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Brief Communication

Production of butter fat rich in *trans*10-C18:1 for use in biomedical studies in rodents

Alexandre ROY, Anne FERLAY, Yves CHILLIARD*

Herbivore Research Unit INRA-Theix, 63122 St-Genès-Champanelle, France

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Abstract – *Trans* fatty acids are suspected to be detrimental to health, particularly to cardiovascular function. *Trans* fatty acids include a wide range of fatty acids, with isomers of C18:1, conjugated and non-conjugated C18:2 as major components. A vaccenic acid (*trans*11-C18:1) + rumenic acid (*cis*9,*trans*11-CLA)-rich butter has been shown previously to exhibit health beneficial effects, but less is known concerning another *trans*-C18:1 present in hydrogenated vegetable oil-based products and sometimes in milk fat, the *trans*10-isomer. The present experiment was conducted to produce butters from milk of variable fatty acid composition for use in biomedical studies with rodents, with the overall aim of evaluating the specific effect of *trans*10-C18:1 and *trans*11-C18:1 + *cis*9,*trans*11-CLA on cardiovascular function. Milks from lactating dairy cows fed two types of maize-based diets supplemented (5% of dry matter) – or not – with sunflower oil were collected, and used to manufacture butters either rich in *trans*10-C18:1 (14% of total fatty acids, 64.5% of fat content) or rich in *trans*11-C18:1 + *cis*9,*trans*11-CLA (7.4 and 3.1% of total fatty acids, respectively, 68.5% of fat content), or with standard fatty acid composition (70% of fat content). Additionally, total saturated fatty acid percentage was reduced by more than one third in the enriched butters compared with the standard butter. An understanding of the role of nutrition on milk fatty acid composition in cows allows for the production of dairy products of variable lipid content and composition for use in biomedical studies in animal models and human subjects.

***trans*10-C18:1 / *trans*11-C18:1 / conjugated linoleic acid / butter**

1. INTRODUCTION

Trans fatty acids (TFA) in human nutrition are discussed for their potential adverse effects on health [1, 2]. A number of studies have investigated the relationship between dietary intake of TFA and coronary heart disease (CHD), and linked an intake of 2% of energy as TFA with an increased risk of CHD [3, 4]. However, in some studies the link between TFA intake and the risk of CHD is still equivocal [5, 6]. Besides the design of studies (epidemiological vs. controlled intervention studies), one explanation for this inconsistency may be due to the dietary source of TFA, since CHD has been positively associated with TFA from partially hydrogenated vegetable oils (PHVO) rather than with the intake of TFA from animal products [7, 8].

The most common TFA in the human diet are *trans*-C18:1, and vaccenic acid (VA, *trans*11-C18:1) is the major TFA in milk fat

* Corresponding author: Yves.Chilliard@clermont.inra.fr

(1.7% of total FA, [9]). *Trans*11-C18:1 is formed in the rumen as an intermediate during the biohydrogenation of polyunsaturated FA. It is also the precursor of rumenic acid (RA, *cis*9,*trans*11-CLA) in the mammary gland, the major isomer of conjugated linoleic acids (CLA) in milk fat, a family of positional and geometric isomers of octadecadienoic acid containing a conjugated double bond [10, 11]. The levels of TFA (especially the *trans*11 and *trans*10 isomers of C18:1, and *cis*9,*trans*11-CLA) in milk fat are highly dependent on the nutrition of lactating dairy cows. Indeed, feeding a forage-based diet supplemented with plant oil or oilseeds results principally in enhanced levels of *trans*11-C18:1 and *cis*9,*trans*11-CLA, while feeding a cereal-based diet supplemented with oil increases *trans*10-C18:1 and to a lesser extent *trans*10,*cis*12-CLA [10, 12].

Research with rodents has demonstrated a wide range of beneficial health effects for CLA used as purified or semi-purified, single or mixture of isomers [13]. In particular, a *cis*9,*trans*11-rich CLA-mixture added to a butter fat-containing diet was found to reduce the early steps of atherogenesis in the hamster [14]. Additionally, it was shown that the consumption of a *trans*11-C18:1+*cis*9,*trans*11-CLA-rich butter reduced total and pro-atherogenic cholesterol levels in the hamster [15] and cancer risk in the rat [16]. However, nothing is known about the potential impact of the consumption of *trans*10-C18:1-rich products on the risk of CHD or on the metabolic syndrome, since the specific effects of this FA have never been tested in animal studies, to our knowledge. However, *trans*10-C18:1 and *trans*9-C18:1 are the major TFA in PHVO [9] and were positively associated (P = 0.03 and 0.04, respectively) to coronary artery disease in one study [8].

