Long-Chain (n-3) Polyunsaturated Fatty Acids Prevent Metabolic and Vascular Disorders in Fructose-Fed Rats

Vanessa Robbez Masson, Anthony Lucas, Anne-Marie Gueugneau, Jean-Paul Macaire, Jean-Louis Paul, Alain Gryenberg, and Delphine Rousseau*

Abstract

The crossover relationship between cardiometabolic risk, in terms of insulin resistance and vascular dysfunction, and the fatty acid (FA) profile of insulin-sensitive tissues as well as the dietary FA impact has almost never been explored in the same experiment. In this study, the intake of α-linolenic acid (ALA) alone and/or with its higher metabolites, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were evaluated in a nonobese, hypertriglycerideremic and insulin-resistant rat model, that exhibits the 2 main characteristics of metabolic syndrome. Wistar rats were fed either a cornstarch and (n-6) PUFA-based diet (C-N6) or a 66% fructose diet over a 10-wk period. Fructose-fed rats received a diet containing ALA alone (F-ALA group) or ALA plus EPA and DHA (F-LC3 group) or no (n-3) PUFA (F-N6 group). The 10-wk high-fructose diet (F-N6) induced an insulin-resistant state, as assessed by glucose and insulin tolerance tests. Insulin resistance was linked to a specific FA pattern in insulin-sensitive tissues, which probably involved modifications of Δ9, Δ6, and Δ5-desaturases. This pathological status was related to high cardiovascular risk as assessed by increases in systolic and diastolic blood pressures and particularly by the increase of pulse pressure, an index of vascular stiffness obtained from telemetry investigations. The (n-3) experimental diets prevented changes in the FA patterns in insulin-sensitive tissues, insulin resistance, and vascular dysfunction. This beneficial effect was large with an intake of long chain (n-3) PUFA (ALA+EPA+DHA) and to a lesser extent with dietary ALA alone. J. Nutr. 138: 1915–1922, 2008.

Introduction

Insulin resistance, impaired glucose tolerance and/or hyperglycemia, high blood serum triglycerides (TG), low HDL cholesterol, high blood pressure (BP), and abdominal adiposity are characteristics of metabolic syndrome. The association of 3 (or more) of these factors leads to an increased risk of type 2 diabetes and cardiovascular diseases, including hypertension and atherosclerosis (1,2), often referred to as “cardiometabolic risk” (3). However, it is not clearly established whether the development of cardiovascular diseases results from insulin resistance alone or from the combination of insulin resistance and obesity. Dietary long-chain PUFA (LCPUFA) are considered prevention factors in cardiovascular diseases and associated with metabolic disorders, including insulin resistance and dyslipidemia (4,5). High carbohydrate diet-induced insulin resistance was correlated with a specific fatty acid (FA) pattern in insulin-sensitive tissues (6–8). Alterations in desaturase activity, particularly an increase of Δ9 and Δ6 and a decrease of Δ5, have been suggested to induce specific changes in the FA tissue profile observed in insulin resistance (9). However, as far as we know, the relationship between cardiometabolic risk (in terms of insulin resistance and vascular dysfunction) and the membrane phospholipid (PL) FA composition and the impact of dietary FA have not been investigated conjunctively. In animals as well as in humans, dietary (n-3) LCPUFA affect the cardiovascular system by decreasing BP and improving cardiac function (10–14). Moreover, increases in (n-3) LCPUFA, namely eicosapentaenoic acid [20:5(n-3), EPA] and docosahexaenoic acid [22:6(n-3), DHA], have been closely related to positive...
Materials and Methods

Animals. Male Wistar rats (Charles River), 6 wk old, were housed individually at 23 ± 1°C, with a 12-h light:dark cycle. Rats were initially fed a standard rat diet (Safe A04). After a 1-wk acclimation period, the rats were randomly assigned to 4 groups and telemetry surgery was performed on the animals from only 3 groups. After a 1-wk recovery period, all rats were fed experimental diets, with food and water freely available. The investigations were carried out in agreement with the NIH guidelines for the care and use of Laboratory Animals (NIH pub. no. 85–23, revised 1996). The animal holding facility was registered (agreement no. A 92–019–01) and the main participants were authorized to manage experiments (agreement level I, no. 92–261). The protocol was approved by the Animal Care and Use Committee of the Faculty of Pharmacy, University Paris-Sud XI.

