

## Gender-related response of lipid metabolism to dietary fatty acids in the hamster

Anne Morise<sup>1</sup>, Jacques Mourot<sup>2</sup>, Carole Boué<sup>3</sup>, Nicole Combe<sup>3</sup>, Ghislaine Amsler<sup>1</sup>, Daniel Gripois<sup>1</sup>, Annie Quignard-Boulangé<sup>4</sup>, Laurent Yvan-Charvet<sup>4</sup>, Evelyne Féart<sup>5</sup>, Pierre Weill<sup>6</sup> and Dominique Hermier<sup>7\*</sup>

<sup>1</sup>Laboratoire d'Endocrinologie de la Nutrition, Université Paris Sud, 91405 Orsay Cedex, France

<sup>2</sup>INRA, SENAH, Saint-Gilles, 35590 L'Hermitage, France

<sup>3</sup>ITRG-Nutrition, 35405 Talence, France

<sup>4</sup>INSERM 465, 75005 Paris, France

<sup>5</sup>ONIDOL, 75008 Paris, France

<sup>6</sup>La Messayais, 35210 Combourtille, France

<sup>7</sup>UMR Physiologie de la Nutrition et du Comportement Alimentaire, INRA-INA-PG, 16 rue Claude Bernard, 75231 Paris Cedex 05, France

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Gender and dietary fatty acids are involved in the regulation of lipid metabolism, disturbances of which can lead to pathologies such as metabolic syndrome or CVD. Possible interactions between these factors were investigated in male and female hamsters fed diets rich in either saturated fatty acids ('butter' diet) or in  $\alpha$ -linolenic acid ('linseed oil' diet). Gender effect predominated over the diet effect on cholesterol (CH) metabolism; compared to males, females exhibited lower concentrations of plasma total CH ( $-20\%$ ,  $P < 0.001$ ), LDL-CH ( $-40\%$ ,  $P < 0.001$ ) and HDL-CH ( $-16\%$ ,  $P < 0.001$ ), together with higher LDL receptor ( $+40\%$ ) and lower HDL receptor ( $-60\%$ ) hepatic content. Triacylglycerol (TG) metabolism was affected by diet above all: compared to animals fed the 'butter' diet, those fed the 'linseed oil' diet exhibited lower plasma ( $-23\%$ ,  $P = 0.046$ ) and liver TG ( $-20\%$ ,  $P = 0.026$ ) concentration which may result from both an increased  $\beta$ -oxidation ( $P < 0.001$ ), without any change in PPAR $\alpha$  mRNA, and a decreased hepatic lipogenesis ( $P = 0.023$ ), without increased sterol response element binding protein 1c (SREBP1c) mRNA. The response to diet was much more pronounced in males than in females, without gender effect on the transcription level of PPAR $\alpha$  and SREBP1c. Finally, the 'linseed oil' diet decreased the insulin resistance index ( $-80\%$ ,  $P < 0.001$ ) with a more marked effect in males, in relation to their higher hepatic PPAR $\gamma$  expression ( $+90\%$ ,  $P = 0.012$ ). In conclusion, in our model, the response of either TG or CH to dietary fatty acids is modulated differently by gender. The possible relevance of these interactions to dietary practice should be taken into account in man.

### $\alpha$ -Linolenic acid: Gender: Lipid metabolism: Dietary fatty acids

Gender-associated responsiveness to diet has been involved in various pathologies linked to a disturbance of lipid homeostasis such as metabolic syndrome (Couillard *et al.* 1999), insulin resistance (Noonan & Banks, 2000) or CVD (Cobb *et al.* 1993; Li *et al.* 2003). Lipid metabolism is clearly influenced by sex hormones. Compared to non-menopausal women, men have a more marked accumulation of fat in the intra-abdominal depots, which results in disturbances of lipid homeostasis and contributes to insulin resistance (Couillard *et al.* 1999). Men exhibit also a more atherogenic lipoprotein profile with higher concentrations of plasma LDL-cholesterol (LDL-CH) and triacylglycerols (TG) and a lower concentration of HDL-CH (Carlson & Ericsson, 1975; Bonithon-Kopp *et al.* 1990; Couillard *et al.* 1999; van Beek *et al.*

1999). Before their menopause, women are therefore less susceptible to lipid-associated pathologies. For instance, the life-time risk of developing a CHD at age 40 years is 33% in women *v.* 50% in men (Lloyd-Jones *et al.* 1999). After menopause, their lipoprotein profile becomes similar to that of men (Bonithon-Kopp *et al.* 1990; van Beek *et al.* 1999). These modifications are probably due to the lack of oestrogen (Stampfer *et al.* 1991), and is attenuated by estrogenic treatment (Matthews *et al.* 1989).

Lipid homeostasis is not only modulated by sex hormones, but also by dietary fatty acids. The beneficial role of PUFA, and notably *n*-3 PUFA, has been largely described, especially through the regulation of genes controlling lipid metabolism (De Caterina & Zampolli, 2001). Therefore, interactions of

**Abbreviations:** AA, arachidonic acid; ACC, acetyl CoA carboxylase; ACO, acyl CoA oxidase; ALA,  $\alpha$ -linolenic acid; CE, cholesteryl ester; CH, cholesterol; CPT, carnitine palmitoyl transferase; FAS, fatty acid synthase; FC, free cholesterol; G6PDH, glucose-6-phosphate dehydrogenase; HOMA, homeostasis model assessment; LA, linoleic acid; PL, phospholipid; SFA, saturated fatty acids; SR-B1, scavenger receptor class B type 1; SREBP, sterol response element binding protein; TC, total cholesterol; TG, triacylglycerol.

\* **Corresponding author:** Dr Dominique Hermier, fax +33 1 44 08 72 46, email [hermier@inap.inra.fr](mailto:hermier@inap.inra.fr)

dietary fatty acids with sex hormones in lipid metabolism are most likely, but remain poorly known. A meta-analysis of five human studies using high and low PUFA to saturated fatty acids (SFA) ratios concluded that normolipidaemic men and women show similar reduction in LDL-CH following dietary change from a high to a low SFA to PUFA ratio. By contrast, the response of HDL-CH was gender-specific, with females showing a greater fall in the potentially beneficial HDL-CH than males (Cobb *et al.* 1993). In response to dietary SFA and CH restriction, mildly hypercholesterolaemic men showed a more pronounced reduction in CH and plasma apoB than post-menopausal women (Li *et al.* 2003).

The above studies were performed in human subjects, which did not enlighten about the mechanisms by which gender influences the response of lipid metabolism to dietary fatty acids. Relevant animal studies are few and focused, as in man, on the lipoprotein profile of the guinea pig (Fernandez, 2001) and the hamster (Wilson *et al.* 1999; Morise *et al.* 2004b). The present study was therefore designed to compare, in male and female rodents, the response of fatty acid and lipid metabolism to dietary fatty acids. The hamster has been chosen as a validated model for assessing the effect of dietary fat on lipid metabolism (Surette *et al.* 1992; Goulinet & Chapman, 1993; Spady *et al.* 1999). The fatty acid profiles of the diets were radically different: the first one was rich in SFA (of which palmitic acid accounted for more than 50%) and thus representative of atherogenic diets consumed in western countries. The second one was rich in  $\alpha$ -linolenic acid (ALA). Indeed, even if long-chain *n*-3 PUFA are best known to be protective against CVD (De Caterina & Zampolli, 2001), some similarities in the precursor and its long-chain derivatives can be found in the literature as regards their effects on lipid risk factors of CVD as well as on CVD epidemiology. Indeed, in man, dietary ALA intake was associated with: (1) bioconversion into 20:5*n*-3, which improves the balance of eicosanoids derived from 20:5 fatty acids (Pawlosky *et al.* 2001); (2) decreased triglyceridaemia in some patients exhibiting CVD risk factors (Wilkinson *et al.* 2005) or in an epidemiological survey (Djousse *et al.* 2003); and (3) protective effect on the relative risk from fatal CHD (Ascherio *et al.* 1996; Hu *et al.* 1997). Besides, in the male hamster, dietary ALA has been shown to reduce hepatic lipogenesis and triglyceridaemia (Morise *et al.* 2005). The aim of the study was to determine (1) whether the lipoprotein profile and the lipid risk factors of metabolic syndrome and CVD were influenced by fatty acids to the same extent in females as in males, and (2) which were the key enzymes and compartments of lipid metabolism, and the major transcription factors involved in the regulation of lipid metabolism by dietary fatty acids, that are involved in the gender-related differences.