The objective of this study was to evaluate the different diets given to dairy cows with the aim of naturally producing a butter with a high level of *trans*10-C18:1 (*trans*10 butter). This would allow future studies on the effects of its intake on CHD risk factors, as compared to the intake of a *trans*11-C18:1+*cis*9,*trans*11-CLA-rich butter (VA/RA butter) in rodent studies as experimental models of atherosclerosis.

2. MATERIALS AND METHODS

2.1. Experimental diets for cows

The experiment was performed from February to May 2003, using a protocol approved by the Care and Use Committee of the Institut National de la Recherche Agronomique. All experimental procedures were conducted in accordance with French recommendations for the use of experimental animals including welfare and appropriate conditions (Guidelines April 18th 1988). The experiment was divided into a 3-wk pre-experimental period (P1) in order to adapt the cows to a high concentrate diet, and a 3-wk experimental period (P2). Twenty-nine lactating Holstein cows were divided into 2 groups of cows. From previously published trials [17, 18], 2 diets allowing large enhancements of either *trans*11-C18:1 + *cis*9,*trans*11-CLA or *trans*10-C18:1 concentrations in milk fat were formulated. During P1, one group of 17 cows received a high-concentrate/maize silage (75/25) diet (CS diet), and the second group of 12 cows a mixed diet with maize silage/long cut natural grassland hay/concentrate (31/15/54) (CSH diet). During P2, 5% of dry matter (DM) as sunflower oil (containing 68% of linoleic acid in total FA, Auvergne Trituration, Lezoux, France) were added to the CS diet (CSO diet, 17 cows) and to the CSH diet (CSHO diet, 12 cows). The ingredients and chemical composition of the diets are presented in Table I. The amount of sunflower oil in the diet was progressively increased (+ 200 $g \cdot d^{-1}$) throughout the first five days of P2 until reaching 5% of DM intake.

2.2. Making and fatty acid composition of butters

Butters with standard FA composition (S butter) were made with the daily pooled

Table I. Ingredient and chemical composition of the diets used for the making of butters.

Diets	CSO	CSHO	CSH
Ingredients, % DM			
Hay[1]	–	14.5	14.5
Maize silage	26.6	34.6	35.2
Maize grain	51.7	25.7	30.4
Soybean meal	14.9	17.5	17.5
Sunflower oil	4.7	5.1	–
Urea	0.6	–	–
Limestone	–	0.7	0.7
Dicalcium phosphate	0.5	–	–
Sodium bicarbonate	0.8	0.5	0.4
Mineral-vitamin mix	0.2	1.4	1.3
Composition, % DM			
CP	13.6	15.1	15.6
OM	95.0	93.1	95.6
NDF	18.0	27.3	28.2
ADF	8.3	14.0	14.4
Starch	49.7	32.6	36.2
EE	7.8	8.0	3.1
Total fatty acids	6.7	5.9	1.4
Fatty acids, % of total FA			
C14:0	0.1	0.1	0.2
C16:0	9.0	8.4	15.8
*cis*9-C16:1	0.1	0.1	0.2
C18:0	3.6	4.0	2.5
*cis*9-C18:1	21.0	19.6	19.9
*cis*11-C18:1	0.6	0.6	0.9
C18:2*n*-6	62.9	63.7	48.8
C18:3*n*-3	1.0	1.8	7.8

CP = crude proteins, OM = organic mater, NDF = neutral detergent fiber, ADF = acid detergent fiber, EE = ether extract.
[1] Long cut from natural grassland.

milk from the 12 cows fed the CSH diet during the last week of P1. At each morning milk session during the first week following the start of oil supplementation (P2), a sample of pooled milk from cows fed the CSHO diet was taken to determine the percentage of *cis*9,*trans*11-CLA. Since *trans*11-C18:1 and *cis*9,*trans*11-CLA percentages in milk fat are positively linked according to a precursor-product relationship [10, 11], the concentration of *cis*9,*trans*11-CLA allows to predict the concentration of *trans*11-C18:1. The VA/RA butter was made from the morning + evening pooled milk when the percentage of *cis*9,*trans*11-CLA in the morning milk was higher or equal to 3.0% of total FA, i.e. 5, 6 and 7 d after the start of oil supplementation, in accordance with [17, 19]. From previous results obtained by our team [20], the milk fat *trans*10-C18:1 percentage was the highest after 2–3 weeks of feeding of the CSO diet. Thus, butter rich in *trans*10-C18:1 was made 16, 17, 18, 19 and 20 d after the start of oil supplementation, with milk pooled daily from cows fed the CSO diet.

