

Effect of dietary supply of butters rich either in *trans*-10-18:1 or in *trans*-11-18:1 plus *cis*-9, *trans*-11-18:2 on rabbit adipose tissue and liver lipogenic activities

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(Received 10 January 2006 – Revised 18 April 2006 – Accepted 25 April 2006)

Experimental butters with a high content of *trans*-18:1 fatty acids and/or *cis*-9,*trans*-11-18:2 (rumenic acid; RA) were fed to thirty-six New Zealand White rabbits to investigate their effects on adipose tissue (AT) and liver lipogenic activities. Animals received one of three atherogenic (0.2% cholesterol) diets containing 12% butter with either a standard fatty acid composition (rich in saturated fatty acids), rich in *trans*-10-18:1 (T10 diet) or in *trans*-11-18:1 plus RA (T11 + RA diet) for 6 or 12 weeks. The ingestion of butters rich in *trans* fatty acids and/or RA for 6 weeks had little or no effect on liver and AT lipogenesis. The ingestion for 12 weeks of butter rich in T11 + RA decreased perirenal AT weight, lipogenic enzyme and lipoprotein lipase activities, without affecting liver lipid concentration or lipogenic activities except for a decrease in glycerol-3-phosphate dehydrogenase activity. Similar trends, but of a lower magnitude, were observed in rabbits fed the T10 diet for 12 weeks. Ingestion of the T10 or T11 + RA diets for 6 or 12 weeks had no significant effect on plasma metabolites and hormones except for glucose which increased at 6 weeks in the T10 group. Plasma leptin concentration was positively correlated with AT weight but did not differ between the three diets. In conclusion, the supply of butters rich in either T10 or T11 + RA in an atherogenic diet for 12 weeks decreased rabbit AT lipogenesis, with a more marked effect of the T11 + RA diet, but had no effect on liver lipogenesis.

Trans fatty acids: Lipogenesis: Adipose tissue: Liver: Rabbits

Studies on animal models have shown that dietary fats, and especially saturated and *trans* fatty acids, affect insulin sensitivity and lipid metabolism (Saravanan *et al.* 2005). In North American and North European countries, most of the dietary *trans* fatty acids are provided by partially hydrogenated vegetable oils, with *trans*-9 and *trans*-10-18:1 as the principal *trans* fatty acids, whereas dairy products are the main source of dietary saturated fatty acids (SFA). Furthermore, dairy products typically provide a high amount of *trans*-11-18:1, and are also characterised by the presence of specific conjugated linoleic acids (CLA) containing a *trans* double bond, especially with the *cis*-9,*trans*-11 as the major CLA isomer (rumenic acid; RA), whereas the *trans*-10,*cis*-12 CLA isomer is always very low (<0.1% total fatty acids) (Piperova *et al.* 2000). Moreover, chemically synthesised RA and *trans*-10,*cis*-12 isomers have been shown, as an equimolar mixture or as purified isomers, to exhibit physiological properties in various models. Indeed, the RA isomer seems to have anticarcinogenic effects (Wahle *et al.* 2004) and to protect against the development and progression of atherosclerosis in rabbits (Kritchevsky *et al.* 2002) and hamsters (Wilson *et al.* 2000) and to improve insulin sensitivity and glucose metabolism in

rats (Belury, 2003), whereas a mixture of CLA decreases body-fat mass in mice, growing rats, pigs and hamsters (Wahle *et al.* 2004). Anti-lipogenic activity has been reported to be specific to *trans*-10,*cis*-12 CLA, but this CLA isomer has also been associated with deleterious side-effects, including development of insulin resistance, hyperinsulinaemia and fatty liver in mice (Wahle *et al.* 2004).

The levels of *trans* fatty acids and CLA in milk fat can be largely modulated by supplementing the cows' diet with plant oils, which generally results in lower milk fat concentrations of SFA and allows concomitant increases in *trans*-11-18:1 plus RA isomer concentrations. However, with starch-rich diets used sometimes in intensive dairy systems, plant oil supplementation results in increases in *trans*-10-18:1 and, to a very limited extent, *trans*-10,*cis*-12 CLA (for reviews, see Bauman *et al.* 2003; Chilliard & Ferlay, 2004). However, little is known concerning the respective effects of either *trans*-10 or *trans*-11-18:1 on fat deposition and tissue lipogenic activities.

Hence, butters either rich in *trans*-10-18:1 or *trans*-11-18:1 plus RA have been prepared using milks from cows fed different basal diets supplemented with plant oils

Abbreviations: AT, adipose tissue; CLA, conjugated linoleic acid; G3PDH, glycerol-3-phosphate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; LPL, lipoprotein lipase; ME, malic enzyme; RA, rumenic acid; SFA, saturated fatty acid.