Experimental design. Four groups of rats (n = 6) were fed for 10 wk with different semipurified diets, containing 8% fat (80 g/kg of diet). The control group received a diet containing cornstarch + sucrose as carbohydrate sources and (n-6) PUFA as PUFA source (C-N6). The experimental groups were fed diets containing 66% fructose and one of the following PUFA supplies: (n-6) PUFA (F-N6), (n-3) PUFA as ALA (F-ALA), or (n-3) PUFA as a mix of ALA, EPA, and DHA (F-LC3) (Table 1). In the 4 groups, an i.v. glucose tolerance test (IVGTT) was performed after 0, 4, and 8 wk and an insulin tolerance test (ITT) was performed after 10 wk. BP was measured using either tail-cuff (wk 0 and 7, in the 4 groups) or telemetry (in the 4 fructose groups). At 0800, food was removed, and 6 h later (1400), rats were anesthetized (50 mg/kg intraperitoneal) and the blood was collected by aorta puncture for serum glucose, insulin, TG, and FA profile determination. The rats were killed as previously described (12). BP and ECG were monitored 24 h/d for 10 s at 10-min intervals, 3 d/wk for 8 wk in unrestrained conditions. Night recordings were separated from day recordings and the stressful (2 h) periods of night/day transition were removed. SBP and diastolic BP (DBP) have been analyzed and pulse pressure (PP) was calculated from SBP and DBP.

Metabolic studies: IVGTT and ITT. At 0800, food was removed. At 1400, the rats received a bolus of either glucose (0.5 g/kg) for IVGTT or insulin (3.5 mmol/kg iv) for ITT. IVGTT was performed according to Valensi et al. (25) and ITT according to Furuya et al. (26). The blood glucose disappearance (KITT) was determined by the linear regression of the neperian logarithm of blood glucose values (27). Blood glucose was determined using a glucose analyzer (Analox GM9D, Analox Instruments).

Biochemical investigations. Serum TG was enzymatically determined with a LX20 analyzer (Beckman-Coulter) and insulinemia was determined using the Rat Insulin ELISA kit (Mercodia). Blood samples and

<table>
<thead>
<tr>
<th>TABLE 1 Composition of the experimental diets</th>
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<tbody>
<tr>
<td>C-N6</td>
</tr>
<tr>
<td>Soy protein isolate</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Cornstarch</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Agar-agar</td>
</tr>
<tr>
<td>Maltodextrin</td>
</tr>
<tr>
<td>L-Cystine</td>
</tr>
<tr>
<td>Choline bitartrate</td>
</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Mineral mixture</td>
</tr>
<tr>
<td>Vitamin mixture</td>
</tr>
</tbody>
</table>

1 The individual basal mix components in g/kg of diet were prepared as jellied cubes as described earlier (12) according to the AIN-93 (56).
2 MP Biomedicals 905456.
3 Louis François.
4 Vitamin AIN-93VX and mineral AIN-93M mixtures were prepared according to AIN-93 (56), MP Biomedicals.
5 Barry-Callebaut.
6 Lesieur.
7 Ch. Daudry Van Cauwenberge & Fils.
8 TG40/90, EPA + DHA, Phosphotech.
9 As determined by analysis of the manufactured diets.