Raw milk was heated to 30–35 °C (milk heater, Geneform, Lempdes, France) and separated into cream and skim milk (cream separator, Alfa Laval, Sweden). Cream was then cooled to 14–15 °C for 2 h. A lyophilised lactic ferment mix (1 g mix for 5 L cream, MM100, Rhodia Food, Dangé-Saint-Romain, France) was added and the cream was then stored at 20–25 °C for 15 h, during which the pH of the cream was measured. Matured cream (a pH range from 4.8 to 5.4) was cooled at 14 °C before churning (Elba 50 Churn, Elecrem, Châtillon, France). Butter-milk was drained off, and butter was rinsed with 4 °C water, and then stored at –20 °C [21]. Although no physical measurements were carried out, the sole difference observed was the consistency of *trans*-rich C18:1 butters which were softer compared with the S butter, certainly due to their lower content in saturated FA and higher content in mono- and poly-unsaturated FA. Personal tests concerning the flavour and odour did not show any marked difference among the butters.

For FA analysis, the samples of butters made over the days described above were pooled for each type of butter. Butter fat was extracted and trans-methylated [22], and fatty acid methyl esters (FAME) were separated and identified [23] using a gas chromatograph (Trace GC 2000 Series, ThermoFinnigan, France) equipped with a flame-ionisation detector, automatic injector, split injection port and a 100 m fused silica capillary column (i.d., 0.25 mm) coated with 0.2 µm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier gas operated at constant pressure (125 kPa). Injector and detector temperatures were maintained at 255 °C and 260 °C, respectively. The column temperature was maintained at 70 °C for 4 min, increased at a rate of 5 °C·min^{-1} to 100 °C, raised to 175 °C at a rate of 10 °C·min^{-1}, held at 170 °C for 40 min, increased at 5 °C·min^{-1} to a final temperature of 225 °C which was maintained for 22 min. Peaks were routinely identified by comparison of retention times with FAME standards (GLC 463, Nu-Check-Prep Inc., Elysian, MN, USA; Reference mixture 47885, Supelco, Bellefonte PA, USA) and a mixture of C18:1, C18:2 and CLA isomers. Correction factors for C4:0 to C10:0 were determined on each day of analysis using a butter oil reference standard (CRM 164, Community Bureau of Reference, Brussels, Belgium).

3. RESULTS AND DISCUSSION

Milk fat *cis*9,*trans*11-CLA and *trans*11-C18:1 percentages were increased by 8 and more than 6 fold in the VA/RA butter compared with the S butter, respectively (Tab. II). The percentages of these FA in milk fat are in agreement with those (3.7% and 7.3% of total FA, respectively) obtained by [17].

The butter fat percentage of *trans*10-C18:1 was notably higher (43 fold) in the *trans*10 butter than in the S butter to account for almost 14% of total FA, i.e. much higher than the highest concentration reported previously in milk fat (9.2% of total milk FA, [18]) among 13 reviewed studies [23]. Although cow characteristics (breed, days in milk) and diet composition (forage to concentrate ratio, ingredients, level of added oil, starch amount) were comparable between the present study and the study of [18], one factor differed, i.e. the type of added oil: sunflower in the present study *vs* soybean oil in [18]. Sunflower oil used in the present study, compared with soybean oil, contained higher linoleic acid (68% vs. 54% of total FA, respectively) and lower linolenic acid (0.1% vs. 7.5% of total FA, respectively) [24]. Since *trans*10-C18:1 is an important intermediate in the ruminal biohydrogenation pathway of linoleic acid with high-concentrate diets [25], a higher intake of linoleic acid should have increased the formation of *trans*10-C18:1 in the present study. Furthermore, the rumen biohydrogenation of linolenic acid does not involve *trans*10-C18:1 as an intermediate [25].

Table II. Fatty acid composition of butters made from milk fat from cows fed the CSO (*trans*10-C18:1-rich butter), CSHO (RA/VA-rich butter) and CSH (S butter) diets.

