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Accumulation and apparent oxidation of *cis*,*trans*-18 : 2 isomers relative to linoleic acid in rats

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Dietary *cis*,*trans*-18 : 2 isomers impair desaturation and elongation of linoleic acid (Δ9*cis*,12*cis*-18 : 2), but little is known of their proportional partitioning between accumulation and oxidation. The present study was therefore designed to assess the accumulation and apparent oxidation of *cis*,*trans*-18 : 2 isomers compared with that of *trans*-18 : 1 isomers and Δ9*cis*,12*cis*-18 : 2 in rats. Accumulation is defined as whole-body increase in a fatty acid during a given period (i.e. final body content − initial body content). The apparent oxidation (disappearance) is defined as whole-body utilization of a fatty acid relative to its intake for a given period (intake − excretion − accumulation − longer-chain products)/intake×100). The animals were fed on a diet containing 15 % (w/w) partially hydrogenated rapeseed oil with 1·72 % energy as *cis*,*trans*-18 : 2 isomers and varying amounts of Δ9*cis*,12*cis*-18 : 2. The apparent oxidation of total *cis*,*trans*-18 : 2 isomers (72–76 % dietary intake) was greater than that of Δ9*cis*,12*cis*-18 : 2 (38–51 % dietary intake) but it was similar to that of total *trans*-18 : 1 isomers (78–82 % dietary intake). Among the four isomers, the apparent oxidation of Δ9*trans*,12-*trans*-18 : 2 was greater than that of the other isomers including Δ9*trans*,12*cis*-18 : 2, Δ9*cis*,12*trans*-18 : 2 and Δ9*cis*,13*trans*-18 : 2. Accumulation of Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4 and Δ5*cis*,8*cis*,11*cis*,14*trans*-20 : 4 derived from chain-elongation and desaturation of Δ9*cis*,13*trans*-18 : 2 and Δ9*cis*,12*trans*-18 : 2 was decreased when the dietary Δ9*cis*,12*cis*-18 : 2 supply was increased.

Linoleic acid: Oxidation: *cis*,*trans*-18 : 2 isomers: Partially hydrogenated rapeseed oil: *trans*-Fatty acids: *trans*-Linoleic acids

Several *cis*,*trans*-isomers of linoleic acid (Δ9*cis*,12-*cis*-18 : 2) present in the Western diet are formed during refining or partial hydrogenation of vegetable oils. These isomers include mainly Δ9*cis*,13*trans*-18 : 2, Δ9*cis*,12-*trans*-18 : 2, and Δ9*trans*,12*cis*-18 : 2 (Ratnayake & Pelletier, 1992; Ratnayake *et al.* 1993). Previous investigations have found that human adipose tissue and breast milk contain these *cis*,*trans*-18 : 2 isomers and have a similar isomeric distribution to that in partially hydrogenated vegetable oils (Chen *et al.* 1995*a*,*b*). This finding suggests that these isomers can accumulate in the body and transfer from the maternal diet to the breast milk.

cis,*trans*-18 : 2 isomers may interfere with the metabolism of essential fatty acids (Holman *et al.* 1991). First, they impair elongation and desaturation of Δ9*cis*,12*cis*-18 : 2, thus reducing the formation of arachidonic acid (Δ5*cis*,8*cis*,11*cis*,14*cis*-20 : 4). Second, some of these dietary *cis*,*trans*-18 : 2 isomers can be desaturated and chain-elongated to form unusual *cis*,*trans*-20 : 4 isomers (Ratnayake *et al.* 1994). In fact, Δ9*cis*,12*trans*-18 : 2 and Δ9*cis*,13*trans*-18 : 2 have been shown to be desaturated and elongated to form two unusual 20 : 4 isomers, i.e. Δ5*cis*,8*cis*,11*cis*,14*trans*-20 : 4 and Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4 in the rat liver (Hill *et al.* 1990; Koletzko, 1992; Ratnayake *et al.* 1994). Subsequently, these *cis*,*trans*-20 : 4 isomers may, via lipoxygenase and cyclooxygenase pathways, serve as precursors of unusual eicosanoids with unknown structure and functions (Berdeaux *et al.* 1996). Thus, it is important to understand the metabolic fate and tissue deposition of these *cis*,*trans*-18 : 1 isomers.

Despite several decades of research on the metabolism of *trans*-fatty acids and their possible influence on the metabolism of essential fatty acids, little is known about their partitioning between accumulation and oxidation in whole animals. Studies in rat heart homogenates and liver mitochondria showed that *trans*-18 : 1 isomers were oxidized more slowly than their *cis*-counterparts (Lawson

Abbreviations: FAME, fatty acid methyl esters; LCP, longer-chain products; PHRO, partially hydrogenated rapeseed oil.