BP measurement. BP was measured using either the tail-cuff technique or telemetry (12,22). For the tail-cuff technique, each rat was conditioned before the measurements; this was done using a Powerlab system (ADInstruments) and a BP sensor (Phymep). These values are reported as the average of 5 individual measurements on conscious rats maintained at 28°C in a restraining tube. When the heart rate was higher than 400 bpm, the measurement was not taken into consideration. The systolic BP (SBP) changes were evaluated by calculating the Delta (individual change) between wk 0 and 7 of nutritional treatment. The telemetry system (DSI) consisted of 18 radiotransmitters (TL11M2-C50-PXT), 18 individual receivers (RLA1020), and the Dataquest ART 3.1 (DSI) software for data analysis. Surgery was performed on rats under pentobarbital anesthesia (50 mg/kg intraperitoneal) and flunixin analgesia (0.34 mL/kg subcutaneous). The transmitter catheter for BP measurement and the subcutaneous electrodes for ECG recording were implanted as previously described (12). BP and ECG were monitored 24 h/d for 10 s at 10-min intervals, 3 d/wk for 8 wk in unrestrained conditions. Night recordings were separated from day recordings and the stressful (2 h) periods of night/day transition were removed. SBP and diastolic BP (DBP) have been analyzed and pulse pressure (PP) was calculated from SBP and DBP.

Biochemical investigations. Serum TG was enzymatically determined with a LX20 analyzer (Beckman-Coulter) and insulinemia was determined using the Rat Insulin ELISA kit (Mercodia). Blood samples and

health benefits associated with insulin sensitivity, glucose metabolism, and cardiovascular diseases (4,12,15,16). However, meeting the population’s need for increased intake of (n-3) LCPUFA through fish supply may raise several ecological and economical concerns. The plant-derived shorter chain (n-3) PUFA, α-linolenic acid (ALA), can be converted to longer and more unsaturated chain PUFA in several species, although the level of DHA synthesis from ALA is extremely low in humans (17), as well as in mammalian cardiac cells (18). Unfortunately, dietary ALA has rarely been studied in the context of cardiometabolic risk (19–21). The fructose-fed rats are described as a nonobese model of insulin resistance, displaying hyperinsulinemia, hypertriglyceridemia, mild hypertension, and alterations in blood serum as well as in heart FA profile (12,16,22–24). This model was chosen to evaluate the changes in membrane FA profile induced by high dietary fructose and to investigate the specific effects of (n-3) PUFA chain length on the relationship between insulin resistance and the associated cardiometabolic risk factors.
TABLE 2  Morphometric and biochemical variables in male rats fed a cornstarch or high-fructose diet enriched with ALA or ALA+EPA+DHA (LC3) for 10 wk

<table>
<thead>
<tr>
<th></th>
<th>C-N6</th>
<th>F-N6</th>
<th>F-ALA</th>
<th>F-LC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>447 ± 9a</td>
<td>445 ± 5a</td>
<td>454 ± 8a</td>
<td>457 ± 13a</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>865 ± 27a</td>
<td>895 ± 20a</td>
<td>864 ± 10b</td>
<td>870 ± 17a</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>10.5 ± 0.25b</td>
<td>11.6 ± 0.33ab</td>
<td>11.5 ± 0.41b</td>
<td>12.2 ± 0.51b</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>11.6 ± 0.60b</td>
<td>11.6 ± 0.70b</td>
<td>12.8 ± 0.89b</td>
<td>13.4 ± 1.02b</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>346 ± 20b</td>
<td>471 ± 27b</td>
<td>418 ± 25ab</td>
<td>323 ± 29b</td>
</tr>
<tr>
<td>I/G ratio</td>
<td>30 ± 2.1b</td>
<td>41 ± 2.3b</td>
<td>34 ± 3.3ab</td>
<td>27 ± 1.7b</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.7 ± 0.06b</td>
<td>1.2 ± 0.26b</td>
<td>0.7 ± 0.11b</td>
<td>0.6 ± 0.06b</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM, n = 6. Means in a row without a common letter differ, P < 0.05.

