Effect of Fructose Overfeeding and Fish Oil Administration on Hepatic De Novo Lipogenesis and Insulin Sensitivity in Healthy Men

David Faeh,[1,2] Kaori Minehira,[1] Jean-Marc Schwarz,[3,4] Raj Periasami,[4] Park Seongsu,[3] and Luc Tappy[1]

High-fructose diet stimulates hepatic de novo lipogenesis (DNL) and causes hypertriglyceridemia and insulin resistance in rodents. Fructose-induced insulin resistance may be secondary to alterations of lipid metabolism. In contrast, fish oil supplementation decreases triglycerides and may improve insulin resistance. Therefore, we studied the effect of high-fructose diet and fish oil on DNL and VLDL triglycerides and their impact on insulin resistance. Seven normal men were studied on four occasions: after fish oil (7.2 g/day) for 28 days; a 6-day high-fructose diet (corresponding to an extra 25% of total calories); fish oil plus high-fructose diet; and control conditions. Following each condition, fasting fractional DNL and endogenous glucose production (EGP) were evaluated using [1-^{13}C]sodium acetate and 6,6-$^{2}H_2$ glucose and a two-step hyperinsulinemic-euglycemic clamp was performed to assess insulin sensitivity. High-fructose diet significantly increased fasting glycemia (7 ± 2%), triglycerides (79 ± 22%), fractional DNL (sixfold), and EGP (14 ± 3%, all $P < 0.05$). It also impaired insulin-induced suppression of adipose tissue lipolysis and EGP ($P < 0.05$) but had no effect on whole-body insulin-mediated glucose disposal. Fish oil significantly decreased triglycerides (37%, $P < 0.05$) after high-fructose diet compared with high-fructose diet without fish oil and tended to reduce DNL but had no other significant effect. In conclusion, high-fructose diet induced dyslipidemia and hepatic and adipose tissue insulin resistance. Fish oil reversed dyslipidemia but not insulin resistance. *Diabetes* 54:1907–1913, 2005

From the [1]Department of Physiology, University of Lausanne, Lausanne, Switzerland; the [2]University Institute of Social and Preventive Medicine, Lausanne, Switzerland; the [3]Department of Medicine, University of California at San Francisco, San Francisco, California; and [4]Basic Science, Touro University, Mare Iland, California.

Address correspondence and reprint requests to Prof. Luc Tappy, Département de physiologie, 7 rue du Bugnon, 1005 Lausanne, Switzerland. E-mail: luc.tappy@unil.ch.

Received for publication 25 January 2005 and accepted in revised form 28 March 2005.

ChREBP, carbohydrate response element–binding protein; DNL, de novo lipogenesis; EGP, endogenous glucose production; NEFA, nonesterified fatty acid.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Over the past decades, per capita consumption of high-fructose corn syrup has increased dramatically. Several authors suggest that increased fructose ingestion may be responsible for the present epidemic of obesity and the increased incidence of metabolic syndrome and diabetes (1). Diets rich in simple sugars, particularly fructose, have been shown to be associated with hypertriglyceridemia both in humans (2) and rodents (3). This may be due to stimulation of hepatic de novo lipogenesis (DNL) and increased secretion of triglyceride-rich particles by the liver or to decreased extrahepatic clearance of triglyceride particles (4,5). Moreover, there is evidence that high-fructose diets can lead to insulin resistance in rodents (3,6). To further delineate the metabolic consequences of fructose overconsumption, we measured fractional hepatic DNL and insulin sensitivity in the liver, adipose tissue, and at the whole-body level. This was performed in a group of healthy male volunteers after 6 days of either fructose overfeeding or an isoenergetic, low-fructose diet. Since n-3 polyunsaturated fatty acids are known to prevent hypertriglyceridemia and the development of insulin resistance in dietary models of obesity in rats (6) and may suppress hepatic lipogenic enzymes (7), each participant was also studied after a 4-week fish oil supplement.

RESEARCH DESIGN AND METHODS

Seven healthy male volunteers without family history of diabetes were recruited by advertisement. They were aged 22–31 years and had BMIs of 20.2–25.4 kg/m^2 (Table 1). All subjects were in apparent good health, were nonsmokers, and took no medications. The study was approved by the ethical committee of Lausanne University School of Medicine and a written consent was obtained from each subject after the nature of the study was explained.

Anthropometry and body composition measurements. Standing height was measured using a stadiometer. Body weight and hip and waist circumferences were measured before the last meal preceding each study (8). Body composition was estimated from subcutaneous skin fold thickness measurements at the biceps, triceps, subscapular, and suprailiac sites as described by Durnin and Womersley (9).

