissue

Treatment	Control† (nmoles/min mg prot^{-1})	CLA†	P‡
	Mammary gland (n=8)§		
FAS	35±2·0	20±0·8	***
G6PDH	365±14	239±14	***
6PGDH	91±2·6	66±2·9	***
NADP malate dehydrogenase	121±4·4	122±5·5	NS
	Liver (n=5)§		
FAS	16±0·7	5±1·6	***
G6PDH	75±12	19±8·1	***
6PGDH	107±8·2	51±13·6	***
NADP malate dehydrogenase	22±2·8	19±7·6	NS
	Adipose tissue (n=4)§		
FAS	1·1±0·22	0·5±0·1	**
G6PDH	4·7±2·6	1·35±2·2	NS
6PGDH	32±3·1	25±2·1	*
NADP malate dehydrogenase	11±2·5	10±1·1	NS

† Mean ± SE
‡ Probability of difference between overall treatment means
§ Number of tissue samples
NS, $P>0·10$; *, $P<0·10$; **, $P<0·05$; ***, $P<0·01$

short chain fatty acids ($P>0·05$). On the other hand, the concentration of long chain fatty acids was increased by 11% ($P<0·05$) after CLA treatment. Additionally, CLA treatment drastically increased the concentration of cis-9, trans-11 (6-fold) and trans-10, cis-12 (15-fold) isomers in milk fat.

The mammary gland tissue presented the same response to CLA treatment as observed for milk fat (data not shown). The concentration of myristic acid (C14 : 0) in the mammary gland of dams fed CLA was 22% lower compared with control dams ($P=0·01$). The palmitic acid percentage in the mammary gland of CLA treated dams decreased when compared with control diet (17·7 × 19·5%, $P<0·05$). Palmitic and stearic acids were the fatty acids present in higher concentrations in liver tissue (data not shown). Interestingly, CLA treatment did not affect the fatty acid profile of adipose tissue from the lactating dams ($P>0·05$, data not shown). However, CLA treatment drastically increased the concentration of cis-9, trans-11 and trans-10, cis-12 isomers in all studied tissues (with exception to adipose tissue).

Enzyme activities: mammary gland, liver and adipose tissue

CLA treatment affected FAS activity in all tissues studied ($P<0·05$, Table 3). These effects could be observed even in adipose tissue of dams in peak lactation, despite the very low activities in this tissue (Table 3).

The activities of G6PDH and 6PGDH (NADPH generating enzymes) were reduced in mammary gland and liver. However, the activities of these enzymes were not reduced in adipose tissue. The activity of NADP-malate dehydrogenase was not changed by CLA supplementation in the analysed tissues. Data on G6PDH, 6PGDH and NADP-malate enzymes suggest that the production of NADPH was high enough to support much higher rates of fatty acid synthesis. So, CLA effects on FAS might be the rate limiting step regulating synthesis. Additionally, CLA effect occur through an inhibition on acetyl CoA carboxylase (ACC) and a number of other steps involved in regulation of lipid synthesis. CLA causes a reduction in ACC mRNA (as observed in cows; Baumgard et al. 2002) and in FAS mRNA levels as observed in lactating rats (Ringseis et al. 2004). Effects of CLA on other genes involved in long-chain fatty acid metabolism have also been observed. It is difficult to conclude a casual relationship as reductions in enzyme gene expression could be a result of inhibition of another step in the pathway of lipid synthesis. A number of recent results (Brown & McIntosh, 2003) demonstrating effects of CLA isomers on peroxisome proliferator-activated receptor (PPARs) and sterol response element binding proteins (SREBPs) pathways suggest that improving our understanding of the direct interactions of these bioactive lipids with transcription factors should be a future goal in this area of research.

References

Azain MJ, Hausman DB, Sisk MB & Flatt WP 2000 Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. *Journal of Nutrition* **130** 1548–1554

Baumgard LH, Corl BA, Dwyer DA, Saebo A & Bauman DE 2000 Identification of the conjugated linoleic acid isomer that inhibits fat synthesis. *American Journal of Physiology- Regulatory Integrative Comparative Physiology* **278** R179–R184

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA & Bauman DE 2002 Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *Journal of Dairy Science* **85** 2155–2163

Brandorff NP 1980 The effect of dietary fatty on the fatty acid composition of lipids secreted in rat milk. *Lipids* **15** 276–278

Brown JM & McIntosh MK 2003 Conjugated linoleic acid in humans: regulation of adiposity and insulin sensitivity. *Journal of Nutrition* **133** 3041–3046

Chin SF, Storkson JM, Abright KJ, Cook ME & Pariza MW 1994 Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *Journal of Nutrition* **124** 2344–2349

Christie WW 1982 A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *Journal of Lipid Research* **23** 1072–1075