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& Holman, 1981; Lanser *et al.* 1986). In contrast, human heart homogenates were found to oxidize *trans*-18 : 1 and oleic acid (Δ9*cis*-18 : 1) at a similar rate (Lanser *et al.* 1986), which is in agreement with the results of another study in human subjects fed ^{13}C-labelled fatty acids, that showed no difference in the oxidation rate of *trans*-18 : 1 and *cis*-18 : 1 (Delany & Bray, 1993). However, limited data are available regarding the oxidation rate of *cis*,*trans*-18 : 2 isomers relative to 18 : 2*n*-6 except one study that demonstrated that the former were oxidized more readily in isolated rat liver (Ide & Sugano, 1984).

The results of *in vivo* oxidation of *trans*- fatty acids using radioisotopic methods reflect their utilization in intact organisms during the time after administration of the isotopically labelled *trans*-fatty acids. In contrast, the balance method measures the long-term utilization of a *trans*-fatty acid. The method of whole-body fatty acid balance was used previously to assess the partitioning between oxidation and accumulation of Δ9*cis*,12*cis*-18 : 2 and linolenic acid (Δ9*cis*,12*cis*,15*cis*-18 : 3) under various conditions (Ide & Sugano, 1984; Yang *et al.* 1994; Chen *et al.* 1996). To our knowledge, data on whole-body retention and oxidation of *cis*,*trans*-18 : 2 isomers relative to their intake are not currently available. Thus, the present study used the balance method to determine oxidation, retention, elongation and desaturation of *cis*,*trans*-18 : 2 isomers in whole animals.

Materials and methods

Diet

Rats were fed one of five diets containing 15 % (w/w) rapeseed oil (diet A) or partially hydrogenated rapeseed oil (PHRO; diet B) supplemented with free Δ9*cis*,12*cis*-18 : 2 at 0·25 (diet C), 0·50 (diet D) and 1·25 % energy (diet E). Other components of the diets are shown in Table 1. The diets were prepared every 2 weeks and stored at −4°C. The PHRO was supplied by Canamera Food (Toronto, Ont., Canada) and the *trans*-fatty acid content was 27·8 % total fatty acids. Non-hydrogenated rapeseed oil was purchased locally in Hong Kong (Lam Soon Oils & Fats Co., Hong Kong, China). The *trans*-isomer content of non-hydrogenated rapeseed oil was found to be 0·59 % total fatty acids (reference diet A), as they can be generated during the refining process. Other ingredients were purchased from Harlan Teklad (Madison, WI, USA) except for choline bitartrate and DL-methionine which were obtained from Sigma (St Louis, MO, USA). The fatty acid contents of the diets (g/kg), as determined by, are GLC, are given in Table 1.

Table 1. Composition of experimental diets (g/kg diet)

Diets…	A	B	C	D	E
Casein	235·0	235·0	234·6	234·3	233·4
Maize starch	288·0	288·0	287·5	287·1	286·1
Sucrose	243·0	243·0	242·6	242·3	241·4
Cellulose	32·0	32·0	31·9	31·9	31·8
Mineral mix (AIN-76)	35·0	35·0	34·9	34·9	34·8
Vitamin mix (AIN-76A)	10·0	10·0	10·0	10·0	9·9
Choline bitartrate	4·0	4·0	4·0	4·0	4·0
DL-Methionine	3·0	3·0	3·0	3·0	3·0
Fat*	150·0	150·0	151·5	152·5	155·6
16 : 0	8·6	8·4	8·4	8·4	8·4
18 : 0	3·8	10·1	10·1	10·1	10·1
20 : 0	1·1	0·4	0·4	0·4	0·4
22 : 0	< 0·1	0·4	0·4	0·4	0·4
Total saturates	13·5	19·2	19·2	19·2	19·2
16 : 1*n*-7	0·4	0·3	0·3	0·3	0·3
18 : 1*n*-9	83·5	73·1	73·1	73·1	73·1
Other 18 : 1	4·9	10·4	10·4	10·4	10·4
20 : 1*n*-9†	2·9	1·4	1·4	1·4	1·4
22 : 1*n*-9	0·5	0·5	0·5	0·5	0·5
Total monounsaturates	92·2	85·7	85·7	85·7	85·7
18 : 2*n*-6	30·9	2·6	4·2	5·5	9·3
18 : 3*n*-3	10·9	< 0·1	< 0·1	< 0·1	< 0·1
Total PUFA	41·8	2·7	4·3	5·6	9·4
trans-18 : 1	< 0·1	33·3	33·3	33·3	33·3
Δ9*trans*,12*trans*-18 : 2	< 0·1	0·4	0·4	0·4	0·4
Δ9*cis*,12*trans*-18 : 2	< 0·1	1·6	1·6	1·6	1·6
Δ9*trans*,12*cis*-18 : 2	0·3	1·9	1·9	1·9	1·9
Δ9*cis*,13*trans*-18 : 2‡	0·3	2·1	2·1	2·1	2·1
Total *cis*,*trans*-18 : 2	0·6	6·0	6·0	6·0	6·0

PUFA, polyunsaturated fatty acids.
* Rapeseed oil or partially hydrogenated rapeseed oil with or without addition of 18 : 2*n*-6.
† Δ11*cis*-20 : 1 may overlap with 18 : 3 isomers.
‡ May overlap with Δ8*trans*,12*cis*-18 : 2.

