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# Effects of dietary alpha linolenic acid on cholesterol metabolism in male and female hamsters of the LPN strain

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**Abstract**

N-3 polyunsaturated fatty acids and estrogens are recognized as protective factors of atherosclerosis, however their interactions on cholesterol metabolism remain unclear. Male and female hamsters were fed for 9 weeks diets containing 12.5% lipids and rich in either $\alpha$-linolenic acid ("linseed" diet) or saturated fatty acids ("butter" diet). Hamsters fed the "linseed" diet exhibited lower plasma concentrations of cholesterol (-29%), total LDL (-35%) and HDL (-17%), glucose (-20%), insulin (-40%) and of the LDL-cholesterol/HDL-cholesterol ratio (-27%) than those fed the "butter" diet. In the liver, cholesterol content was 2.7-fold lower in response to the "linseed" diet, whereas the concentration of HDL receptor (SR-BI) and the activities of HMGCoA reductase and cholesterol 7$\alpha$-hydroxylase were 30 to 50% higher than with the "butter" diet. By contrast, the LDL receptor concentration did not vary with the diet. Females exhibited higher concentration of LDL (+24%), lower concentration of plasma triglycerides (-34%), total VLDL (-46%) and VLDL-cholesterol (-37%) and of biliary phospholipids (-19%). Besides, there was also an interaction between gender and diet: in males fed the "butter" diet, plasma triglycerides and VLDL concentration, were 2 to 4 fold higher than in the other groups. These data suggest that gene and/or metabolic regulations by fatty acids could interact with that of sex hormones and explain why males are more sensitive to dietary fatty acids.

*Key words:* $\alpha$-linolenic acid; Gender; Hamster; Cholesterol; Dietary lipids 

## 1. Introduction

For a number of years, n-3 polyunsaturated fatty acids (PUFAs) have been recognized as protective factors of atherosclerosis and subsequent cardiovascular diseases (CVD) [1–3]. The first evidence relies on epidemiological studies of human populations characterized by their high intake of n-3 long-chain (LC) PUFAs of marine origin [4,5]. Beneficial reported effects of n-3 LC-PUFAs include reduced plasma lipids [6–8], thrombogenesis [9] and possibly blood pressure [8].

Besides direct dietary intake, n-3 LC-PUFAs can also derive from the desaturation and elongation of the precursor fatty acid of plant origin, $\alpha$-linolenic acid (ALA, 18:3n-3). Recent epidemiological data demonstrate inverse associations between dietary ALA and risk of CVD [10–13]. In the human, ALA and its LC derivatives exhibited similar positive effects on hemostatic factors and thrombogenesis [1] and on blood pressure [14]. The impact of dietary intake of ALA on plasma lipids and lipoproteins is more controversial : no effects [1] or decrease in total cholesterol (TC) and LDL-cholesterol (LDL-C) [15–17]. In all human studies, and contrary to its LC derivatives, an ALA-rich diet failed to decrease plasma triglycerides (TG) [1,18,19]. Much less is known concerning its metabolic targets and mechanisms of action (direct or through its transformation into LC derivatives).

In addition to nutritional agents, hormonal status has an effect on the etiology of CVD. In the human, susceptibility to coronary heart disease is higher in men than in age-matched women. After menopause, the incidence of CVD in women tend to reach that of men, together with an increase in TC, LDL-C, TG and apoB, and a decrease in HDL-cholesterol (HDL-C) [20–22]. The underlying mechanisms are related to the known effects of estrogens on lipid metabolism, such as a decrease in HDL catabolism via a decrease in hepatic lipase activity and an increase in LDL catabolism via an increase in the number of LDL receptors [23].

In consequence, hormonal influences on lipid metabolism may interact with the impact of nutritional factors and influence the responsiveness to dietary treatment of males

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and females. Among animal models suitable for investigating the interactions between gender and dietary PUFAs in the susceptibility to CVD, the golden Syrian hamster (*Mesocricetus auratus*) is probably one of the most relevant. Indeed, among rodents, its lipid metabolism is the less different from that of humans, especially as concerns hepatic cholesterol synthesis [24,25], plasma lipoprotein profile [26,27], and bile acid metabolism [28]. Moreover, the hamster is a recognized model for atherosclerosis research, because some strains develop vascular lesions together with hyperlipidemia in response to a diet rich in cholesterol and saturated FA [29–31]. This effect is more pronounced in males than in females, which supports the hypothesis of the modulation of the response of lipid metabolism to dietary FA by hormonal status [32,33]. In the hamster, cholesterol metabolism is sensitive to dietary LC-PUFAs however, to our knowledge, the effects of dietary ALA on lipid metabolism has not been investigated in this species.

The present study was therefore designed to determine, in the hamster: 1/ The potential beneficial effects of dietary ALA on lipid metabolism, 2/ The gender-related differences in this metabolism, and 3/ The possible interactions between dietary FA and gender.