Study design. Each subject was studied on four occasions (Fig. 1). On one occasion, volunteers received 7.2 g of fish oil (1.2 g eicosapentaenoic acid and 0.8 g docasahexaenoic acid; Biorganic Omega-3, Gisand, Bern, Switzerland) per day for 28 days. It has been documented that such supplementation with fish oils leads to marked increases in n-3 fatty acids in serum phospholipids (10,11). On another occasion, subjects ingested 3 g of fructose (D-Fructose; Fluka Chemie Gmbh, Buchs, Switzerland) per kilogram of body weight per day (high-fructose diet) as a 20% fructose solution with the three main meals during the 6 days before the test. On the third occasion, fish oil supplementation was combined with high-fructose diet. Each subject also underwent a

TABLE 1
Subject characteristics

	Controls	Fish oil	High-fructose diet	High-fructose diet plus fish oil
Protein/fat/carbohydrate (%)*	15/35/50	15/35/50	11/26/63	11/26/63
Body weight (kg)	71.5 ± 4.0	72.6 ± 3.7	72.1 ± 4.1	73.1 ± 4.1
Body fat (%)	16.5 ± 0.7	17.2 ± 0.7	16.5 ± 0.8	17.2 ± 1.0
Waist circumference (cm)	80.0 ± 2.9	81.1 ± 3.3	81.0 ± 2.7	81.2 ± 2.6
Fasting NEFA (μmol/l)	392 ± 43	375 ± 48	243 ± 43†	212 ± 26†
Percent of controls	100 ± 0	101 ± 14	61 ± 6†	55 ± 5†
Fasting insulin (pmol/l)	53 ± 7	49 ± 6	61 ± 9	58 ± 4
Percent of controls	100 ± 0	96 ± 11	117 ± 14	116 ± 13
Fasting glucose (mmol/l)	4.6 ± 0.1	4.7 ± 0.1	5.0 ± 0.1†	5.0 ± 0.1†
Percent of controls	100 ± 0	101 ± 4	107 ± 3†	108 ± 3†
Fasting lactate (mmol/l)	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1†	1.0 ± 0.1†
Percent of controls	100 ± 0	107 ± 9	158 ± 12†	141 ± 9†

Data are means ± SE. *Percentage of total energy from protein, fat, and carbohydrate. †Significantly different ($P < 0.05$).

control test. Volunteers were instructed to avoid certain foods and to follow a balanced, isoenergetic diet, which was controlled by a food diary during an initial 3-day period. Thereafter, during the 3 days preceding each test, the subject followed a provided isoenergetic diet (15% proteins, 35% lipids, 40% starch, 10% mono- and disaccharides) partitioned into three meals at 0700, 1200, and 1900 and two snacks at 0900 and 1500. During the high-fructose diet, subjects received the same diet supplemented with 3 $g \cdot kg^{-1} \cdot day^{-1}$ fructose, resulting in an hyperenergetic (800–1,000 kcal/day) diet containing 11% proteins, 26% lipids, 30% starch, 8% glucose and disaccharides, and 25% fructose. Subjects were told to avoid vigorous physical activity during the 6 days preceding the tests.

The order by which the four dietary conditions were applied was randomized, with an interval of 12 weeks after the two tests with fish oil administration to allow wash-out of fish oil between the experiments.

Fasting hepatic DNL and endogenous glucose production. After each dietary condition, subjects underwent an overnight 13-h study (from 2200 to 1100 of the next morning), during which they stayed in bed and slept between 2200 and 0600. On the evening of the study, they took their last meal at 1830. At 2030, two indwelling catheters were inserted: one into a right wrist vein for blood sampling; the other into a vein of the controlateral forearm for infusions. From 2200 to 0730, 0.5 g/h of [1-^{13}C]sodium acetate (10 mg/ml in NaCl 0.9%) was infused constantly. Whole-body glucose turnover was assessed with 6,6-$^2H_2$ glucose infusion (bolus: 2 mg/kg; continuous: 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) between 0500 and 1100. Basal blood samples were obtained at 0600, 0700, and 0730 for determination of hepatic DNL, endogenous glucose production (EGP), insulin, glucose, nonesterified fatty acid (NEFA), and triglyceride concentrations (referred as "fasting condition").

Hyperinsulinemic-euglycemic clamp. To assess insulin sensitivity, insulin-mediated glucose disposal (6,6-$^2H_2$ glucose, "hot infusion model") (12), inhibition of EGP, and suppression of lipolysis (plasma NEFA concentrations) and lipid oxidation (indirect calorimetry), a two-step (0.2 $mU \cdot kg^{-1} \cdot min^{-1}$ from $t = 0$ to $t = 90$, then 0.5 $mU \cdot kg^{-1} \cdot min^{-1}$ from $t = 90$ to $t = 180$) hyperinsulinemic-euglycemic clamp (13) was performed between 0800 and 1100 (Fig. 1). Blood samples were collected at 30-min intervals to measure insulin, NEFA, and triglycerides concentrations. The glucose infusion rate (insulin resistance) during the clamp was used to evaluate insulin-mediated glucose disposal. Indirect calorimetry was performed using a ventilated canopy as described previously (14) from 0700 until 1100 (Fig. 1). Energy expenditure and substrate oxidation rates were measured using the equation of Livesey and Elia (15).