Animals

Male Sprague-Dawley rats (2 months old, weight 67 ± 6 g, Laboratory Animal Service Centre, The Chinese University of Hong Kong) were divided randomly into five groups (ten rats per group) and housed in stainless-steel wire-bottomed cages in an animal room maintained at 25°C with 12 h light-dark cycles and 50 % humidity. Food intake was measured daily and body weight was recorded every second day. Animals had free access to food for 3 weeks. Six animals were killed immediately before the experiment after overnight fasting to provide a baseline value. The rats fed one of the experimental diets were then killed by exsanguination under CO_2 anaesthesia after an overnight fast. Blood was collected via the abdominal aorta. The whole carcass (whole body−blood) was then stored at −20°C.

Whole-carcass fatty acid analysis

The whole carcass was blended three times using a meat grinder and mixed thoroughly as described previously (Yang *et al.* 1994; Chen *et al.* 1996). Total lipids were extracted from three aliquots (2 g each) using 20 ml chloroform−methanol (2:1, v/v) containing 0·02 % (v/v) butyrated hydroxytoluene (Sigma, St Louis, MO, USA) as an antioxidant (Folch *et al.* 1957). At the same time, heptadecanoic acid (17 : 0, 99 % purity; Sigma) was added as an internal standard to quantify the total carcass fatty acids. After homogenization, the bottom layer was taken and the three extracts were pooled and dried under a gentle stream of N_2. The method used for extraction yielded > 95 % recovery of tissue lipids (Folch *et al.* 1957; Ways & Hanahan, 1964; Christie, 1982). The carcass total lipids (20 mg) were then converted to their corresponding fatty acid methyl esters (FAME) using 2 ml 14 % (v/v) BF_3 in methanol and 1 ml toluene at 90°C for 40 min under N_2. FAME were then extracted by adding 5 ml hexane and 1 ml

saline (9 g NaCl/l). After centrifugation, the hexane layer was taken and evaporated to 1 ml under a gentle stream of N_2. In some cases, the carcass lipids were transmethylated using 2 ml methanolic hydrogen chloride. The results of the two methods of transmethylation were similar, as described previously (Zhang & Chen, 1997); the data presented here were obtained by the BF_3–methanol methylation method.

FAME in hexane were analysed initially by using an SP-2560 flexible fused silica capillary column (100 × 0·25 mm i.d., 20 μm film thickness; Supelco, Beffefonte, PA, USA) in a Hewlett-Packard 5980 Series II gas chromatograph equipped with a flame-ionization detector (Hewlett-Packard, Palo Alto, CA, USA). The column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 20 min. Injection and detector temperatures were set at 250°C, and the column head pressure was set at 15 psi. The individual fatty acids were then quantified according to the amount of heptadecanoic acid added (Chen *et al.* 1996). We found that > 96 % of the heptadecanoic acid can be recovered. It should be pointed out that *cis*,*trans*-18 : 2 isomers, Δ9*cis*,12*cis*-18 : 2 and *trans*-18 : 1 isomers extracted from tissue would have the same recovery relative to the amount of internal standard added.

Individual *cis*,*trans*-18 : 2 isomers and *cis*,*trans*-20 : 4 isomers were identified and determined as described previously (Ratnayake *et al.* 1994). In brief, the total *trans*- and *cis*-FAME were separated by $AgNO_3$ TLC. The *cis*- or *trans*-FAME from the carcass lipids were saponified and acidified to the free fatty acids and then converted to their 2-alkenyloxazoline derivatives. The double-bond positions were confirmed by GLC–MS analysis of the 2-alkenyloxazoline derivatives. Determination of total *trans*-18 : 1 fatty acids was carried out by using $AgNO_3$ TLC in conjunction with capillary GLC, as described previously (Ratnayake & Bears-Rogers, 1990; Chen *et al.* 1995*b*) because a single-step and direct GLC cannot determine the total *trans*-18 : 1 fatty acids due to overlap of the high Δ*trans*-18 : 1 isomer (Δ12*trans*-18 : 0 to Δ16*trans*-18 : 1) with the *cis*-18 : 1 isomer peak. The preparative $AgNO_3$ TLC plates were prepared as described previously (Ratnayake & Bears-Rogers, 1990). FAME (15 mg) were subjected to a silica TLC plate in hexane solution and then developed in toluene at −20°C for 3 h. The separated *trans*-18 : 1 band was visualized under u.v. light (254 nm) and then scraped from the plate and extracted with hexane–chloroform (1:1, v/v). After drying under N_2, the *trans*-18 : 1 fraction was redissolved in hexane, and analysed by GLC as described earlier. The proportion of *trans*-18 : 1 isomers (Δ12*trans*-18 : 1 to Δ16*trans*-18 : 1) that overlapped with the *cis*-18 : 1 isomer peaks was calculated by comparing the 18 : 1 region of the GLC chromatogram of the isolated *trans*-18 : 1 fraction with that of the parent FAME mixture before $AgNO_3$ fractionation. For this purpose, the *trans*-18 : 1 isomer peaks that were well separated from the *cis* peak serve as an internal standard. The total *trans*-18 : 1 content was then calculated by summing the proportions of the *trans*-18 : 1 isomers (Δ12*trans*-18 : 1 to Δ16*trans*-18 : 1) that overlapped with the *cis*-18 : 1 isomers and the well separated *trans*-18 : 1 isomers in the direct FAME analysis.

