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

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Cis 9, *trans* 11 (*c* 9, *t*11)-18 : 2 and *trans* 10, *cis* 12 (*t*10, *c*12)-18 : 2 are the major conjugated linoleic acid (CLA) isomers in dietary supplements which reduce milk fat content in nursing women. The present study evaluated the effects of each CLA isomer or vaccenic acid on body composition and tissue fatty acids during lactation in mice. Dams were fed 30 g rapeseed oil (control)/kg diet or 20 g control plus 10 g 18 : 0, *trans* 11-18 : 1 (*t*11-18 : 1), *c* 9, *t*11-18 : 2, or *t*10, *c*12-18 : 2. Dietary *t*10, *c*12-18 : 2 reduced food intake by 18 % and carcass fat weight of the dams by 49 % compared with the other treatments. Milk fat percentage ranked by treatment was $18 : 0 > t11\text{-}18 : 1 = c\,9$, $t11\text{-}18 : 2 > t10$, $c12\text{-}18 : 2$. The sum of saturated 12 : 0 to 16 : 0 in milk fat was lower when *c* 9, *t*11-18 : 2 was fed compared with the control, 18 : 0, or *t*11-18 : 1 treatments. Dietary *t*10, *c*12-18 : 2 caused further reductions in milk fat 12 : 0 to 16 : 0. The proportion of CLA isomers was 3-fold greater in milk fat than in the carcasses of the dams. The pups nursing from the dams fed *t*10, *c*12-18 : 2 had the lowest body weights and carcass fat, protein, and ash contents. Nursing from the dams fed *c* 9, *t*11-18 : 2 also resulted in lower carcass fat compared with the 18 : 0 or *t*11-18 : 1 treatments. The ratios of *cis* 9-16 : 1:16 : 0 or *cis* 9-18 : 1:18 : 0, proxies for Δ^9-desaturase activity, were markedly lower in the carcasses of the dams and pups fed *t*10, *c*12-18 : 2. The ratio of 20 : 4*n*-6:18 : 2*n*-6, a proxy for Δ^6- and Δ^5-desaturase and elongase activity, in the liver of the dams and pups fed *t*10, *c*12-18 : 2 also was lower. Dietary *t*11-18 : 1 enhanced the content of *c* 9, *t*11-18 : 2 in milk fat and carcasses. As in previous studies, the reduction in food intake by *t*10, *c*12-18 : 2 could not entirely account for the marked decrease in carcass fat content and milk fat concentration. *T*10, *c*12-18 : 2 probably had a negative effect on Δ^9-desaturase and mammary *de novo* fatty acid synthesis. Although these effects need to be confirmed in lactating women, the results suggest that the consumption of supplements containing *t*10, *c*12-18 : 2 should be avoided during the nursing period.

Conjugated linoleic acids: Vaccenic acid: Body composition: Milk fat: Lactation

Conjugated linoleic acids (CLA) represent a special class of polyunsaturated fatty acids (Ntambi *et al.* 2002). The *cis* 9, *trans* 11 (*c* 9, *t*11) isomer of CLA, derived from the biohydrogenation of linoleic acid in the rumen and the desaturation of vaccenic acid (*trans* 11-18:1; *t*11-18:1) in adipose, liver, or mammary tissue, is the primary CLA found in bovine milk fat (Bauman *et al.* 2001). Feeding CLA mixtures enriched to varying degrees with *trans* 10, *cis* 12 (*t*10, *c*12)-18:2 reduced carcass fat in growing mice (Park *et al.* 1999) and hamsters (De Deckere *et al.* 1999), whereas milk-fat-derived *c* 9, *t*11-18:2 prevented the growth of human mammary cancer cells more effectively than synthetic *t*10, *c*12-18:2 (O'Shea *et al.* 2000). In those and other (DeLany *et al.* 1999) experiments, CLA mixtures or *t*10, *c*12-18:2 decreased food intake compared with control groups. The metabolic responses to *c* 9, *t*11-18:2 or *t*10, *c*12-18:2 may differ substantially, but both isomers may have implications for human health.

Mixtures of CLA produced synthetically are readily available in 'health-food' stores in the USA, with *c* 9, *t*11-18:2 plus *t*10, *c*12-18:2 making up 60 to 80 % total fatty acids (Masters *et al.* 2002). Consumption of a CLA supplement (60 % total CLA) by lactating women increased the incorporation of *c* 9, *t*11-18:2 and *t*10, *c*12-18:2 in milk fat, but resulted in a 23 % reduction in milk fat percentage (Masters *et al.* 2002). Loor & Herbein (1998) showed for the first time that exogenous CLA mixtures caused milk-fat depression in dairy cows by reducing *de novo* fatty acid synthesis and desaturation of 18:0. It is now evident that anti-lipogenic effects in bovine mammary tissue are associated with synthetic *t*10, *c*12-18:2 rather than *c* 9, *t*11-18:2 (Bauman *et al.* 2001; Loor & Herbein, 2003).

It is important to determine the extent to which CLA isomers may affect body composition and fatty acid profiles during lactation in single-stomached animals, which may serve as models for man. Since the lactating mammary gland is capable of extensive desaturation, it also may be an important source of dietary c9, t11-18:2 for the neonate during the suckling period. Lactating mice and their pups were chosen as the animal model because of the simplicity of working with total tissues and the high cost of pure c9, t11-18:2, t10, c12-18:2, and t11-18:1. The first objective of the present study was to evaluate the extent to which pure sources of c9, t11-18:2 and t10, c12-18:2 affect the body composition and fatty acid profiles of lactating mice and their pups. The second objective was to evaluate the enrichment of c9, t11-18:2 in response to a supply of vaccenic acid.

Materials and methods

Animals and experimental design

Pregnant CD-1 mice were purchased from Harlan Teklad (Harlan, Madison, WI, USA) and were assigned to one of five dietary groups from day 4 to 15 postpartum. A Harlan Teklad mouse-breeder diet containing the required complement (National Research Council, 1995) of nutrients for lactation was used as a carrier for one of five fatty acid treatments (Table 1). Diets were prepared the day before parturition and stored at 4°C (Assumpcão *et al.* 2002; Loor *et al.* 2002; Terpstra *et al.* 2002). Supplemental fatty acids included 30 g high-oleic (control; 79·0 g *cis* 9-18:1/100 g total fatty acids) rapeseed oil (Columbus Foods Co., North Albany, IL, USA)/kg diet, or 20 g control plus 10 g 18:0 (>99 % purity, N-18-A; Nu-Check Prep, Elysian, MN, USA), 10 g t11-18:1 (>99 % purity, U-49-A; Nu-Check Prep, Elysian, MN, USA), 10 g c9, t11-18:2 (90·7 g c9, t11-18:2/100 g and 1·0 g t10, c12-18:2/100 g; Natural Lipids, Oslo, Norway), or 10 g t10, c12-18:2 (1·8 g c9, t11-18:2/100 g and 96·2 g t10, c12-18:2/100 g; Natural, Oslo, Norway).