Analytical procedures. Blood samples were immediately centrifuged at 4°C to separate plasma, which was then frozen at −20°C until testing. Plasma glucose and lactate concentrations were measured with a glucose-lactate analyzer YSI 2300 STAT Plus (Yellow Springs, OH). Plasma NEFA and triglycerides concentrations were analyzed by a colorimetric method using commercial kits for NEFA (NEFA C; Wako Chemicals, Freiburg, Germany) and for triglycerides (Biomérieux Vitek, Switzerland). Commercial radioimmunoassay and enzyme-linked immunosorbent assay kits were used for determination of plasma insulin (Biochem Immunosystems, Freiburg, Germany), adiponectin (Linco Research, St. Charles, MO), and resistin (Human Resistin ELISA, BioVendor Laboratory Medicine, Czech Republic). During the clamp, plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma 6,6- $^2H_2$ glucose isotopic enrichment was measured by gas chromatography–mass spectrometry (Model 5973; Hewlett-Packard, Palo Alto, CA) as described (16). Plasma VLDL-^{13}C palmitate enrichment and mass isotopomers were measured as described by Hellerstein et al. (17). Gas chromatography–mass spectrometry was used for analysis of isotopic enrichments of plasma fatty acid-methyl esters from VLDL. For fatty acid-methyl esters analysis, a 25-m fused DB-1 silica column was used, with electron impact ionization ion at m/z 270–272 representing the parent M0 through the M2 isotopomers (17). Fractional DNL was calculated by the isotopomer distribution analysis technique. The ratio of excess double-labeled to excess of single-labeled species (EM2/EM1) in VLDL palmitate reveals the isotope enrichment of the true precursor pool for lipogenesis (hepatic cytosolic acetyl-CoA) by application of probability logic based on the multinomial expansion. The fractional contribution from DNL to VLDL fatty acid can then be calculated by the precursor-product relationship (17).

Statistical analysis. Data are expressed as means ± SE. Statistical analyses were performed by using STATA version 8.2 (StataCorp, College Station, TX)

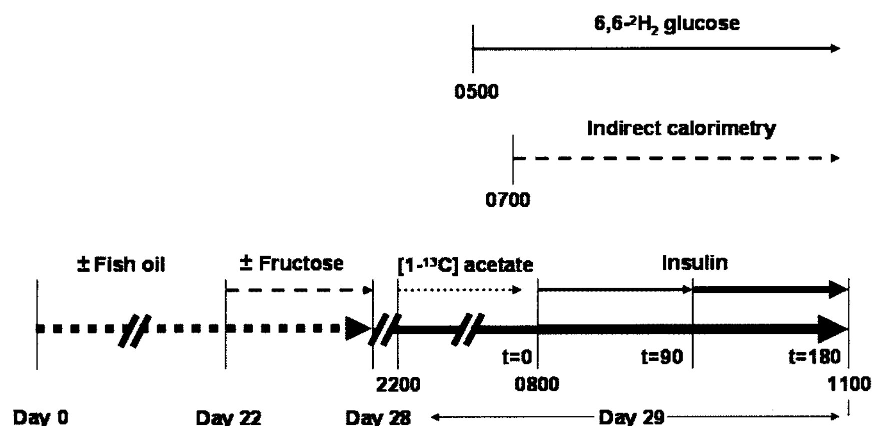

FIG. 1. Experimental protocol. After each of the four types of dietary intervention, a 13-h metabolic study was started at 2200. At 2200, 0.5 g/h of [1-^{13}C]acetate was perfused until 0730. 6,6-$^2H_2$ glucose (bolus: 2 mg/kg; continuous: 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was perfused between 0500 and 1100. Indirect calorimetry was carried out from 0700 to 1100. Between 0800 ($t = 0$ min) and 1100 ($t = 180$ min), a two-step (0.2 $mU \cdot kg^{-1} \cdot min^{-1}$ and 0.5 $mU \cdot kg^{-1} \cdot min^{-1}$) hyperinsulinemic-euglycemic (4.9 ± 0.1 mmol/l) clamp was performed.

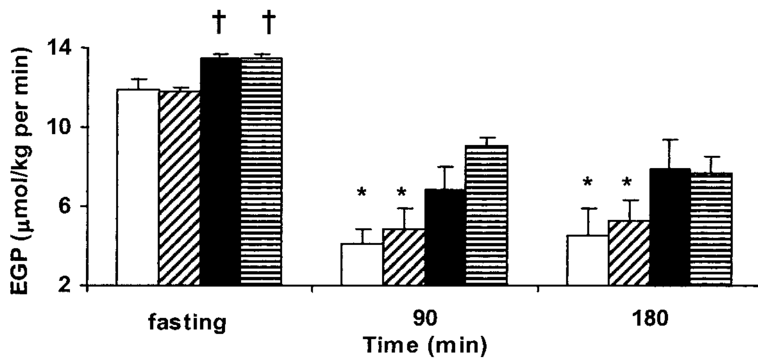

FIG. 2. EGP in fasting conditions and at 90 and 180 min of euglycemic-hyperinsulinemic clamping after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. *Significant suppression ($P < 0.05$) vs. fasting control subjects and fish oil alone. †$P < 0.05$ vs. fasting control subjects and fish oil alone.

with $P < 0.05$ as level of significance. Global mean difference among the various conditions was tested using the Friedman's test. When a significant difference was found, multiple comparisons between two conditions were performed using the paired Wilcoxon test. To test the existence of a trend, we performed the Page's test (18).

RESULTS

Baseline. Mean fasting parameters are summarized in Table 1. The subjects' body weights and compositions were not affected significantly by high-fructose diet or fish oil supplementation, respectively. Insulin levels did not differ significantly between the four conditions. Fasting glycemia and lactatemia significantly increased after high-fructose diet (7 ± 2 and 58 ± 12%, respectively; $P < 0.05$) compared with control condition, whereas fish oil had no influence.