Butters	*trans*10	VA/RA	S
	% of total fatty acids		
Fatty acids			
C4:0	2.17	2.84	3.60
C6:0	1.31	1.60	2.60
C8:0	0.79	0.86	1.74
C10:0	1.87	1.93	3.93
C12:0	2.53	2.34	4.46
C14:0	9.48	8.24	13.24
*cis*9-C14:1	1.20	0.78	1.00
C15:0	1.01	0.88	0.93
C16:0	21.17	19.68	33.43
*cis*9-C16:1	1.89	1.18	1.45
C17:0	0.47	0.44	0.53
*cis*9-C17:1	0.25	0.15	0.14
C18:0	7.46	10.39	8.70
*cis*9,*cis*12-C18:2	3.30	2.97	1.85
*cis*9,*trans*13-C18:2[1]	0.23	0.33	0.10
*cis*9,*trans*11-CLA[2]	0.86	3.05	0.38
*trans*9,*cis*11-CLA[3]	0.12	< 0.01	< 0.01
*cis*9,*cis*12,*cis*15-C18:3	0.13	0.20	0.18
C20:0	0.07	0.08	0.07
Total *cis*-C18:1	19.72	24.09	15.67
*cis*9	18.27	22.34	14.73
*cis*11	0.98	0.89	0.69
c*is*12	0.33	0.73	0.25
c*is*13	0.15	0.13	0.06
Total *trans*-C18:1[4]	18.90	14.01	2.68
*trans*4	0.05	0.05	< 0.01
*trans*5	0.07	0.04	< 0.01
*trans*6, 7, 8	1.08	0.92	0.19
*trans*9	0.46	0.65	0.18
*trans*10	13.69	2.28	0.32
*trans*11	2.09	7.38	1.18
*trans*12	0.68	1.61	0.30
*trans*13, 14	0.79	1.08	0.51
Other fatty acids	5.06	3.97	3.32

[1] Other minor non-conjugated, *trans* double bond-containing C18:2 were likely to account for less than 0.81%, 0.63% and 0.16% of total FA in *trans*10, RA/VA and S butters, respectively.
[2] The *trans*10, *cis*12-CLA was not detected.
[3] The identification of this peak on the chromatogram was done according to [26].
[4] According to our analysis parameters, *cis*14- and *cis*15-C18:1 co-elute with *trans*16- and *trans*17-C18:1, respectively. However, these two *trans*-C18:1 that co-elute with *cis*-isomers are in very low level in milk fat [18].

Interestingly, the VA/RA and *trans*10 butters both presented similar concentrations of saturated FA, which were much lower than in the S butter, except for C18:0 (Tab. II). Total saturated FA (C4:0 to C20:0) represented 73.2% of total FA in the S butter, while it represented only 49.3% and 48.4% of total FA in the VA/RA and *trans*10 butters, respectively. Furthermore, butter fat concentrations of linoleic and oleic acids were higher in enriched butters, and total *trans*-C18:1 percentage was increased by more than 5 and 7 fold in the RA/VA and *trans*10 butters, respectively, compared with the S butter. The sum of total C18:1 + C18:2 containing a *trans* double bond (conjugated or not) was increased from 3.2% in the S butter to 17.7% or 20.7% of total FA in RA/VA or *trans*10 butters, respectively. These modifications in FA composition of butter fats are in accordance with the decrease in saturated FA and the increase in TFA generally observed in milk fat from cows fed oil-supplemented diets [10, 12, 26].

Since fat content of milk from cows fed the CSO diet was significantly lower than with the CSH and CSHO diets (1.4% vs. 4.2% and 3.1% of milk fat content, respectively, $P < 0.05$), in agreement with [17, 18], the number of cows and days of milk collect for further butter making were managed to be higher for the CSO diet. Practically, 400–450 L of raw milk were used for the making of 16 kg of either S or VA/RA butter, while 1400 L of raw milk were necessary to make 16 kg of *trans*10 butter. Nevertheless, the wringing out of the butters was adapted to obtain similar fat contents among butters (70%, 68.5% and 64.5% of fat content in S, RA/VA and *trans*10 butters, respectively). With the dietary strategies used here, the decrease in milk fat yield was accompanied by an increase in milk fat *trans*10-C18:1 percentage, suggesting that *trans*10-C18:1, or other *trans* isomers such as *trans*9,*cis*11-CLA detected only in the CSO butter fat, could be related directly or indirectly to anti-lipogenic activities [10, 23, 26]. Several animal studies have shown that *trans*10,*cis*12-CLA, the CLA-isomer precursor of *trans*10-C18:1 in the rumen [25], decreased body fat mass or adiposity [27] although this was not observed in some animal models [28]. However, this CLA isomer was not detected in the present study, even in the *trans*10 butter. It could be suggested either that almost all the *trans*10,*cis*12-CLA has been hydrogenated into *trans*10-C18:1 in the rumen, or that any absorbed *trans*10,*cis*12-CLA would have been less taken up and/or secreted by the mammary gland.

This is the first report on the practical way to produce a butter with a very high *trans*10-C18:1 content, which is a suitable material to evaluate the effect of the consumption of *trans*10-C18:1-rich dairy products on risk factors involved in CHD [29]. However, butter could also help appreciate the effect of this TFA on body composition and energy metabolism in animal models, or on in vitro metabolic activities in cell models, after isolation of this isomer from milk fat, which is feasible since it represented more than 70% of total *trans*-C18:1.

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