**Abstract**

Glucose intolerance and dyslipidaemia are independent risk factors for endothelium dysfunction and CVD. The aim of the present study was to analyse the preventive effect of n-3 PUFA (EPA and DHA) on lipid and carbohydrate disturbances and endothelial dysfunction. Three groups of adult hamsters were studied for 20 weeks: (1) control diet (Control); (2) high-fat diet (HF); (3) high-fat diet enriched with n-3 PUFA (HFn-3) groups. The increase in body weight and fat mass in the HF compared to the Control group (P<0·05) was not found in the HFn-3 group. Muscle TAG content was similar in the Control and HF groups, but significantly lower in the HFn-3 group (P=0·008). Glucose tolerance was impaired in the HF compared to the Control group, but this impairment was prevented by n-3 PUFA in the HFn-3 group (P<0·001). Plasma TAG and cholesterol were higher in the HF group compared to the Control group (P<0·001), but lower in the HFn-3 group compared to the HF group (P<0·001). HDL-cholesterol was lower in the HFn-3 group compared to the Control and HF groups (P<0·0005). Hepatic secretion of TAG was lower in the HFn-3 group compared to the HF group (P<0·005), but did not differ from the Control group. Hepatic gene expression of sterol regulatory element-binding protein-1c, diacylglycerol O-acyl-transferase 2 and stearyl CoA desaturase 1 was lower in the HFn-3 group, whereas carnitine palmitoyl transferase 1 and scavenger receptor class B type 1 expression was higher (P<0·05). In adipocytes and adipose macrophages, PPARγ and TNFα expression was higher in the HF and HFn-3 groups compared to the Control group. Endothelium relaxation was higher in the HFn-3 (P<0·001) than in the HF and Control groups, and was correlated with glucose intolerance (P=0·03) and cholesterol (P=0·0003). In conclusion, n-3 PUFA prevent some metabolic disturbances induced by high-fat diet and improve endothelial function in hamsters.

**Key words:** n-3 PUFA: Dyslipidaemia: Glucose intolerance: Endothelial function: Hamsters

The metabolic syndrome is related to an increase in incidence of type 2 diabetes and CVD(1). The disturbances associated with obesity (glucose intolerance, dyslipidaemia and post-prandial hyperlipaemia) are independent risk factors for endothelium dysfunction, which is considered as an early marker of atherosclerosis(2).

Among fatty acids, n-3 PUFA, especially EPA and DHA found in sea fish, have been reported to reduce the risk of arrhythmia and myocardial infarction in animal models(3,4). Effect of n-3 PUFA on lipid metabolism is widely reported in rodents(4,5), and in human subjects(6,7), with various findings on LDL- and HDL-cholesterol (HDLc) depending on the type of study and animal species. Postprandial lipaemia response to n-3 PUFA also showed inconsistent data(8,9). Glucose intolerance and insulin resistance are associated with a high risk of developing diabetes(10). Results from human epidemiological studies indicated that n-3 PUFA reduce the development of insulin resistance and diabetes, whereas those from intervention studies in animal models present some divergences(11,12).

**Abbreviations:** AUC, area under the curve; BW, body weight; CD11b, cluster of differentiation molecule 11b; Control, control diet; HDLc, HDL-cholesterol; HF, high-fat diet not enriched with n-3 PUFA, HFD, high-fat diet; HFn-3, high-fat diet enriched with n-3 PUFA; LPL, lipoprotein lipase; MDA, malondialdehyde; PE, phenylephrine; RCT, reverse cholesterol transport; SCD1, stearyl CoA desaturase 1; SR-B1, scavenger receptor class B type 1; SREBP-1c, sterol regulatory element-binding protein-1c.