Delany JP, Blohm F, Truett AA, Scimeca JA & West DB 1999 Conjugated linoleic acid rapidly reduces body fat contend in mice without affecting energy intake. *American Journal of Physiology* **276** R1172–R1179

Glock GE & McLean P 1953 Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochemical Journal* **55** 400–408

https://doi.org/10.1017/S002202990600224X Published online by Cambridge University Press

Gornall AG, Bradawill CT & David MM 1949 Determination of serum protein by means of biuret reagent. *Journal of Biological Chemistry* **177** 751–766

Hara A & Radin NS 1978 Lipid extraction of tissues with low-toxicity solvent. *Analitical Biochemistry* **90** 420–426

Ingle DL, Bauman DE, Mellenberger RW & Johnson DE 1973 Lipogenesis in the ruminant: effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. *Journal of Nutrition* **103** 1479–1488

Keen CL, Lonnerdal B, Sloan MV & Hurley LS 1980 Effects of milking procedure on rat milk composition. *Physiology & Behaviour* **24** 613–615

Loor JJ & Herbein JH 1998 Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis. *Journal of Nutrition* **128** 2411–2419

Loor JJ, Lin X & Herbein JH 2003 Effects of dietary cis 9, trans 11–18 : 2, trans 10, cis 12–18 : 2, or vaccenic acid (trans 11–18 : 1) during lactation on body composition, tissue fatty acid profiles, and litter growth in mice. *British Journal of Nutrition* **90** 1039–1048

McGuire MA & McGuire MK 2000 Conjugated Linoleic acid (CLA). A ruminant fatty acid with beneficial effects on human health. Proceedings of the American Society of Animal Science. Available at: http://www.asas.org.jas/symposia/proceedings/0938.pdf

Medeiros SR 2002 Conjugated Linoleic Acid: Content in foodstuffs and its use to increase milk production, milk protein content and to alter fatty acids profile. Ph.D São Paulo University, Piracicaba, SP

Mellenberger RW, Bauman DE & Nelson DR 1973 Metabolic adaptations during lactogenesis. *Biochemistry Journal* **136** 741–748

Moore CE, Hafliger HC, Mendivil OB, Sanders SR, Bauman DE & Baumgard LH 2004 Increasing amounts of conjugated linoleic acid progressively reduces milk fat synthesis immediately postpartum. *Journal of Dairy Science* **87** 1886–1895

National Research Council 1985 Guide for the Care and Use of Laboratory Animals. Publication 85–23 (rev.). National Institutes of Health, Washington, DC

Nutrient Requirements of Laboratory Animals 1995 Nutrient requirements for the laboratory rat. Publication no. 4 (rev.). National Academic of Science, Washington, DC

Ostrowska E, Muralitharan M, Cross RF, Bauman DF & Dunshea FR 1999 Dietary conjugated linoleic acid increase lean tissue and decrease fat deposition in growing pigs. *Journal of Nutrition* **129** 2037–2042

Park Y, Albright KL, Liu W, Storkson JM, Cook ME & Pariza MW 1997 Effect of conjugated linoleic acid on body composition in mice. *Lipids* **32** 853–858

Park Y, Storkson JM, Albright KJ, Liu W & Pariza MW 1999 Evidence that the trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **34** 235–241

Perfield JW, Bernal-Santos G, Overton TR & Bauman DE 2002 Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. *Journal of Dairy Science* **85** 2609–2617

Peterson DG, Matitashvili EA & Bauman DE 2004 The inhibitory effect of trans-10, cis-12 CLA on lipid synthesis in bovine mammary epithelial cells involves reduced proteolytic activation of the transcription factor SREBP-1. *Journal of Nutrition* **134** 2523–2527

Picciano MF 1978 Mineral contend of human milk during a single nursing. *Nutrition Reports International* **18** 5–10

Ringseis R, Saal D, Muller A, Steinhart H & Eder K 2004 Dietary conjugated linoleic acids lower the triacylglycerol concentration in the milk of lactating rats and impair the growth and increase the mortality of their suckling pups. *Journal of Nutrition* **134** 3327–3334

Roche HM, Noone E, Nugent A & Gibney MJ 2001 Conjugated linoleic acid: a novel therapeutic nutrient? *Nutrition Research Reviews* **14** 173–187

Statistical Analysis System – SAS® User's Guide: Statistics, version 6.18. 2000. SAS Inst, Inc., Cary, N.C.

Williamson DH 1980 Integration of metabolism in tissues of the lactating rat. *FEBS Letters* **117** K93–K105

Yang L, Yeung YV, Huang Y, Wang HQ & Chen Z 2002 Preferential incorporation of trans-conjugated linoleic acid isomers into the liver of suckling rats. *British Journal of Nutrition* **87** 253–260