The dams were fed the control diet during the first 3 d of lactation at 08.00 hours. On day 4 postpartum, five lactating mice were randomly assigned to the control or 18:0 groups and six lactating mice to the t11-18:1, c9, t11-18:2, or t10, c12-18:2 group. Our original target was to have six dams in each treatment group. However, the final distribution was necessary because not all purchased mice were pregnant. The litters were reduced to eight pups each. Quite unexpectedly, two dams receiving t10, c12-18:2 reduced their food intake to <5 g/d between days 12 and 14, became agalactic, and had to be removed from the study. The mice were kept in a mouse colony, maintained at 23°C with a 12 h light–dark cycle daily, and housed in individual polypropylene cages with access to water at all times. The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University animal care committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1995).

Measurements and sampling

Food intake was monitored daily between days 3 to 14 postpartum. The accurate assessment of food intake was achieved by thoroughly collecting spilled food from the cages. Cardboard shavings were used as bedding and were changed daily. Based on the previous day's intake, a sufficient amount of diet was offered to ensure at least 10 % refusal. Diet samples were collected on days 3, 8, and 15 and stored at −20°C. At the end of the experiment, samples from each treatment were combined and stored at −20°C before fatty acid analysis. The dams and their litters were weighed before feeding on days 4, 9, and 15 postpartum to assess body-weight gain and growth. On day 14 postpartum, the pups were separated from the dams for 2 h before feeding to ensure maximal accumulation of milk in the mammary gland. Subsequently, all dams were milked (1–2 ml) by suction. Milk fat was isolated by centrifugation at 3000 g for 10 min, and stored at −20°C until fatty acid analysis.

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

Fatty acids	Control	18:0	t11-18:1	c9, t11-18:2	t10, c12-18:2
12:0	0·1	0·1	0·1	0·1	0·1
14:0	0·9	0·9	0·9	0·9	0·9
16:0	15·6	15·6	15·7	15·8	15·7
c9-16:1	1·6	1·6	1·6	1·6	1·6
18:0	9·3	16·6	9·4	9·5	9·4
c9-18:1	50·1	45·1	45·4	45·4	45·2
t11-18:1	0·1	0·1	6·3	0·1	0·1
18:2n-6	17·7	16·5	16·7	16·6	16·6
c9, t11-18:2	0·1	0·1	0·7	6·3	0·2
t10, c12-18:2	0·0	0·0	0·0	0·2	6·6
18:3n-3	1·0	0·8	0·9	0·8	0·9
20:3n-6	0·1	0·1	0·6	0·5	0·6
20:4n-6	0·2	0·2	0·2	0·2	0·2
20:5n-3	<0·1	<0·1	<0·1	<0·1	<0·1
22:6n-3	<0·1	<0·1	<0·1	<0·1	<0·1
Other	3·1	2·3	1·5	2·0	1·9
Total (mg/g diet as fed)	105	106	106	106	106

t, trans; *c*, cis.

Lactating mice and their pups were killed by cervical dislocation in the morning of day 15 postpartum. Blood samples (1 ml) from the lactating mice were obtained by heart puncture immediately after death. The blood was transferred to tubes containing 150 IU heparin in 100 µl sterile saline, then centrifuged at 3000 *g* for 15 min for harvesting of the plasma. The plasma was stored at −20°C.

The livers from lactating mice and their pups were excised and weighed. Mammary gland tissue from the dams also was completely removed and weighed. Livers and mammary tissue were stored at −20°C. The dam and pup carcasses were stripped of skin, head, and remaining organs. The empty carcasses were stored at −20°C. Subsequently, the empty carcasses were lyophilised (Dura-Top freeze dryer; FTS Systems, Inc., Stone Ridge, NY, USA).

Sample analyses

The lyophilised carcasses were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093; Tecator, Hoganas, Sweden) before analyses. Total N and crude fat contents were analysed using standard procedures (Association of Official Analytical Chemists, 1990). Plasma glucose concentration was measured using a commercial kit (Sigma-Aldrich, St Louis, MO, USA). Lipids were extracted from the diets, liver, and carcasses with chloroform–methanol (Folch *et al.* 1957). The milk fat percentage was estimated by creamatocrit (Lucas *et al.* 1978).

The fatty acids in the diets, liver, carcasses, and milk fat were methylated by *in situ* transesterification with 0·5 N-NaOH in methanol at 50°C for 30 min, followed by 14 % boron trifluoride in methanol also at 50°C for 30 min (Christie *et al.* 2001). Undecenoate (Nu-Check Prep, Elysian, MN, USA) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionisation detector (Hewlett Packard, Sunnyvale, CA, USA). Methyl esters were separated on a 100 m × 0·25 mm internal diameter fused silica capillary column (CP-Sil 88; Chrompack, Middleburg, The Netherlands). A customised mixture, thoroughly described by Loor & Herbein (2003), made with pure methyl ester standards (Nu-Check Prep, Elysian, MN, USA; Supelco Inc., Bellefonte, PA, USA; Sigma, St Louis, MO, USA) was used to identify peaks and determine the response factors for individual fatty acids. The chromatographic conditions during fatty acid analysis were the same as those reported by Loor & Herbein (2003).

Statistical analysis

The data are reported as least squares means with standard errors for each dietary group. All data were analysed as a completely randomised block design using the MIXED procedure of SAS® (2000). Food intake over time was analysed using repeated measures. The differences between treatment means were established using the Tukey–Kramer adjustment for multiple comparisons when the *F* value was significant. Correlation analysis between the proportion of vaccenic acid and *c* 9, *t*11-18 : 2 in tissues and milk fat was conducted using the CORR procedure of SAS® (2000). Overall differences between treatment means were considered to be significant when $P \leq 0.05$.