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(Roy *et al.* 2006). The objective of the present study was to investigate the effects of the ingestion of these butters on lipogenesis and fat deposition in rabbits fed an atherogenic diet. The experimental butters were compared with a standard butter (low in *trans* fatty acids and rich in SFA). The rabbit has been chosen as the experimental model because, in a parallel study, we studied the effect of the ingestion of these butters on CVD risk factors, such as plasma lipid and lipoprotein profiles, and lipid deposition in the aorta (Chardigny *et al.* 2005). Moreover, to our knowledge there is no published work on the effects of the nature of dietary fatty acids on lipogenic enzyme activities and fat deposition in the rabbit. Lipogenic enzyme activities were measured in liver and adipose tissue (AT), which are the main sites of *de novo* fatty acid synthesis in rabbits (Gondret, 1999). Other parameters putatively linked to lipogenesis and adiposity, such as plasma glucose, NEFA, insulin and leptin, are also reported.

Materials and methods

Animals and diets

Thirty-six male New Zealand White rabbits (9.6 (SE 0.5) weeks old, weighing 2.1 (SE 0.1) kg), purchased from the Elevage des Dombes (Chatillon sur Chalaronne, France), were randomly divided into six groups of six animals having the same average initial body weight (2.08 (SE 0.01) kg). The animals were housed in individual stainless steel cages in a temperature-controlled room (18°C) maintained on a 12 h light–dark cycle. The institution's guidelines for the care and use of laboratory animals were applied. The rabbits were allowed free access to water and fed one of three diets daily at 17.00 hours: one with 12 % butter with a standard fatty acid composition (standard diet), one with 12 % butter rich in *trans*-10-18:1 (diet T10) or one with 12 % butter rich in *trans*-11-18:1 plus RA (diet T11 + RA) for 6 weeks (6-week trial; six animals for each diet) or 12 weeks (12-week trial; six animals for each diet). These two experimental period durations have been chosen in order to estimate the lag-time before any putative effect of *trans*-fatty acids on body fat or lipogenic activities. The preparation and composition of the three butters have been described by Roy *et al.* (2006). The three diets were prepared and pelleted by INRA (Toulouse, France) and contained (g/kg DM): lucerne flour, 305; soya cake, 233; butter, 120; sucrose, 102; beet pulp, 100; barley, 65; sunflower-seed cake, 50; cholesterol, 2; maize oil, 8; sodium chloride, 5; calcium hydrogen phosphate, 5; vitamin and mineral mix, 5. The DM concentration of these diets was 845 g/kg feed and the composition of DM was (g/kg DM): organic matter, 921; crude cellulose, 171; protein (N × 6.05), 231; lipids, 134; fatty acids, 114. The fatty acid composition of the three diets is presented in Table 1. Total lipids in the diets were extracted according to the method of Folch *et al.* (1957) and saponified overnight in an ethanolic potassium hydroxide solution (100 g/l). The fatty acid present in these samples were methylated at room temperature by the BF₃–sodium methanolate method (Sébédio *et al.* 1999), and subsequently analysed by GLC using a DI 200 chromatograph (Perichrom, Saulx-les-Chartreux, France) equipped with a CP-Sil 88 glass capillary column (length 100 m, internal diameter 0.25 mm). Conditions for GLC analysis have been described previously (Scislowski

Table 1. Fatty acid composition (weight % total fatty acid methyl esters) of the three diets enriched with 12 % butter with standard fatty acid composition (standard), rich in *trans*-10-18:1 (T10) or rich in *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA)

Fatty acids (%)	Diets		
	Standard	T11 + RA	T10
<12	6.0	3.8	3.5
12:0	3.8	2.0	2.1
14:0	10.7	6.8	7.8
16:0	29.4	19.3	21.5
Σ 16:1	1.7	1.4	2.0
18:0	7.7	9.3	7.0
<i>Trans</i> -9-18:1	0.3	0.9	0.6
<i>Trans</i> -10-18:1	0.4	2.2	11.8
<i>Trans</i> -11-18:1	1.1	7.0	1.8
<i>Trans</i> -13-18:1	0.4	0.8	0.5
<i>Cis</i> -9-18:1	16.6	20.9	16.8
<i>Cis</i> -11-18:1	0.7	0.9	0.9
<i>Cis</i> -12-18:1	0.2	0.6	0.3
<i>Cis</i> -13-18:1	0.1	0.1	0.1
<i>Cis</i> -9, <i>cis</i> -12-18:2	9.8	11.0	10.1
<i>Cis</i> -9, <i>cis</i> -12, <i>cis</i> -15-18:3	2.2	2.4	2.0
<i>Cis</i> -9, <i>trans</i> -11-18:2	0.5	2.6	0.8
Other fatty acids	8.4	8.0	10.4
Σ Saturated fatty acids	57.6	41.2	41.9
Σ <i>Trans</i> -18:1	2.2	10.9	14.7
Σ <i>Cis</i> -18:1	17.6	22.5	18.1