As shown in Fig. 2, fasting EGP was significantly higher (14 ± 3%, $P < 0.05$) after the high-fructose diet (13.5 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and the high-fructose diet plus fish oil (13.5 ± 1.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) compared with fish oil alone (11.8 ± 1.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and control subjects (11.9 ± 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Fish oil did not change EGP significantly. Fasting NEFAs (Table 1) were significantly lower (−39 ± 6%, $P < 0.05$) after high-fructose diet (243 ± 43 μmol/l) compared with control subjects (392 ± 43 μmol/l). Again, fish oil had no significant impact on fasting NEFA concentrations. Fasting adiponectin and resistin concentrations were similar under all four conditions (adiponectin: control subjects, 7.7 ± 1.3; fish oil alone, 7.3 ± 1; high-fructose diet, 7.9 ± 1.4; high-fructose diet plus fish oil, 7.6 ± 1.5 μg/ml; resistin: control subjects, 2.2 ± 0.7; fish oil alone, 2.1 ± 0.4; high-fructose diet, 2.0 ± 0.5; high-fructose diet plus fish oil, 2.4 ± 0.5 ng/ml). Fasting glucose oxidation was higher (13.7 ± 1.8 and 13.8 ± 1.4 vs. 9.2 ± 2.2 and 7.7 ± 0.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and lipid oxidation lower (0.24 ± 0.12 and 0.23 ± 0.09 vs. 0.36 ± 0.12 and 0.61 ± 0.07 $mg \cdot kg^{-1} \cdot min^{-1}$) after high-fructose diet and high-fructose diet plus fish oil than after fish oil alone or control subjects ($P < 0.05$ for both). High-fructose diet increased fasting triglycerides (1.13 ± 0.21 mmol/l) by 79 ± 22% ($P < 0.05$) compared with control subjects (0.63 ± 0.07 mmol/l), as shown in Fig. 3. Fasting triglycerides concentrations were significantly lower (37%, $P < 0.05$) in the high-fructose diet plus fish oil (0.83 ± 0.12 mmol/l) compared with the high-fructose diet alone, but both remained significantly higher than fish oil alone, and control subjects ($P < 0.05$). Figure 4 shows the percentage of fractional DNL. After fish oil alone and control subjects, DNL was very low (1.9 ± 0.44% and 1.6 ± 0.34%, respectively). In contrast, DNL significantly increased after the high-fructose diet to 9.4 ± 2.8% ($P < 0.05$). Mean DNL tended to be lower after the high-fructose diet plus fish oil (mean DNL: 7.5 ± 1.8%) compared with the high-fructose diet alone but the difference was not significant. The relationship between DNL and fasting

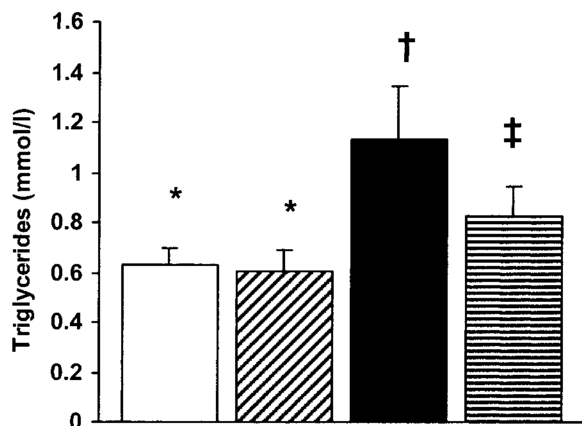

FIG. 3. Mean fasting triglyceride concentration after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. Values not sharing the same superscripts are significantly different ($P < 0.05$).

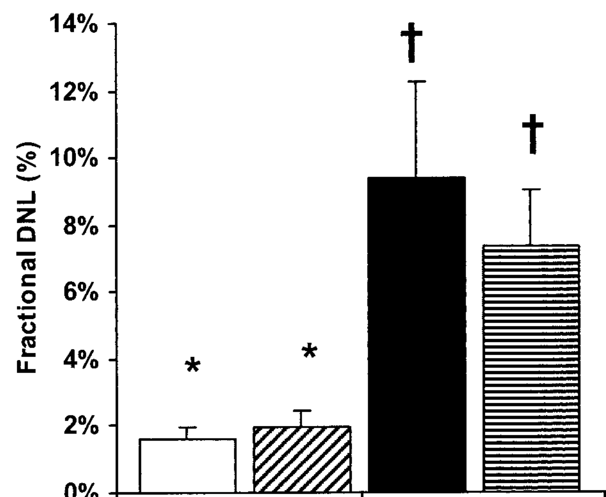

FIG. 4. Mean baseline fractional hepatic DNL after control condition (□), after 28 days of fish oil supplementation (▨), after 6 days of high-fructose diet (■), and after 28 days of fish oil supplementation plus high-fructose diet (▤) in seven men. Values are means ± SE represented by vertical bars. Values not sharing the same superscripts are significantly different ($P < 0.05$).