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Although controversial, clinical studies with fish or fish oils have also shown a decrease in cardiovascular events in human subjects\(^{(13)}\). Several mechanisms have been reported to explain this effect\(^{(14,15)}\). Whether or not fish intake is associated with future development of the metabolic syndrome and its related endothelium dysfunction has not been carefully evaluated. Endothelium dysfunction is related to the metabolic syndrome, and a diet enriched with DHA has been reported to enhance vasodilation mechanisms and to attenuate constriction responses in hyperlipidaemic, overweight men\(^{(16)}\). However, in experimental models, the influence of EPA and DHA on endothelium-dependent vasorelaxation has been controversial and seems to depend mostly on the experimental conditions and the animal species. Preincubation of aortic rings with EPA reduced the vessel-relaxing response to carbachol under similar conditions and the animal species. Preincubation of aortic rings with EPA reduced the vessel-relaxing response to carbachol in healthy rats\(^{(17)}\). On the same line, DHA-enriched diet with EPA reduced the vessel-relaxing response to carbachol in spontaneously hypertensive rats\(^{(18)}\) and in Tokushima Fatty rats\(^{(20)}\), whereas EPA improved the endothelium-dependent aortic vasorelaxation in spontaneously hypertensive rats\(^{(17)}\) and in streptozotocin diabetic rats\(^{(19)}\), whereas EPA improved the endothelium-dependent relaxation of Otsuka Long-Evans Tokushima Fatty rats\(^{(20)}\). The effect of n-3 has already been assessed in metabolic disturbances associated with obesity. However, few studies evaluated the involved parameters in the same animal and in the same conditions, and none of these used the hamster model. The present study is the first one that integrated different metabolic disturbances associated with obesity and the metabolic syndrome, in the same condition and using the hamster animal model. The hamster has been used as a valuable model because of its similarities to human subjects with regard to lipoprotein cholesterol metabolism\(^{(21)}\) and development of atherosclerotic lesions\(^{(22)}\).

In this study, we investigated the effect of n-3 PUFA on the prevention of some clinical and metabolic disturbances associated with obesity (dyslipidaemia, glucose intolerance, body composition) as well as endothelial dysfunction in hamsters fed with a high-fat diet (HFD).

### Materials and methods

#### Animals

A total of thirty-six male golden Syrian hamsters were obtained from Janvier (Le Genest-St-Isle, France) at 8 weeks of age, weighing 80–90 g. They were housed in colony cages with wood litter (four hamsters per cage) in a controlled environment (22°C, 12 h light–12 h dark cycle) and received water and diet ad libitum. All experiments were performed according to the regulations for animal welfare of the French Ministry of Food, Agriculture and Fisheries. The experimental protocol adhered to European Union guidelines and was approved by the local animal use and care advisory committee.

#### Diets

Two HFD (21 % fat, w/w), either enriched (HF-n-3) or not (HF) with n-3 PUFA, and a control chow diet (5 % fat, w/w; Control) were used. Each of the HFD were designed with the same composition and contained 387 % starch, 24.7 % proteins, 21 % lipids, 8.5 % minerals, 6 % cellulose and 1.2 % vitamins. The lipid mixture consisted of 15 % lard, 3.5 % palm oil and 2.5 % maize oil for HF. In the HF-n-3 diet, 10 % of lard (1.5 g) was replaced by an equivalent amount of n-3 PUFA oil mixture (Pierre Fabre Santé, Castres, France). In both diets, lipids provided about 45 % of total energy content. The Control diet contained 23 % protein, 58 % starch, 5 % lipids (2 % maize oil and 3 % palm oil), 8.5 % minerals, 6 % cellulose and 1.2 % vitamins, and 13 % of energy intake was provided by lipids. The experiments were performed in three groups of animals for a period of 20 weeks of consumption of one of the three diets. The fatty acid composition of the diets was analysed using a gas chromatograph with DB225 capillary column (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA; 60 m × 0.25 mm × 0.25 µm; Table 1).

### Body composition

Hamsters were intraperitoneally injected with 150 mg/kg body weight (BW) \(^{2}H\)-labelled water (99-9 % D/H; Eurisotop, Gif-sur-Yvette, France). Blood samples were collected before and 2 h after administering tracer injection. Plasma samples were frozen at −20°C until analysis. Plasma \(^{2}H\) enrichment was analysed by Fourier-transformed IR spectroscopy liquid using the sampling cell system (Perkin-Elmer, Les Ulis, France). Total body water was calculated from the dilution space of the isotope. A 74.4 % fat-free mass hydration rate was assumed. Fat-free mass was thus calculated as total body water/0.744\(^{(25)}\).

### Glucose tolerance test

An intraperitoneal glucose tolerance test was performed at 09:00 hours after being unfed for 18 h. Eight hamsters of each group received 1 g of glucose per kg BW intraperitoneally.