et al. 2004). The oven temperature was held constant for 30 s at 70°C, then increased from 70 to 175°C at 20°C/min, held at 175°C for 25 min, increased again from 175 to 215°C at 10°C/min, and was finally held at 215°C for 41 min. The carrier gas was H₂ (1.1 ml/min) in conditions of split injection (1/50). Injector and detector temperatures were 235 and 250°C, respectively. Fatty acids were identified by comparing their retention times with those of fatty acid standards (Supelco Park, Bellefonte, PA, USA). Chromatographic signals were analysed using the Wininlab II Chromatography data systems software (Perichrom, Saulx-les-Chartreux, France). All rabbits were killed at the end of the experiment during the post-absorptive period (15 h after and 9 h before the regular daily feeding time). Truncal blood was collected in tubes containing EDTA and centrifuged. Plasma was frozen at –20°C until the analysis of NEFA, glucose, insulin and leptin concentrations. Perirenal AT and liver were quickly excised, frozen in liquid N₂ and stored at –80°C until the analysis of lipogenic enzyme and lipoprotein lipase (LPL) activities and hepatic lipid content.

One rabbit of the group receiving the standard diet for 6 weeks died accidentally after 3 weeks of the experiment and was not replaced.

Plasma metabolites and hormones

Plasma glucose and NEFA concentrations were determined spectrophotometrically by the glucose dehydrogenase method (Glucose RTU kit; BioMérieux, Lyon, France) and the acyl-CoA synthetase method (Wako-Unipath NEFA-C kit; Oxoid, Dardilly, France), respectively.

Plasma concentration of leptin was assayed using a multi-species commercial RIA kit (Linco Research, Inc., Charles,

MO, USA), with a guinea-pig anti-human-leptin antibody, human [¹²⁵I]leptin and human leptin as standard. As recommended by the manufacturer, quantification was carried out in 100 µl plasma and all samples were tested in duplicate. The within- and between-assay variations were 3.4 and 8.7 %, respectively.

The plasma concentration of insulin was assayed using a porcine commercial RIA kit (Cis Bio International, Gif sur Yvette, France). This analysis was performed using a guinea-pig anti-porcine insulin antibody, porcine [¹²⁵I]insulin and porcine insulin as standard. As recommended by the manufacturer, quantification was carried out in 100 µl plasma and all samples were tested in duplicate. The within- and between-assay variations were 6 and 8.6 %, respectively.

Tissue enzyme activities

LPL activity was measured in perirenal AT using an artificial emulsion containing [³H]triolein after a detergent (Deoxycholate-Nonidet P₄₀; Sigma Chemicals, Saint-Quentin-Fallavier, France) extraction procedure (Faulconnier *et al.* 1994). The glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), fatty acid synthase and glycerol-3-phosphate dehydrogenase (G3PDH) activities were assayed spectrophotometrically in perirenal AT and liver as described previously (Chilliard *et al.* 1991).

Enzyme activities were expressed as IU per g tissue. The IU was either 1 nmol released fatty acid (LPL) or 1 nmol reduced (G6PDH, ME) or oxidised (fatty acid synthase, G3PDH) nucleotides per min.

Hepatic lipid content

Total lipids in the liver were determined gravimetrically after their extraction by mixing 5 g fresh tissue with a chloroform–methanol mixture (2 : 1, v/v) according to the method of Folch *et al.* (1957).

Statistical analysis

Results are expressed as means with their standard errors. Data presented in Tables 2, 3, 4 and 5 for each length of treatment (either after 6 weeks of diet or after 12 weeks of diet) were analysed with the ANOVA procedure of SAS (1985; SAS Institute Inc., Cary, NC, USA). The differences between two treatments were tested using the Fisher protected least-squares difference test. Values were considered to be significantly different if *P* values were less than 0.05. Tendencies with *P*<0.10 are also indicated, but only in the discussion.