organisms were stored at −20°C in chloroform-methanol (2:1) until FA determination. After lipid extraction (28), the primary PL component of the tissue membranes, were separated from nonphospholipid (NL) (12). FA were transmethylated with BF3-methanol and the FAME were analyzed by GC as previously described (12). Desaturase activity (DAI) were estimated as the product:precursor ratios of individual FA according to the following equations: D = [16:1(n-7)/16:0] and [18:1(n-9)/18:0], D = [18:3(n-6)/18:2(n-6)], and D = [20:4(n-6)/20:3(n-6)] (29,30). Because the (n-3) groups received D and D end-products, the D and D DAI were calculated as well, but only on the basis of the (n-6) PUFA series, particularly the dietary 18:2(n-6). Indeed, the (n-3) LCPUFA were supplied by the diet and directly entered into the membrane PL without utilizing the elongase-desaturase pathway. The values are presented for indication only.

Statistical analyses. The data are expressed as means ± SEM. Data were evaluated using 1-way ANOVA followed by a Newman-Keuls test when significance applied (P < 0.05). For longitudinal time course studies, data obtained from each group were tested at each time point with a 2-way ANOVA (time, nutritional treatment) followed by a Newman-Keuls test when significance applied (P < 0.05). All statistical analyses were conducted using NCSS60 software.

Results

Morphometric and biochemical data
Body weight and heart weight were not modified by the diets, whereas liver weight was greater in rats fed high-fructose diets than in the C-N6 group (Table 2). At the end of the experiment (wk 10), blood glucose concentrations were similar in the 4 groups. High-fructose diets induced a significant increase in both serum insulin and TG concentrations; only LC3 intake prevented the increase in both. The ALA diet prevented the increase in TG only but did not affect hyperinsulinemia. The insulin:glucose (I/G) ratio was used as a marker of insulin resistance. The fructose diet induced a 77% increase in the I/G (P < 0.05). Moreover, in the fructose groups, the LC3 diet was the only one to normalize the I/G ratio.

Insulin and glucose responses in the IVGTT
Glucose and insulin homeostasis were similar in the 4 groups before the beginning of the nutritional treatment (Table 3, wk 0). At wk 4 (Fig. 1A; Table 3), glucose homeostasis was not affected by nutritional treatments, whereas insulin homeostasis (insulin secretion and tissue insulin sensitivity) was significantly affected by the high-fructose diet. The insulin homeostasis was normalized with dietary (n-3) PUFA of every chain length (Table 3). After 8 wk of nutritional treatment, glucose homeostasis was affected by the (n-3) LCPUFA intake, especially by the intake of ALA+EPA+DHA (Table 3). The insulin homeostasis of F-N6 rats was similar after 8 wk of nutritional treatment when compared with the same F-N6 rats at wk 4. However, at wk 8, the insulin homeostasis of F-N6 rats did not differ from those of C-N6 rats, which exhibited a higher level of response at that time (P = 0.12). In fact, the insulin homeostasis of C-N6 rats tended to deteriorate between wk 4 and 8 of nutritional treatment (P = 0.058; Table 3).

Glucose response in the ITT
Fructose feeding impaired the glucose response compared with C-N6 (Fig. 2A). The absolute value of KITT was lower (P < 0.05) in the F-N6 group compared with the C-N6 group (Fig. 2B), indicating a marked decrease in insulin sensitivity. The (n-3) LCPUFA intake, especially LC3 (P < 0.05), prevented the decrease of insulin sensitivity induced by fructose feeding, as evaluated by the KITT (Fig. 2B).