## Experimental methods

### Experimental procedure

**Animals.** Twenty-six male and twenty-six female golden Syrian hamsters were obtained from Janvier (Centre d'élevage Janvier, Le Genest-St Isle, France) at 4 weeks of age. They were housed in colony cages with wood litter (four or five per cage) in a controlled environment (22°C, 14 h light–10 h dark cycle) and received *ad libitum* distilled water, and a ground commercial diet (UAR 113 containing, by weight,

72.0% cereals, 17.8% soy meal, 6.0% fish meal, 4.2% vitamins and minerals mix, and providing 5.1% lipids and 19.3% proteins; UAR 113, Villemoisson, France). At 8 weeks of age, the hamsters were housed in colony cages with a wire floor, weighed weekly and fed experimental diets during 7 weeks.

**Diets.** The two experimental diets consisted (in weight) of 85% of the above commercial ground pellets, and either 12.5% butter and 2.5% sunflower oil in the 'butter' diet, or 12.9% linseed oil (Valorex, Javené, France), and 2.1% water and 0.031% CH (5-cholesten-3 $\beta$ -ol; Sigma, St Louis, MO, USA) in the 'linseed oil' diet. The calculated composition (in weight) of the two diets was 16.4% protein, 52.9% carbohydrate, 17.8% lipid, 10.9% water and 4.6% minerals. Lipids provided about one-third (36.5%) of total energy intake. The lipid and CH content of the diets and their fatty acid profile was determined as described later and are shown in Table 1. The experimental diets were formulated in order to differ mostly in their SFA and ALA content, while keeping oleic and linoleic acid (LA) proportion as close as possible.

**Experimental design.** Five days before the hamsters were killed, four per group were housed in individual cages with a wire floor in order to harvest their faeces. They received a single dose of two radiolabelled sterols ([<sup>14</sup>C]CH and [<sup>3</sup>H] $\beta$ -sitostanol) to measure their dietary CH absorption as described later. The others (nine in each group) were kept in colony cages. At 15 weeks of age, after 7 weeks on the experimental diets, all hamsters were fasted overnight, then weighed and anaesthetized by intramuscular injection of Zoletil 50 (Virbac, Carros, France) at a dose of 4 mg/100 g body weight. First, a limited blood sample (about 250  $\mu$ l) was taken as fast as possible by intracardiac puncture, using a heparinized syringe (10 units heparin/ml blood), for determination of glucose and insulin concentrations, which are very sensitive to the stress induced by anaesthesia. Immediately

**Table 1.** Lipid, cholesterol and vitamin E content and fatty acid profile of the experimental diets

	'Butter' diet	'Linseed oil' diet
Lipid content (% by wt of the diet)	14.6	14.8
Cholesterol (% by wt of the diet)	0.068	0.067
Vitamin E (mg/kg)	10.2	8.6
Fatty acid (% of total fatty acids)		
10:0	1.01	ND
12:0	2.37	ND
14:0	8.25	0.14
14:1	0.66	ND
15:0	0.79	ND
15:1	0.18	ND
16:0	24.31	7.68
16:1	1.12	0.20
17:0	0.49	0.10
18:0	7.92	3.82
18:1 <i>n</i> -9	24.60	19.00
18:1 <i>n</i> -7	0.49	0.49
18:2 <i>n</i> -6t	0.35	ND
18:2 <i>n</i> -6	22.72	24.11
18:3 <i>n</i> -3	1.91	43.78
20:0	0.17	0.15
20:1 <i>n</i> -9	0.55	0.26
20:5 <i>n</i> -3	0.30	0.24
22:6 <i>n</i> -3	0.25	0.23
<i>n</i> -6: <i>n</i> -3	9.27	0.55

ND, not detected.

after, a larger blood sample was taken under the same conditions. Plasma was separated from blood by centrifugation for 20 min at 4°C and 1700g then stored at -20°C. Erythrocytes were washed with saline, and stored at -80°C. After blood sampling, the abdominal cavity was opened surgically and bile was taken from the gall bladder and stored at -20°C. Liver was then carefully removed and weighed. Two samples of 1.0 and 0.3 g were immediately frozen in liquid N<sub>2</sub> for the measurement of lipogenic enzyme activity and RNA extraction, respectively. Two other samples of 0.3 and 0.5 g were stored at -20°C for further analysis of lipid composition and quantification of lipoprotein receptors, respectively. A 1 g sample was kept on ice for immediate isolation of microsomes, on the one hand, and mitochondria + peroxisomes, on the other hand (Souidi *et al.* 1999). Hamsters were then killed by section of the jugular vein. A red muscle (*Vastus lateralis*) was removed from the left leg and frozen immediately in liquid N<sub>2</sub> for measurement of  $\beta$ -oxidation activity. Gonadal adipose tissue were removed and weighed. Lipids from plasma and liver were analysed in all hamsters (thirteen per group) in order to check whether the type of housing could modify lipid metabolism. In this respect, hamsters kept in collective cages (nine per group) did not differ significantly from those placed in individual cages for 5 d in order to measure CH absorption (four per group). However, results in the text and the corresponding tables and figures are from the nine hamsters kept in collective housing. All other biochemical measurements were performed in hamsters kept in collective cages throughout (*n* 9). The present work was carried out in agreement with the French legislation on animal experimentation and with the authorization of the French Ministry of Agriculture (Animal Health and Protection Directorate).

### Analyses

**Lipid components and fatty acid profile.** *Diet:* lipids were extracted according to Folch's method (Folch *et al.* 1957), dried under N<sub>2</sub> and weighed for the calculation of total lipid content. They were then trans-methylated with BF<sub>3</sub> in methanol (14%) according to the method of Morisson & Smith (1964). Fatty acid methyl esters were analysed by GLC (Fisons 8000; Thermo Products, Les Ulis, France) as described previously (Morise *et al.* 2004a). Neutral sterols (containing CH) were extracted with petroleum ether after hydrolysis in basic conditions and CH was separated and quantified by GLC as described by Boehler *et al.* (1999). Vitamin E content in the diets was determined as described by the international standard organization (ISO9936:1997).

**Erythrocytes:** 1.5 ml erythrocytes were treated according to the method of Folch *et al.* (1957). Total lipids were separated by TLC on 0.3 mm thick silica-gel (Kieselgel H 60; Merck, Darmstadt, Germany) laboratory-coated plates using the solvent mixture hexane-diethyl ether-acetic acid (90:10:1, by vol.). Fatty acid methyl esters from phospholipids (PL) were prepared by trans-methylation of individually isolated fractions in the presence of silica-gel and in the same conditions as described by Morise *et al.* (2004a). Fatty acid methyl esters were analysed according to Boué *et al.* (2000).

**Dietary cholesterol absorption.** Dietary CH absorption was measured in four hamsters per group, according to the method of radioactive faecal balance (Quintao *et al.* 1971) adapted to the

hamster. This method is based on the ingestion of a single dose of two radiolabelled sterols (2  $\mu$ Ci [<sup>14</sup>C]CH (Perkin Elmer Life Sciences, Boston, MA, USA) and 1  $\mu$ Ci [<sup>3</sup>H] $\beta$ -sitostanol (American Radiolabeled Chemicals, St Louis, MO, USA)) incorporated into the diet. Since  $\beta$ -sitostanol is considered not to be absorbed significantly, it was used as internal standard for CH absorption calculation according to the formulae:

$$\text{Dietary CH absorption} = 1 - ((^3\text{H}/^{14}\text{C})_{\text{diet}} / (^3\text{H}/^{14}\text{C})_{\text{faeces}}).$$

The <sup>3</sup>H/<sup>14</sup>C ratio in faeces was determined after extraction of neutral sterol as described for dietary CH (Boehler *et al.* 1999).