Results

The three experimental diets were well accepted by the rabbits in the 6- and 12-week trials, except for two rabbits fed the T10 diet in the 12-week trial. Indeed, these two rabbits (T10 anorexic sub-group; T10a), compared with the others in the same experimental group (T10 group), had significantly (*P*<0.05) lower (–64 %) food consumption during the week before slaughter (Table 2). Moreover, the T10a rabbits were clearly different from the others rabbits of the T10 group, and presented numerical decreases in LPL and ME activities in

Table 2. Effects of *trans*-10-18:1 (T10) and *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA) diets on body weight and perirenal adipose tissue weight, and food intake, during experimental periods, and during the week before slaughter (Mean values with their standard errors)

Diet...	6-Week trial						12-Week trial							
	Standard (n 5)		T11 + RA (n 6)		T10 (n 6)		Standard (n 6)		T11 + RA (n 6)		T10 (n 4)		T10a (n 2)*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Final weight of rabbits (kg)	3.15	0.05	3.07	0.05	3.13	0.04	3.70 ^a	0.05	3.47 ^b	0.06	3.50 ^b	0.09	3.29 ^b	0.08
Perirenal adipose tissue weight (g)	54.0	5.0	55.7	2.9	54.2	3.4	113.1 ^a	6.3	88.4 ^b	3.7	86.0 ^b	9.3	86.6 ^b	20.1
Perirenal adipose tissue weight (% body weight)	1.72	0.17	1.82	0.08	1.73	0.11	3.07 ^a	0.20	2.55 ^b	0.10	2.46 ^b	0.27	2.62 ^{a,b}	0.55
Cumulative food intake during period (g)	4564	3	4570	0	4570	0	9919 ^a	18	9860 ^a	54	9925 ^a	13	9264 ^b	239
Food intake during the week before slaughter (g)	838	2	840	0	840	0	869 ^a	20	856 ^a	13	875 ^a	13	319 ^b	144

^{a,b} Mean values within a row and for the same length of treatment (either 6 or 12 weeks) with unlike superscript letters were significantly different (*P*<0.05).

* Anorexic rabbits in *trans*-10-18:1 diet group.

Table 3. Effects of *trans*-10-18:1 (T10) and *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA) diets on plasma non-esterified fatty acids, glucose, insulin and leptin concentrations

(Mean values with their standard errors)

Diet...	6-Week trial						12-Week trial							
	Standard (n 5)		T11 + RA (n 6)		T10 (n 6)		Standard (n 6)		T11 + RA (n 6)		T10 (n 4)		T10a (n 2)*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NEFA (mM)	0.35 ^{a,b}	0.08	0.61 ^b	0.17	0.27 ^a	0.07	0.41	0.15	1.27	0.52	0.79	0.24	1.20	0.09
Glucose (mM)	8.7 ^a	0.5	9.9 ^a	0.9	13.0 ^b	1.2	11.9 ^a	1.3	9.6 ^a	1.2	8.2 ^a	0.3	23.6 ^b	5.5
Insulin (μ U/ml)	10.7	1.5	14.4	2.2	11.6	1.2	18.5	1.9	25.6	4.0	20.8	6.0	13.9	1.0
Leptin (ng/ml)	2.60	0.25	3.14	0.28	3.18	0.40	4.11	0.30	4.14	0.48	3.39	0.38	2.63	0.30

^{a,b} Mean values within a row and for the same length of treatment (either 6 or 12 weeks) with unlike superscript letters were significantly different ($P < 0.05$).* Anorexic rabbits in *trans*-10-18:1 diet group.

perirenal AT and in all lipogenic activities in liver, decreases in plasma insulin and leptin and increases in plasma glucose, NEFA and liver lipid concentrations (Tables 3, 4 and 5).

Effect of trans-10-18:1 diet or trans-11-18:1 plus ruminic acid diet on body weight, perirenal adipose tissue weight, food intake and plasma metabolites and hormones

Rabbits fed the three experimental diets in the 6-week trial showed similar final body weights and similar perirenal AT weights, and consumed the same amount of the diets (Table 2). In contrast, rabbits fed the T10 or T11 + RA diets in the 12-week trial had significantly ($P < 0.05$) lower body weights (-5 and -6% for the T10 and T11 + RA groups, respectively) and lower perirenal AT weights (-24 and -22% for the T10 and T11 + RA groups, respectively), when compared with rabbits fed the standard diet (Table 2). The same differences were noted if perirenal AT weight was expressed as a percentage of body weight. These decreases in body and AT weights were observed whereas rabbits consumed the same amount of diets during the 0–12-week period (Table 2).

In the 6-week trial, plasma NEFA concentration was significantly greater ($+126\%$; $P < 0.05$) in rabbits fed the T11 + RA diet compared with rabbits fed the T10 diet (Table 3). Moreover, plasma glucose concentration was significantly ($P < 0.05$) higher in rabbits fed the T10 diet compared with rabbits fed the standard ($+49\%$) or T11 + RA ($+31\%$) diet (Table 3). Ingestion of the T10 or T11 + RA diets had no significant effect on plasma insulin and leptin concentrations in the 6- and 12-week trials. A significant positive correlation ($r = 0.54$; $n = 33$; $P < 0.01$) was observed between the plasma leptin concentrations and the weight of perirenal AT, across experimental periods, feeding groups and individual rabbits (Fig. 1).