#### Table 1. Lipid diet composition (% of total fatty acids) measured by GC

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>HF group</th>
<th>HF-n-3 group</th>
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<tr>
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<td>1.25</td>
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<tr>
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<td>–</td>
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<tr>
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<td>–</td>
</tr>
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<td>16:1 n-9</td>
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<td>0.3</td>
<td>0.25</td>
</tr>
<tr>
<td>16:1 n-7</td>
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<td>2.5</td>
<td>2.07</td>
</tr>
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<tr>
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<td>2.1</td>
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<td>0.69</td>
<td>0.82</td>
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<td>24.7</td>
<td>9.1</td>
<td>16.4</td>
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<td>20:4 n-6</td>
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<td>16.7</td>
</tr>
<tr>
<td>PUFA n-3</td>
<td>0.5</td>
<td>0.4</td>
<td>4.5</td>
</tr>
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</table>

Control, control diet; HF, high-fat diet; HF-n-3, high-fat diet enriched with n-3 PUFA.
Blood samples were obtained by retro-orbital puncture (under isoflurane-induced anaesthesia) at 0 (basal), 10, 20, 30, 60 and 120 min and glycaemia was measured immediately using fresh blood (glucometer: Accu-Chek active; Roche Diagnostics, Mannheim, Germany). Areas under the curves (AUC) were calculated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

**Plasma lipids**

Hamsters were food deprived for 18 h and blood was obtained by retro-orbital puncture to determine plasma lipids. Plasma was then separated by centrifugation (4°C, 10 min, 3000 g). The following components were quantified in plasma: TAG, cholesterol and NEFA using enzymatic kits (Bio-Merieux, Marcy-l’Etoile, France and Wako Chemicals, Richmond, VA, USA). Cholesterol and TAG profiles were performed using fast protein liquid chromatography (AKTA FPLC SYSTEM; GE Healthcare, Piscataway, NJ, USA).

**Post-prandial lipaemia**

A measure of 450 μl of olive oil per 100 g BW was given to animals unfed for 18 h. Blood was taken from the eye orbital venous plexus every 2 h for 10 h. Plasma was separated, TAG concentration was measured and AUC was calculated.

**Measurement of the in vivo secretion of VLDL TAG**

The effect of n-3 PUFA on the secretion of VLDL TAG by the liver was determined using tyloxapol (Sigma-Aldrich Chemicals, Lyon, France), a non-ionic surfactant that inhibits the liver was determined using tyloxapol (Sigma-Aldrich Healthcare, Piscataway, NJ, USA)(24).

Evaluation of lipid peroxidation

The measurement of malondialdehyde (MDA) concentration in plasma was performed by using an MDA kit (Sobioda, Montbonnot-Saint-Martin, France).

**Preparation of aortic rings and functional procedures**

A total of three or four hamsters from each group were anaesthetised with pentobarbital (60 mg/kg BW). The thoracic aorta was dissected. The blood vessels were cleaned of connective tissue and cut into 2-mm rings. The rings were suspended in organ chambers of Mulvany myograph (Danish Mio Technology, Aarhus, Denmark). The arterial rings were challenged with KCl (60 mmol/l) to evaluate their functional integrity and then contracted with increasing doses of phenylephrine (PE, 10⁻⁹ to 10⁻⁴ mol/l; Sigma-Aldrich Chemicals). The concentration of PE inducing 90% maximal contraction of the rings was used to contract the arteries. The rings were then relaxed with increasing doses of carbobachol (10⁻⁹ to 10⁻⁴ mol/l; Sigma-Aldrich Chemicals).

**Separation of adipose tissue components**

Gonadal depots were transferred in a Falcon tube filled with Dulbecco’s modified Eagle’s media. The adipose tissue was then chopped thoroughly and resuspended in 10 ml digestion solution (7 ml Hanks’ solution, 3 ml of 7·5 % BSA and 20 mg collagenase type II; Sigma-Aldrich Chemicals). The digestion was performed at 37°C using a shaker at 100 rpm for 20 min. Then, the samples were kept at room temperature for 5 min. After that, the adipocyte fraction (floating) was isolated, and the solution containing the stroma vascular fraction was filtered through a 100-mm cell strainer (BD Falcon, Isère, France), collected in a new tube and then centrifuged at 1500 rpm, 4°C for 5 min. The stroma vascular fraction pellet was suspended in 1 ml of selection buffer (PBS, 2 mM-EDTA, 0·5 % BSA), centrifuged again and then the cluster of differentiation molecule 11b (CD11b)-positive cells were selected using CD11b micro-beads (Miltenyi Biotec, Bergisch Gladbach, Germany).