**Biochemical analyses. Plasma composition:** glycaemia was determined colorimetrically using an enzymatic kit (Biochem, Aix-en-provence, France; Trinder, 1969) and insulinemia using a RIA kit (sensitive rat insulin RIA; Linco, St Charles, MO, USA) and validated in the hamster in our laboratory. The insulin resistance index was calculated by the homeostasis model assessment (HOMA; Matthews *et al.* 1985).

TG, total cholesterol (TC) and PL were determined in plasma by colorimetric enzymatic methods (Richmond, 1973; Takayama *et al.* 1977; Fossati & Prencipe, 1982; respectively), using the kits provided by Bio-Merieux (Marcy-l'Etoile, France). Analyses were performed with an automatic analyser (Abbott-VP, Rungis, France). Free cholesterol (FC) was determined manually using a procedure adapted from Richmond (1973). The concentration of cholesteryl esters (CE) was calculated with the formulae: CE = TC - FC.

Plasma vitamin E concentration was measured according to the method of Menke *et al.* (2000). Briefly, after addition of 2,6-di-*tert*-butyl-*p*-cresol, vitamin E was extracted with hexane and separated by HPLC. Quantification was realized by adding  $\alpha$ -tocopherol as internal standard. Plasma malondialdehyde concentration was measured according to the method of Lapenna *et al.* (2001).

**Lipoprotein isolation:** lipoproteins were isolated from plasma by ultracentrifugation in a density gradient, specifically adapted for the hamster (Ferezou *et al.* 1997) and modified by Morise *et al.* (2004b). The density limits (*d*) were as described previously (Morise *et al.* 2004b), i.e. VLDL, *d* < 1.010; intermediate-density lipoprotein, 1.010 < *d* < 1.015; LDL, 1.015 < *d* < 1.050; and HDL, 1.050 < *d* < 1.190. In each lipoprotein class, TC, FC, PL and TG were assayed as in plasma, and proteins were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Lipoprotein concentrations were calculated by summing up the concentrations of the individual protein and lipid components.

**Bile and liver lipids composition:** biliary PL and TC were assayed with the same procedures as described above for plasma lipids, after bile dilution in distilled water (1:10). Bile acids were determined by an enzymatic manual procedure after bile dilution in distilled water (1:20) (Turley & Dietschy, 1978). Lithogenic index was calculated as described previously (Morise *et al.* 2004b).

Hepatic lipids were determined as described above for plasma lipids after extraction in isopropanol from a 150 mg liver sample as described by Loison *et al.* (2002). The pellet was kept and dissolved in 1 M-NaOH for further determination of hepatic proteins according to Lowry *et al.* (1951).

**Enzyme activities.** Hepatic hydroxymethyl-glutaryl CoA reductase (EC 1.1.1.34) activity was determined in



microsomal fractions in the presence of alkaline phosphatase using Phillip and Shapiro's radioisotopic technique (Phillip & Shapiro, 1979).

The activity of the key enzymes of fatty acid oxidation, carnitine palmitoyl transferase (CPT, EC 2.3.1.21) and acyl CoA oxidase (ACO, EC 1.3.3.6), was determined in the hepatic mitochondrial-peroxisomal fraction. CPT activity was also determined in a muscle homogenate as described by Takada *et al.* (1994). Total mitochondrial CPT activity was measured according to Bieber *et al.* (1972). Briefly, the assay is based on the spectrophotometric measurement of reduced CoA resulting from the transformation of palmitoyl CoA into palmitoylcarnitine, which is catalysed by CPT. ACO activity was measured according to Lazarow & De Duve (1976). The assay is based on the measurement of the NADH produced when an acyl CoA is oxidized by ACO. Potassium cyanate was added to the media in order to inhibit mitochondrial oxidation and to measure the peroxisomal activity only. CPT activity was also determined in a muscle homogenate as described by Takada *et al.* (1994).

Activities of the lipogenic enzymes were determined in the liver as described by Mourou *et al.* (2000). Briefly, tissues were homogenized in 0.25 M-sucrose and centrifuged at 40 000 g for 40 min. The supernatant cytosolic fractions were analysed for malic enzyme (EC 1.1.1.40) and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) using modifications (Gandemer *et al.* 1983) of the methods of Fitch *et al.* (1959) and Hsu & Lardy (1969), respectively. Acetyl CoA carboxylase (ACC; EC 6.4.1.2) was assayed by the  $H^{14}CO_3$ -fixation method (Chang *et al.* 1967; Chakrabarty & Leveille, 1969). Fatty acid synthase (FAS; EC 2.3.1.85) was measured as described by Lavau *et al.* (1982). FAS, malic enzyme and G6PDH activities were expressed as  $\mu$ mol NADPH produced or used per min per total liver and per mg protein. ACC activity was expressed as nmol bicarbonate incorporated per min per total liver and per mg of protein.

**Lipoprotein receptor quantification.** Relative hepatic amounts of LDL receptor and scavenger receptor class B type I (SR-BI), shown to be an HDL receptor (Acton *et al.* 1996), were determined as described previously (Milliat *et al.* 2000). Briefly, Western blots of membrane proteins from hamster liver were performed using polyclonal antibodies raised in the rabbit against the LDL receptor of the bovine adrenal cortex (gift from P. Roach, Adelaide, Australia) and against the terminal part of murine SR-BI (fifteen amino acids, gift from A. Mazur, Theix, France). The detection was achieved in a chemiluminescence reagent (ECL; Amersham, Little Chalfont, UK). Films were scanned with a laser densitometer (Ultrascan 2222; LKB, Bromma, Sweden). Peak areas allowed quantification of the antigen response. Receptor levels were expressed in arbitrary units, and the value 100 was arbitrarily attributed to the group with the highest value.

**RNA analysis.** Total RNA was isolated from liver using a TRIzol kit (Invitrogen, Carlsbad, CA, USA) adapted from the Chomczynski and Sacchi method (Chomczynski *et al.* 1986). cDNA was synthesized from 1  $\mu$ g total RNA using RT Superscript II RNase (Invitrogen) in the conditions described by the manufacturers. To check whether any contamination with genomic DNA occurred, a control without RT Superscript II was included for each RNA sample. Real-time quantitative RT-PCR analyses were performed starting with 25 ng

reverse-transcribed total RNA, in a final volume of 20  $\mu$ l, using a qPCR Master Mix for Sybr Green I No ROX kit (Eurogentech, Angers, France) in a thermocycler MyiQ Real Time detection system (Biorad, Marnes-La-Coquette, France). Optimized PCR consisted of forty cycles of amplification (95°C for 30 s, 60°C for 30 s and 72°C for 30 s). Relative quantification for a given gene was calculated after normalization to 18S ribosomal RNA. Design of specific primers was performed using Applied Biosystem (Foster City, Canada) software. Sense and antisense primers were: GAGA-AAGCAAAGTCAAAGCAGAGA and GAAGGGCGGGT-TATTGCTG for PPAR $\alpha$ , CACTCCCATTCCCTTTGACATCA and AGCTGGGCCTTTTCAGAATAATAA for PPAR $\gamma$ ; GAACATCTCTTGGAGAGAGACTG and GGTGGAAGC-CATGCTGGA for sterol response element binding protein 1c (SREBP1c); TGATGATGGGACAAGAGAAAGTTC and TTTGTGCATCTTGGCATCTGTT for SREBP2, GTGGGC-CTGCGGCTTAAT and GCCAGAGTCTCGTTCGTTATC for 18S; GCAACTCAATGATGCCGAGTT and CGTGGGA-ACATCAGTCGGTC for liver X receptor  $\alpha$ ; and AGGCTGC-AAGGCTTTCTTCA and CTCTCCGGTCTTTCCGTATCC for oestrogen receptor.