Effect of trans-10-18:1 diet or trans-11-18:1 plus ruminic acid diet on lipogenic enzyme and/or lipoprotein lipase activities in perirenal adipose tissue and liver

In the 6-week trial, the ingestion of the T10 or T11 + RA diet had no significant effect, compared with the standard diet, on lipogenic enzyme and LPL activities in perirenal AT, except for G3PDH activity which was significantly ($P < 0.05$) decreased in rabbits fed the T10 diet (-44%) (Table 4).

In the 12-week trial, the ingestion of the T10 diet significantly ($P < 0.05$) decreased, compared with the standard diet, G6PDH (-12%) and G3PDH (-51%) activities (Table 4). In rabbits fed the T11 + RA diet, compared with the standard diet, there was a significant ($P < 0.05$) decrease in LPL (-39%), G6PDH (-15%) and ME (-48%) activities. AT lipogenic enzyme and LPL activities did not differ between rabbits fed the T10 and T11 + RA diets in the 12-week trial except for ME activity which was significantly ($P < 0.05$) greater ($+63\%$) in the T10 than in the T11 + RA group (Table 4).

In the 6-week trial, the ingestion of the T10 diet had no significant effect, compared with the standard diet, on lipogenic enzyme activities in liver. The ingestion of the T11 + RA diet had no significant effect, compared with the standard diet, but significantly ($P < 0.05$) decreased liver ME (-29%) and G3PDH (-29%) activities compared with the T10 diet (Table 5).

In the 12-week trial, the ingestion of the T11 + RA diet significantly ($P < 0.05$) decreased liver G3PDH activity (-49%) compared with the standard diet. However, the lipid concentration in liver did not differ between the three experimental diets in the 6- or 12-week trial of treatment (Table 5).

Discussion

Rabbits fed the three experimental diets in the 6- and 12-week trials showed similar food intake except for two rabbits in the T10 group with a significantly lower intake during the last week of the 12-week trial. Logically, these two rabbits (T10a sub-group) also showed decreases in lipogenic enzyme activities in perirenal AT and liver, plasma insulin and leptin concentrations and an increase in plasma NEFA concentration, which are typically observed during undernutrition in rodents (Kochan *et al.* 1997). Thus, these two animals were analysed separately as an 'anorexic sub-group'. Furthermore, the elevated plasma glucose concentration in these animals could be related to an inhibition of glucose uptake by the peripheral tissues and/or stimulated hepatic neoglucogenesis due, at least, in part, to low plasma insulin and high plasma NEFA concentrations and a putative insulin resistance outcome as observed in non-insulin-dependent diabetes mellitus in rodents (Plum *et al.* 2000). It could be hypothesised that the T10 diet induced fatty liver (Table 5) and dyslipidaemia

Table 4. Effects of *trans*-10-18:1 (T10) and *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA) diets on lipogenic enzyme and lipoprotein lipase activities (IU/g adipose tissue) in perirenal adipose tissue (Mean values with their standard errors)

Diet...	6-Week trial				12-Week trial									
	Standard (n 5)		T11 + RA (n 6)		T10 (n 6)		T11 + RA (n 6)		T10 (n 4)		T10a (n 2)*			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
Lipoprotein lipase	263	47	192	26	230	38	89 ^a	14	54 ^b	4.3	60 ^{a,b}	13	20 ^b	6.1
Glucose-6-phosphate dehydrogenase	1006	76	1026	120	1236	68	1033 ^a	35	883 ^b	48	906 ^b	9	970 ^{a,b}	4.2
Malic enzyme	133	32	100	14	123	18	170 ^a	15	89 ^b	11	145 ^a	16	71 ^b	5.6
Fatty acid synthase	47	2.8	60	5.9	50	5.7	50	4.9	40	3.1	41	4.3	37	3.3
Glycerol-3-phosphate dehydrogenase	8015 ^a	1507	5675 ^{a,b}	901	4517 ^b	647	6649 ^a	180	4104 ^{a,b}	892	3238 ^b	588	4006 ^{a,b}	340

^{a,b} Mean values within a row and for the same length of treatment (either 6 or 12 weeks) with unlike superscript letters were significantly different ($P < 0.05$).

* Anorexic rabbits in *trans*-10-18:1 diet group.