**Measurement of lipid content in liver and muscle**

About 30 mg of hepatic tissue and 50 mg of muscle were used to extract lipid using the Folch method(20). Briefly, the tissue was homogenised with chloroform–methanol (2:1) and then centrifuged to recover the liquid phase. The solvent was washed with 0.9 % NaCl solution. After centrifugation, the lower chloroform phase containing lipids was evaporated and lipids were suspended in 0.5 ml of ethanol. TAG and cholesterol were then measured using Biomérieux kit reagents (TAG: PAP 150, cholesterol RTU; Biomérieux, Marcy-l’Etoile, France).

RNA extraction from liver, adipocyte and macrophage and gene expression analysis

Tissue samples for mRNA analysis were homogenised, and RNA was isolated using Trizol reagent (Invitrogen, Villebon sur Yvette, France). Real-time quantitative PCR analysis was performed as follows: 1 μg of total RNA was reverse-transcribed using 100 U (100 mol/min) of Moloney murine leukaemia virus RT (Promega, Charbonnières-les-Bains, France). Real-time quantitative PCR was performed on the 7000 Sequence Detection System (GeneTool, Silicone Valley,
CA, USA) with SYBR green MESAGREEN Master Mix Plus (Eurogentec, Angers, France). The reaction contained 10 ng of reverse-transcribed total RNA, 500 nM forward and reverse primers and 5 £ 10⁻⁹ SYBR green mix. Primer sequences are available on request. All reactions were performed at least in duplicate and cyclophilin RNA amplification was used as a reference. Each couple of primers was tested in successive dilutions of complementary DNA to analyse and validate its efficiency.

The expression of sterol regulatory element-binding protein-1c (SREBP-1c), stearyl CoA desaturase 1 (SCD1), diacylglycerol O-acyltransferase 2, fatty acid synthase, PPARγ, carnitine palmitoyl transferase 1, microsomal TAG transfer protein, scavenger receptor class B type I (SR-B1), LPL and TNFα was measured in liver.

The expression of CD11b (present only in macrophage membrane), SCD1, PPARγ, fatty acid synthase, LPL and TNFα was measured in adipocytes and adipose tissue macrophages.

Statistics

Results were expressed as mean values and standard errors of the mean. Statistical analyses were performed using Statview software (SAS Institute Inc., Cary, NC, USA). Two-way repeated measures ANOVA followed by Fisher’s protected least significant difference (PLSD) test were performed to estimate the effect of group and n-3 PUFA enrichment diet on obtained values. A simple regression was used to evaluate the correlation among variables. Differences were considered significant at P < 0.05.

Results

Effects of dietary n-3 PUFA on body weight, body fat percentage, muscle lipid content and glucose tolerance

At the end of 20 weeks, the HF group had a higher BW than the Control group (P < 0.05), and the BW of the HF-n-3 group was lower than that of the HF group (P < 0.05) and did not

| Table 2. Effects of dietary n-3 PUFA on weight, percentage of fat mass, basal glycaemia, plasma lipid concentrations VLDL cholesterol (VLDLCH), LDL cholesterol (LDLCH) and HDL cholesterol (HDLCH) (Mean values with their standard errors) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Control group   | HF group        | HFn-3 group     |                |                |                |                |
|                | Mean SEM        | Mean SEM        | Mean SEM        | P              |                |                |                |
| Weight (g)     | 123.7 2.8       | 134.9* 3.2      | 125.1† 3.2      | P < 0.01       |                |                |                |
| Fat (%)        | 13.6 1.6        | 17.60 1.5       | 13.9 1.0        | P = 0.09       |                |                |                |
| Glycaemia (g/l)| 0.96 0.07       | 1.30* 0.08      | 1.1† 0.07       | P < 0.01       |                |                |                |
| TAG (mmol/l)   | 1.37 0.1        | 2.64* 0.24      | 1.83† 0.14      | P < 0.0001     |                |                |                |
| CH (mmol/l)    | 3.31 0.18       | 4.06* 0.54      | 3.29† 0.46      | P < 0.0005     |                |                |                |
| NEFA (mmol/l)  | 0.77 0.06       | 0.66 0.04       | 0.68 0.06       | NS             |                |                |                |
| VLDLCH (mmol/l)| 0.208 0.003     | 0.596* 0.03     | 0.592* 0.031    | P < 0.0001     |                |                |                |
| LDLCH (mmol/l) | 0.52 0.009      | 0.697* 0.035    | 0.646* 0.033    | P < 0.0005     |                |                |                |
| HDLCH (mmol/l) | 2.608 0.046     | 2.831 0.142     | 1.988† 0.104    | P < 0.0001     |                |                |                |

Control, control diet; HF, high-fat diet not enriched with n-3 PUFA; HF-n-3, high-fat diet enriched with n-3 PUFA.