Results

Food intake, plasma glucose concentration in dams, milk fat concentration, and body weight of dams and pup litters

Food intake ranged from 18·3 to 20·9 g/d in the dams fed the control, *c* 9, *t*11-18 : 2, *t*11-18 : 1, and 18 : 0 (Table 2). The dams fed the control and *c* 9, *t*11-18 : 2 consumed on average 14 % less food than those fed 18 : 0. Feeding *t*10, *c*12-18 : 2 caused reductions of 16 to 25 % in food intake compared with the other treatments, and these differences in intake were apparent throughout lactation (Fig. 1). Food intake as a percentage of live body weight (data not shown), however, was similar for all groups. At the beginning of the experiment the body weight of all dams averaged 42·6 g (Fig. 2 (B)) but the final body weight averaged 41·5 g for the dams fed *t*10, *c*12-18 : 2 compared with 45·7 g for the other dams (Table 2). Plasma glucose concentration ranged from 7·79 mmol/l (*c* 9, *t*11-18 : 2) to 10·24 mmol/l (control) and was not markedly different between the groups. Milk fat concentration was greater in the dams fed 18 : 0 compared with those fed *t*11-18 : 1, *c* 9, *t*11-18 : 2, or *t*10, *c*12-18 : 2 (Fig. 3). Feeding *t*10, *c*12-18 : 2 resulted in the lowest concentration of milk fat.

The body weight of the pup litters averaged 31·3 g at the onset of the experiment (Fig. 2 (A)). At the end of the study, the litters nursing from the dams fed *t*10, *c*12-18 : 2 weighed 28 % (49·2 g) less than the other litters (68·7 g). The liver weight of the pups nursing from the dams fed *t*10, *c*12-18 : 2 also was lower compared with the other pups (Table 2).

Chemical composition of dam and litter carcasses

The crude protein content of the carcasses was greater when *t*10, *c*12-18 : 2 was fed (19·3 %) compared with the other diets (18·7 %) (Table 2). The total weight of crude protein in the carcasses of the dams did not differ due to diets and averaged 2·29 g. The crude fat content was drastically reduced when *t*10, *c*12-18 : 2 (2·73 %) was fed compared with *c* 9, *t*11-18 : 2 (6·35 %) or the other diets (4·69 %). The total weight of crude fat in the carcasses also was decreased in response to feeding *t*10, *c*12-18 : 2 (0·31 g) compared with the other diets (0·61 g).

The litters nursing from the dams fed *t*10, *c*12-18 : 2 had greater water (71·9 %) and crude protein (17·8 %) but lower crude fat content (3·4 %) compared with the other litters, but the weight of each of these carcass components was lower (Table 2). The litters in the *c* 9, *t*11-18 : 2 group also had a lower content of crude fat in the carcasses (6·9 %) compared with the litters in the 18 : 0 or *t*11-18 : 1 group (9·3 %). The content of ash plus other remaining components in the carcasses was greater in the litters receiving *t*10, *c*12-18 : 2-rich milk (6·9 %), intermediate in the control, 18 : 0, or *c* 9, *t*11-18 : 2 groups (5·8 %), and lower in the *t*11-18 : 1 group (5·4 %). However, total

Table 2. Body weight, tissue weight, food intake, and carcass chemical composition in lactating mice and their litters fed a control diet or the control diet plus 18:0, *trans* 11-18:1, *cis* 9, *trans* 11-18:2, or *trans* 10, *cis* 12-18:2*

(Mean values with their standard errors; residual degrees of freedom 21)

	Control		18:0		*t*11-18:1		*c*9, *t*11-18:2		*t*10, *c*12-18:2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	5		5		6		6		4	
Dams										
Final live body weight (g)	45·1[a]	1·30	45·3[a]	1·30	46·8[a]	1·10	45·4[a]	1·10	41·5[b]	1·40
Food intake (g/d)	18·26[b]	0·90	20·92[a]	0·90	19·46[ab]	0·82	18·45[b]	0·82	15·71[c]	1·00
Plasma glucose (mmol/l)	10·24[a]	0·46	9·37[ab]	0·46	9·62[ab]	0·36	7·79[b]	0·36	8·94[ab]	0·51
Liver weight (g)	3·21	0·21	3·19	0·21	3·33	0·18	3·56	0·18	3·31	0·24
Mammary-gland weight (g)	5·66	0·61	5·53	0·61	6·11	0·58	5·73	0·58	6·52	0·65
Carcass										
Water content (% wet carcass weight)	69·71	0·64	69·44	0·64	69·57	0·59	68·09	0·59	70·53	0·68
Water weight (g)	8·41	0·32	8·45	0·32	8·52	0·29	8·36	0·29	8·11	0·36
Protein content (% wet carcass weight)	18·93[b]	0·25	18·75[b]	0·25	18·79[b]	0·23	18·57[b]	0·23	19·33[a]	0·27
Protein weight (g)	2·28	0·11	2·28	0·11	2·30	0·09	2·37	0·09	2·22	0·12
Fat content (% wet carcass weight)	4·58[b]	0·74	4·82[ab]	0·74	4·67[b]	0·67	6·35[a]	0·67	2·73[c]	0·80
Fat weight (g)	0·56[a]	0·08	0·59[a]	0·08	0·58[a]	0·07	0·69[a]	0·07	0·31[b]	0·09
Ash + other content (% wet carcass weight)	6·78[b]	0·23	6·99[ab]	0·23	6·97[ab]	0·21	6·99[ab]	0·21	7·41[a]	0·25
Ash + other weight (g)	0·81	0·04	0·85	0·04	0·85	0·03	0·86	0·03	0·85	0·05
Litters										
Final live body weight (g)	65·7[a]	3·51	71·9[a]	3·51	71·8[a]	3·22	65·0[a]	3·22	49·3[b]	3·70
Liver weight (mg/pup)	348[a]	29	359[a]	29	342[a]	26	321[a]	26	238[b]	32
Carcass										
Water content (% wet carcass weight)	69·97[b]	0·56	69·07[b]	0·56	69·08[b]	0·50	70·49[ab]	0·50	71·93[a]	0·60
Water weight (g)	14·57[a]	0·94	15·33[a]	0·94	15·25[a]	0·86	13·61[a]	0·86	9·84[b]	1·00
Protein content (% wet carcass weight)	16·42[b]	0·20	15·91[c]	0·20	16·05[c]	0·17	16·61[b]	0·17	17·76[a]	0·21
Protein weight (g)	3·42[a]	0·23	3·53[a]	0·23	3·55[a]	0·21	3·21[a]	0·21	2·42[b]	0·25
Fat content (% wet carcass weight)	7·92[ab]	0·83	9·18[a]	0·83	9·46[a]	0·76	6·97[b]	0·76	3·41[c]	0·90
Fat weight (g)	1·71[ab]	0·26	2·06[a]	0·26	2·11[a]	0·24	1·40[b]	0·24	0·48[c]	0·28
Ash + other content (% wet carcass weight)	5·69[bc]	0·21	5·84[bc]	0·21	5·41[c]	0·18	5·93[b]	0·18	6·90[a]	0·23
Ash + other weight (g)	1·19[a]	0·09	1·29[a]	0·09	1·19[a]	0·07	1·14[ab]	0·07	0·94[b]	0·10