#### Statistical analyses

Power analysis was calculated on the basis of a two-way ANOVA by using the Power and Precision program (SPSS Inc., Chicago, IL, USA). Main outcome values were plasma risk factors of metabolic syndrome and CVD, i.e. concentrations of TG, CH, LDL-CH and HDL-CH. Data were analysed using the Statview 4.5 program (Abacus Concept, Berkeley, CA, USA). Differences between treatments were assayed with a two-way ANOVA with diet and gender as factors. Differences between means of the four groups were determined by *post hoc* Fisher test and considered to be significant at  $P < 0.05$ .

## Results

### Body weight and adiposity

At the beginning of the experiment (8 weeks of age), males were 6% heavier than females, whereas they were 19% lighter at the end (13 weeks of age) (Table 2). As a consequence, body weight gain was much more important for females than males (43 v. 17 g). Diet, however, affected neither body weight, nor the total and relative weight of gonadal adipose tissue.

### Fatty acid incorporation into erythrocytes

As expected, the fatty acid profile of erythrocyte PL exhibited a high PUFA content (40%), most of them from the *n*-6 family (Table 3). Diet influenced the proportion of all fatty acids: in animals fed the 'butter' diet, SFA, MUFA, *n*-6 PUFA (LA) and more surprisingly DHA, were in higher proportion than in those fed the 'linseed oil' diet, whereas total PUFA, LA and *n*-3 PUFA (except DHA) were in lower proportion. Gender also affected the fatty acid profile of erythrocytes. Females exhibited higher percentages than males of total SFA, ALA and 22:5*n*-3, and lower percentages of

**Table 2.** Effect of gender and diet on body weight gain and adiposity\*  
(Mean values with their standard errors for nine hamsters per group)

	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Initial body wt (g)	85.4 <sup>b</sup>	1.7	84.7 <sup>b</sup>	1.3	91.1 <sup>a</sup>	0.9	90.8 <sup>a</sup>	1.7	<0.001	0.894	0.747
Final body wt (g)	128 <sup>a</sup>	2	129 <sup>a</sup>	3	111 <sup>b</sup>	3	105 <sup>b</sup>	2	<0.001	0.344	0.186
EAT or PUAT wt (g)	1.08 <sup>b</sup>	0.11	1.21 <sup>ab</sup>	0.13	1.52 <sup>a</sup>	0.14	1.34 <sup>ab</sup>	0.08	0.021	0.854	0.201
(% body wt)	0.84 <sup>b</sup>	0.08	0.93 <sup>b</sup>	0.08	1.35 <sup>a</sup>	0.09	1.27 <sup>a</sup>	0.06	<0.001	0.897	0.302

EAT, epididymal adipose tissue; PUAT, periuterine adipose tissue.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>ab</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

MUFA, *n*-6 PUFA, 22:5*n*-6, LA and EPA. However, as concerns *n*-3 PUFA (except DHA), most of these gender-related effects were more pronounced in animals fed the 'linseed oil' diet.

As expected, LA:ALA, arachidonic acid (AA):EPA and *n*-6:*n*-3 PUFA ratios were higher in animals fed the 'butter' diet than in those fed the 'linseed oil' diet. Moreover, LA:ALA and *n*-6:*n*-3 PUFA ratios, but not the AA:EPA ratio, were also higher in males than in females.

#### Plasma parameters

Plasma TG concentration was not affected by gender (Fig. 1(A)). It was lower in animals fed the 'linseed oil' diet than in those fed the 'butter' one, and the difference was more pronounced in males (−58%) than in females (−14%). The variation in VLDL-TG concentration tended to parallel that of total TG,

but the differences were not significant. LDL-TG concentration was not responsive to diet, but was lower in males than in females. This difference was essentially due to the males fed the 'linseed oil' diet, in which LDL-TG concentration was about 40% lower than in the three other groups. As for HDL-TG, neither gender nor diet influenced their concentration. CH concentration was affected by gender mainly (Fig. 1(B)), males exhibiting higher plasma CH (+22%), LDL-CH (+81%) and HDL-CH (+19%) and lower VLDL-CH concentration (−22%) than females. Diet affected only HDL-CH concentration that was lower in 'linseed oil'-fed animals, and the difference was more pronounced in males than in females (−13% *v.* −3%). PL concentration paralleled that of CH; males exhibited higher values of total PL (+18%), LDL-PL (+62%) and HDL-PL (+18%) and lower values of VLDL-PL (−28%) than females (Fig. 1(C)). Moreover, diet affected plasma-, LDL- and HDL-PL concentrations which were,

**Table 3.** Effect of gender and diet on fatty acid composition of erythrocytes (as % total fatty acids)\*

(Mean values with their standard errors for nine hamsters per group)