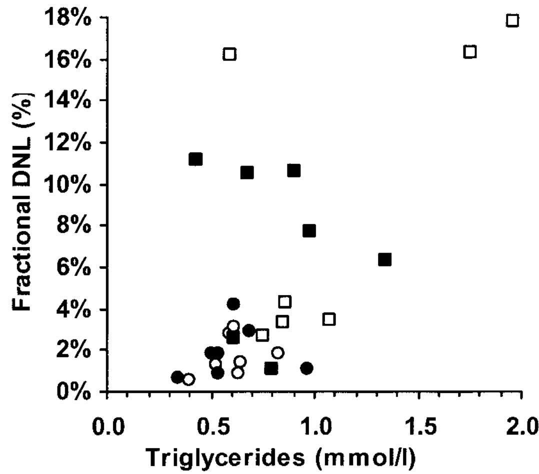

FIG. 5. Relationship between fractional hepatic DNL and triglyceride concentration after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men.

plasma triglycerides in all four conditions is illustrated in Fig. 5.

Hyperinsulinemic-euglycemic clamp. Figure 6 shows plasma glucose and insulin concentrations as well as insulin resistance during hyperinsulinemic-euglycemic clamp. Glycemia was maintained at 4.9 ± 0.1 mmol/l and did not differ between the four conditions. Insulin concentrations and insulin resistance did not vary significantly

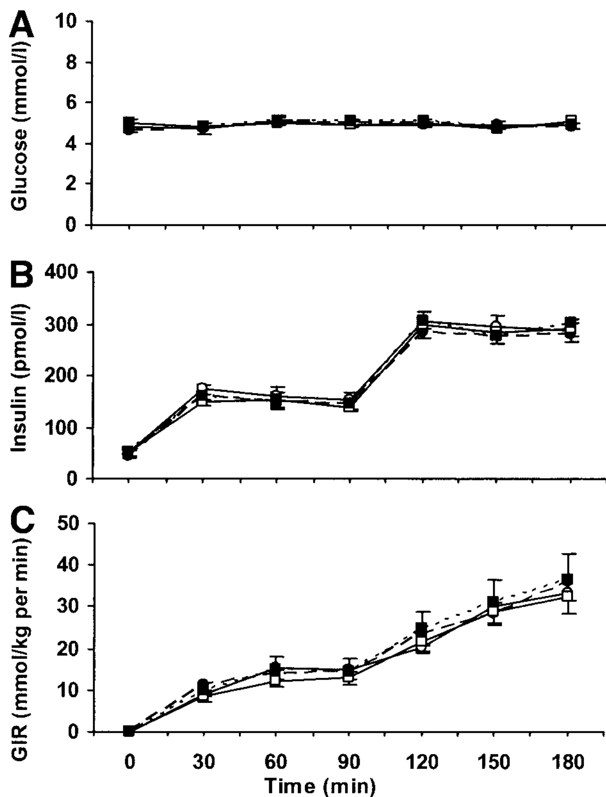

FIG. 6. Plasma glucose (*A*), insulin concentrations (*B*), and glucose infusion rate (*C*) during euglycemic-hyperinsulinemic clamping after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men. Values are means ± SE represented by vertical bars.

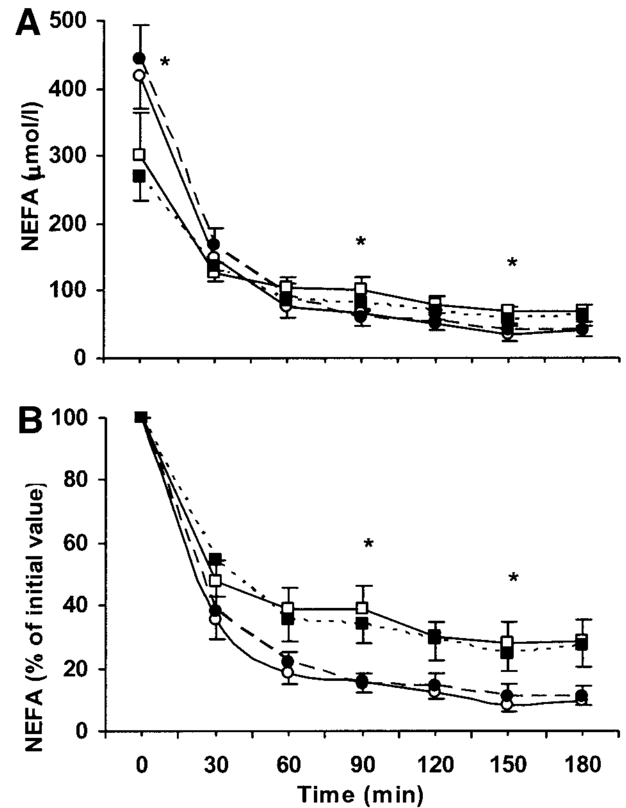

FIG. 7. NEFAs expressed as absolute values (*A*) and in percentage of the baseline value (*B*) during euglycemic-hyperinsulinemic clamping after control condition (○), after 28 days of fish oil supplementation (●), after 6 days of high-fructose diet (□), and after fish oil supplementation plus high-fructose diet (■) in seven men. Values are means ± SE represented by vertical bars. *$P < 0.05$ high-fructose diet versus control.

between the four conditions. Similarly, glucose oxidation at $t = 180$ (control subjects, 16.0 ± 1.0; fish oil alone, 16.3 ± 1.3; high-fructose diet, 18.8 ± 0.6; high-fructose diet plus fish oil, 19.6 ± 0.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and lipid oxidation at $t = 180$ (control subjects, −0.09 ± 0.06; fish oil alone, −0.22 ± 0.08; high-fructose diet, −0.14 ± 0.10; high-fructose diet plus fish oil, −0.22 ± 0.06 $mg \cdot kg^{-1} \cdot min^{-1}$) did not differ significantly under all four conditions.