Statistics

Data are expressed as means and standard deviations. ANOVA followed by a least-significant difference test was used repeatedly for statistical evaluation of the differences among *trans*-18 : 1 isomers, Δ9*cis*,12*cis*-18 : 2, Δ9*trans*, 12*trans*-18 : 2, Δ9*cis*, 13*trans*-18 : 2, Δ9*cis*,13*trans*-18 : 2, Δ9*trans*,12*cis*-18 : 2 and total *cis*,*trans*-18 : 2 isomers within the same diet. This analysis was performed using ANOVA software on a personal computer (PC ANOVA for the IBM Personal Computer, version 1.1; IBM, Armonk, NY, USA).

Results

Fatty acid composition of dietary fat

Dietary group A was fed a reference diet containing rapeseed oil. The fatty acid content (g/kg diet) of diets B–E was the same except for the amount of Δ9*cis*,12*cis*-18 : 2 (Table 1), which was increased from 2·6 to 9·3 g/kg. Dietary Δ9*cis*,12*cis*-18 : 2 expressed as a percentage of total energy was 0·56 in diet B, 0·81 in diet C, 1·06 in diet D, and 1·81 in diet E. The total *cis*,*trans*-18 : 2 isomers accounted for 6·0 g/kg diet (1·72 % total energy), with Δ9*cis*,13-*trans*-18 : 2 being a major isomer followed by Δ9*trans*,12-*cis*-18 : 2 and Δ9*cis*,12*trans*-18 : 2. In contrast, the total *trans*-18 : 1 isomers accounted for 33·3 g/kg diet (7·17 % total energy), whereas Δ9*cis*-18 : 1 was 73·1 g/kg diet (15·7 % total energy).

Food consumption and body weight

The rats fed on different diets with varying levels of Δ9*cis*,12*cis*-18 : 2 supplementation consumed similar amounts of food (17·0–18·5 g diet/rat per d). No significant differences in body-weight gain expressed as either g/d or g/g diet were detected between the dietary groups (Table 2).

Apparent oxidation of cis,trans-18 : 2 isomers

Accumulation is defined as the whole-body increase in levels of a fatty acid, whereas apparent oxidation is the whole-body utilization (disappearance) in relation to its intake for a given period. The following equations were used to calculate the accumulation and apparent oxidation of *cis*,*trans*-18 : 2 isomers in the whole body:

$$\text{accumulation (mg/rat)} = (\text{final body content} - \text{initial body content}),$$

$$\text{apparent oxidation (\% dietary intake)} = (\text{intake} - \text{excretion} - \text{accumulation} - \text{LCP})/\text{intake} \times 100,$$

where excretion was assumed to be 2 % of the total dietary fatty acids (Yang *et al.* 1994); LCP is the amount of the longer-chain products in the desaturation and elongation pathway. In this regard, the LCP for Δ9*cis*,12*cis*-18 : 2 would include Δ6*cis*,9*cis*,12*cis*-18 : 3, Δ11*cis*,14*cis*-20 : 2, Δ8*cis*,11*cis*,14*cis*-20 : 3, Δ5*cis*,8*cis*,11*cis*,14*cis*-20 : 4, Δ7*cis*,

Table 2. Body weight and daily food intake of rats fed diets containing non-hydrogenated (diet A) or partially hydrogenated rapeseed oil with 1·72 % energy as *cis*,*trans*-18 : 2 isomer (diet B) and varying amounts of Δ9 *cis*,12*cis*-18 : 2 (diets C–E)

(Mean values and standard deviations for ten rats)

Diet…*	A		B		C		D		E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial body wt (g)	67	6	65	5	66	4	64	4	67	6
Final body wt (g)	230	11	211	13	237	17	219	2	216	15
Body-wt gain (g/d per rat)	7·8	0·4	7·0	0·5	8·1	0·6	7·4	0·4	7·1	0·5
Food intake (g)	17·0	4·3	18·0	3·5	18·5	3·8	17·5	4·0	18·2	4·3
Body-wt gain (g/rat per g diet)	0·46	0·11	0·39	0·08	0·44	0·09	0·42	0·10	0·39	0·09

* For details of diets and procedures, see Table 1 and p. 250.