*t, trans; c, cis.*
[a,b,c] Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).
* For details of diets and procedures, see Table 1 and p. 1040.
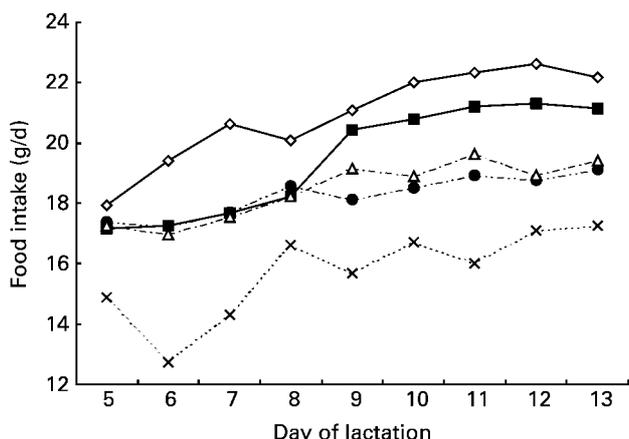
Fig. 1. Pattern of daily food intake by dams during days 5 to 13 of lactation. Dams that were fed *trans* 10, *cis* 12-18:2 (− × −) had lower ($P<0.05$) overall food intake compared with the other treatments. (−●−), Control treatment; (−◇−), 18:0 treatment; (−■−), *trans* 11-18:1 treatment; (−△−), *cis* 9, *trans* 11-18:2 treatment.
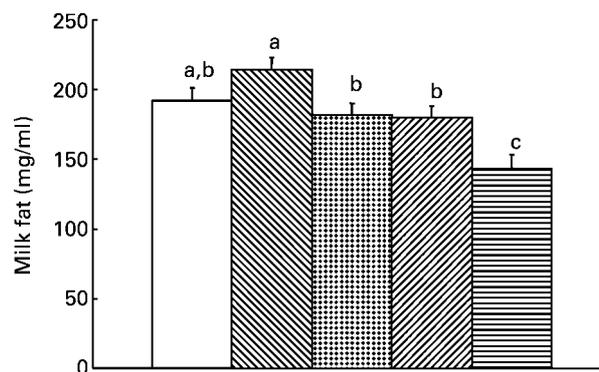
Fig. 3. Fat concentration in milk. (□), Control treatment; (▨), 18:0 treatment; (▦), *trans* 11-18:1 treatment; (▨), *cis* 9, *trans* 11-18:2 treatment; (▤), *trans* 10, cis 12-18:2 treatment. Values are means, with their standard errors represented by vertical bars. [a,b,c]Mean values with unlike superscript letters were significantly different ($P<0.05$).

weight in the carcasses was lower with *t*10, *c*12-18:2 (0·94 g) compared with the control, 18:0, or *t*11-18:1 treatments (1·22 g).

Fatty acid profiles in the carcasses and milk fat of lactating dams

The individual fatty acid profiles in the tissues of the dams are shown in Table 3. Total fatty acid concentration in the carcasses of the dams fed the control, 18:0, *t*11-18:1, or *c*9, *t*11-18:2 averaged 133 mg/g dry tissue, and was 68 % greater compared with the dams fed *t*10, *c*12-18:2 (79 mg/g dry tissue). In milk fat the sum of the percentages of saturated 12:0 to 16:0 was lower in response to feeding *t*10, *c*12-18:2 (26·2 %) compared with the control, 18:0, or *t*11-18:1 treatments (37·8 %). Although feeding *c*9, *t*11-18:2 resulted in higher percentages of 12:0 to 16:0 in milk fat compared with *t*10, *c*12-18:2, they were still lower (33·2 %) compared with the control, 18:0, or *t*11-18:1 treatments.

The palmitoleic acid percentage in the carcasses of the dams was more than doubled when *c*9, *t*11-18:2 (4·90 %) was fed compared with *t*10, *c*12-18:2 (2·19 %), but was intermediate due to feeding the control, 18:0, or *t*11-18:1 (3·57 %). The stearic acid percentage in the carcasses was greater in response to feeding *t*10, *c*12-18:2 (11·21 %) compared only with *c*9, *t*11-18:2 (8·05 %). Feeding the control, 18:0, and *t*11-18:1 resulted in an intermediate percentage of 18:0 (9·69 %). As in the carcasses, the percentage of 18:0 in milk fat was greater due to feeding *t*10, *c*12-18:2 (5·94 %) compared with the other diets (4·99 %). The oleic acid percentage in milk fat was 46·4 in response to feeding *t*10, *c*12-18:2 compared with 40·1 for the other treatments.

The vaccenic acid percentage in tissues and milk fat was proportional to dietary availability, and was greater in response to feeding *t*11-18:1 (2·0 or 2·8 % in carcass or milk fat) compared with other diets (0·07 or 0·11 % in carcass or milk fat). Although feeding *c*9, *t*11-18:2 led to greater overall percentages of *c*9, *t*11-18:2 (1·3 or 3·5 % in carcass or milk fat), feeding *t*11-18:1 increased *c*9, *t*11-18:2 (0·31 or 0·33 % in carcass or milk fat) compared
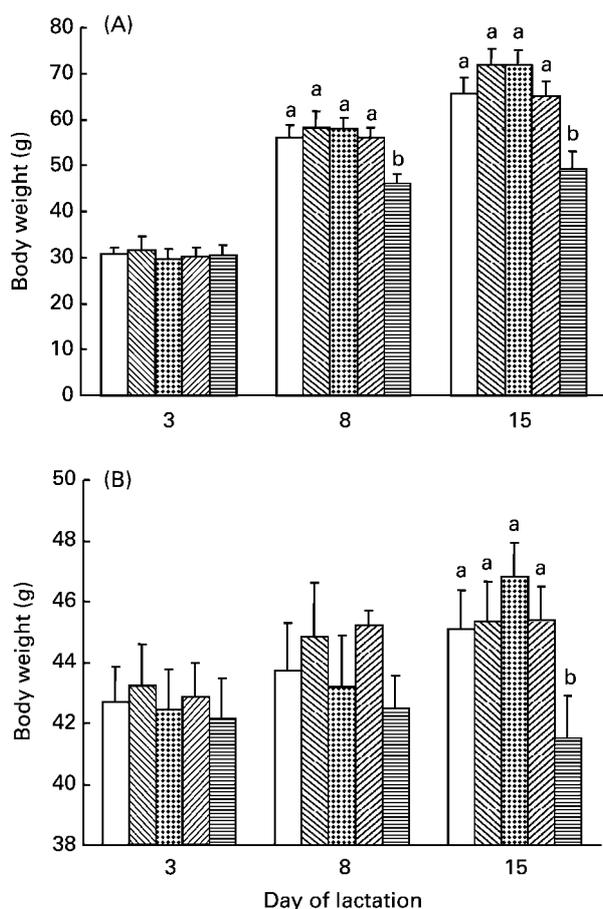
Fig. 2. Pattern of body-weight gain for nursing pups (A) and their dams (B). (□), Control treatment; (▨), 18:0 treatment; (▦), *trans* 11-18:1 treatment; (▨), *cis* 9, *trans* 11-18:2 treatment; (▤), *trans* 10, *cis* 12-18:2 treatment. Values are means, with their standard errors represented by vertical bars. [a,b]Mean values, within a day of lactation, with unlike superscript letters were significantly different ($P<0.05$).