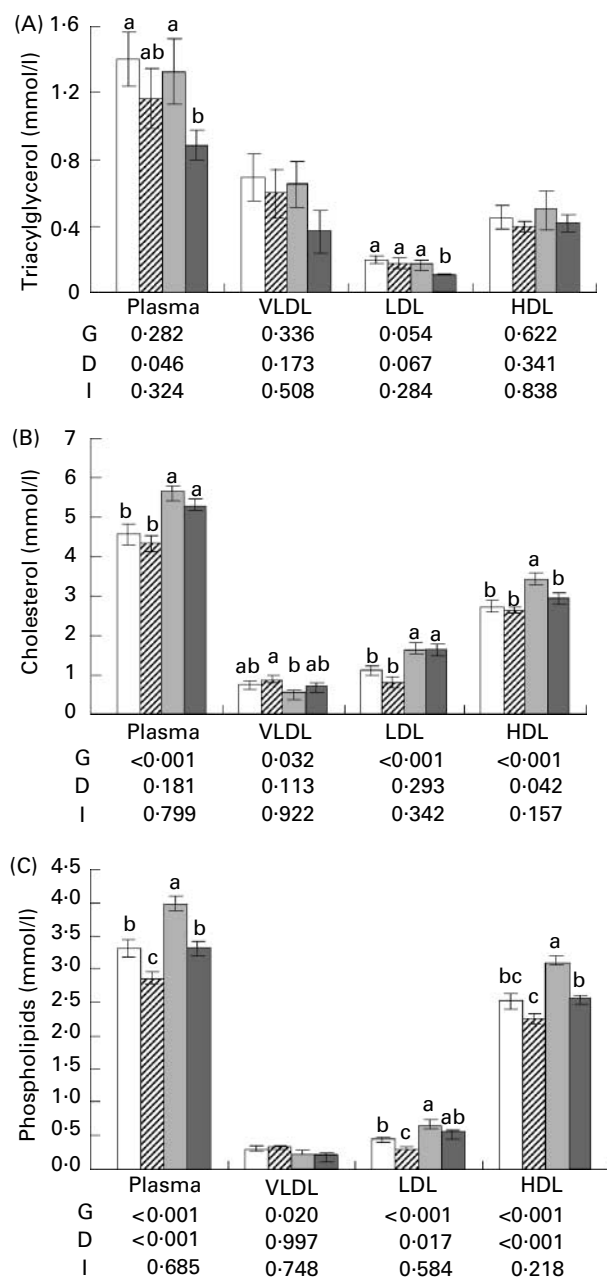
	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
ΣSFA	40.97 <sup>a</sup>	0.56	39.75 <sup>ab</sup>	0.46	39.17 <sup>b</sup>	0.25	38.54 <sup>b</sup>	0.47	0.021	0.047	0.510
ΣMUFA	18.29 <sup>bc</sup>	0.17	17.88 <sup>c</sup>	0.18	19.33 <sup>a</sup>	0.16	18.59 <sup>b</sup>	0.19	<0.001	0.003	0.369
18:1 <i>n</i> -9	13.19 <sup>b</sup>	0.08	12.59 <sup>c</sup>	0.13	13.91 <sup>a</sup>	0.16	13.28 <sup>b</sup>	0.16	<0.001	<0.001	0.907
ΣPUFA	39.94 <sup>c</sup>	0.52	41.89 <sup>ab</sup>	0.44	40.77 <sup>bc</sup>	0.25	42.52 <sup>a</sup>	0.51	0.112	<0.001	0.821
Σ <i>n</i> -6	33.10 <sup>b</sup>	0.36	29.37 <sup>d</sup>	0.30	34.08 <sup>a</sup>	0.18	30.72 <sup>c</sup>	0.21	<0.001	<0.001	0.493
18:2 <i>n</i> -6	15.93 <sup>d</sup>	0.17	17.29 <sup>b</sup>	0.19	16.49 <sup>c</sup>	0.17	18.39 <sup>a</sup>	0.16	<0.001	<0.001	0.131
20:4 <i>n</i> -6	12.80 <sup>a</sup>	0.20	9.63 <sup>b</sup>	0.16	13.24 <sup>a</sup>	0.18	9.92 <sup>b</sup>	0.22	0.068	<0.001	0.693
22:4 <i>n</i> -6	2.61 <sup>a</sup>	0.08	0.84 <sup>b</sup>	0.04	2.60 <sup>a</sup>	0.04	0.94 <sup>b</sup>	0.05	0.363	<0.001	0.332
22:5 <i>n</i> -6	0.37 <sup>a</sup>	0.02	0.15 <sup>b</sup>	0.01	0.39 <sup>a</sup>	0.01	0.17 <sup>b</sup>	0.01	0.040	<0.001	0.752
Σ <i>n</i> -3	6.84 <sup>c</sup>	0.19	12.52 <sup>a</sup>	0.21	6.70 <sup>c</sup>	0.13	11.79 <sup>b</sup>	0.34	0.069	<0.001	0.214
18:3 <i>n</i> -3	0.16 <sup>c</sup>	0.00	3.04 <sup>a</sup>	0.04	0.15 <sup>c</sup>	0.00	2.77 <sup>b</sup>	0.04	<0.001	<0.001	<0.001
20:5 <i>n</i> -3	0.40 <sup>c</sup>	0.01	1.64 <sup>b</sup>	0.03	0.44 <sup>c</sup>	0.01	1.77 <sup>a</sup>	0.06	0.022	<0.001	0.192
22:5 <i>n</i> -3	1.32 <sup>c</sup>	0.05	4.08 <sup>a</sup>	0.07	1.53 <sup>c</sup>	0.02	3.58 <sup>b</sup>	0.11	0.052	<0.001	<0.001
22:6 <i>n</i> -3	4.96 <sup>a</sup>	0.14	3.76 <sup>b</sup>	0.09	4.58 <sup>a</sup>	0.11	3.67 <sup>b</sup>	0.16	0.069	<0.001	0.265
18:2 <i>n</i> -6:18:3 <i>n</i> -3	102.2 <sup>b</sup>	2.3	5.7 <sup>c</sup>	0.1	108.2 <sup>a</sup>	2.1	6.6 <sup>c</sup>	0.1	0.033	<0.001	0.114
<i>n</i> -6: <i>n</i> -3	4.86 <sup>b</sup>	0.11	2.35 <sup>d</sup>	0.04	5.10 <sup>a</sup>	0.1	2.62 <sup>c</sup>	0.07	0.004	<0.001	0.829
AA:EPA	31.88 <sup>a</sup>	0.77	5.90 <sup>b</sup>	0.11	30.21 <sup>a</sup>	1.08	5.62 <sup>b</sup>	0.13	0.152	<0.001	0.303

AA, arachidonic acid; SFA, saturated fatty acids.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>abc</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).



**Fig. 1.** Distribution of plasma triacylglycerol (A), total cholesterol (B) and phospholipids (C) in lipoprotein fractions obtained by density-gradient ultracentrifugation of plasma from females fed the 'butter' diet (□), females fed the 'linseed oil' diet (▨), males fed the 'butter' diet (■) and males fed the 'linseed oil' diet (▩). For details of procedures, see pp. 710–711. Values are means with their standard errors depicted by vertical bars (nine hamsters per group). <sup>a,b</sup>Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). D, effect of diet (butter v. linseed); G, effect of gender (females v. males); I, interaction between gender and diet effects.

respectively, 15, 24 and 14 % higher in animals fed the 'butter' diet.

These effects of gender and diet on plasma lipids were the reflection of differences in concentration and composition of lipoproteins (Table 4). Plasma VLDL concentration was significantly affected neither by gender nor by diet. In contrast, its composition was responsive to the diet. Whereas VLDL from hamsters fed the 'butter' diet contained as expected mostly

TG, those from hamsters fed the 'linseed oil' diet were enriched in CE (+40 %) to the detriment of TG. Therefore, the main lipid component of the VLDL core was TG in animals fed the 'butter' diet and CE in those fed the 'linseed oil' one, whereas the sum of TG and CE proportions remained the same (70 %) in both groups. In contrast to VLDL, LDL were influenced by gender: in males, LDL were in higher concentration than in females and they were richer in CE (43.6 % v. 38.6 %) and poorer in TG (4.25 % v. 10.7 %). Moreover, these gender-related differences were more pronounced in animals fed the 'linseed oil' diet. Plasma total HDL concentration was affected by both gender and diet, mostly because it was 25 % higher in males fed the 'butter' diet than in the other three groups. HDL composition was affected neither by gender nor by the diet.

As a result of concomitant variations due to gender and diet in lipoprotein concentration and composition, the non-HDL-CH:HDL-CH ratio was identical in all groups.

Glycaemia was affected by both gender and diet (Table 5). It was 10 % higher in females than in males and 11 % higher in hamsters fed the 'butter' diet than in those fed the 'linseed oil' one. As a consequence, females fed the 'butter' diet were markedly hyperglycaemic when compared to the other three groups. Insulinaemia and HOMA, the insulin resistance index, did not differ with gender and were, respectively, 71 and 87 % higher in animals fed the 'butter' diet than in those fed the 'linseed oil' one. As concerns oxidative status in plasma, the concentration of malondialdehyde (a product of peroxidation) was 28 % higher in females fed the 'butter' diet than in the three other groups. Vitamin E concentration in plasma was 36 % lower in hamsters fed the 'linseed oil' diet than in those fed the 'butter' diet, regardless of gender. It was also affected by gender, but only in animals fed the 'butter' diet, in which females exhibited 20 % lower plasma vitamin E concentration than males.

#### Liver and bile composition

Total liver weight (in g), was 9 % lower in males than in females, and this difference was more pronounced in the 'linseed oil' diet (Table 6). However, when expressed as a percentage of total body weight, the liver proportion was 9 % higher in males than in females, especially in those fed the 'butter' diet. Hepatic CE and TG content were, respectively, 52 and 37 % higher in males than in females and, respectively, 26 and 22 % higher in animals fed the 'butter' diet than in those fed the 'linseed oil' one. FC and PL contents were 35 and 7 % higher in males than in females. These effects remained the same when the results were expressed as mmol per mg proteins or per whole liver (data not shown).

Bile concentrations of TC and PL were, respectively, 52 and 33 % higher in males than in females and were 18 and 49 % higher in animals fed the 'linseed oil' diet than in those fed the 'butter' one. Bile acid concentration tended to be higher in males and was not affected by the diet. All these effects resulted in a higher lithogenic index in males.

#### Cholesterol absorption and synthesis

The percentage of absorbed dietary CH was high in all groups and varied between 77.5 (SEM 0.9) and 83.4 (SEM 2.4), without any effect of gender or diet.