BP studies
At the beginning of the experimental feeding period, all rats displayed a normal SBP (119.7 ± 2.6 mm Hg) as investigated by the tail-cuff technique. After 7 wk of dietary treatment, the SBP remained unchanged in the C-N6 group (Delta SBP between wk 0 and 7 = 3.4 ± 3.2 mm Hg). The SBP of the rats fed a high-fructose diet was significantly increased compared with those fed a control diet (Fig. 3A). This increase was prevented in the F-ALA and F-LC3 groups (Fig. 3B). BP was also investigated by telemetry in the 3 F groups. Heart rates did not differ irrespective of dietary treatment (data not shown). The increase in SBP, induced by fructose, was confirmed (Fig. 4A,B) but was less pronounced than that measured by tail-cuff. In the F-N6 group, DBP remained constant (Fig. 4C,D), whereas PP increased as a consequence of SBP increase (Fig. 4E,F). The increase of SBP and PP was significantly reduced with an intake of ALA+EPA+DHA

TABLE 3  IVGTT glucose and insulin AUC from male rats fed a cornstarch or high-fructose diet enriched with ALA or ALA+EPA+DHA (LC3) for 10 wk

<table>
<thead>
<tr>
<th>Week</th>
<th>C-N6</th>
<th>F-N6</th>
<th>F-ALA</th>
<th>F-LC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>430 ± 16ad</td>
<td>414 ± 19ad</td>
<td>414 ± 24ad</td>
<td>423 ± 20ad</td>
</tr>
<tr>
<td>4</td>
<td>16032 ± 1953bd</td>
<td>13501 ± 1490ad</td>
<td>13385 ± 1877ad</td>
<td>12275 ± 2687ad</td>
</tr>
<tr>
<td>8</td>
<td>14188 ± 692bd</td>
<td>22342 ± 2028bd</td>
<td>15384 ± 1198bd</td>
<td>14873 ± 2251bd</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM, n = 6. Means in a row without a common letter differ, P < 0.01. Means in a column without a common symbol differ, P < 0.01.
2 AUC of blood glucose or serum insulin during the time of IVGTT.

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On the contrary, intake of ALA alone did not normalize BP parameters.

**FA composition**

**Heart.** The FA profile of cardiac membrane PL was significantly affected by fructose feeding (Table 4), exhibiting a decrease of the (n-3) PUFA content and a 25% increase in the (n-6):(n-3) FA ratio. The high-fructose diet significantly increased Δ9-DAI and decreased Δ5-DAI. The ALA diet induced a strong increase in (n-3) PUFA content and a decrease in (n-6) PUFA content [mainly arachidonic acid (AA)]. The LC3 intake amplified this phenomenon, inducing a strong increase in EPA, docosapentaenoic acid, and mainly DHA as well as a further decrease in AA of the membrane.

**Blood serum.** The blood serum total FA profile was affected: fructose feeding increased the (n-6):(n-3) FA ratio (Supplemental Table 1). In the F-N6 rats, Δ9-DAI (C18) increased compared with the C-N6 rats, whereas Δ6-DAI decreased. ALA and LC3 diets strongly affected the blood serum FA profile, increasing the (n-3) PUFA content and decreasing the (n-6) PUFA. Both (n-6):(n-3) FA and P:S ratios were significantly decreased by dietary (n-3) PUFA, regardless of the chain length.

**Gastrocnemius muscle.** In skeletal muscle, the F-N6 diet decreased AA, increased linoleic acid (LA), and increased the (n-6):(n-3) FA ratio in membrane PL (Supplemental Table 2). The Δ9-DAI was higher, whereas Δ5-DAI was lower in F-N6 rats compared with the C-N6 group. Dietary ALA and LC3 induced a significant increase in (n-3) PUFA at the expense of (n-6) PUFA. DHA was the major (n-3) PUFA incorporated in muscle PL.