Table 3. Selected fatty acid composition (g/100 g total fatty acids) of carcass and milk fat from lactating mice fed a control diet or the control diet plus 18 : 0, *trans* 11-18 : 1, *cis* 9, *trans* 11-18 : 2, or *trans* 10, *cis* 12-18 : 2*

(Mean values with their standard errors; residual degrees of freedom 21)

	Control		18 : 0		*t*11-18 : 1		*c*9, *t*11-18 : 2		*t*10, *c*12-18 : 2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	5		5		6		6		4	
Carcass										
12 : 0 + 14 : 0 + 16 : 0	27·9	0·96	26·11	0·96	27·44	0·88	28·33	0·88	26·90	1·07
*c*9-16 : 1	3·43[b]	0·43	3·50[b]	0·43	3·77[b]	0·39	4·90[a]	0·39	2·19[c]	0·48
18 : 0	9·51[bc]	0·72	10·33[ab]	0·72	9·25[bc]	0·66	8·05[c]	0·66	11·21[a]	0·80
*c*9-18 : 1	47·82	1·05	49·31	1·05	47·10	0·96	46·84	0·96	46·85	1·18
*t*11-18 : 1	0·07[b]	0·05	0·06[b]	0·05	1·98[a]	0·05	0·06[b]	0·05	0·09[b]	0·06
18 : 2*n*-6	7·22	0·52	6·86	0·52	6·79	0·47	7·00	0·47	7·30	0·58
*c*9, *t*11-18 : 2	0·06[c]	0·08	0·05[c]	0·08	0·31[b]	0·07	1·27[a]	0·07	0·07[c]	0·09
*t*10, *c*12-18 : 2	0·00[b]	0·02	0·00[b]	0·02	0·00[b]	0·02	0·05[b]	0·02	1·00[a]	0·03
18 : 3*n*-3	0·13[a]	0·01	0·11[a]	0·01	0·10[a]	0·01	0·09[b]	0·01	0·12[a]	0·01
20 : 3*n*-6	0·24[ab]	0·01	0·26[a]	0·01	0·24[ab]	0·01	0·21[b]	0·01	0·16[c]	0·02
20 : 4*n*-6	0·70	0·11	0·60	0·11	0·47	0·10	0·54	0·10	0·84	0·12
20 : 5*n*-3	0·04[ab]	0·01	0·04[ab]	0·01	0·02[b]	0·01	0·03[ab]	0·01	0·05[a]	0·01
22 : 6*n*-3	0·38	0·09	0·33	0·09	0·21	0·08	0·30	0·08	0·46	0·10
Total (mg/g dry tissue)	119·9[b]	15·9	126·1[b]	15·9	125·3[b]	14·6	161·7[a]	14·6	79·3[c]	17·8
Milk fat										
12 : 0 + 14 : 0 + 16 : 0	36·90[a]	1·16	38·41[a]	1·16	38·22[a]	1·06	33·20[b]	1·06	26·21[c]	1·29
*c*9-16 : 1	1·39	0·09	1·67	0·09	1·44	0·08	1·67	0·08	1·39	0·10
18 : 0	4·95[b]	0·27	5·06[b]	0·27	4·86[b]	0·25	5·12[b]	0·25	5·94[a]	0·30
*c*9-18 : 1	40·97[b]	1·19	40·24[b]	1·19	38·11[b]	1·09	41·20[b]	1·09	46·40[a]	1·32
*t*11-18 : 1	0·09[b]	0·07	0·09[b]	0·07	2·83[a]	0·06	0·11[b]	0·06	0·15[b]	0·08
18 : 2*n*-6	11·93[b]	0·23	10·73[c]	0·23	10·94[c]	0·21	11·32[bc]	0·21	12·20[a]	0·26
*c*9, *t*11-18 : 2	0·05[c]	0·05	0·02[c]	0·05	0·33[b]	0·05	3·45[a]	0·05	0·13[c]	0·06
*t*10, *c*12-18 : 2	0·00[b]	0·09	0·00[b]	0·09	0·00[b]	0·08	0·14[b]	0·08	3·02[a]	0·09
18 : 3*n*-3	0·43[a]	0·02	0·35[b]	0·02	0·36[b]	0·02	0·38[ab]	0·02	0·39[ab]	0·02
20 : 3*n*-6	0·35[a]	0·03	0·38[a]	0·03	0·33[a]	0·03	0·30[ab]	0·03	0·20[b]	0·04
20 : 4*n*-6	0·46[b]	0·08	0·51[b]	0·08	0·44[b]	0·08	0·57[b]	0·08	0·93[a]	0·09
20 : 5*n*-3	0·14	0·04	0·08	0·04	0·07	0·04	0·14	0·04	0·11	0·05
22 : 6*n*-3	0·07[b]	0·01	0·08[b]	0·01	0·07[b]	0·01	0·08[b]	0·01	0·11[a]	0·01

t, *trans*; *c*, *cis*

[a,b,c] Mean values within a row with unlike superscript letter were significantly different ($P<0.05$).

* For details of diets and procedures, see Table 1 and p. 1040.

with feeding the control, 18 : 0, or *t*10, *c*12-18 : 2 (0·06 or 0·07 % in carcass or milk fat). The *t*10, *c*12-18 : 2 percentage was 1·0 or 3·0 in carcass or milk fat due to feeding *t*10, *c*12-18 : 2.