10*cis*,13*cis*,16*cis*-22 : 4 and Δ4*cis*,7*cis*,10*cis*,13*cis*,16*cis*-22 : 5, whereas the LCP for Δ9*cis*,13*trans*-18 : 2 and Δ9*cis*,12*trans*-18 : 2 would include Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4 and Δ5*cis*,8*cis*,11*cis*,14*trans*-20 : 4, respectively (Kwan *et al.* 1998; Ratnayake *et al.* 1994). No other LCP for *cis*,*trans*-18 : 2 isomers and *trans*-18 : 1 isomers was found in the carcass lipids.

Values for accumulation and apparent oxidation of total *trans*-18 : 1 isomers, total *cis*,*trans*-18 : 2 isomers and Δ9*cis*,12*cis*-18 : 2 are summarized in Table 3. About 78–82 % of the total *trans*-18 : 1 isomers were apparently oxidized in dietary groups B–E; this means that only 18–22 % of the total dietary *trans*-18 : 1 isomers were accumulated in the body during the 3 weeks of the experiment. For Δ9*cis*,12*cis*-18 : 2, 67 % of the total dietary intake in dietary group A (the reference diet) was apparently oxidized in contrast with 38–51 % in dietary groups B–E. For Δ9*trans*,12*trans*-18 : 2, about 85–92 % of the dietary intake was apparently oxidized in dietary groups B–E. For Δ9*cis*,13*trans*-18 : 2, 68–72 % of the dietary intake was apparently oxidized. For Δ9*cis*,12*trans*-18 : 2 and Δ9*trans*,12*cis*-18 : 2, the apparent oxidation was similar (71–78 % of the dietary intake). Overall, about 72–76 % of the total dietary intake of *cis*,*trans*-18 : 2 isomers were apparently oxidized (Table 3).

Compared with that of Δ9*cis*,12*cis*-18 : 2, the apparent oxidation of all *cis*,*trans*-18 : 2 isomers was greater (72–76 % *v.* 38–51 %), but it was similar to that of total *trans*-18 : 1 isomers except for Δ9*trans*,12*trans*-18 : 2 (Table 3). Among four *cis*,*trans*-18 : 2 isomers, the apparent oxidation of Δ9*trans*,12*trans*-18 : 2 was greatest, while those of Δ9*cis*,13*trans*-18 : 2, Δ9*cis*,12*trans*-18 : 2 and Δ9*trans*,12*cis*-18 : 2 were very similar.

Two *cis*,*trans*-18 : 2 isomers, Δ9*cis*,13*trans*-18 : 2 and Δ9*cis*,12*trans*-18 : 2, can be chain-elongated and desaturated to form Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4 and Δ5*cis*,8*cis*,11*cis*,14*trans*-20 : 4 respectively (Kwan *et al.* 1998; Ratnayake *et al.* 1994). Among groups B–E the whole-body partitioning of Δ9*cis*,13*trans*-18 : 2 was 68–72 % to apparent oxidation, 25–31 % to accumulation, and 0·9–2·7 % to the LCP. Similarly, the whole-body partitioning of Δ9*cis*,12*trans*-18 : 2 was 71–76 % to apparent oxidation, 23–28 % to accumulation, and 0·2–1·8 % to LCP. In contrast, the whole-body partitioning of Δ9*cis*,12*cis*-18 : 2 in groups B–E was 38–51 % to apparent oxidation, 3–17 % to accumulation and 31–57 % to LCP corresponding to its amount in diet, i.e. a higher amount in the diet is associated with a higher percentage partitioning to apparent oxidation and a lower percentage partitioning to accumulation.

The accumulation of LCP for *cis*,*trans*-18 : 2 isomers decreased with increasing dietary Δ9*cis*,12*cis*-18 : 2 (Table 3). Accumulation of the LCP (Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4) derived from Δ9*cis*,13*trans*-18 : 2 decreased from 22 to 7 mg/rat when dietary Δ9*cis*,12*cis*-18 : 2 was increased from 0·56 to 1·81 % total energy. Similarly, the accumulation of the LCP (Δ5*cis*,8*cis*,11*cis*,14*trans*-20 : 4) derived from Δ9*cis*,12*trans*-18 : 2 decreased from 11 to < 1 mg/rat when Δ9*cis*,12*cis*-18 : 2 was gradually increased in the diet.