Table 5. Effects of *trans*-10-18:1 (T10) and *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA) diets on lipogenic enzyme activities (IU/g liver) and lipid content in liver (Mean values with their standard errors)

Diet...	6-Week trial				12-Week trial									
	Standard (n 5)		T11 + RA (n 6)		T10 (n 6)		T11 + RA (n 6)		T10 (n 4)		T10a (n 2)*			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
Total lipid (mg/g liver)	61.0	4.0	60.0	5.5	57.8	3.1	70.1 ^a	7.7	80.7 ^{a,b}	11.7	56.2 ^a	2.1	115.6 ^b	6.5
Glucose-6-phosphate dehydrogenase	3321	475	3658	381	4208	303	4704	833	4285	197	4681	227	2553	437
Malic enzyme	993 ^{a,b}	86	839 ^b	63	1188 ^a	115	1319	305	903	118	1039	109	637	97
Fatty acid synthase	264	27	327	31	346	38	339 ^{a,b}	50	373 ^{a,b}	32	491 ^a	76	263 ^b	51
Glycerol-3-phosphate dehydrogenase	9326 ^{a,b}	1423	7968 ^b	1141	11212 ^a	700	15134 ^a	1244	7737 ^b	596	14748 ^a	2084	4593 ^b	1162

^{a,b} Mean values within a row and for the same length of treatment (either 6 or 12 weeks) with unlike superscript letters were significantly different ($P < 0.05$).

* Anorexic rabbits in *trans*-10-18:1 diet group.

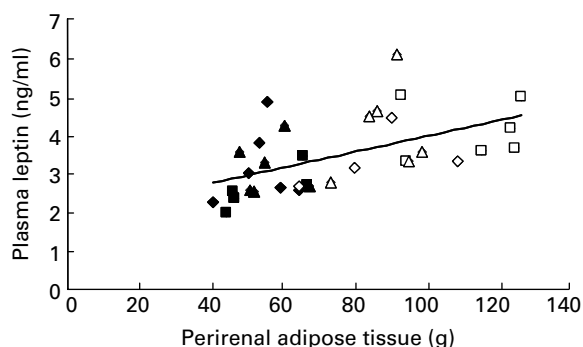


Fig. 1. Relationship between plasma leptin concentrations and perirenal adipose tissue weights ($y = 0.020x + 1.93$; $r = 0.54$). (■), standard diet for 6 weeks; (◆), *trans*-10-18:1 (T10) diet for 6 weeks; (▲), *trans*-11-18:1 plus *cis*-9,*trans*-11-18:2 (T11 + RA) diet for 6 weeks; (□), standard diet for 12 weeks; (◇), T10 diet for 12 weeks; (△), T11 + RA diet for 12 weeks ($n = 33$).

(Chardigny *et al.* 2005; A Roy, JM Chardigny, D Bauchart, A Ferlay, S Lorenz, D Durand, D Gruffat, Y Faulconnier, JL Sébédio and Y Chilliard, unpublished results) and could have led to anorexic behaviour in these two rabbits after 11 weeks on this diet. Further studies are needed with more animals and longer experimental periods to unravel this observation.

The present study shows that ingestion of the T10 or T11 + RA diets in the 6-week trial had no effect on AT and liver lipogenic enzyme activities in growing New Zealand White rabbits receiving isoenergetic atherogenic diets. However, ingestion of the T11 + RA diet in the 12-week trial, compared with the standard butter, significantly decreased the activity of enzymes involved in NADPH generation for *de novo* lipogenesis (G6PDH and ME), and in fatty acid uptake (LPL) and tended ($P < 0.10$) to decrease activities of enzymes involved in fatty acid synthesis and esterification (G3PDH) in perirenal AT in adult rabbits. Similar trends, but to a lower extent, were observed for G6PDH, G3PDH and LPL activities in rabbits fed the T10 diet in the 12-week trial. This is consistent with the decrease in perirenal AT weight observed in rabbits fed the T10 or T11 + RA diets, which could explain, at least in part, the decrease in the body weight of these rabbits. Moreover, these effects occurred despite the absence of any difference between the three experimental diets in cumulative food intake measured either during the week before slaughter or during the 12 weeks of the experiment. Thus, the present study shows that the experimental period duration must be sufficiently long (more than 6 weeks) to induce a significant body fat-lowering effect of *trans*-fatty acids, which is due, at least in part, to reduced lipogenesis in rabbits receiving the same amount of food. Lipogenic enzyme activities were greater in liver than in AT of rabbits, in agreement with the results of Gondret (1999), showing that the liver is the major site of fatty acid synthesis in rabbits. The absence of any difference between the three diets in hepatic lipid content and the decrease in G3PDH activity (enzyme involved in fatty acid esterification), despite the observed trend for an increase in plasma NEFA concentration in rabbits fed the T11 + RA diet, indicate that the experimental diets, compared with the standard diet, did not induce liver steatosis development in our rabbits.