**Table 4.** Plasma lipoprotein concentration (g/l) and composition (%) of female and male hamsters fed 'butter' or 'linseed oil' diets\* (Mean values with their standard errors for nine hamsters per group)

	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
VLDL	1.41	0.18	1.47	0.22	1.18	0.18	1.01	0.20	0.090	0.791	0.561
VLDL-FC	3.90	0.36	3.16	0.41	3.81	0.35	3.99	0.32	0.317	0.449	0.214
VLDL-CE	28.85 <sup>b</sup>	2.39	39.47 <sup>a</sup>	3.66	27.15 <sup>b</sup>	2.25	45.77 <sup>a</sup>	4.18	0.482	<0.001	0.226
VLDL-TG	41.32 <sup>ab</sup>	3.37	31.19 <sup>bc</sup>	4.53	45.45 <sup>a</sup>	2.99	23.82 <sup>c</sup>	5.29	0.699	<0.001	0.175
VLDL-PL	17.56 <sup>ab</sup>	0.51	18.00 <sup>a</sup>	0.76	16.01 <sup>b</sup>	0.66	17.96 <sup>a</sup>	0.68	0.234	0.078	0.259
VLDL-PR	8.37	0.53	8.18	0.41	7.58	0.65	8.46	0.45	0.632	0.509	0.307
LDL	1.48 <sup>bc</sup>	0.13	1.06 <sup>c</sup>	0.15	2.14 <sup>a</sup>	0.24	1.91 <sup>ab</sup>	0.14	<0.001	0.066	0.561
LDL-FC	5.59	0.35	4.83	0.21	5.56	0.31	5.70	0.41	0.855	0.724	0.895
LDL-CE	37.6 <sup>b</sup>	1.4	38.8 <sup>b</sup>	2.0	40.6 <sup>ab</sup>	1.8	44.8 <sup>a</sup>	1.4	0.006	0.237	0.233
LDL-TG	12.6 <sup>ab</sup>	2.5	15.4 <sup>a</sup>	2.5	8.0 <sup>bc</sup>	1.4	5.0 <sup>c</sup>	0.35	<0.001	0.974	0.134
LDL-PL	23.7	0.9	22.2	1.1	23.5	0.6	22.5	0.6	0.909	0.128	0.749
LDL-PR	20.6	1.6	18.9	1.3	22.3	1.5	22.0	1.2	0.096	0.493	0.637
HDL	5.89 <sup>b</sup>	0.30	5.53 <sup>b</sup>	0.13	7.34 <sup>a</sup>	0.41	6.11 <sup>b</sup>	0.16	<0.001	0.007	0.120
HDL-FC	4.24	0.30	3.77	0.26	3.63	0.18	3.97	0.16	0.393	0.789	0.095
HDL-CE	22.79 <sup>b</sup>	0.61	23.93 <sup>ab</sup>	0.71	23.73 <sup>ab</sup>	0.38	24.45 <sup>a</sup>	0.56	0.213	0.114	0.721
HDL-TG	6.49	0.84	6.23	0.48	5.79	1.15	5.90	0.65	0.537	0.930	0.819
HDL-PL	33.36	0.62	31.46	0.64	32.92	1.29	32.39	0.56	0.769	0.154	0.412
HDL-PR	33.13	0.78	34.61	0.70	33.94	2.02	33.28	0.65	0.827	0.731	0.372
Non-HDL-TC : HDL-TC	0.68	0.06	0.64	0.06	0.67	0.07	0.78	0.04	0.280	0.488	0.178

CE, cholesteryl esters; FC, free cholesterol; PL, phospholipids; PR, proteins; TC, total cholesterol; TG, triacylglycerols.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>abc</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

As compared to the 'butter' diet, the 'linseed oil' diet provoked an increase in hydroxymethyl-glutaryl CoA reductase specific activity but this effect was significant in females only (Table 7). Expressed as total activity in the whole liver, the diet effect was still significant and due to the females fed the 'linseed oil' diet, which exhibited a 27% lower activity than the other three groups (data not shown).

#### Fatty acid synthesis and oxidation

Specific and total activities per liver of key enzymes of lipogenesis are reported in Table 7. ACC and FAS specific activities were affected neither by gender nor by diet. Since the liver was heavier in females than in males, total ACC activity was higher in the former. FAS tended to respond like ACC, although

neither differences between groups nor the gender effect were significant. In contrast, malic enzyme, a NADPH provider, was affected by both gender and diet. Males had more than twice higher specific and total activities than females, and the gender effect was more pronounced in animals fed the 'butter' diet. Moreover, animals fed the 'linseed oil' diet exhibited about 50% weaker activities than those fed the 'butter' one, the diet effect being more pronounced in males. The activity of G6PDH, another NADPH provider, was 2–3-fold weaker than that of malic enzyme. Expressed as specific activity or per total liver, G6PDH was not affected by any treatment.

Specific hepatic activity of ACO was lowered by the 'linseed oil' diet but only in males (–20%; Table 7). By contrast, specific activity of CPT was higher in animals fed the 'linseed oil' diet than in those fed the 'butter' one and this

**Table 5.** Effect of gender and diet on glycaemic and oxidative status\* (Mean values with their standard errors for nine hamsters per group)

	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Glucose (mmol/l)	6.44 <sup>a</sup>	0.20	5.69 <sup>b</sup>	0.22	5.77 <sup>b</sup>	0.19	5.29 <sup>b</sup>	0.20	0.020	0.007	0.649
Insulin (mU/l)	35.3 <sup>ab</sup>	5.6	25.6 <sup>bc</sup>	4.2	38.3 <sup>a</sup>	4.8	17.5 <sup>c</sup>	1.8	0.467	<0.001	0.224
HOMA	10.34 <sup>a</sup>	1.59	6.55 <sup>bc</sup>	1.13	9.82 <sup>ab</sup>	1.22	4.20 <sup>c</sup>	0.55	0.234	<0.001	0.448
Vitamin E (pmol/μl)	81.0 <sup>b</sup>	5.0	58.2 <sup>c</sup>	2.0	100.7 <sup>a</sup>	5.7	58.1 <sup>c</sup>	2.2	0.022	<0.001	0.021
MDA (μmol/l TBARS)	1.84 <sup>a</sup>	0.08	1.39 <sup>b</sup>	0.12	1.46 <sup>b</sup>	0.12	1.46 <sup>b</sup>	0.06	0.126	0.030	0.029

HOMA, homeostasis model assessment; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>abc</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).



**Table 6.** Effect of gender and diet on composition of liver and bile\*  
(Mean values with their standard errors for nine hamsters per group)

	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Liver wt (g)	4.51 <sup>a</sup>	0.12	4.71 <sup>a</sup>	0.17	4.42 <sup>a</sup>	0.18	3.99 <sup>b</sup>	0.05	0.007	0.425	0.033
Liver wt (% body wt)	3.52 <sup>c</sup>	0.06	3.65 <sup>bc</sup>	0.09	3.98 <sup>a</sup>	0.08	3.81 <sup>ab</sup>	0.05	<0.001	0.703	0.046
Liver (μmol/g)											
TC	55.1 <sup>c</sup>	1.8	49.3 <sup>c</sup>	3.4	88.8 <sup>a</sup>	4.0	66.8 <sup>b</sup>	3.9	<0.001	<0.001	0.023
FC	6.0 <sup>c</sup>	0.2	6.3 <sup>c</sup>	0.3	8.9 <sup>a</sup>	0.5	7.7 <sup>b</sup>	0.3	<0.001	0.302	0.049
CE	49.1 <sup>c</sup>	1.7	43.0 <sup>c</sup>	3.2	79.9 <sup>a</sup>	3.6	59.1 <sup>b</sup>	3.5	<0.001	<0.001	0.025
TG	10.0 <sup>bc</sup>	0.9	8.6 <sup>c</sup>	0.3	14.2 <sup>a</sup>	1.3	11.3 <sup>b</sup>	0.9	<0.001	0.026	0.414
PL	23.4 <sup>c</sup>	0.3	24.0 <sup>ab</sup>	0.4	25.3 <sup>a</sup>	0.9	25.3 <sup>a</sup>	0.5	0.011	0.607	0.532
Bile (mmol/l)											
TC	1.84 <sup>b</sup>	0.18	2.15 <sup>b</sup>	0.14	2.77 <sup>a</sup>	0.15	3.30 <sup>a</sup>	0.24	<0.001	0.030	0.562
PL	9.59 <sup>c</sup>	1.39	15.1 <sup>b</sup>	1.31	17.4 <sup>b</sup>	1.1	25.1 <sup>a</sup>	1.7	<0.001	<0.001	0.420
BA	142 <sup>b</sup>	14	160 <sup>ab</sup>	15	169 <sup>ab</sup>	9	171 <sup>a</sup>	8	0.068	0.328	0.441
LI	0.17 <sup>bc</sup>	0.01	0.16 <sup>c</sup>	0.01	0.19 <sup>ab</sup>	0.01	0.19 <sup>a</sup>	0.01	<0.001	0.593	0.217

BA, bile acid; CE, cholesteryl esters; FC, free cholesterol; LI, lithogenic index (a ratio, thus without unit); PL, phospholipids; TC, total cholesterol; TG, triacylglycerols.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>abc</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

was more pronounced in males (+130%) than in females (+40%). As in the liver, specific CPT activity in the muscle was higher in animals fed the 'linseed oil' diet than in those fed the 'butter' one, but in males only (+16%).