* Mean values were significantly different from those of the Control group (P < 0.05).

† Mean values were significantly different for the HF-n-3 group from those of the HF group (P < 0.05).

Fig. 1. Effect of different diets on glycaemia after an intra-peritoneal injection of glucose (1 g/kg body weight). Values were measured in control diet (Control, ), high-fat diet (HF, ), high-fat diet enriched with n-3 PUFA (HF+n-3, ) groups at t0, t10, t20, t30, t60 and t120 min. Area under the curve (AUC) values are given as means with their standard errors represent by vertical bars for a group of six to eight hamsters. * Mean values were significantly different from those of the Control group (P < 0.05). † Mean values were significantly different for the HF-n-3 group from those of the HF group (P < 0.05).
differ from the Control group (Table 2). A higher percentage of fat body mass was observed in the HF group compared to the Control group ($P=0.048$), whereas fat percentage tended to be lower in the HFn-3 group compared to the HF group ($P=0.07$) and did not differ from the Control group. Muscle TAG content was similar in the Control group (4.01 (SEM 0.80) mg/g) and HF group (4.76 (SEM 0.23) mg/g), but was significantly lower in the HFn-3 group (2.29 (SEM 0.29) mg/g), with $P=0.008$. No difference was observed in cholestrol content among groups.

Basal blood glucose was higher in the HF group than in the Control and HFn-3 groups ($P<0.05$; Table 2). After intraperitoneal injection of glucose, the HF group showed an impairment of glucose tolerance assessed by glycaemia AUC compared to both Control ($P<0.0001$) and HFn-3 ($P<0.0005$) groups. No significant difference was observed between the Control and HFn-3 groups (Fig. 1).

**Effects of dietary n-3 PUFA on plasma lipids**

The effect of diets on plasma lipids is shown in Table 2. Plasma TAG and cholesterol were higher in the HF group ($P<0.001$) compared to the Control group, whereas the HFn-3 group showed a lower concentration of TAG ($P<0.01$) and cholesterol ($P<0.001$) compared to the HF group. TAG concentration was higher in the HFn-3 group compared to the Control group. No difference was observed in plasma NEFA concentrations.

Data from fast protein liquid chromatography are shown in Table 2. Cholesterol level was higher in VLDL and LDL in both HF and HFn-3 groups compared to the Control group ($P<0.005$), whereas it was lower in HDL of the HFn-3 group compared to both Control and HF groups ($P<0.0005$). Diet enriched with n-3 PUFA significantly decreased VLDL TAG. No change was observed in LDL and HDL TAG content among groups.

**Effect of dietary n-3 PUFA on endogenous TAG secretion and liver lipid content**

*In vivo* TAG secretion rate was measured after administering an intravenous injection of tyloxapol (Fig. 2). The results show that plasma TAG was higher in the HF group compared to Control and HFn-3 groups at t30 ($P<0.05$) and t120 ($P<0.01$) after tyloxapol injection. No significant difference was observed at t180 between the Control and HF groups ($P=0.11$), whereas plasma TAG in HFn-3 group was lower than that in the HF group ($P=0.002$) and tended to be lower compared to the Control group ($P=0.06$). The HFn-3 group presented a lower TAG secretion rate (1.19 (SEM 0.14) mmol/l per h) compared to the HF group (1.84 (SEM0.22) mmol/l per h; $P=0.006$) and tended to decrease compared to the Control group (1.46 (SEM2.0) mmol/l per h; $P=0.098$). Liver TAG content was higher in the HFn-3 group compared to the Control group ($P<0.05$), but lower compared to the HF group ($P<0.05$). No difference was observed in hepatic cholesterol content among the three groups.

**Post-prandial lipaemia**

AUC of plasma TAG after olive oil oral administration was higher in the HF group compared to the Control group ($P<0.05$; Fig. 3) and was not significantly different from that of the HFn-3 group ($P=0.11$). Nevertheless, plasma TAG concentration was significantly lower in the HFn-3 group at 6, 8 and 10h after oral administration compared to the HF group, although it did not differ from the Control group at these time periods.