**Liver.** The high-fructose diet significantly decreased SFA and increased primarily (n-6) PUFA (Supplemental Table 3). Moreover, it increased significantly the Δ9- and Δ6-DAI and decreased the Δ5-DAI. The LC3 diet, but not the ALA diet, prevented changes of the SFA and PUFA content in liver PL. Both ALA and LC3 diets increased the (n-3) PUFA content, but this increase was more pronounced with the LC3 diet. In the liver, the NL were considered as well (data not shown). Fructose feeding induced a 44% increase in monounsaturated FA (MUFA) and a 27% decrease in PUFA. When the fructose-fed rats received an (n-3) PUFA diet, especially LC3, the FA qualitative distribution was close to that of the C-N6. Surprisingly, although liver weight increased after fructose feeding, the NL content was similar in F-N6 rats (2.1 ± 0.05% of the tissue mass) and the C-N6 rats (2.5 ± 0.50%).
FIGURE 3 Changes of systolic blood pressure (Δ SBP), obtained from tail-cuff measurements at wk 0 and 7 of dietary treatment, in male rats fed a cornstarch or high-fructose diet enriched with ALA or ALA+EPA+DHA (LC3). Values are means ± SEM, n = 6. Means without a common letter differ, P < 0.01.

Epididymal WAT. With respect to the PL of adipose tissue, the high-fructose diet induced an increase of AA and a decrease of (n-3) PUFA, mainly EPA (Supplemental Table 4). ALA and LC3 diets decreased (n-6) PUFA and increased (n-3) PUFA. As a consequence, the (n-6):(n-3) FA ratio was significantly decreased. NL were also considered (data not shown) and were qualitatively but not quantitatively affected by fructose. Similarly, the dietary FA affected the PUFA profile in NL in a qualitative manner, mainly through the LA:ALA ratio, as the longer chain PUFA fraction in this lipid class was extremely low.

Discussion

This study was designed to evaluate the changes in membrane FA profile induced by a high-fructose diet. Specific effects of ALA alone or ALA associated with longer chain (n-3) PUFA on these changes and on cardiometabolic risk factors were investigated in a hypertriglyceridemic and insulin-resistant rat model. This study focused on insulin resistance, independent of obesity, known for its high correlation with hypertension and cardiovascular consequences (31). As expected in this model, the fructose treatment did not affect body weight or total NL content in epididymal WAT. In rats (7,32) and humans (30), insulin resistance associated with abdominal obesity was correlated to a specific FA pattern in the target tissues of insulin action, characterized by increased AA and reduced LA content. Our results demonstrate that fructose diet increased blood serum insulin and TG in association with insulin resistance. It also significantly affected the FA patterns before the onset of obesity. The Δ9-DAI increased in blood serum, heart, skeletal muscle, liver, and epididymal WAT, whereas the Δ5-DAI decreased significantly. Similar results in humans documented that insulin resistance is associated with high Δ9-DAI and low Δ5-DAI in serum and skeletal muscle (9,29). A recent review suggested that the defect in Δ6 and Δ5 desaturase activity could be a factor that favors insulin resistance (33). Insulin resistance rather than obesity may thus be the key factor behind the changes in the LA:AA ratio reported above, through the alteration of desaturases. For the same reason, and because the desaturases are not specific to the PUFA series, our results show that fructose feeding induced a strong increase in the (n-6):(n-3) FA ratio of blood serum lipids as well as in cardiac and skeletal muscle membranes. Such an increase has been reported to play a critical role in insulin resistance (34,35). Other studies have shown that dietary-induced changes of membrane FA profiles are stabilized in 4 wk (36). Previous studies showed similar profiles to the present ones after 8 wk of similar dietary treatments (12) as well as in longer-term studies (14). In all the FA profiles reported here, DHA and other (n-3) PUFA increased with the ALA diet and to a greater extent with the LC3 diet. These results confirm that (n-3) LCPUFA need to be supplied for their potential to prevent a dramatic increase of the (n-6):(n-3) FA ratio, particularly in the heart (37), in the context of diabetes.