In Fig. 2, EGP is displayed in fasting condition and at $t = 90$ and $t = 180$ into the clamp. Although suppression of EGP in high-fructose diet (28% after $t = 180$) and high-fructose diet plus fish oil (24% after 180 min) were not significant, suppression in control subjects (44% at $t = 180$) and fish oil (36% at $t = 180$) were both significant ($P < 0.05$).

Figure 7 shows NEFA concentrations expressed as absolute values (Fig. 7*A*) and as percent of fasting values ($t = 0$) (Fig. 7*B*). During the clamp, NEFAs were higher after high-fructose diet compared with control subjects and fish oil alone. At times $t = 90$ and $t = 150$, differences between high-fructose diet and control subjects were significant ($P < 0.05$). Under high-fructose diet plus fish oil, NEFA concentrations tended to be lower compared with high-fructose diet alone throughout the clamp, but the difference did not reach statistical significance (Fig. 7*A*). In all four conditions, NEFA concentrations were significantly suppressed ($P < 0.01$) during the clamp.

However, NEFA suppression was significantly stronger ($P < 0.05$) after control subjects (90% at $t = 180$) and after fish oil alone (91% at $t = 180$) compared with high-fructose diet (76% at $t = 180$) and high-fructose diet plus fish oil (77% at $t = 180$) (Fig. 7*B*).

DISCUSSION

Fructose overfeeding for 6 days led to the development of several features of the metabolic syndrome in these healthy, normal-weight volunteers. First, fructose overfeeding significantly increased plasma triglycerides concentration by 79%. This increase was associated with a sixfold stimulation of fractional hepatic DNL. Although the kinetics of VLDL particles were not assessed, it is likely that stimulation of hepatic DNL by fructose contributed to the observed hypertriglyceridemia (Fig. 5). Previous studies have indeed observed significant correlations between stimulation of fractional hepatic DNL and plasma triglycerides levels in humans fed high simple carbohydrate diets (4,19). Second, fructose increased fasting glycemia and EGP and blunted suppression of EGP during the clamp experiment, indicating hepatic insulin resistance (Fig. 2). Similar observations have been made in rodents after 2 weeks of high-sucrose diet (20). In rodents, as in our study, this increase in EGP was not associated with fasting hyperinsulinemia, suggesting that hepatic insulin resistance alone does not stimulate insulin secretion. Third, and quite unexpectedly, fructose impaired the suppression of plasma NEFA during low and moderate insulin infusion rate, indicating adipose tissue insulin resistance (Fig. 7).

The mechanisms by which fructose exerts these various effects cannot be determined from the present experiments and can only be speculated. Changes in adiponectin and resistin concentrations could have been suspected given the effects of these adipokines on whole body and hepatic insulin resistance (21,22). However, their concentrations were not altered by high-fructose diet or fish oil.

Fructose overfeeding exposes the liver to a huge carbohydrate load. The fructose carbons can be converted into glucose and glycogen, thus leading to increased hepatic glycogen stores (23,24). This process may contribute to enhanced EGP and to its impaired suppression by insulin (25). The suppression of glucose production during a hyperinsulinemic clamp may, however, not truly reflect what occurs during oral feeding when the liver will be exposed to higher concentrations of glucose due to portal glucose delivery. A portion of fructose carbons can be also converted into lactate, leading to hyperlactatemia and possible extrahepatic metabolic effects in muscle and adipose tissue (26,27). Finally, carbon atoms can be converted into fatty acids, thus enhancing hepatic DNL (28–30). However, these acute effects of fructose administration are unlikely to account for our observation of increased EGP, DNL, triglyceridemia, and lactatemia after an overnight fast (i.e., more than 10 h after ingestion of fructose). These effects are most likely explained by alterations of hepatic gene expression by high-fructose diet (28). In this regard, it is documented that high carbohydrates diets stimulate carbohydrate response element–binding protein (ChREBP), a hepatic transcription factor that upregulates the expression of lipogenic and glycolytic enzymes (31). ChREBP also regulates the expression of key enzymes of hepatic fructose metabolism. Although ChREBP is primarily regulated by glucose through the increase in intrahepatic glucose metabolites, it may possibly be stimulated as well during high-fructose diet. In addition, recent findings show that hepatic stress may mediate hepatic insulin resistance associated to high-fructose diet (32,33).

Regarding the effects of fructose overfeeding on adipose tissue, we have no mechanistic explanation to offer. Although it is generally accepted that fructose is nearly completely metabolized at first pass in the gut and the liver (34), it remains possible that some fructose reached the systemic circulation and exerted metabolic effects directly at the level of adipocytes. Alternatively, fructose metabolism in the liver may possibly exert effects on adipose tissue through the release of metabolites such as lactate (24). It could also be speculated that fructose overfeeding activated the sympathetic nervous system, as it has been demonstrated in rodents (35) and hence stimulated lipolysis.