Discussion

The balance method has been shown to be as accurate as indirect calorimetry for determining energy expenditure (Rothwell & Stock, 1982). The present study applied the balance method to the estimation of the apparent oxidation of *cis*,*trans*-18 : 2 isomers relative to that of Δ9*cis*,12*cis*18 : 2. We are unaware of any studies using either radiolabelled fatty acids as tracers, or other methods of estimating whole-body oxidation and accumulation of *cis*,*trans*-18 : 2 isomers. The present study was restricted to the estimation of whole-body partitioning of *cis*,*trans*-18 : 2 isomers (accumulation, elongation and desaturation to LCP and apparent oxidation) relative to that for Δ9*cis*,12*cis*-18 : 2 and *trans*-18 : 1 isomers, because none of these fatty acids can be synthesized by mammals and their intake represents only the net change in their availability. The estimation of whole-body partitioning of saturates and monounsaturates (18 : 0, Δ9*cis*-18 : 1, 16 : 0, Δ9*cis*-16 : 1) is inappropriate, because it is impossible to differentiate the proportion diverting to oxidation and accumulation from the diet or *de novo* synthesis.

The present study demonstrated that whole-body partitioning of *cis*,*trans*-18 : 2 isomers is different from that of Δ9*cis*,12*cis*-18 : 2. First, 72–76 % of the dietary *cis*,*trans*-18 : 2 isomers but only 41–52 % of the dietary Δ9*cis*,12*cis*-18 : 2 in groups B–E was diverted to oxidation ($P < 0·05$; Table 3), suggesting that *cis*,*trans*-18 : 2 isomers are more likely to be oxidized than their *cis* counterpart Δ9*cis*,12*cis*-18 : 2. Second, < 3 % of the dietary Δ9*cis*,13*trans*-18 : 2 and Δ9*cis*,12*trans*-18 : 2 were diverted to LCP as compared with dietary Δ9*cis*,12*cis*-18 : 2, 31–57 % of which was chain-elongated and desaturated to LCP in groups B–E ($P < 0·05$); this finding indicates that Δ9*cis*,12*cis*-18 : 2 is a better substrate than *cis*,*trans*-18 : 2

Table 3. Intake, whole-body accumulation and apparent oxidation of linoleic acid (Δ9*cis*,12*cis*-18 : 2), total *trans*-18 : 1 and *cis-trans*-18 : 2 isomers during a 21 d experimental period in rats fed diets containing non-hydrogenated (diet A) or partially hydrogenated rapeseed oil with 1·72 % energy as *cis*,*trans*-18 : 2 isomers (diet B) and varying amounts of Δ9*cis*,12*cis*-18 : 2 (diets C–E)

(Mean values and standard deviations)

	Initial intake (mg/rat) *		Final intake (mg/rat)		Accumulated (mg/rat)		Ingested (mg/rat)†		Accumulated as LCP (mg/rat)‡		Oxidized			
											mg/rat		% ingested§	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diet A														
trans-18 : 1	39	22	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Δ9*cis*,12*cis*-18 : 2	760	67	3417	613	2657	476	11316	2241	1125	178	7534	669	67	6
Δ9*trans*,12*cis*-18 : 2	< 1	–	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Δ9*cis*,13*trans*-18 : 2	< 1	–	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Δ9*cis*,12*trans*-18 : 2	4	1	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Δ9*trans*,12*cis*-18 : 2	4	1	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Total *cis*,*trans*-18 : 2	9	2	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
Diet B														
trans-18 : 1	39	22	2623	341	2593	336	14047	2583	0	–	11454	422	82^{ab}	3
Δ9*cis*,12*cis*-18 : 2	760	67	786	87	26	4	996	189	575	104	396	129	40^{d}	13
Δ9*trans*,12*cis*-18 : 2	< 1	–	20	14	19	13	159	30	0	–	140	13	88^{a}	9
Δ9*cis*,13*trans*-18 : 2	< 1	–	202	39	201	38	791	150	22	5	568	41	72^{c}	5
Δ9*cis*,12*cis*-18 : 2	4	1	147	29	144	27	616	117	11	2	461	32	75^{bc}	5
Δ9*trans*,12*cis*-18 : 2	4	1	170	25	166	23	752	142	0	–	586	68	78^{b}	3
Total *cis*,*trans*-18 : 2	9	2	539	93	530	91	2318	436	34	6	1754	72	76^{b}	3
Diet C														
trans-18 : 1	39	22	3221	629	3182	621	14314	2871	0	–	11132	566	78^{b}	4
Δ9*cis*,12*cis*-18 : 2	760	67	939	151	242	39	1641	328	784	171	615	209	38^{d}	15
Δ9*trans*,12*cis*-18 : 2	< 1	–	16	10	15	9	151	29	0	–	136	13	90^{a}	13
Δ9*cis*,13*trans*-18 : 2	< 1	–	245	64	244	63	787	157	15	5	528	58	67^{c}	8
Δ9*cis*,12*trans*-18 : 2	4	1	163	46	159	46	621	124	7	2	455	50	73^{bc}	8
Δ9*trans*,12*cis*-18 : 2	4	1	209	44	205	42	768	154	0	–	563	46	74^{bc}	6
Total *cis*,*trans*-18 : 2	9	2	633	156	623	151	2328	465	23	6	1682	167	72^{bc}	7
Diet D														
trans-18 : 1	39	22	2876	543	2837	536	13892	2926	0	–	11055	557	80^{ab}	4
Δ9*cis*,12*cis*-18 : 2	760	67	102	164	266	43	2061	431	844	172	951	310	46^{c}	15
Δ9*trans*,12*cis*-18 : 2	< 1	–	21	16	20	13	131	28	0	–	111	20	85^{a}	15
Δ9*cis*,13*trans*-18 : 2	< 1	–	226	49	225	48	735	154	13	5	497	52	68^{b}	7
Δ9*cis*,12*trans*-18 : 2	4	1	16	34	166	33	580	122	5	2	409	36	71^{b}	6
Δ9*trans*,12*cis*-18 : 2	4	1	184	39	180	39	719	151	0	–	539	37	75^{b}	5
Total *cis*,*trans*-18 : 2	9	2	600	122	591	119	2166	455	18	7	1557	129	72^{b}	6
Diet E														
trans-18 : 1	39	22	2818	496	2779	515	13527	3114	0	–	10748	541	80^{b}	4
Δ9*cis*,12*cis*-18 : 2	760	67	1349	208	589	91	3470	764	1108	215	1773	348	51^{c}	10
Δ9*trans*,12*cis*-18 : 2	< 1	–	14	11	13	9	153	34	0	–	140	20	92^{a}	11
Δ9*cis*,13*trans*-18 : 2	< 1	–	209	54	208	43	752	165	7	4	537	54	71^{b}	7
Δ9*cis*,12*trans*-18 : 2	4	1	147	57	143	56	588	129	1	1	444	50	76^{b}	10
Δ9*trans*,12*cis*-18 : 2	4	1	175	39	171	39	724	159	0	–	553	37	77^{b}	5
Total *cis*,*trans*-18 : 2	9	4	545	138	535	136	2217	487	8	5	1674	133	76^{b}	6