Table 1
Lipid content (as % by weight of the diet) and fatty acid profile of the experimental diets (as % of total fatty acids)

| | "butter" diet | "linseed" diet |
|---|---|---|
| Lipid content (%) | 14.2 | 15.3 |
| Fatty acid: | | |
| C10:0 | 1.04 | 0.00 |
| C12:0 | 2.73 | 0.00 |
| C14:0 | 9.91 | 0.00 |
| C14:1 | 1.39 | 0.00 |
| C15:0 | 0.99 | 0.00 |
| C15:1 | 0.19 | 0.00 |
| C16:0 | 32.54 | 6.57 |
| C16:1 | 1.81 | 0.00 |
| C17:0 | 0.50 | 0.00 |
| C18:0 | 8.15 | 3.53 |
| C18:1 n-9 | 22.30 | 16.08 |
| C18:1 n-7 | 0.57 | 0.45 |
| C18:2 n-6 | 15.51 | 21.79 |
| C18:3 n-3 | 1.79 | 47.51 |
| C20:0 | 0.23 | 0.17 |
| C20:1 n-9 | 0.35 | 0.22 |
| C21:0 | 0.00 | 0.72 |
| C22:0 | 0.00 | 0.21 |
| C23:0 | 0.00 | 1.41 |
| n-6/n-3 | 8.66 | 0.46 |

## 2. Methods and materials

### *2.1. Animals and diets*

Golden Syrian hamsters (*Mesocricetus auratus*) were males and females born in our breeding unit (LPN strain). After weaning at 3 weeks of age, 18 hamsters of each gender were bred in colony cages in a temperature- (22°C) and light- (14 hrs day/10 hrs night) controlled room. Males and females were separated, and sterile food pellets and distilled water were provided *ad libitum*. This commercial diet (UAR113, Villemoisson, France) contained 72.0% cereals, 17.8% soy meal, 6.0% fish meal, 4.2% vitamins and minerals mix and provided 5.1% lipids and 19.3% proteins. At 8 weeks of age (average weight 72g, identical in both genders), the hamsters were housed in colony cages (4 or 5 in each cage) with wired floor (to avoid the consumption of litter and feces), weighed weekly and fed experimental diets for 9 weeks. Experimental diets consisted of 87.5% of the above commercial pellets that were grinded and supplemented with 0.04% cholesterol (5-cholesten-3$\beta$-ol, Sigma, Saint Louis, USA) and 12.5% of either butter ("butter" diet) or linseed oil ("linseed" diet). Final lipid content of the diet was 14 to 15% and that of CT was $<$0.1%. The cholesterol content of the "linseed" diet (0.064%) was not adjusted to that of the "butter" diet (0.097%) because the LPN strain is known to exhibit a low metabolic responsiveness to dietary cholesterol [34]. Moreover, previous studies in the hamster showed that, in this range of cholesterol content, the effects of dietary cholesterol alone are minor in regard to that of the saturated vs. polyunsaturated FA [35,36]. Each diet was provided *ad libitum* to 9 males and 9 females. The present work was carried out in agreement with the French legislation on animal experimentation and with the authorisation of the French Ministry of Agriculture (Animal Health and Protection Directorate).

In order to study the FA composition of the diets, lipids were extracted according to Folch's method [37], dried and weighed. After methylation, fatty acids were analyzed by gaz chromatography fitted with an "on column" injector and a flame ionization detector (BPX70, SGE, Villeneuve St Georges, France; 60m lenght, internal diameter: 0.32 mm, film thickness: 25 $\mu$m). Fatty acid composition of the experimental diets is shown on Table 1.

### *2.2. Experimental procedure*

At 17 weeks of age, after 9 weeks on the experimental diets, all hamsters were fasted overnight, then weighed and anesthetized by intramuscular injection of Zoletil 50 (Virbac, Carros, France) at a dose of 4 mg/100g of 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, that are very sensitive to the stress induced by anesthesia. Immediately after, a larger blood sample was taken under the same conditions. Plasma was separated from blood samples by centrifugation for 20 min at 4°C and 1700 g, then stored at $-20$°C.

After blood sampling, the abdominal cavity was opened chirurgically and the hamsters were killed by section of the

cardiac apex. Bile was taken from the gallbladder and stored at −20°C. The liver was carefully removed, and kept on ice. A sample of about 0.5g was stored at −80°C for further determination of lipoprotein receptors, another sample of about 0.5 g was stored at −20°C for further analysis of lipid composition, and another of about 1g was kept on ice for immediate isolation of microsomes.

### 2.3. Biochemical analyses

#### 2.3.1. Plasma glucose and insulin

Glycaemia was determined colorimetrically using the enzymatic kit provided by Biochem (Aix-en-provence, France) [38] and insulinemia using a radioimmunassay kit (sensitive rat insulin RIA) provided by Linco (St Charles, USA) and validated in the hamster in our laboratory. The insulin resistance index was calculated by the homeostasis model assessment (HOMA) [39].

#### 2.3.2. Plasma, bile and liver lipids

The following components were quantified in plasma: TG, TC, phospholipids (PL) by colorimetric enzymatic methods using the kits provided by Bio-Merieux (Marcy-l'Etoile, France) [40–42]. Analyses were performed with an automatic analyzer (Abott-VP, Rungis, France). Free cholesterol (FC) was determined manually using a procedure adapted from Richmond [41]. The amount of cholesteryl esters (CE) was calculated using the formulae: CE = (TC – FC) x 1.67. In this calculation, it was assumed that the factor for the ratio of the average molecular weight of CE to FC (i.e., 1.67), was the same in the human and hamsters.