Despite the observed hepatic and adipose tissue insulin resistance, whole-body insulin-stimulated glucose disposal was not decreased after six days of fructose overfeeding. Because skeletal muscle glucose metabolism is the major determinant of whole-body glucose disposal under conditions of hyperinsulinemia (36), this strongly suggests that muscle insulin sensitivity was not impaired. Similar observations were made in rodents after 2 weeks of a diet rich in sucrose (20). Rodents, however, were shown to subsequently develop whole-body insulin resistance after several weeks of high-sucrose feeding (20), and it is therefore likely that the duration of fructose overfeeding was too short to produce effects in skeletal muscle.

We observed that fish oil supplementation significantly blunted fructose-induced hypertriglyceridemia. The hypotriglyceridemic effect of fish oil in animals (37) and humans (38) is well known. The mechanisms by which this effect was attained can only be speculated on the basis of our data. Although hepatic DNL was not significantly reduced after fish oil supplementation, we cannot exclude that the number of subjects studied was too small to detect such an effect. Alternatively, fish oil may have reduced the secretion of triglycerides-rich particles and/or stimulated their extrahepatic clearance (39,40). Whatever the mechanism, the hypotriglyceridemic effect of fish oil was not associated with a reduction of EGP or with improved insulin-induced NEFA suppression (Fig. 2 and 7), indicating that it failed to impact the metabolic steps involved in hepatic and adipose tissue insulin resistance. In rats however, fish oil has been shown to prevent whole body insulin resistance (6) after several weeks of high sucrose feeding (41). This suggests that fish oil could have the potential to prevent development of whole-body insulin resistance induced by chronic fructose overfeeding in humans.

Our study design involved administration of an extra amount of fructose while leaving the other dietary intakes unchanged. It therefore resulted in both energy and fructose overfeeding. As such, it is representative of a condition where increased dietary fructose intake would not be compensated by a reduction of calories from other sources. It cannot, however, truly differentiate the effects

of high-fructose intake per se and of energy total carbohydrate overfeeding. Only comparative studies involving subjects overfed with fructose versus starch or glucose will be able to address this issue.

In conclusion, we found a hepatic phenotype that was characterized by hepatic (and adipose tissue) insulin resistance, hypertriglyceridemia, and increased DNL after 6 days of fructose overfeeding. However, whole-body insulin-mediated glucose disposal remained unchanged, suggesting normal muscle insulin sensitivity. Experiments done in rodents, however, suggest that exposure to large amounts of fructose for several weeks may impair muscle insulin sensitivity as well. Despite its significant hypotriglyceridemic effect, fish oil supplementation did not improve the other metabolic alteration induced by fructose. Therefore, the overall consequences of fructose overfeeding over longer periods of time and the potential preventive properties of fish oil in the development of whole-body insulin resistance remain to be evaluated.

ACKNOWLEDGMENTS

This study was funded by Grant 3200–067787 from the Swiss National Science Foundation (L.T.) and by the Nutricia Research Foundation.

Part of this work was presented as an abstract at the 1st International Congress on "Prediabetes" and the Metabolic Syndrome, Berlin, Germany, 13–16 April 2005.

The authors are indebted to Christiane Ruffieux and Guy van Melle for their precious help in statistical analyses.