LCP, longer-chain products; ND, not determined.

[a,b,c,d]Means within a dietary group with different superscript letters were significantly different ($P < 0·05$).

* The initial values are mean of six rats killed before the experimental period, while the remaining values were means for ten rats.

† Assuming that 2 % of the dietary fatty acids was excreted.

‡ LCP of linoleic acid (Δ9*cis*,12*cis*-18 : 2) include Δ6*cis*,9*cis*,12*cis*-18 : 3, Δ11*cis*,14*cis*-20 : 2, Δ8*cis*,11*cis*,14*cis*-20 : 3, Δ5*cis*,8*cis*,11*cis*,14*cis*-20 : 4, Δ7*cis*,10*cis*,13*cis*,16*cis*-22 : 4 and Δ4*cis*,7*cis*,10*cis*,13*cis*,16*cis*-22 : 5. LCP of Δ9*cis*,13*trans*-18 : 2 includes Δ5*cis*,8*cis*,11*cis*,15*trans*-20 : 4. LCP of Δ9*cis*,12*trans*-18 : 2 includes Δ5*cis*,8,*cis*,11*cis*,14*trans*-20 : 4.

§ Oxidized (% ingested) = (intake − excretion − accumulation − LCP)/intake × 100.

isomers for desaturase and elongase. Third, 22–24 % of the total dietary *cis*,*trans*-18 : 2 isomers was accumulated in the body. In contrast, only 3–17 % of the dietary Δ9*cis*,12-*cis*-18 : 2 was retained in the body. Among the four *cis*,*trans*-18 : 2 isomers, it appears that the apparent oxidation of Δ9*trans*,12*trans*-18 : 2 was greater than that of the other three isomers including Δ9*cis*,13*trans*-18 : 2, Δ9*cis*,12*trans*-18 : 2 and Δ9*trans*,12*cis*-18 : 2.

The oxidation of *trans*-fatty acids *in vivo* and *in vitro* has been studied extensively by using radioisotopic methods (Lawson & Holman, 1981; Menon & Dhopeshwarkar, 1983; Ide & Sugano, 1984; Lanser *et al.* 1986; Delany & Bray, 1993; Berdeaux *et al.* 1998; Bretillon *et al.* 1998; Beyer & Emken, 1991). Lawson & Holman (1981) found that rat heart and liver mitochondria oxidized *cis*-18 : 1 isomers significantly more rapidly than their respective *trans*-isomers. Similarly, Lanser *et al.* (1986) demonstrated that oleic acid was oxidized 35–40 % faster than elaidic acid by rat