**Lipid peroxidation**

The measurement of MDA in plasma of the different groups showed no difference between the Control (11.59 (SEM 1.19) μmol/l) and HF (13.75 (SEM1.59) μmol/l) group. MDA concentration in the HFn-3 group (27.64 (SEM2.21) μmol/l) was higher compared to both the Control and HF groups ($P<0.001$).

**Endothelial function**

The contraction produced in response to PE was the same in all the three groups, whereas carbachol added to
pre-constricted rings with $10^{-6}$ M of PE showed significant difference of relaxation between the HF-n-3 group compared to both the Control and HF groups at $10^{-6}$, $10^{-5}$ and $10^{-4}$ M-carbachol with $P<0.01$. Vessels of HF-n-3 hamsters were more sensitive to carbachol ($10^{-6}$ M)-induced relaxation (47.5 (SEM 5.0)%) compared to the Control (22.13 (SEM 5.7)%; $P=0.01$) and HF groups (7.28 (SEM 4.85)%; $P<0.0005$). Control and HF groups did not differ significantly ($P=0.09$). At $10^{-5}$ and $10^{-4}$ M of carbachol, HF-n-3 hamsters vessel relaxation remained higher ($P<0.005$), whereas no difference was observed between the Control and HF groups (Fig. 4).

Endothelium-dependant vasorelaxation correlated negatively with glucose intolerance ($r^2=0.591$, $P=0.033$) and with total cholesterol concentration ($r=-0.84$, $P=0.0003$).

**Gene expression in liver, adipocytes and macrophages**

Hepatic SREBP-1c mRNA expression was strongly activated in the HF group compared to the Control group ($P<0.005$), whereas it reduced in the HF-n-3 group ($P<0.005$). Diacylglycerol O-acyltransferase 2 and SCD1 expression was lower in the HF-n-3 group compared to both the Control and HF groups ($P<0.05$). There was no significant difference for fatty acid synthase ($P=0.08$). PPARγ mRNA expression showed no significant increase with n-3 PUFA compared to the Control and HF groups. Carnitine palmitoyl transferase 1 gene expression was higher in the HF-n-3 group compared to the Control group, whereas it did not differ from the Control group. Hepatic mRNA expression of LPL and TNFα genes was not affected by diet. SR-B1 gene expression was higher in the HF-n-3 group compared to both the Control and HF groups ($P<0.05$; Fig. 5).

In macrophages, the expression of PPARγ and TNFα was higher in the HF and HF-n-3 groups compared to the Control group ($P<0.05$). No difference was showed for fatty acid synthase, LPL and SCD1. In adipocyte, expression of these genes was not different among the three groups.

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**Table 1.** Effect of different diets on TAG after gavages with olive oil and area under the curve (AUC) measurement. Values are means, with their standard errors represented by vertical bars. * Mean values were significantly different for the high-fat diet (HF, ---) group from those of the control diet (Control, ---); $P<0.05$) group. † Mean values were significantly different for the HF group from those of the high-fat diet enriched with n-3 PUFA (HF-n-3, ---); $P<0.05$) group.

<table>
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<tr>
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<th>HF-n-3 group</th>
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</table>

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**Fig. 3.** Effect of different diets on TAG after gavages with olive oil and area under the curve (AUC) measurement. Values are means, with their standard errors represented by vertical bars. * Mean values were significantly different for the high-fat diet (HF, ---) group from those of the control diet (Control, ---); $P<0.05$) group. † Mean values were significantly different for the HF group from those of the high-fat diet enriched with n-3 PUFA (HF-n-3, ---); $P<0.05$) group.

**Fig. 4.** Dose–response curves to (a) phenylephrine (Phe) and (b) carbachol (CCH) of control diet (Control, ---), high-fat diet (HF, ---) and high-fat diet enriched with n-3 PUFA (HF-n-3, ---) groups. Values are means with their standard errors represented by vertical bars ($n=4$). * Mean values were significantly different for the HF-n-3 group from those of the HF and Control groups ($P<0.001$).
Discussion

In the present study, we investigated the preventive effect of n-3 PUFA on the development of metabolic syndrome-associated disturbances induced by a HFD in golden Syrian hamster. We reported that n-3 PUFA enrichment ameliorated metabolic syndrome-associated cardiovascular risk by preventing BW gain, dyslipidaemia, high postprandial lipaemia and glucose intolerance. Despite the fact that HFD did not impair endothelial-dependent vasorelaxation in the HF group compared to the Control group, n-3 PUFA improved it in the HFn-3 group.