Bile PL and TC were assayed with the same procedures 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) [43]. Lithogenic index was calculated according to Hofmann method [44], using the TC, PL and bile acid concentrations.

Hepatic lipids were determined as plasma lipids after extraction in isopropanol from a 150 mg liver sample as described by Loison et al. [45]. The pellet was kept and solved in NaOH 1M for further determination of hepatic proteins (PR) (see below).

### 2.4. Lipoprotein isolation

Lipoproteins were isolated from plasma by ultracentrifugation in a density gradient specifically adapted for the hamster [46] and modified by the addition of 0.8 mL of solution d 1.24 in the bottom of the ultracentrifugation tube (Ultra-Clear, Beckman, Gagny, France). Distribution of TC in the density gradient from one plasma in each group was determined after fractionation of the gradient into 0.5 mL fractions (Fig. 1). This distribution was identical in all groups as concerns the density limits and corresponding volumes of the successive lipoprotein classes, i. e.: VLDL, fraction 1, 0.5 mL, d <1.010; IDL, fraction 2, 0.5 mL, 1.010<d <1.015; LDL, fractions 3 to 8, 3 mL, 1.015<d <1.050; and HDL, fractions 9 to 19, 5.5 mL, 1.050<d <1.190. Due to technical modification of the density gradient, the recovery volumes of the successive lipoprotein classes were slightly different from those described previously [46], however the density limits were identical in both studies.

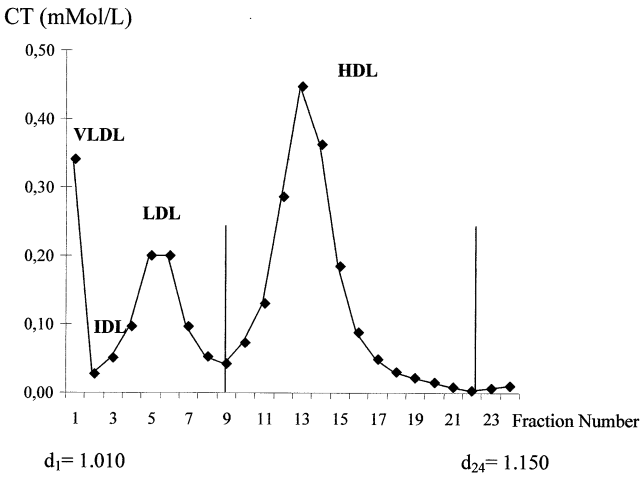



Fig. 1. Density distribution of the hamster plasma lipoproteins as determined by the cholesterol content of successive fractions from a representative density gradient.

In a second step, lipoproteins from the 36 plasma samples were fractionated on these density and volume bases. IDL, that were in very low concentration, were pooled with LDL. In each lipoprotein class, TC, FC, PL and TG were assayed as in plasma, and PR were determined by the method of Lowry et al. [47] using bovine serum albumin as standard. Lipoprotein concentrations were calculated by summing up the concentrations of the individual protein and lipid components.

### 2.5. Hepatic enzyme activities and lipoprotein receptors

Hepatic enzymes activities were determined in microsomal fractions for HMGCoA reductase and for cholesterol 7$\alpha$-hydroxylase (CYP7A1) as described previously by Souidi et al. [48]. Briefly, HMGCoA reductase activity was determined in the presence of alkaline phosphatase using Phillip and Shapiro's radioisotopic technique [49]. CYP7A1 was assayed according to a radioisotopic method using [4-$^{14}$C]cholesterol, solubilized and carried out by hydroxypropyl-$\beta$-cyclodextrin.

Relative hepatic amounts of LDL receptor and scavenger receptor class B type I (SR-BI), shown to be an HDL receptor [50], were determined according to a previously described technique [51]. 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

Table 2
Plasma parameters

| | | "butter" diet | | "linseed" diet | | |
|---|---|---|---|---|---|---|
| | unit | Male | Female | Male | Female | effect |
| Glucose | mMol/L | 5.65 ± 0.22 | 6.01 ± 0.27 | 4.15 ± 0.22† | 4.92 ± 0.20† | D |
| Insulin | pMol/L | 186.3 ± 20.2 | 140.4 ± 10.9 | 93.2 ± 23.1† | 101.5 ± 11.0† | D |
| Insulin resistance | | 6.72 ± 2.53 | 5.33 ± 1.31 | 2.65 ± 2.26† | 3.23 ± 1.32† | D |
| TC | | 2.30 ± 0.13 | 2.49 ± 0.12 | 1.64 ± 0.10† | 1.69 ± 0.10† | D |
| FC | | 0.53 ± 0.03 | 0.51 ± 0.02 | 0.28 ± 0.03† | 0.34 ± 0.04† | D |
| CF | mMol/L | 1.78 ± 0.10 | 1.98 ± 0.11 | 1.36 ± 0.09† | 1.35 ± 0.07† | D |
| PL | | 2.18 ± 0.12 | 2.15 ± 0.06 | 1.74 ± 0.04† | 1.67 ± 0.09† | D |
| TG | | 1.26 ± 0.20 | 0.67 ± 0.05* | 0.68 ± 0.09† | 0.61 ± 0.04 | D G I |
| VLDL | g/L | 1.04 ± 0.16 | 0.38 ± 0.06* | 0.30 ± 0.05† | 0.32 ± 0.04 | D G I |
| LDL | g/L | 0.76 ± 0.06 | 0.92 ± 0.08 | 0.47 ± 0.05† | 0.60 ± 0.07† | DG |
| HDL | g/L | 3.90 ± 0.15 | 4.08 ± 0.27 | 3.28 ± 0.27 | 3.36 ± 0.20 | D |
| VLDL-C | | 0.24 ± 0.03 | 0.11 ± 0.02* | 0.06 ± 0.01† | 0.06 ± 0.01† | D G I |
| LDL-C | mMol/L | 0.54 ± 0.06 | 0.64 ± 0.08 | 0.27 ± 0.03† | 0.34 ± 0.05† | D |
| HDL-C | | 1.69 ± 0.11 | 1.74 ± 0.09 | 1.22 ± 0.11† | 1.31 ± 0.11† | D |
| LDL-C/ HDL-C | | 0.32 ± 0.02 | 0.36 ± 0.03 | 0.21 ± 0.02† | 0.26 ± 0.02† | D |