Despite the BP increase, the fructose-fed rats did not display cardiac hypertrophy. When BP was evaluated by tail-cuff, the BP increase after 7 wk was significant, in accordance with the literature (12,38). When BP was monitored by telemetry, we observed an increase in both SBP and PP with high-fructose diet. The BP increase was drastically lower when recorded by telemetry as opposed to tail-cuff, as already reported by our group and others (12,38). The restraint and thermal stress required for tail-cuff measurements lead to an increased sympathetic output and thus a rise in BP. Whatever the method, the fructose-induced BP increase was reduced by (n-3) PUFA intake, although ALA was clearly less efficient than LC3. This effect is probably complex and may involve the modification of the prostanoid balance that affects the constriction/dilatation balance (39), the incorporation of DHA in cardiac membranes that affects adrenergic function, the membrane fluidity (12,14,40), or the level of cytokines and growth factors (41). PP is governed by both the ventricular contractility and the intrinsic properties of the vascular tree, including stiffness (42). This parameter is viewed as an independent predictor of cardiovascular events and was reported to reflect the arterial stiffness increase in metabolic syndrome patients (43,44). In this study, PP increased significantly in fructose-fed rats within 4 wk and continued to increase slightly thereafter. This increase was limited by dietary (n-3) PUFA in the resting period (more in F-LC3 than in F-ALA) and only by LC3 in the activity period. EPA and DHA may exert complementary effects, with a role for EPA in the peripheral vasculature as a precursor of prostaglandins and a role for DHA in cardiac function (12,14).

In this model, intake of ALA with longer chain EPA and DHA partially altered glucose homeostasis. The F-LC3 diet increased glucose response to IVGTT after 8 wk of nutritional treatment but without any significant effect on basal blood glucose. This adverse effect on blood glucose control was sometimes reported in type 2 diabetic patients consuming large amounts of fish oil (45), although several clinical studies reported no glucose concentration alteration with low amounts of (n-3) LCPUFA (46). TG concentrations were significantly higher with the fructose diet. With LC3, hypertriglyceridemia was prevented, as already reported in hypertriglyceridemic and type 2 diabetic patients (47,48); ALA exhibited the same effect. In this study, the fructose diet also induced hyperinsulinemia, and dietary (n-3) PUFA intake, LC3 mainly, limited this alteration. Starting from wk 4, the high-fructose diet increased IVGTT insulin area under curve (AUC) values; (n-3) PUFA intake prevented this increase. As proven by ITT, the high-fructose diet induced insulin resistance that was partially prevented by ALA and almost totally prevented by LC3. Several experimental studies have demonstrated the efficacy of fish oil in preventing (49,50), and sometimes in reversing (51), insulin resistance. Several parameters resulting from the modification of membrane FA profile in insulin target tissues (e.g. changes in membrane fluidity or diacylglycerol signaling function) were considered as critical factors that influence
insulin secretion and biological activities (49,52). In rodents and humans, insulin sensitivity in skeletal muscle was negatively correlated with muscle TG accumulation and positively correlated with the (n-3) LCPUFA content in muscle PL (6,49,53). Clinical investigations suggested that (n-3) LCPUFA may prevent insulin resistance and reduce the risk of impaired glucose tolerance in type 2 diabetes (46,54,55).

In conclusion, the results of the present study suggest that insulin resistance alone (without obesity) is associated with a specific FA pattern in insulin-sensitive tissues that likely involves alterations of the \( \Delta^9 \) and \( \Delta^5 \) desaturase activities. This pathological status is related to an increased cardiovascular risk as estimated by the increase in SBP and PP in particular. As expected, the (n-3) experimental diets were shown here to be important preventive effectors in the relationship between cardiometabolic risk (in terms of insulin resistance and vascular dysfunction) and the FA profile of insulin-sensitive tissues (mainly in the muscle and the liver). Under our experimental conditions, ALA alone decreased TG concentrations but failed to prevent insulin resistance and BP increases. ALA associated with EPA and DHA induced stronger effects on FA profiles, significantly increasing DHA, docosapentaenoic acid, and EPA in the PL of tissue membranes and decreasing the (n-6):(n-3) FA ratio. Moreover, the ALA+EPA+DHA mix significantly prevented insulin resistance and PP increase.

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**Literature Cited**