We showed that HFD induced obesity (as BW gain and increase in fat mass percentage) in the HF group compared to the Control group, but n-3 PUFA enrichment attenuated it in the HFn-3 group. Data from studies on the effect of n-3 PUFA on BW reduction are inconsistent. In human studies, the effect on BW was only effective when n-3 PUFA were combined with a BW loss regimen (29,30). Some studies described a limited extension of adipose tissue in rats (31,32), mice (33,34) and salmon (35) explained by different mechanisms, whereas in other studies no effect was observed (36) or observed only in mice fed with high-fat/high-sucrose diet (37). In our study, we showed a lower hepatic SCD1 gene expression in the HFn-3 group. The inhibition of SCD1 is described to protect from HFD-induced obesity related to an up-regulation of genes involved in β-oxidation and a down-regulation of lipogenesis gene expression, as shown in mice SCD (−/−) (38). However, another study in mice reported a decrease in SCD1, but with no consequence on BW (39).

Impaired glucose tolerance is widely described as a cardiovascular risk factor (10). In our study, the effect of EPA and DHA enrichment on glucose tolerance, as assessed by fasting glycaemia and AUC, was impaired in the HF group and prevented by n-3 PUFA (HFn-3) groups. The same result was reported in rodents (40,41) and human subjects, and was related to an increasing membrane fluidity and GLUT4 transport (42) or affecting insulin receptor and insulin receptor substrate expression and protein abundance (43). The prevention of BW gain associated with low muscle lipid content in the HFn-3 group can also explain the improvement of glucose tolerance measured in this group. Indeed, it was reported that BW loss induced by energy restriction decreased muscle lipid content in obese individuals with and without type 2 diabetes (44), in conjunction with enhancement of insulin sensitivity (45).

Obesity is associated with an increase in the recruitment of adipose tissue macrophages (46,47). Interventions that inhibit the recruitment of macrophages (48,49) or decrease the pro-inflammatory activity (50,51) of macrophages improve...
the insulin sensibility in obesity. In our study, the expression of genes relative to lipid metabolism and TNFα in adipocytes and in adipose macrophages was measured. The level of TNFα gene expression did not differ among groups when measured in adipocytes. However, we observed an increase in the level of gene expression of TNFα and PPARγ in macrophages of both HF and HFn-3 groups, with no effect of n-3 PUFA against inflammatory and pro-adipogenic activity of macrophages. In other studies, n-3 PUFA were described to have a protective effect against adipose tissue inflammation induced by a HFD in obese diabetic mice(52) and against TNFα production(55).

In this study, the HFD led to a trend to increase HDL₃ when compared to the control diet. This result is in accordance with what we and others have reported in hamsters fed with a HFD or cholesterol-enriched diet, with increases in TAG, total cholesterol and HDL₃. We showed that EPA and DHA decrease fasting and postprandial plasma TAG and HDL₃. The decrease of total cholesterol has been reported in other studies in hamsters(57,58) and mice(59) to be associated with a decrease in HDL₃. It was reported that this decrease was associated with an overexpression of SR-B1 (50,60), whereas a study in obese rats reported an increase in SR-B1 activity, but with no change in HDL₃ level(61). Most studies of reverse cholesterol transport (RCT) are conducted in mice or rats. These species do not express cholesteryl ester transfer protein, a protein that transfers cholesteryl ester from HDL to VLDL/LDL, followed by a hepatic uptake of these lipoproteins. As this pathway is the major route of RCT in human subjects, a cholesteryl transfer protein-expressing species, such as the hamster, represents a convenient preclinical model for investigating novel therapies for the treatment of dyslipidaemia in human subjects. It has been revealed that the overexpression of hepatic SR-B1 increases RCT and has an anti-atherosclerotic role, despite low HDL₃ concentrations(62–64). We recently reported in hamsters fed with a cholesterol-enriched diet a high HDL₃ and low SR-B1 gene expression associated with a decrease in RCT efficiency(56). In the present study, we found an increase of SR-B1 expression in liver. These data suggested that n-3 PUFA stimulates one step in the rate of cholesteryl ester transport from peripheral tissues to the liver, probably by increasing the amount of SR-B1. The level of LDL and VLDL cholesterol did not change with n-3 PUFA enrichment, whereas some studies described an increase in LDL-cholesterol in fish oil-fed hamsters(65) and in type 2 diabetic patients(66).