REFERENCES

1. Bray GA, Nielsen SJ, Popkin BM: Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr* 79:537–543, 2004
2. Bantle JP, Raatz SK, Thomas W, Georgopoulos A: Effects of dietary fructose on plasma lipids in healthy subjects. *Am J Clin Nutr* 72:1128–1134, 2000
3. Huang YJ, Fang VS, Juan CC, Chou YC, Kwok CF, Ho LT: Amelioration of insulin resistance and hypertension in a fructose-fed rat model with fish oil supplementation. *Metabolism* 46:1252–1258, 1997
4. Parks EJ, Hellerstein MK: Carbohydrate-induced hypertriacylglycerolemia: historical perspective and review of biological mechanisms. *Am J Clin Nutr* 71:412–433, 2000
5. Pagliassotti MJ, Horton TJ: Sucrose, insulin action and biologic complexity. *Recent Res Devel Physiol* 2:337–353, 2004
6. Podolin DA, Gayles EC, Wei Y, Thresher JS, Pagliassotti MJ: Menhaden oil prevents but does not reverse sucrose-induced insulin resistance in rats. *Am J Physiol* 274:R840–R848, 1998
7. Xu J, Nakamura MT, Cho HP, Clarke SD: Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem* 274:23577–23583, 1999
8. Callaway CW, Chumlea WC, Bouchard C, Himes JH, Martin AD, Mitchell CD, Mueller WH, Roche AF, Seefeldt VD: Circumferences. In *Anthropometric Standardization Reference Manual.* Lohman TJ, Roche AF, Martonell R, Eds. Champaign, IL, Human Kinetics, 1988, p. 44–46
9. Durnin JV, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32:77–97, 1974
10. Stark KD, Park EJ, Maines VA, Holub BJ: Effect of a fish-oil concentrate on serum lipids in postmenopausal women receiving and not receiving hormone replacement therapy in a placebo-controlled, double-blind trial. *Am J Clin Nutr* 72:389–394, 2000
11. Buckley R, Shewring B, Turner RC, Yaqoob P, Minihane AM: Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br J Nutr* 92:477–483, 2004
12. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic euglycemic glucose clamps: comparison of labelled and unlabelled glucose infusates. *Diabetes* 36:914–924, 1987
13. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
14. Jallut D, Tappy L, Kohut M, Bloesch D, Munger R, Schutz Y, Chioléro R, Felber JP, Livio JJ, Jéquier E: Energy balance in elderly patients after surgery for a femoral neck fracture. *J Parenter Enter Nutr* 14:563–568, 1990
15. Livesey G, Elia M: Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am J Clin Nutr* 47:608–628, 1988
16. Schneiter P, Gillet M, Chioléro R, Jéquier E, Tappy L: Hepatic nonoxidative disposal of an oral glucose meal in patients with liver cirrhosis. *Metabolism* 48:1260–1266, 1999
17. Hellerstein MK, Christiansen M, Kaempfer S: Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* 87:1841–1852, 1991
18. Hollander M, Wolfe DA: The two way lay-out. In *Nonparametric Statistical Methods.* New York, John Wiley & Sons, 1973, p. 138–184
19. Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD, Hirsch J: Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J Lipid Res* 41:595–604, 2000
20. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA: Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. *Am J Physiol* 271:R1319–1326, 1996
21. Havel PJ: Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Op Lipidol* 13:51–59, 2002
22. Muse ED, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, Scherer PE, Rossetti L: Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 114:232–239, 2004
23. Mayes PA: Intermediary metabolism of fructose. *Am J Clin Nutr* 58:754S–765S, 1993
24. Tounian P, Schneiter P, Henry S, Jéquier E, Tappy L: Effects of infused fructose on endogenous glucose production, gluconeogenesis and glycogen metabolism in healthy humans. *Am J Physiol* 267:E710–E717, 1994
25. Schwarz JM, Acheson KJ, Tappy L, Piolino V, Müller MJ, Felber JP, Jéquier E: Thermogenesis and fructose metabolism in humans. *Am J Physiol* 262:E591–E598, 1992
26. Ferrannini E, Natali A, Brandi LS, Bonadonna R, De Kreutzemberg SV, Del Prato SDS: Metabolic and thermogenic effects of lactate infusion in humans. *Am J Physiol* 265:E504–E512, 1993
27. Paquot N, Schneiter P, Cayeux MC, Chioléro R, Temler E, Jéquier E, Tappy L: Effects of infused sodium lactate on glucose and energy metabolism in healthy humans. *Diabete Metab* 21:345–352, 1995
28. Sebokova E, Klimes I, Gasperikova D, Bohov P, Langer P, Lavau M, Clandinin MT: Regulation of gene expression for lipogenic enzymes in the liver and adipose tissue of hereditary hypertriglyceridemic, insulin-resistant rats: effect of dietary sucrose and marine fish oil. *Biochim Biophys Acta* 1303:56–62, 1996
29. Schwarz J-M, Neese RA, Turner S, Dare D, Hellerstein MK: Short-term alterations in carbohydrate energy intake in humans: striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *J Clin Invest* 96:2735–2743, 1995
30. Uyeda K, Yamashita H, Kawaguchi T: Carbohydrate responsive element-binding protein (ChREBP): a key regulator of glucose metabolism and fat storage. *Biochem Pharmacol* 63:2075–2080, 2002
31. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K: Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc Natl Acad Sci U S A* 101:7281–7286, 2004
32. Kelley GL, Allan G, Azhar S: High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology* 145:548–555, 2004
33. Wei Y, Pagliassotti MJ: Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance. *Am J Physiol Endocrinol Metab* 287:E926–E933, 2004
34. Björkman O, Gunnarsson R, Hagström E, Felig P, Wahren J: Splanchnic and renal exchange of infused fructose in insulin-deficient type 1 diabetic patients and healthy controls. *J Clin Invest* 83:52–59, 1989
35. Young JB, Weiss J, Boufath N: Effects of dietary monosaccharides on sympathetic nervous system activity in adipose tissues of male rats. *Diabetes* 53:1271–1278, 2004
36. Shulman JI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171–176, 2000

37. Harris WS: n-3 fatty acids and serum lipoproteins: animal studies. *Am J Clin Nutr* 65:1611S–1616S, 1997
38. Harris WS: n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 65:1645S–1654S, 1997
39. Haussinger D, Lang F, Gerok W: Regulation of cell function by the cellular hydration state (Review). *Am J Physiol* 267:E343–E355, 1994
40. Chan DC, Barrett PH, Watts GF: Lipoprotein transport in the metabolic syndrome (Part II): pathophysiological and interventional studies employing stable isotopy and modelling methods. *Clin Sci* 107:233–249, 2004
41. D'Alessandro ME, Lombardo YB, Chicco A: Effect of dietary fish oil on insulin sensitivity and metabolic fate of glucose in the skeletal muscle of normal rats. *Ann Nutr Metab* 46:114–120, 2002