Fatty acid profiles in carcasses and liver of pup litters

The individual fatty acid profiles in the tissues of the pups are shown in Table 4. Compared with the pups nursing from the dams fed 18 : 0 or *t*11-18 : 1 (183 mg/g dry tissue), total fatty acid concentration in the carcasses of the pups nursing from the dams fed *c*9, *t*11-18 : 2 (151 mg/g) and *t*10, *c*12-18 : 2 (89 mg/g) was reduced by 18 and 51 %, respectively. The sum of percentages of saturated 12 : 0 to 16 : 0 in the carcasses and liver of the pups nursing from the *c*9, *t*11- and *t*10, *c*12-18 : 2-fed dams was lower compared with those in the 18 : 0 or *t*11-18 : 1 groups. This corresponded to decreases of 11 to 29 % in the carcasses, and 6 to 16 % in the liver in response to nursing from the dams fed *c*9, *t*11-18 : 2 or *t*10, *c*12-18 : 2.

The palmitoleic acid percentage in the carcasses, but not liver, was reduced by 56 % in the pups nursing from the dams fed *t*10, *c*12-18 : 2 (0·92 *v.* 2·11 %) compared with the other treatments. The stearic acid percentage was more than doubled in the carcasses of the pups nursing

*t*10, *c*12-18 : 2-enriched milk (17·08 %) compared with the other pups (8·30 %). The oleic acid percentage in the carcasses (47·27 *v.* 44·85 %) or liver (40·55 *v.* 28·57 %) of the litters nursing from the dams fed *t*10, *c*12-18 : 2 was greater compared with those fed 18 : 0 or *t*11-18 : 1. In the carcasses, but not liver, the pups nursing from the dams fed the control or *c*9, *t*11-18 : 2 had a similar percentage of oleic acid compared with those in the *t*10, *c*12-18 : 2 group.

The vaccenic acid percentage in the carcasses and liver of the pups nursing from the *t*11-18 : 1-fed dams (1·82 and 1·46 % for carcasses and liver) was markedly greater compared with the pups in the other groups (0·07 % for carcasses or liver). The *c*9, *t*11-18 : 2 percentage in the carcasses and liver of the pups nursing from the *c*9, *t*11-18 : 2-fed dams (1·08 and 1·26 % for carcasses or liver) was greater compared with the pups in the other groups. However, the percentage of *c*9, *t*11-18 : 2 averaged 0·33 or 0·23 in the carcasses or liver of the pups nursing from the dams fed *t*11-18 : 1 compared with < 0.1 for the pups in the control, 18 : 0, or *t*11-18 : 1 groups. The pups nursing from the dams fed *t*10, *c*12-18 : 2 had a similar percentage of *c*9, *t*11-18 : 2 in the liver compared with those in the *t*11-18 : 1 group. The response in the *t*10, *c*12-18 : 2 litter group may have been due to the transfer

Table 4. Selected fatty acid composition (g/100 g total fatty acids) of carcass and liver tissue of pups nursing from dams fed a control diet or the control diet plus 18:0, *trans* 11-18:1, *cis* 9, *trans* 11-18:2, or *trans* 10, *cis* 12-18:2*

(Mean values with their standard errors for eight pups per litter; residual degrees of freedom 21)

	Control		18:0		t11-18:1		c9, t11-18:2		t10, c12-18:2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	5		5		6		6		4	
Liver										
12:0 + 14:0 + 16:0	35.31^{ab}	0.85	38.04^a	0.85	37.81^a	0.77	34.44^b	0.77	26.51^c	0.94
c9-16:1	0.80	0.10	0.89	0.10	0.98	0.10	0.98	0.10	0.71	0.12
18:0	26.00	1.33	26.24	1.33	25.04	1.22	25.20	1.22	24.36	1.49
c9-18:1	31.05^b	2.01	29.16^b	2.01	27.97^b	1.83	30.32^b	1.83	40.55^a	2.25
t11-18:1	0.09^b	0.06	0.08^b	0.06	1.46^a	0.06	0.08^b	0.06	0.07^b	0.07
18:2n-6	2.71^{bc}	0.31	1.97^c	0.31	2.39^{bc}	0.28	3.24^{ab}	0.28	4.00^a	0.34
c9, t11-18:2	0.00^c	0.05	0.00^c	0.05	0.23^b	0.04	1.26^a	0.04	0.21^b	0.05
t10, c12-18:2	0.00^b	0.01	0.00^b	0.01	0.00^b	0.01	0.00^b	0.01	0.29^a	0.04
18:3n-3	0.13^a	0.01	0.14^a	0.01	0.13^a	0.01	0.10^{bc}	0.01	0.08^c	0.01
20:3n-6	0.09	0.02	0.05	0.02	0.09	0.01	0.09	0.01	0.07	0.02
20:4n-6	0.78^a	0.30	0.59^{ab}	0.30	1.02^a	0.27	1.19^a	0.27	0.43^b	0.33
20:5n-3	0.00	0.02	0.00	0.02	0.06	0.02	0.08	0.02	0.00	0.02
22:6n-3	0.35	0.12	0.32	0.12	0.46	0.11	0.44	0.11	0.18	0.13
Total (mg/g wet tissue)	19.2	1.10	18.9	1.10	18.6	1.00	19.0	1.00	21.0	1.23
Carcass										
12:0 + 14:0 + 16:0	29.81^{ab}	0.81	32.24^a	0.81	31.87^a	0.74	28.60^b	0.74	22.68^c	0.91
c9-16:1	1.86^a	0.19	2.25^a	0.19	2.24^a	0.18	2.10^a	0.18	0.92^b	0.21
18:0	8.65^{bc}	0.71	7.86^{bc}	0.71	7.35^c	0.65	9.35^b	0.65	17.08^a	0.79
c9-18:1	47.32^a	0.55	45.10^c	0.55	44.59^c	0.50	47.22^a	0.50	47.27^a	0.61
t11-18:1	0.06^b	0.03	0.05^b	0.03	1.82^a	0.02	0.07^b	0.02	0.08^b	0.03
18:2n-6	7.96^a	0.32	8.28^a	0.32	7.94^a	0.29	7.08^b	0.29	5.94^c	0.36
c9, t11-18:2	0.04^c	0.04	0.04^c	0.04	0.33^b	0.04	1.08^a	0.04	0.08^c	0.04
t10, c12-18:2	0.00^b	0.04	0.00^b	0.04	0.00^b	0.04	0.04^b	0.04	0.54^a	0.04
18:3n-3	0.13^a	0.01	0.14^a	0.01	0.13^a	0.01	0.10^b	0.01	0.08^c	0.01
20:3n-6	0.24^{ab}	0.01	0.26^a	0.01	0.24^{ab}	0.01	0.21^b	0.01	0.16^c	0.02
20:4n-6	0.94^b	0.13	0.98^b	0.13	0.89^b	0.12	1.13^b	0.12	1.60^a	0.14
20:5n-3	0.06^{ab}	0.01	0.04^b	0.01	0.04^b	0.01	0.05^b	0.01	0.07^a	0.01
22:6n-3	0.23^{bc}	0.04	0.22^{bc}	0.04	0.19^c	0.03	0.30^{ab}	0.03	0.42^a	0.04
Total (mg/g dry tissue)	163.1^{ab}	9.5	182.3^a	9.5	183.5^a	8.7	151.0^b	8.7	88.7^c	10.6

t, *trans*; *c*, *cis*.
[a,b,c] Mean values within a row with an unlike superscript letter were significantly different ($P<0.05$).
* For details of diets and procedures, see Table 1 and p. 1040.