Results are mean ± SEM of 9 hamsters in each group, except for the insulinemia and insulin resistance in males fed the "linseed" diet (n = 8) and for the lipoproteins in females fed the "butter" diet (n = 8). Insulin resistance is calculated by HOMA.

TC: total cholesterol; FC: free cholesterol; CE: cholesteryl esters; PL: phospholipids; TG: triglycerides.

The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with *t*-tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males vs females).

* Significant effect of gender within each diet.

† Significant effect of diet within each gender.

SR-BI (15 amino acids, gift from A. Mazur, Theix, France). The detection was achieved in a chemiluminescence reagent (ECL, Amersham, Little Chalfont, England). Films were scanned with a laser densitometer (Ultroscan 2222 LKB, Bromma, Sweden). Peak areas allowed quantifying the antigen response. Receptor levels were expressed in arbitrary units, and the value 100 was arbitrarily attributed to the "butter males" group.

### 2.6. Statistical analyses

The data were analyzed by using the Statview 4.5 program (Abacus Concept, Berkeley, CA, USA). The data of the four dietary groups were statistically analyzed with a two-way Analysis of Variance (ANOVA) with diet and gender as factors. When ANOVA indicated a significant effect ($P < 0.05$), the following groups were compared pairwise with correction for multiple comparisons (*t*-test with the Bonferroni adaptation): 1) butter diet vs. linseed diet within each gender 2) males vs. females within each diet. Thus, each group was used for two comparisons, and therefore, the level of significance for these multiple comparisons was preset at $P < 0.025$ (= 0.05/2).

## 3. Results

### 3.1. Plasma parameters

Hamsters fed the "butter" diet exhibited higher glycaemia and insulinemia, and a higher insulin resistance index calculated by HOMA than hamsters fed the "linseed" diet (Table 2). By contrast, there were no gender-related differences in fasting glycaemia and insulinemia. Plasma concentrations of all lipids (except TG) were significantly lower in animals fed the "linseed" diet, and identical in males and females. Plasma TG concentration was twice higher in males fed the "butter" diet than in the 3 other groups, in which mean values did not differ significantly.

HDL was the main lipoprotein class in all groups (3 to 4 g/L), whereas VLDL and LDL concentrations were 4 to 10 fold lower (Table 2). VLDL concentration paralleled that of TG, and was therefore dramatically higher in the males fed the "butter" diet than in the other groups. VLDL composition did not differ markedly among groups; however, ANOVA indicated a diet effect, VLDL from hamsters fed the "linseed" diet being significantly poorer in CE. Moreover, VLDL particles from the females fed the "butter" diet contained significantly less TG and more PL and PR than

males on the same diet (Fig 2). In hamsters fed the "linseed" diet, LDL and HDL concentrations were lower, and LDL contained less CE and more TG (Fig. 2). LDL concentration was higher in females than in males, but HDL concentration was similar in both genders. (Table 2). The LDL-C/HDL-C ratio was lower in hamsters fed the "linseed" diet than in those fed the "butter" diet, but did not differ significantly between males and females (Table 2).

### *3.2. Bile and liver composition.*

The concentration of bile lipids is reported on Table 3. Bile TC concentration was identical in all groups, and PL concentration was significantly higher in males than in females. Bile acids were the main sterols in hamster bile. Their concentration was lower in hamsters fed the "linseed" diet, but did not differ with gender. The lithogenic index was modified neither by the diet nor by the gender.

The body weights did not differ between groups (Table 4). However, ANOVA indicated that the liver proportion -as related to body weight- was significantly lower in females than in males, the difference being more pronounced in the group fed the "butter" diet (Table 4). TG were the main storage lipids, with similar concentration in all groups. In contrast, ANOVA indicated a diet effect on CE storage, that was considerably higher in hamsters fed on the "butter" diet. There was an interaction between gender and diet since the diet effect was considerably higher in males than in females. As concerns membrane lipids, the concentration of FC did not differ with gender, but was lower in hamsters fed the "linseed" diet, whereas that of PL was lower in females.