The decrease in AT lipogenesis, especially in rabbits fed the T11 + RA diet, and the absence of a marked effect on liver lipogenesis could be due in part to differences in saturated and/or unsaturated fatty acid intake between the three experimental diets. Indeed, the T10 and T11 + RA diets both presented similar concentrations of SFA (41.7 and 40.8% of total fatty acids in the T10 and T11 + RA diets, respectively) which were much lower than in the standard diet (56.6% of total fatty acid). It is well known that the degree of saturation of dietary fat (especially SFA *v.* PUFA) can alter AT and liver lipogenesis. In rodents, *de novo* lipogenesis in liver was clearly reduced by PUFA but not by SFA, whereas data on AT lipogenesis suggest that dietary SFA interact less with liver lipogenesis because they are more channelled towards AT (for a review, see Chilliard, 1993). Moreover, several studies in rodents reported that the intake of high-fat diets rich in *n*-3 PUFA limited the hypertrophy of fat depots compared with diets rich in SFA, which increased fatty acid esterification and decreased lipolysis in AT (for a review, see Chilliard, 1993).

There are few published studies comparing the effects of MUFA *v.* SFA on lipid metabolism. In the liver, the activities of lipogenic enzymes, acetyl-CoA carboxylase and fatty acid synthase, were similar between rats fed a diet enriched with either beef tallow rich in SFA or olive oil rich in *cis*-9-18:1 (Hillgartner *et al.* 1995) in agreement with the absence of any marked effect of the butters on liver lipogenesis in the present study. In AT, Lhuillery *et al.* (1988) observed that the incorporation of fatty acids into adipocyte acylglycerols was lower in rats fed a diet rich in *cis*-9-18:1 compared with diets containing either hydrogenated coconut oil or cacao butter rich in medium-chain SFA, which is in agreement with the decrease of G3PDH activity in AT in our rabbits fed the T10 or T11 + RA diet. In other respects, Mourot *et al.* (1995) showed that pigs fed a rapeseed oil-supplemented diet rich in *cis*-18:1 and *cis*-9,*cis*-12-18:2 had greater AT lipogenesis and carcass fatness than pigs fed a diet rich in SFA from bovine milk fat.

The high content of *trans* fatty acids and/or RA in our experimental butters could also be involved in the present results. Reports on the effects of dietary *trans* fatty acids or RA on AT or liver lipid metabolism are limited and, to our knowledge, there is no published work on the effects of dietary supply of butters naturally enriched in either T10 or T11 + RA on AT lipid metabolism. However, the decrease of perirenal AT weight in our rabbits is in agreement with the results of Atal *et al.* (1994) showing that a diet containing a mixture of shortenings rich in *trans*-18:1 reduced mouse perirenal and epididymal AT weights. Although the composition of the mixture of *trans*-18:1 isomers was not reported in the study of Atal *et al.* (1994), we could hypothesise that the *trans*-9 and *trans*-10 isomers were the main fatty acids since these *trans*-fatty acids are typical of shortening (Precht & Molkenin, 1995). Similarly, Panigrahi & Sampugna (1993) reported that a mixture of unknown *trans*-18:1 reduced *in vitro* fat accumulation in 3T3 L1 adipocytes. A decrease in body fat due to the *trans*-10,*cis*-12 CLA isomer was demonstrated *in vivo* in rodents (Warren *et al.* 2003; Simon *et al.* 2006), RA being without effect. Thus, the decrease in AT weight in our rabbits fed the T10 diet could suggest a common mechanism between *trans*-10-18:1

and *trans*-10,*cis*-12 CLA. Moreover, the absence of an effect of RA alone on AT weight in mice (Warren *et al.* 2003), rats (Faulconnier *et al.* 2004) or hamsters (Simon *et al.* 2006) suggests that the decrease in AT weight and lipogenesis in rabbits fed the T11 + RA diet could be due to *trans*-11-18:1 rather than to RA or to the simultaneous presence of the *trans*-11-18:1 and RA. This agrees with the *in vitro* study of Cromer *et al.* (1995) showing that *trans*-11-18:1 reduced glucose utilisation for lipogenesis in rat adipocytes. In addition, the decrease in AT LPL activity in rabbits fed the T11 + RA diet corroborates data of Sauer *et al.* (2004) reporting that *in situ* perfusion of pure *trans*-11-18:1 decreased fatty acid arterio-venous differences in Buffalo rat inguinal fat pads.