of dietary *c* 9, *t*11-18:2 via milk fat (Table 3). *T*10, *c*12-18:2 accounted for 0.54 or 0.29 % in the carcasses or liver from the pups nursing from the dams fed *t*10, *c*12-18:2, but was not detected in tissues from the pups in the other groups.

Discussion

Dietary *t*10, *c*12-18:2 reduced food intake (Table 2) and resulted in a slight loss (−1 g) of body weight (Fig. 2). It required 4 d for the dams fed *t*10, *c*12-18:2 to reach 17 g food intake/d, which they maintained until the end of the study (Fig. 1). Reductions in food intake were previously observed in growing mice fed CLA mixtures (DeLany *et al.* 1999; Ntambi *et al.* 2002) or a CLA mixture enriched with *t*10, *c*12-18:2 (Park *et al.* 1999). However, reductions in food intake have been relatively small (less than 17 % reduction) compared with changes in body fat (more than 35 % reduction) (Ntambi *et al.* 2002). Thus, it is unlikely that decreased food intake in past studies and the present one was the sole cause of reduced carcass fat weight or milk fat concentration. This is supported by results from pair-feeding experiments with rats or mice (Ryder *et al.* 2001; Ntambi *et al.* 2002) showing that

CLA mixtures decreased body fat compared with pair-fed animals and controls. In the present study, the dams fed *t*10, *c*12-18:2 had substantially lower carcass fat weight (−50 to −63 %) and total fatty acids in the carcasses (Tables 2 and 3) compared with the other treatments. Dietary *t*10, *c*12-18:2 may have enhanced lipolysis and fatty acid oxidation in adipose tissue (Ntambi *et al.* 2002) once the dams had stabilised their food intake, resulting in reduced carcass fat deposition. Such an effect was previously observed in adipocytes incubated with various doses of *t*10, *c*12-18:2 (Evans *et al.* 2002). Alternatively, the dams fed *t*10, *c*12-18:2 may have had greater rates of energy expenditure (DeLany *et al.* 1999; Ntambi *et al.* 2002) and/or energy excretion in their faeces (Terpstra *et al.* 2002). The body-fat-lowering effect of CLA has been less prominent in human subjects than mice (Terpstra, 2001; Ntambi *et al.* 2002). Theoretical calculations indicated that the reduction in body fat would be higher in mice than human subjects because of their higher metabolic rate/kg body weight (Terpstra, 2001).

The total fat content of human milk was reduced (−24 %) by feeding a CLA mixture containing >20 % *t*10, *c*12-18:2 (Masters *et al.* 2002). The present study found a relative decrease of 29 % in milk fat concentration

in response to dietary t10, c12-18:2, which was similar to a previously reported value in rats fed a diet with 2% synthetic CLA mixture (Yang *et al.* 2002). Compared with the other treatments, the mammary gland tissue of the dams fed t10, c12-18:2 (data not shown) contained lower absolute amounts of total fatty acids (0·526 *v.* 0·615 g) and 12:0, 14:0, plus 16:0 (−30%). The percentage of 12:0, 14:0, plus 16:0 in the milk fat reflected to a large extent those of the mammary tissue, and also were lowest in response to t10, c12-18:2. These results suggest that t10, c12-18:2 may have reduced *de novo* fatty acid synthesis in the mammary gland, potentially by reducing the amount and/or activity of key lipogenic enzymes rather than glucose availability because its concentration in plasma was similar for the dams fed t10, c12-18:2 compared with the other treatments. The abundance of mRNA for a number of lipogenic enzymes in bovine mammary tissue was drastically reduced by an intestinal supply of synthetic t10, c12-18:2 (Baumgard *et al.* 2002), resulting in an 82% reduction in the lipogenic capacity of mammary tissue.

The mice fed t11-18:1 or c9, t11-18:2 in the present study also had lower milk fat concentration compared with 18:0. Blends of *trans*-18:1 isomers from partially hydrogenated vegetable oils reduced milk fat content in mice (Teter *et al.* 1990). In mouse mammary epithelial cells, vaccenic acid (compared with oleic or stearic acid) reduced the activity of fatty acid synthase and acetyl-CoA carboxylase in a dose-dependent fashion leading to a lower concentration of total cellular fatty acids (Jayan & Herbein, 2000). Malic enzyme and ATP–citrate lyase activity in mammary tissue were reduced by feeding a mixture of vaccenic and elaidic acids to lactating rats (Assumpcão *et al.* 2002). The results from the present study and those with lactating cows (Baumgard *et al.* 2002; Loor & Herbein, 2003) and growing mice (Viswanadha *et al.* 2002) suggest that at very low intakes (0·08–0·70% of food intake), dietary t10, c12-18:2 is more potent in reducing lipogenesis than *trans*fatty acids from hydrogenated vegetable oils.

The body-weight gain by the litters in the t10, c12-18:2 group averaged 19 g compared with 38 g for the other litters (Fig. 2). This response was unexpected because previous results with rat pups showed moderately enhanced body-weight gain at weaning when lactating rats consumed a diet supplemented with 0·5% of a CLA mixture (45% c9, t11-18:2 and 46% t10, c12-18:2) (Poulos *et al.* 2001). Recently it was shown that c9, t11-18:2 was associated with enhanced growth in young mice (Pariza *et al.* 2001; Ntambi *et al.* 2002). No differences in litter body weight or liver weight were observed between the c9, t11-18:2 group and the control or 18:0 groups. It is probable, however, that exposure to CLA isomers during the suckling period may result in changes in growth and body composition after weaning. Enhanced muscle and skeletal growth along with decreased fat pads were observed in growing mice that had nursed dams fed a CLA mixture (Poulos *et al.* 2001).