heart homogenates, whereas human heart homogenates oxidized these fatty acids at similar rates. In contrast, Ide & Sugano (1984) found that perfused liver oxidized 9*trans*-octadecenoic acid more rapidly than its *cis*-isomer. These conflicting reports clearly suggest that it is inappropriate to compare these published data; Lawson & Holman (1981) and Lanser *et al.* (1986) used isolated mitochondria or heart and liver homogenates, whereas Ide & Sugano (1984) used the perfused liver, which is more relevant physiologically than the former. The present results are in agreement with those of Ide & Sugano (1984), who also demonstrated that *trans*-octadecadienoic acid is oxidized faster than the *cis*-isomer. This finding further demonstrated that isolated mitochondria and organs may not necessarily reflect the complex interplay of the various metabolic pathways involved in utilization of a fatty acid in an intact animal. Although the balance method used in the present study may have many limitations, it measures the oxidation of *trans*-fatty acids in intact animals and also over a longer period.

Failure to measure the amount of *cis*,*trans*-18 : 2 isomers, *trans*-18 : 1 isomers and Δ9*cis*,12*cis*-18 : 2 present in the digestive system and faeces may lead to overestimation of their accumulation and apparent oxidation. However, the animals were killed after overnight fasting and it was assumed that the digestion and absorption of dietary fat were completed. The present study did not measure the faecal excretion of *cis*,*trans*-18 : 2 isomers, *trans*-18 : 1 isomers or Δ9*cis*,12*cis*-18 : 2. We assume that this factor would not significantly affect the estimation. First, although it cannot transfer directly to the present study, our previous study showed that faecal excretion is $< 2\%$ of the dietary total fat (Yang *et al.* 1994) and it would not significantly affect the estimation of apparent oxidation of these *cis*,*trans*-18 : 2 isomers (2 % intake was lost in faeces *v.* 72–77 % intake was oxidized). Second, the objective of the study was to compare the apparent oxidation of *cis*,*trans*-18 : 2 isomers relative to that of Δ9*cis*,12*cis*-18 : 2 and *trans*-18 : 1 isomers. No data have shown that *cis*,*trans*-18 : 2 isomers were absorbed differently from *trans*-18 : 1 isomers, Δ9*cis*,12*cis*-18 : 2 and other fatty acids. Regarding the effectiveness of extraction, first, previous reports have shown that using methanol–chloroform (2 : 1, v/v) could yield $> 95\%$ recovery of tissue lipids (Folch *et al.* 1957; Ways & Hanahan, 1964; Christie, 1982). Second, there have been no data showing that the effectiveness of extraction of *cis*,*trans*-18 : 2 isomers is different from that of Δ9*cis*,12*cis*-18 : 2 and *trans*-18 : 1 isomers, and so relative oxidation of *cis*,*trans*-18 : 2 isomers would be underestimated to the same extent as that for Δ9*cis*,12*cis*-18 : 2 and *trans*-18 : 1 isomers, if indeed there is any deviation.

The reliability of balance method also depends on accurate GLC measurement of fatty acids in the diet, initial and final body content, and their corresponding LCP. The instrumental error for GLC analysis of Δ9*cis*,12*cis*-18 : 2, total Δ9*cis*,12*cis*-18 : 2 isomers and *trans*-18 : 1 fatty acids in groups B–E was $< 2\%$. In contrast, it was $> 10\%$ for Δ9*cis*,12*cis*,15*cis*-18 : 3 due to a trace amount of this fatty acid in the PHRO. Thus, the reliability of balance method may not apply to the minor fatty acids that cannot be measured accurately by GLC analysis. In this regard, the apparent oxidation and accumulation of *cis*,*trans*-18 : 2 isomers and *trans*-18 : 1 fatty acids were not determined in rats fed diet A containing the non-hydrogenated rapeseed oil, because the quantities of these fatty acids are relatively minor. For the same reason, the apparent oxidation of Δ9*cis*,12*cis*,15*cis*-18 : 3 was not estimated in rats fed diets B–E, because it only accounted for $< 0{\cdot}01\%$ of the total fat. In contrast, for diets B–E containing PHRO in which *trans*-18 : 1 fatty acids and *cis*,*trans*-18 : 2 isomers were 23 and 5·5 % of the total fatty acids respectively, GLC analysis is sensitive enough to measure them accurately. Thus, the data on apparent oxidation of these fatty acids should be reliable. It was noticed that the animals showed no sign of *n*-3 fatty acid deficiency during the 3-week balance period, although diets B–E were low in Δ9*cis*,12*cis*,15*cis*-18 : 3.

From the results for whole-body fatty acid balance presented here, we conclude that most of dietary *cis*,*trans*-18 : 2 isomers ($> 70\%$) is diverted to oxidation, $< 24\%$ is accumulated in the body and only a small percentage (< 3) is desaturated and chain-elongated to the unusual *trans*-LCP.

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