Total ACO activity in whole liver was higher in animals fed the 'linseed oil' diet than in those fed the 'butter' one

(Table 7). Total CPT activity in whole liver was affected by both gender and diet: it doubled in animals fed the 'linseed oil' diet compared to those fed the 'butter' one. The gender effect was essentially due to the males fed the 'linseed oil' diet that exhibited about 100% higher activities than the other three groups. Total CPT in whole muscle was affected neither by gender nor by diet. However, a significant

**Table 7.** Activity of enzymes involved in cholesterol and fatty acid synthesis and oxidation in liver and muscle\*  
(Mean values with their standard errors for nine hamsters per group)

Tissues and enzymes	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Liver (nmol/min per mg protein)											
HMGCoA-R	9.21 <sup>a</sup>	0.69	6.27 <sup>b</sup>	0.40	9.17 <sup>a</sup>	0.66	8.92 <sup>a</sup>	0.51	0.030	0.010	0.025
ACC	0.77	0.19	0.62	0.11	0.47	0.07	0.48	0.08	0.082	0.576	0.547
FAS	1.60	0.14	1.48	0.16	2.00	0.40	1.62	0.18	0.267	0.306	0.606
ME	0.21 <sup>b</sup>	0.04	0.12 <sup>b</sup>	0.02	0.57 <sup>a</sup>	0.13	0.31 <sup>b</sup>	0.06	<0.001	0.023	0.277
G6PDH	0.07	0.01	0.07	0.01	0.07	0.01	0.09	0.02	0.248	0.373	0.327
ACO	23.6 <sup>a</sup>	0.9	26.4 <sup>a</sup>	1.6	24.0 <sup>a</sup>	1.0	19.5 <sup>b</sup>	0.6	0.007	0.457	0.002
CPT	3.86 <sup>b</sup>	0.48	5.47 <sup>b</sup>	0.64	2.91 <sup>b</sup>	0.38	6.60 <sup>a</sup>	1.34	0.690	<0.001	0.057
Liver (nmol/min per liver)											
HMGCoA-R	956 <sup>a</sup>	76	653 <sup>b</sup>	39	881 <sup>a</sup>	69	853 <sup>a</sup>	60	0.327	0.012	0.034
ACC	454 <sup>a</sup>	91	393 <sup>ab</sup>	64	236 <sup>bc</sup>	35	195 <sup>c</sup>	29	0.003	0.504	0.982
FAS	461	52	423	55	407	51	329	39	0.147	0.250	0.697
ME	118 <sup>b</sup>	16	70 <sup>b</sup>	9	283 <sup>a</sup>	55	125 <sup>b</sup>	18	<0.001	<0.001	0.122
G6PDH	32.3	3.4	35.3	1.7	33.8	3.3	35.5	2.9	0.764	0.421	0.824
ACO	1442	70	1601	73	1455	49	1567	64	0.871	0.045	0.723
CPT	241 <sup>b</sup>	36	333 <sup>b</sup>	39	181 <sup>b</sup>	27	533 <sup>a</sup>	92	0.049	<0.001	0.002
Vastus lateralis muscle											
CPT (nmol/min per mg protein)	6.24 <sup>b</sup>	0.41	6.34 <sup>b</sup>	0.24	6.97 <sup>b</sup>	0.30	8.11 <sup>a</sup>	0.30	<0.001	0.059	0.114
CPT (nmol/min per muscle)	257 <sup>b</sup>	17	266 <sup>b</sup>	20	298 <sup>b</sup>	17	353 <sup>a</sup>	26	0.094	0.079	0.028

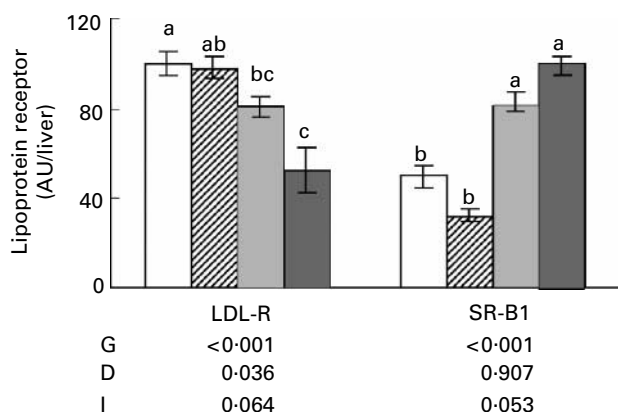
ACC, acetyl CoA carboxylase; ACO, acyl CoA oxidase; CPT, carnitine palmitoyl transferase; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; HMGCoA-R, hydroxymethyl-glutaryl CoA reductase; ME, malic enzyme.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

<sup>abc</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).





**Fig. 2.** Quantity of LDL and HDL receptor in the liver, obtained by Western blot, of females fed the 'butter' diet (□), females fed the 'linseed oil' diet (▨), males fed the 'butter' diet (▩) and males fed the 'linseed oil' diet (■). For details of procedures, see pp. 710–711. Values are means with their standard errors depicted by vertical bars (nine hamsters per group). <sup>a,b</sup>Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). AU, arbitrary units; D, effect of diet (butter v. linseed); G, effect of gender (females v. males); I, interaction between gender and diet effects; LDL-R, LDL receptor; SR-B1, scavenger receptor class B type 1.

interaction ( $P < 0.03$ ) between gender and diet resulted, as in the liver, in a higher value in males fed the 'linseed oil' diet than in the other three groups.

#### Hepatic lipoprotein receptors

The amount of LDL receptors in the whole liver was 50% higher in females than in males, and 20% higher in animals fed the 'butter' diet than in those fed the 'linseed oil' one (Fig. 2). However, the effect of diet was significant in males only. In contrast to the LDL receptor, the amount of hepatic HDL receptors (SR-B1) was twice higher in males than in females and was not affected by the diet. Gender- and diet-related effects were the same when data were expressed per mg membrane protein (data not shown).

#### Gene expression

There were few effects of gender and diet on hepatic expression of transcription factors (Table 8). PPAR $\gamma$  expression was 40% lower in females than in males, whatever

the diet. PPAR $\alpha$ , liver X receptor  $\alpha$  and SREBP2 expression was influenced neither by gender nor by diet. Hepatic mRNA level of SREBP1c was 46% higher, and that of oestrogen receptor was 46% lower in animals fed the 'linseed oil' diet than in those fed the 'butter' one.

#### Discussion

The present study was designed to compare, in male and female hamsters, the response of fatty acids and lipid metabolism to dietary fatty acids. It aimed to determine key factors of CH and TG metabolic pathways that are involved in the gender-related differences, as well as their impact on markers and risk factors of metabolic syndrome and CVD.