The hypotriacylglycerolaemic effect of n-3 PUFA shown in several studies in both rodents(4,5) and human subjects(6,7) was confirmed in this study. We also showed a lower liver TAG content with n-3 PUFA related to a decrease in VLDL secretion. This decrease may be explained by both a decrease in lipid synthesis and catabolism. We measured the gene expression of SREBP-1c, the main transcriptional factor controlling lipid synthesis in the liver. We observed an increase in SREBP-1c expression in the presence of the HFD and a decrease in the presence of n-3 PUFA. We noted that n-3 PUFA reduced diacylglycerol O-acyltransferase 2, an enzyme that catalyses the final step of TAG synthesis. Furthermore, CPT-1, an enzyme involved in the transport of fatty acids into the mitochondria, was higher in the HFn-3 compared to the HF group and did not differ from the Control group. This result suggested an active mitochondrial β-oxidation. The same results were observed in mice fed with a DHA-supplemented diet(67). This was not observed in rats fed with EPA, in which no effect was observed in SREBP-1c expression(68). Unexpectedly, the effect of n-3 PUFA on lipogenesis and hepatic lipid accumulation was not related to the activation of PPARγ, but seems to result from the inhibition of SREBP-1c expression. The same result was obtained recently in mice fed with EPA and was explained by the inhibition of maturation of SREBP-1c by n-3 fatty acids(69).

The decrease of VLDL secretion by the liver was also reported in other studies. In vitro, HepG2 cells treated with DHA resulted in overall reduced apoB100 secretion(70). In rats, DHA decreased VLDL secretion(71,72). Diabetic patients treated with EPA and DHA showed a decrease in VLDL apoB100 concentration related to a decrease in VLDL production(73). Recently, another mechanism implying lipid peroxidation and oxidative stress on ApoB100 degradation via a post-endoplasmic reticulum presecretory proteolysis, was described to be relevant to the hypotriacylglycerolaemic effect of dietary n-3 fatty acids(74,75). Consistent with these data, we showed an increase in the level of MDA in the HFn-3 group compared to the Control and HF groups.

In this study, although the postprandial lipaemia magnitude, as calculated by the AUC, was similar between the HF and HFn-3 groups, the triacylglycerolaemia at 6, 8 and 10 h of kinetic were lower in the HFn-3 group. Postprandial TAG concentration reflects production and clearance of chylomicrons. Harris et al.(9) suggested that n-3 PUFA accelerate chylomicron lipid clearance by facilitating LPL-mediated lipolysis. Similarly, a study in mice also showed an enhancement of blood clearance of TAG-rich particles(70-a). As no difference was observed in LPL gene expression in our study, we suggested that n-3 PUFA lowered postprandial lipaemia by decreasing the release of TAG-rich lipoproteins.

In our experimental conditions, the evaluation of endothelial function did not show a difference between the Control and HF groups despite the difference in glucose tolerance and lipid plasma concentrations. However, in the presence of n-3 PUFA, we observed better endothelium-dependent vascular response in accordance with previously reported data(77). The endothelium relaxation was negatively correlated with the impairment of glucose tolerance and plasma cholesterol concentration. This improvement can be explained by the incorporation of n-3 PUFA into cell membranes, thus affecting function of cell and tissues with subsequent impact on the production of various vasoactive eicosanoids and other mediators(78).

In conclusion, we demonstrated that, although no effect was observed on inflammation (TNFα in macrophages), n-3 PUFA have a preventive effect on the development of BW gain, glucose intolerance and dyslipidaemia induced by a HFD with decrease of hepatic secretion of VLDL TAG by reducing the expression of genes involved in lipogenesis,
especially SREBP-1c and diacylglycerol O-acyltransferase 2. These disturbances induced by the HFD were not associated with endothelial function impairment in the HF group, but we showed a beneficial effect on this function with n-3 PUFA enrichment. Finally, we showed, under n-3 PUFA supplementation, a decrease in HDL$_C$ concentration related to an increase in SR-B1 gene expression, suggesting an improvement of RCT. This later effect needs more investigation to elucidate the effect of n-3 PUFA on HDL turnover and RCT.

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