### *3.3. Hepatic lipoprotein receptors and enzymes*

Representative western blots of LDL receptors and SR-BI are displayed on Fig. 3 (a and b respectively). Relative amounts of these receptors in the various groups are reported on Fig. 3 (c–f). The relative concentration and total amount of LDL receptors in liver membranes were identical in all groups (Fig. 3c and 3e). Those of SR-BI were higher in hamsters fed the "linseed" diet than in those fed the "butter" diet, but did not differ with gender (Fig. 3d and 3f). This dietary effect on SR-BI was more pronounced in males than in females.

The specific activity of HMGCoA reductase exhibited a very high variation within each group (Fig. 4a). ANOVA indicated that the specific activities of HMGCoA reductase and CYP7A1 were lower in hamsters fed the "butter" diet, but did not differ with gender (Fig. 4a-b). For both enzymes, this dietary effect was more pronounced in males than in females, although the difference was significant for CYP7A1 only.

## 4. Discussion

### *4.1. Effects of an ALA-rich diet on lipid metabolism*

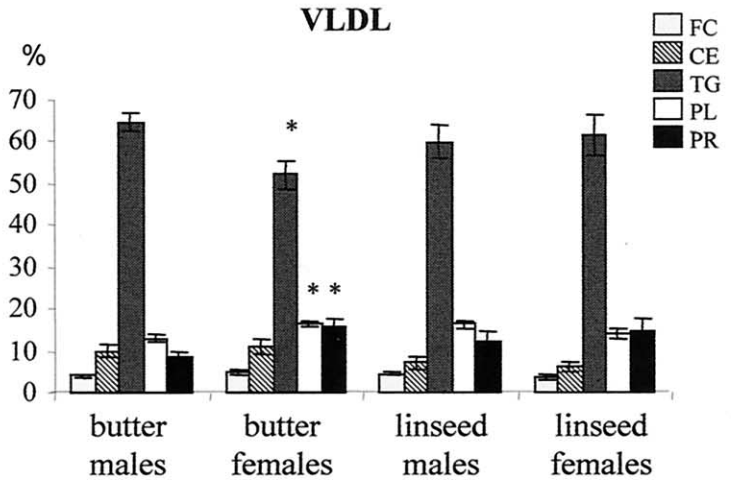
In the present study, the "butter" diet was formulated to present some of the nutritional features of diets in western populations, such as a high caloric content, 32% of total energy brought by lipids and a predominance of saturated fatty acids over PUFAs (Table 1). When compared to those fed this western-type diet, male and female hamsters fed the ALA-rich diet for 9 weeks exhibited lower glycaemia, insulinemia and insulin resistance index (Table 2). Similar effects of an ALA rich diet on glycaemia was previously described in the rat [52]. Our study is also consistent with that of Storlien et al. [53] in the rat, in which dietary ALA was as effective as n-3 LC-PUFAs in improving insulin sensitivity.

The concentration of all plasma lipids was also lower in hamsters on the "linseed" diet (Table 2). Both FC and CE were lower in ALA-fed hamsters. This hypocholesterolemic effect is consistent with a previous study in hamsters fed LC-PUFAS together with a low dietary cholesterol intake [54], and reproduces the hypocholesterolemic effect shown in some human studies in response to ALA [15] or LC-PUFAs [55].

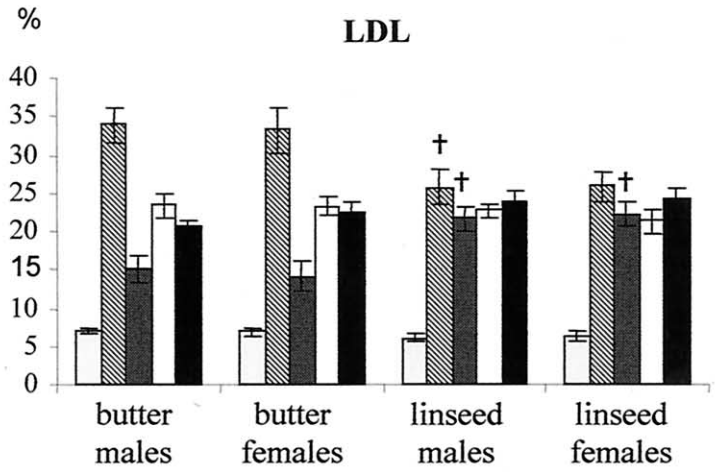
This hypocholesterolemia affected all lipoprotein classes, the plasma concentrations of which being lower than with the "butter" diet (Table 2). This effect was more pronounced on LDL than on HDL, which resulted in a lowered LDL-C/HDL-C ratio and suggests an antiatherogenic effect of dietary ALA, at least in the hamster.

Thus, feeding hamsters an ALA-rich diet resulted in the significant decrease of a number of proatherogenic factors, such as TC, LDL-C, and LDL-C/HDL-C, and that in both genders. This is indicative of modifications in cholesterol metabolism, as evidenced by data in the liver. Indeed, to our knowledge, the present study is the first evidence of an effect of dietary ALA on hepatic cholesterol metabolism in the hamster. In response to the ALA-rich diet, hamsters exhibited a lower accumulation of hepatic CE, as well as higher activities of HMGCoA reductase and CYP7A1 (Fig. 2). These effects differ from the response to dietary n-3 LC-PUFAs that resulted in a decreased expression of HMG-CoA reductase [56]. It can be hypothesized that, in response to dietary ALA, the higher activity of CYP7A1 results in a higher cholesterol secretion into bile, leading to a depletion of the intrahepatic pool of cholesterol (as indicated by the lower cholesterol content of the liver, Table 4), and thus to an increase in cholesterol synthesis and turnover.