#### Cholesterol metabolism

The impact of gender was particularly obvious on CH metabolism, where it predominated over the influence of diet. Indeed, plasma CH, LDL-CH and HDL-CH concentrations were higher in males than in females, whereas VLDL-CH concentration was lower (Fig. 1). This was associated with differences in lipoprotein receptors. Females exhibited more liver LDL receptors (Fig. 2), that could explain their weaker concentration of LDL-CH. As for HDL-CH and total HDL, however, their concentration was higher in males than in females despite a higher hepatic content of SR-B1. This may result from the low cholesteryl ester transfer protein activity found in male hamsters compared to females (Zuckerman & Evans, 1995; Salter *et al.* 1998).

The influence of gender was also observed on hepatic CH. The higher CH content of the liver from males could not be accounted for by differences in dietary CH absorption and hepatic CH synthesis, which were similar in both genders (Table 7). Alternately, it could result from (1) enhanced CH re-uptake due to elevated levels of both HDL-CH and SR-B1 (Figs 1(B) and 2) or (2) impaired hepatic CH secretion into VLDL, in accordance with the lower VLDL-CH concentration found in males (Fig. 1(B)).

In contrast to that of gender, the effect of diet on CH metabolism was slight and significant in males only: males fed the 'linseed oil' diet exhibited lower plasma concentration of HDL-CH, liver CH content and higher bile concentration of

**Table 8.** Effect of gender and diet on the expression of transcription factor and key genes of lipid metabolism (arbitrary units)\* (Mean values with their standard errors for nine hamsters per group)

	Females				Males				Effects†		
	'Butter'		'Linseed oil'		'Butter'		'Linseed oil'		G	D	I
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
PPAR $\alpha$	715 <sup>b</sup>	98	1106 <sup>a</sup>	130	805 <sup>ab</sup>	70	808 <sup>ab</sup>	113	0.324	0.066	0.071
PPAR $\gamma$	15.0 <sup>b</sup>	3.5	21.1 <sup>b</sup>	4.2	24.9 <sup>ab</sup>	4.8	37.5 <sup>a</sup>	6.7	0.012	0.067	0.514
SREBP1c	153	31	242	43	148	26	199	32	0.489	0.050	0.580
SREBP2	50.2	6.7	70.4	10.3	56.4	9.6	69.0	10.4	0.795	0.089	0.686
Liver X receptor $\alpha$	217 <sup>b</sup>	45	318 <sup>ab</sup>	28	302 <sup>ab</sup>	34	340 <sup>a</sup>	54	0.218	0.111	0.464
Oestrogen receptor	4.08 <sup>a</sup>	0.87	2.98 <sup>ab</sup>	0.52	4.68 <sup>a</sup>	0.88	1.72 <sup>b</sup>	0.31	0.646	0.007	0.201

SREBP, sterol response element binding protein.

\* For details of procedures and diets, see pp. 710–711 and Table 1.

† G, D and I are the effects of gender, diet and interaction between both treatments after two-way ANOVA.

Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

CH than males fed the 'butter' diet (Table 6; Fig. 1(B)). Thus, the 'linseed oil' diet could decrease HDL-CH and liver CH by increasing CH excretion via the bile, but only in males.

#### *Triacylglycerol metabolism*

In contrast to CH metabolism, TG metabolism was affected by diet mostly. Indeed, plasma and liver TG concentrations were lower in animals fed the 'linseed oil' diet, which may result from a decreased lipogenesis or an increased fatty acid oxidation in the liver (Table 7). Indeed, two correlations could be established between the concentration of VLDL-TG, on the one hand, and total hepatic activity of ACC and FAS, on the other hand ( $R^2$  0.15,  $P=0.02$  and  $R^2$  0.30,  $P<0.001$ , respectively). However, we could not establish any correlation between triglyceridaemia and hepatic CPT activity. Despite an increased  $\beta$ -oxidation activity in animals fed the 'linseed oil' diet, PPAR $\alpha$  transcription, that is activated by PUFA (Kersten *et al.* 1999), was not increased in the present study with the ALA-rich diet (Table 8). Besides, *n*-3 long-chain PUFA (EPA and DHA) are known to decrease lipogenesis by decreasing SREBP1c mature protein amount and expression (Xu *et al.* 2002). On the contrary, in the present study, dietary ALA increased SREBP1c mRNA levels. It seems therefore that the mechanisms underlying the decrease in lipogenesis by dietary ALA, precursor of EPA and DHA, were different from those of its long-chain derivatives and may not involve SREBP1c. This also suggests that ALA may regulate some key points of lipid metabolism directly, without being converted into its long-chain derivatives. Moreover, decreased lipogenesis and increased  $\beta$ -oxidation in response to the 'linseed oil' diet were much pronounced in males than in females. This gender-specific sensitivity to diet did not seem to be due to hepatic levels of PPAR $\alpha$  and SREBP1c transcripts that were similar in both genders.

Oestrogens are also involved in the regulation of many key enzymes of TG metabolism through the oestrogen receptor. In particular, oestradiol treatment of ovariectomized rats decreased the expression (Gower *et al.* 2002) and the activity (Weinstein *et al.* 1986) of CPT, and increased the activity of FAS and ACC (Abraham *et al.* 1980). In the present study, the transcription of the oestrogen receptor was higher in hamsters fed the 'butter' diet (Table 8). Therefore, these animals could have a higher sensitivity to oestrogens that could contribute to their higher TG content in liver and plasma. On the contrary, whereas dietary fatty acids seemed to influence the transcription of the oestrogen receptor, gender did not affect mRNA levels of PPAR $\alpha$  and SREBP1c (Table 8). Therefore, the much more pronounced response to diet of males may not be linked to the level of PPAR $\alpha$  and SREBP1c transcription.

#### *Other parameters involved in metabolic syndrome and CVD*

In erythrocytes from hamsters fed linseed oil, the total *n*-3 PUFA content was twice higher than in those from hamsters fed butter, whereas *n*-6 to *n*-3 and AA to EPA ratios were beneficially lowered (Table 3). This increase in *n*-3 bioavailability did not result in a higher susceptibility to peroxidation, since malondialdehyde content did not differ with the diet (Table 5).

Besides, among animals fed the 'linseed oil' diet, females had reduced LA to ALA and *n*-6 to *n*-3 PUFA ratios. However, the AA to EPA ratio did not vary with gender,

suggesting that gender did not influence the balance of eicosanoids originating from these two PUFA. Such effect of gender on ALA conversion into its long-chain derivatives has been found in human studies, in which women exhibited a higher capacity of conversion than men (Burdge & Wootton, 2002). The present results did not allow us to confirm this observation in the hamster. However, they highlight the impact of gender on fatty acid incorporation into tissues. Not only lipid metabolism, but also glucose homeostasis, which is involved in metabolic syndrome and CVD, was improved by the 'linseed oil' diet. Indeed, glycaemia, insulinaemia and therefore the insulin resistance index (HOMA) were lower in animals fed the 'linseed oil' diet (Table 5). This confirms the improvement of insulin sensitivity by dietary ALA (Manco *et al.* 2004; Ghafoorunissa *et al.* 2005).

Besides, the decrease in glycaemia in response to the ALA-rich diet was much more pronounced in females, whereas that of insulinaemia was more pronounced in males (Table 5). Since PPAR $\gamma$  agonists like PUFA are insulin sensitizers (Saltiel & Olefsky, 1996; Xu *et al.* 1999), the higher hepatic level of PPAR $\gamma$  transcripts in males could make their glucose control more sensitive to dietary fatty acids (Table 8).

#### **Conclusion**

The present study showed that male and female hamsters responded differently to dietary fatty acids. Moreover, gender and diet exerted differential effects on the metabolism of individual lipid classes. Indeed, CH metabolism was affected by gender mostly, whereas TG metabolism was more sensitive to dietary fatty acids, males being more responsive than females.

As a consequence, male hamsters, which exhibited higher lipid risk factors of CVD and metabolic syndrome than females, seemed to benefit more from an ALA-rich diet. The relevance of these observations has to be investigated in man, which raises the question of taking gender into account when establishing lipid dietary recommendations.

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