The effects on litter carcass composition in response to t10, c12-18:2 paralleled those of their dams, and were similar to those previously observed in growing mice fed restricted or unrestricted amounts of a diet with 10 g

CLA/kg diet (30% c9, t11-18:2 and 30% t10, c12-18:2) (Terpstra *et al.* 2002). Live body weight or body-weight gain were not affected by food restriction, but were lower due to CLA (Terpstra *et al.* 2002). From the results obtained in the present study, it is probable that the litters consuming t10, c12-18:2-enriched milk had lower rates of growth due to the reduced fat content of the milk.

A consistent response to supplementation with synthetic CLA mixtures or t10, c12-18:2 in growing mice (Park *et al.* 1999), pigs (Bee, 2000*a,b*), and lactating cows (Bauman *et al.* 2001; Loor & Herbein, 2003) is an increase in the percentage of stearic acid in tissues or milk fat. This has led to decreases in the oleic acid:stearic acid ratio, a proxy for Δ^9-desaturase activity in adipose, liver, and mammary tissue (Choi *et al.* 2000; Bauman *et al.* 2001; Ntambi *et al.* 2002; Gomez *et al.* 2003), which suggested inhibition of Δ^9-desaturase. In the carcasses of the dams fed t10, c12-18:2, the ratios (calculated from the absolute amounts of each fatty acid in tissue) of *cis* 9-16:1:16:0 and *cis* 9-18:1:18:0 were lower ($P<0·05$) (0·09 and 4·2) compared with the other treatments (0·17 and 5·2). The pups nursing from the dams fed t10, c12-18:2 also had markedly lower ($P<0·05$) ratios of *cis* 9-16:1:16:0 and *cis* 9-18:1:18:0 (0·05 and 2·8) than the other pups (0·10 and 5·6). The down regulation of Δ^9-desaturase gene expression in adipocyte cultures became more pronounced as the level of t10, c12-18:2 in the medium increased (Choi *et al.* 2000), which was associated with a decrease in the ratios of *cis* 9-16:1:16:0 and *cis* 9-18:1:18:0.

As first shown by Pollard *et al.* (1980), Δ^9-desaturase may play a key role in the production of c9, t11-18:2 from t11-18:1. The percentage of c9, t11-18:2 in blood plasma, liver, carcass, mammary gland, and milk fat was enhanced by feeding a diet with 10 g added t11-18:1/kg diet to lactating mice (Loor *et al.* 2002). Growing mice fed a diet enriched with t11-18:1 had greater c9, t11-18:2 in the whole carcass (Santora *et al.* 2000). The present study confirmed earlier results from our laboratory and showed that the percentage of t11-18:1 and c9, t11-18:2 during lactation is closely related across different tissues or milk fat (Fig. 4). Vaccenic acid may increase the synthesis of c9, t11-18:2 in adipose, liver, or mammary tissue as a result of greater Δ^9-desaturase mRNA abundance and activity (Jayan *et al.* 1998; Jayan & Herbein, 2000).

Arachidonic acid, eicosapentaenoic acid, and di-homo-γ-linoleic acid are substrates for eicosanoid synthesis (Sprecher, 2000). These fatty acids are formed from the desaturation of linoleic acid or α-linolenic acid via Δ^6- and Δ^5-desaturation and subsequent elongation. Lower ratios of 20:3n-6:18:2n-6, 20:4n-6:18:2n-6, 20:5n-3:18:3n-3, and 22:6n-3:22:5n-3 suggested (Eder *et al.* 2002) that physiological concentrations of t10, c12-18:2 may reduce the activity of Δ^6- and Δ^5-desaturase, and potentially elongation, in the liver. In the present study, the ratios of 20:3n-6:18:2n-6 and 20:4n-6:18:2n-6 in liver from the dams fed t10, c12-18:2 (data not shown) was lower ($P<0·05$) and averaged 0·017 and 0·040 compared with 0·056 and 0·158 for the other dams. The ratios of 20:3n-6:18:2n-6 and 20:4n-6:18:2n-6 in liver
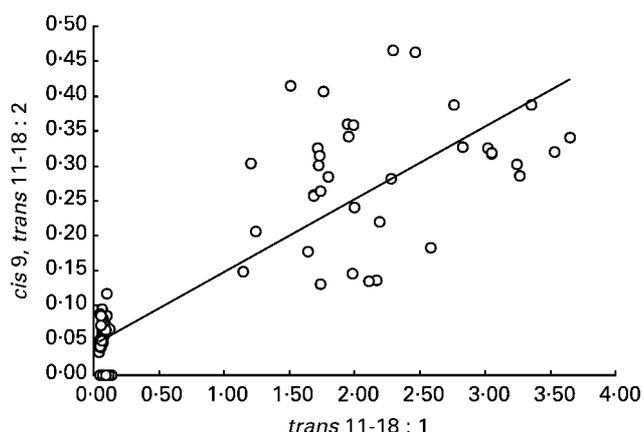
Fig. 4. Relationship between percentage of *trans* 11-18 : 1 and *cis* 9, *trans* 11-18 : 2 in carcass (dams and pups), liver (dams and pups), mammary gland, and milk fat. The regression analysis included values (*n* 96 observations) obtained when feeding the control, 18 : 0, or *trans* 11-18 : 1 diet. *cis* 9, *trans* 11-18 : 2 = 0·105 (*trans* 11-18 : 1) +0·043; *r* 0·87 and *P* < 0·0001.

from the pups nursing from the dams fed *t*10, *c*12-18 : 2 also were lower (*P* < 0·05) and averaged 0·018 and 0·110 compared with 0·031 and 0·348 for the other pups. These results provide additional evidence of the potential inhibitory effect of *t*10, *c*12-18 : 2 on the elongation and desaturation of linoleic acid by the liver first observed *in vitro* (Bretillon *et al.* 1999).

In summary, *t*10, *c*12-18 : 2 appeared to decrease *de novo* fatty acid synthesis in the mammary gland and Δ^9-desaturation in the adipose tissue of both lactating mice and their pups. Fat deposition in the carcass was substantially decreased by *t*10, *c*12-18 : 2. Elongation and desaturation of linoleic acid in the liver also appeared to be reduced by this CLA. Bovine milk fat is an important source of *t*11-18 : 1 and consumption during the nursing period may result in greater *c*9, *t*11-18 : 2 in human milk and tissues. Due to its potential anti-lipogenic effects and the possible adverse effects on food intake, dietary supplements enriched with *t*10, *c*12-18 : 2 should not be consumed during the nursing period. These effects, however, need to be confirmed in human subjects.

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