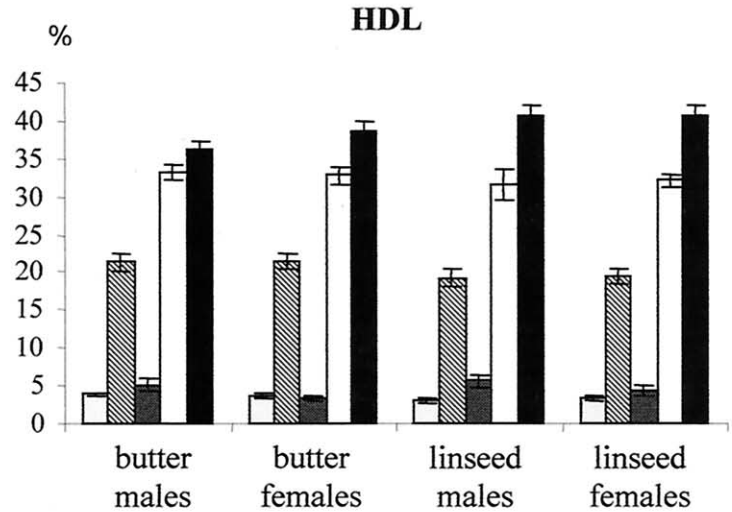
In parallel, the amount of hepatic SR-BI was significantly higher in our hamsters fed the ALA-rich diet (Fig. 3d and 3f). This higher uptake capacity may be in part responsible for the lower plasma concentration of HDL. Besides, there was a negative correlation between CE concentration and the total amount of SR-BI in the liver ($r = -0.336$, $P < 0.05$), as previously found in the hamster by Loison et al. [45]. This suggests a negative feed back by hepatic CE concentration on SR-BI.




| % | effect |
|---|---|
| FC | |
| CE | D |
| TG | |
| PL | I |
| PR | G |




| % | effect |
|---|---|
| FC | |
| CE | D |
| TG | D |
| PL | |
| PR | D |




| % | effect |
|---|---|
| FC | |
| CE | |
| TG | G |
| PL | |
| PR | D |

Fig. 2. Composition of plasma lipoproteins. Results are mean ± SEM of 9 hamsters in each group except for the ≪ butter≫ females group (n=8). The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with t-tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males vs females). * Significant effect of gender within each diet; † Significant effect of diet within each gender. FC: Free Cholesterol; CE: Cholesteryl Esters; TG: Triglycerides; PL: phospholipids; PR: Proteins.

Table 3
Bile lipid concentration and lithogenic index

| | | "butter" diet | | "linseed" diet | | |
|---|---|---|---|---|---|---|
| | unit | Male | Female | Male | Female | effect |
| TC | mMol/L | 2.36 ± 0.20 | 1.71 ± 0.25 | 1.72 ± 0.24 | 171 ± 0.26 | - |
| PL | mMol/L | 13.5 ± 0.8 | 11.0 ± 1.5 | 14.2 ± 1.0 | 12.4 ± 0.8 | G |
| BA | mMol/L | 157.1 ± 7.4 | 143.6 ± 10.2 | 110.6 ± 11.7† | 130.4 ± 10.9 | D |
| LI | | 0.18 ± 0.01 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.16 ± 0.03 | - |

Results are mean ± SEM of 9 hamsters in each group.

TC: total cholesterol; BA: bile acids; PL: phospholipids; LI: lithogenic index.

The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with *t*-tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males vs females).

*Significant effect of gender within each diet.

† Significant effect of diet within each gender.

By contrast to SR-BI, the concentration of LDL receptor did not differ with the diet (Fig. 3c and 3e). This is consistent with a previous study in the rat, in which dietary ALA had no effects on LDL receptor activity, that was increased by n-3 LC PUFAs [57].

Therefore, dietary ALA may influence hepatic cholesterol metabolism differently from its LC-derivatives, especially as concerns the regulation of HMGCoA reductase and LDL receptor. Since only a fraction of ALA is usually converted into LC-PUFAs, it is possible that dietary ALA regulates metabolism directly rather than through its derivatives [58]. However, since these regulations depend on the level of dietary cholesterol intake and of subsequent cholesterolemia, only direct comparison of dietary ALA vs. n-3 LC-PUFAs would properly address this question.

### *4.2. Influence of hormonal status on lipid metabolism.*

#### *4.2.1. Effects on cholesterol metabolism*

Despite of a higher plasma concentration of total LDL in females, there was no difference in plasma LDL-C between males and females. This contrasts with epidemiological data showing that women before menopause exhibit lower LDL-C concentration than age-matched men [59]. In the human, the mechanism leading to a lower LDL-C concentration involves an increase in LDL catabolism under the influence of estrogens [60,61]. In our hamsters, the concentration of hepatic LDL receptors was the same in males and females, which is consistent with the absence of difference in LDL-C. Actually, in the hamster, the effect of gender on LDL-C concentration appears to be strain-dependent. Indeed, females of strains prone to atherosclerosis, such as the F1B, exhibit lower LDL-C than males [32, 33], whereas no gender-related differences were noted in strains prone to biliary cholesterol gallstones, which is the case of the LPN strain used in the present study [62]. The same discrepancy with epidemiological data appears for HDL metabolism. Indeed, there were no gender-related differences in plasma HDL and HDL-C (Table 2) and in hepatic SR-BI concentration (Fig. 3d). This contrasts with the human, in which HDL-C is higher in women than in men [59]. Again, a strain-related sensitivity is to be involved [62,33].