In vivo results on liver lipogenesis are limited to the effects of CLA (*trans*-10,*cis*-12 isomer CLA or CLA mixtures) with conflicting data. Corino *et al.* (2002) showed, in rabbits, a lower activity of acetyl-CoA carboxylase in liver after feeding a mixture of CLA without affecting ME and G6PDH activities. *In vivo* studies noted an increase in hepatic fatty acid synthesis, a reduction of leptinaemia followed by hyperinsulinaemia, and an increase in liver lipid concentration in mice fed the *trans*-10,*cis*-12 isomer (Warren *et al.* 2003). However, other studies observed that the ingestion of *trans*-10,*cis*-12 CLA did not affect liver lipogenic enzyme activities in rats (Faulconnier *et al.* 2004) and even increased fatty acid oxidation and decreased liver lipid content in hamsters (Macarulla *et al.* 2005). These findings suggest the existence of species-specific responses of liver metabolism to *trans*-10,*cis*-12 CLA feeding, with mice being much more sensitive than other rodent species, probably due to differences in the metabolic rate which is considerably higher in mice than in hamsters and rats (Wahle *et al.* 2004). The absence of an effect of RA on hepatic lipogenesis in rodents (Faulconnier *et al.* 2004; Macarulla *et al.* 2005) is in agreement with the absence of an effect of the T11 + RA diet on liver lipogenesis in rabbits. The present results suggest that the rabbit liver is poorly responsive to a high intake of either *trans*-10 or *trans*-11-18:1.

In the present study, plasma leptin was positively correlated with the weight of perirenal AT (Fig. 1) as reported previously for rats (Koba *et al.* 2002), human subjects (Maffei *et al.* 1995) and mice (Frederich *et al.* 1995). Moreover, although differences were not significant (probably because of the low number of rabbits and/or because the diet's effect was partly masked by a reduction in leptinaemia due to the post-absorptive state), ingestion of the T10 diet in the 12-week trial, compared with the standard diet, decreased (−18%) plasma leptin concentration in agreement with the decrease of perirenal AT weight. However, plasma leptin was not decreased at all by the T11 + RA diet in the 12-week trial, despite the fact that perirenal AT weight was significantly lower than with the standard diet. Thus, a specific stimulatory effect of the T11 + RA diet on leptin secretion cannot be ruled out. Studies on the relationship between dietary fat and circulating leptin concentrations in animals showed that change in dietary intake of SFA correlated positively, whereas changes in the intake of PUFA correlated negatively, to changes in plasma leptin levels, both in mice (Wang *et al.* 2002) and rats (Reseland *et al.* 2001). Studies using CLA (*trans*-10,*cis*-12 CLA or CLA mixtures) supplementation showed a reduction in plasma leptin concentrations together

with a decrease in body fatness in rats (Rahman *et al.* 2001) and human subjects (Medina *et al.* 2000), with a specific effect of the *trans*-10,*cis*-12 CLA isomer. However, Corino *et al.* (2002) found that a CLA mixture tended to increase plasma leptin in rabbits despite a decrease in perirenal AT, while Simon *et al.* (2006) observed that the addition of *trans*-10,*cis*-12 CLA to an atherogenic diet decreased subcutaneous, perirenal and epididymal AT without affecting serum leptin levels in hamsters.

In conclusion, the present study is the first to provide evidence that the consumption of butter rich in either T10 or T11 + RA in a 12-week trial, in rabbits fed isoenergetic diets, decreases perirenal AT weight and lipogenic enzyme activities, with a more marked effect of the T11 + RA-rich butter, without affecting liver lipid concentration and lipogenic activities. Moreover, experimental butters did not greatly modify plasma leptin, whose concentration was related to perirenal AT weight, although a stimulatory effect by the T11 + RA butter cannot be ruled out, suggesting that leptin could play a role in the decreased adiposity with this diet. Further studies with purified *trans*-10-18:1, *trans*-11-18:1 and/or SFA are needed to unravel the respective effects of these fatty acid in the regulation of AT lipogenesis in rabbits, and to investigate the molecular mechanisms that could be involved in these effects. Finally, it would be interesting to relate these results obtained in rabbits to future studies on the use of butters enriched with *trans*-10-18:1 or *trans*-11-18:1 + RA in others animal models and human subjects, due to fact that they mimic some aspects of the dietary patterns followed in developed countries.

Acknowledgements

The authors wish to gratefully acknowledge Cyril Labonne, Martine Touret, Carole Delavaud and Didier Bany for their technical assistance during laboratory analyses and Philippe Gaydier for animal management and care.

The present study was funded by the INRA 'Micronutrients in animal products: CLA-B12' project managed by Jean-Louis Sébédio and Dominique Bauchart, and by the French Ministry of Research 'Increase by nutritional means in CLA and *trans* fatty acid content in milk and beef' project of the 'Aliment Qualité Sécurité' programme managed by Michel Doreau.

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