Table 4
Body weight (BW) and hepatic lipid composition

| | | "butter" diet | | "linseed" diet | | |
|---|---|---|---|---|---|---|
| | unit | Male | Female | Male | Female | effect |
| Body weight | | 97.9 ± 2.0 | 97.2 ± 2.1 | 89.1 ± 3.1 | 97.7 ± 2.2 | - |
| Liver | % BW | 3.5 ± 0.1 | 3.1 ± 0.1* | 3.3 ± 0.1 | 3.2 ± 0.1 | G |
| TC | | 15.06 ± 2.34 | 8.78 ± 1.43 | 6.36 ± 0.22† | 6.31 ± 0.33 | D G I |
| FC | mMol/g liver | 5.15 ± 0.27 | 5.01 ± 0.26 | 4.14 ± 0.33 | 3.43 ± 0.17† | D |
| CE | | 9.92 ± 2.38 | 3.77 ± 1.43 | 2.23 ± 0.20† | 2.87 ± 0.31 | D I |
| TG | | 13.5 ± 1.4 | 13.4 ± 1.4 | 12.2 ± 0.8 | 14.8 ± 1.6 | - |
| PL | | 26.5 ± 0.4 | 24.5 ± 0.4* | 26.0 ± 0.5 | 24.9 ± 0.4 | G |

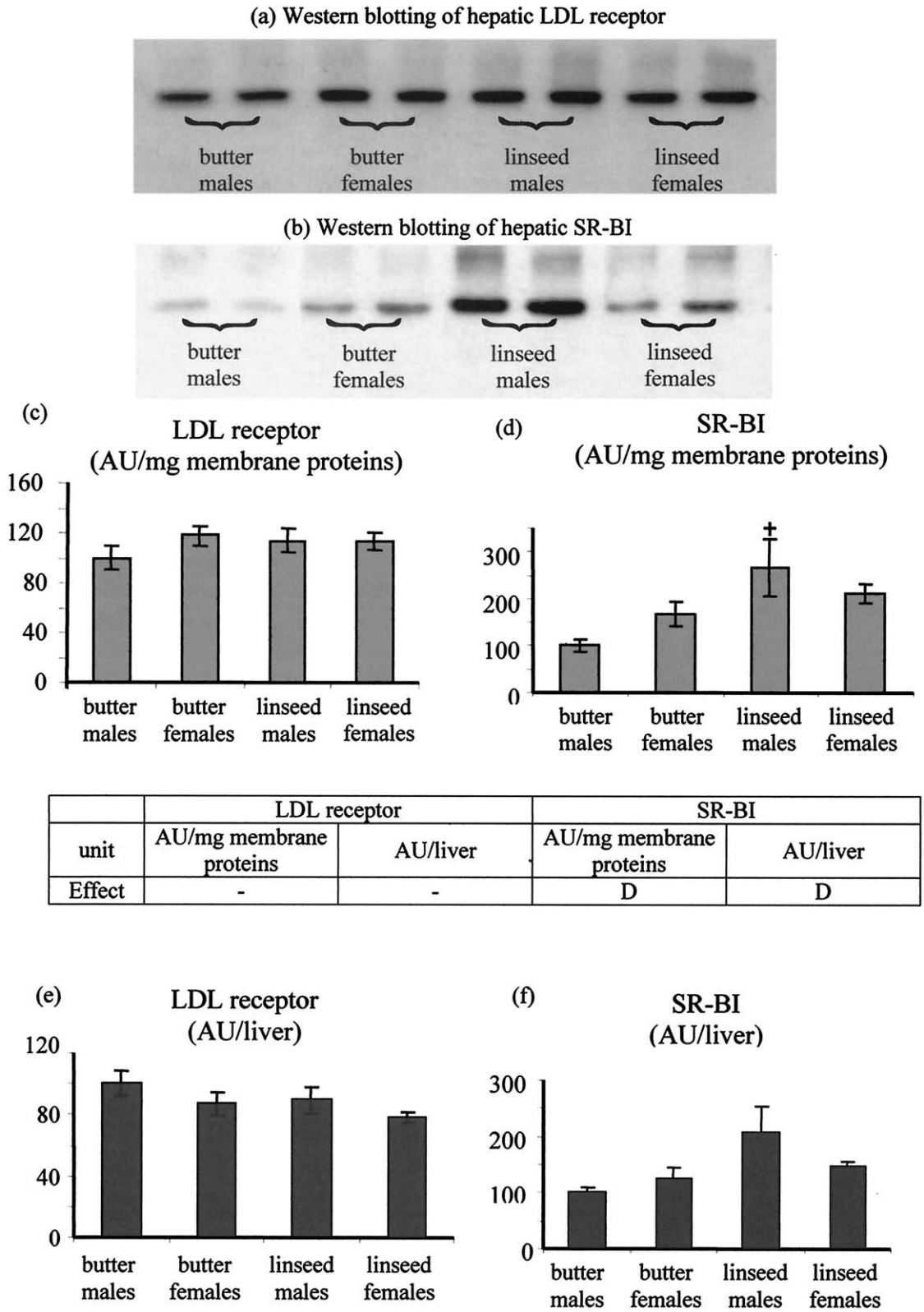
Results are mean ± SEM of 9 hamsters in each group.

TC: total cholesterol; FC: free cholesterol; CE: cholesteryl esters; TG: triglycerides; PL: phospholipids.

The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with *t* - tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males and females).

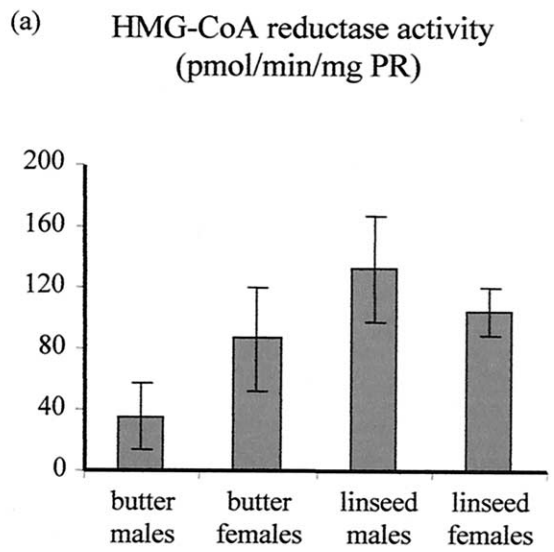
* Significant effect of gender within each diet.

† Significant effect of diet within each gender.




| | LDL receptor | | SR-BI | |
|---|---|---|---|---|
| unit | AU/mg membrane proteins | AU/liver | AU/mg membrane proteins | AU/liver |
| Effect | - | - | D | D |

Fig. 3. Hepatic LDL receptor (a, c, e) and SR-BI (b, d, f). a and b : western blotting pattern from two representative hamsters in each group. c and d : relative concentration (per mg proteins; AU, arbitrary units). e and f : total hepatic amount of LDL receptor and SR-BI. Results are mean ± SEM of 9 hamsters in each group. The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with t-tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males vs females). * Significant effect of gender within each diet; † Significant effect of diet within each gender.




| Enzyme activity | effect |
|---|---|
| HMG-CoA reductase | D |
| CYP7A1 | D |

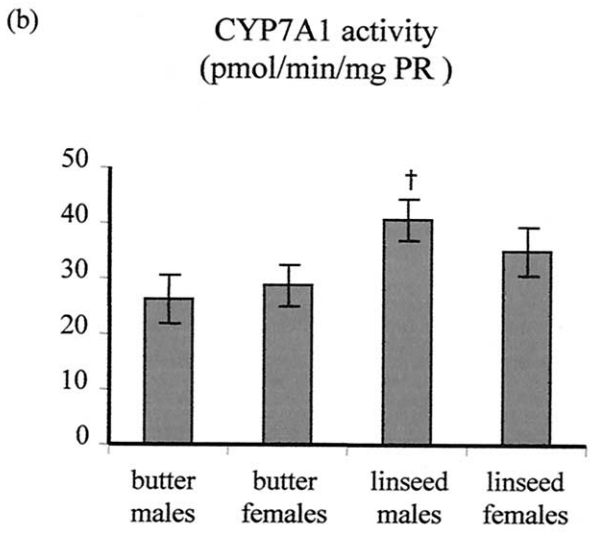



Fig. 4. Specific activity of hepatic CYP7A1 (a) and HMGCoA reductase (b). Results are mean ± SEM of 9 hamsters in each group. The data were analyzed with a two-way (diet and gender as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with t-tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, effect of diet (butter vs linseed); G, effect of gender (males vs females). * Significant effect of gender within each diet; † Significant effect of diet within each gender.

*4.2.2. Interactions between gender and dietary fatty acids*

There was a significant interaction between gender and dietary fatty acids on TG metabolism. Indeed, male hamsters fed the "butter" diet exhibited considerably higher plasma concentration of TG and VLDL than females. The hypotriglyceridemic effect of n-3 LC-PUFAs is well documented, in humans [1,8,63] as well as in animal models like the hamster [64]. The present data indicate that, in this respect, the effects of dietary ALA are similar to those of n-3 LC-PUFAs. However, ALA is effective in hypertriglyceridemic animals only, i.e., males. It failed to decrease TG and VLDL concentration in females, probably because they are already protected from hypertriglyceridemia by their hormonal status.

In conclusion, this study in the hamster provided evidence that dietary ALA exerts beneficial effects on some atherogenic risk factors related to cholesterol metabolism. Besides, the replacement, in the diet, of saturated fatty acids by ALA improved some parameters of the metabolic syndrome, such as insulin resistance and elevated concentrations of glucose, insulin, TG, and VLDL. Because the dietary-related effects were more pronounced in males than in females, and especially for plasma TG and VLDL, ALA was not effective in normolipemic females. The molecular

interactions between dietary fatty acids and hormonal status remain to be explored in the hamster, as well as their relevance in the dietary management of human dyslipidemias.

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