

Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation

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Bentham, Lambertus, Klaasjan Keizer, Coen H. Wiegman, Sietse F. de Boer, Jan H. Strubbe, Anton B. Steffens, Folkert Kuipers, and Anton J. W. Scheurink. Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. *Am J Physiol Endocrinol Metab* 279: E1286–E1293, 2000.—We tested the hypothesis that excessive portal venous supply of long-chain fatty acids to the liver contributes to the development of insulin resistance via activation of the hypothalamus-pituitary-adrenal axis (HPA axis) and sympathetic system. Rats received an intraportal infusion of the long-chain fatty acid oleate (150 nmol/min, 24 h), the medium-chain fatty acid caprylate, or the solvent. Corticosterone (Cort) and norepinephrine (NE) were measured as indexes for HPA axis and sympathetic activity, respectively. Insulin sensitivity was assessed by means of an intravenous glucose tolerance test (IVGTT). Oleate infusion induced increases in plasma Cort ($\Delta = 13.5 \pm 3.6 \mu\text{g/dl}$; $P < 0.05$) and NE ($\Delta = 235 \pm 76 \text{ ng/l}$; $P < 0.05$), whereas caprylate and solvent had no effect. The area under the insulin response curve to the IVGTT was larger in the oleate-treated group than in the caprylate and solvent groups (area = 220 ± 35 vs. 112 ± 13 and 106 ± 8 , respectively, $P < 0.05$). The area under the glucose response curves was comparable [area = 121 ± 13 (oleate) vs. 135 ± 20 (caprylate) and 96 ± 11 (solvent)]. The results are consistent with the concept that increased portal free fatty acid is involved in the induction of visceral obesity-related insulin resistance via activation of the HPA axis and sympathetic system.

insulin resistance; hypothalamus-pituitary-adrenal axis; sympathetic activity; visceral obesity

SYNDROME X IS CHARACTERIZED by a combination of insulin resistance, impaired glucose tolerance, dyslipidemia, sympathetic overactivity, and hypertension (42). It is regarded as a major precursor for the development of non-insulin-dependent diabetes mellitus (NIDDM). There is a distinct relation between the symptoms of syndrome X and weight maintenance. Weight gain decreases insulin sensitivity and glucose tolerance and increases blood pressure, whereas loss of excessive weight normalizes these symptoms (38, 43, 45, 48, 50). Epidemiological studies show that upper body obesity

is associated with a higher incidence of the symptoms of syndrome X than is lower body obesity (4, 22, 32, 49). This is primarily related to the amount of visceral fat rather than to the amount of subcutaneous fat (2–4). Visceral adipose tissue has metabolic characteristics that are unique in comparison with other adipose tissues. This is most pronounced for the regions that drain on the portal vein, i.e., the omental and mesenteric adipose tissues. The fat cells from these regions have increased β_3 -adrenoceptor sensitivity (24) and higher lipolytic activity (28) than other adipocytes. Increased lipolysis in these regions will expose the liver directly to an exaggerated supply of long-chain fatty acids.

Grekin and coworkers (20, 21) recently provided evidence for reflex activation of the sympathoadrenal system and hypothalamus-pituitary-adrenal axis (HPA axis) in rats by increased supply of long-chain fatty acids to the liver. This activation was associated with an increase in heart rate (HR) and blood pressure. In obesity, increased HPA axis activity and sympathetic activity are associated with insulin resistance (12, 26, 27), raising the possibility that reflex activation of the HPA axis and sympathoadrenal system resulting from excess portal venous supply of long-chain fatty acids plays a role in the induction of visceral obesity-related insulin resistance.

It was the aim of the present study to evaluate the impact of the basic observations by Grekin and colleagues (20, 21) concerning the pathophysiological significance of portal venous supply of long-chain free fatty acids (FFAs) to the liver. Specifically, we aimed to investigate whether reflex activation of the HPA axis and sympathetic system by an excessive portal venous supply of long-chain fatty acids to the liver plays a role in the development of insulin resistance. First, central venous plasma norepinephrine (NE) and epinephrine (Epi) levels, as indexes of sympathoadrenal activity, and mean arterial blood pressure (MAP) and HR frequency were determined during a 2-h intraportal infusion of the long-chain fatty acid oleate. Infusion of the

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medium-chain fatty acid caprylate or the solvent, slightly alkaline saline, served as a control. In a second set of experiments, rats received a portal venous infusion of oleate, caprylate, or saline for 24 h. Before the start of the infusion and in the 24th hour of infusion, central venous plasma NE, Epi, and corticosterone (Cort) concentrations were determined to assess sympathoadrenal and HPA axis activity. Additionally, in the 24th hour of infusion, insulin sensitivity was assessed by means of an intravenous glucose tolerance test (IVGTT). In a third series of experiments, hepatic triglyceride (TG) production was measured to check for alterations in hepatic lipid metabolism.

MATERIAL AND METHODS

Animal Care and Surgery

Male Wistar rats weighing 400–475 g at the beginning of the studies were used. They were housed individually in Plexiglas cages (25 × 25 × 30 cm; length × width × height) at room temperature (21 ± 2°C). The animals were maintained on a 12:12-h light-dark cycle (0700–1900 light) and were handled and weighed every morning at 0900.

The animals were provided with silicon catheters through one (*experiments 1 and 3*) or both (*experiment 2*) jugular veins according to Steffens (51), with the tip of the catheters positioned at the entrance of the right atrium. The other end was externalized on top of the skull, where the ends were fixed with acrylic cement. This method allows frequent sampling of well-mixed central venous blood from unanesthetized, undisturbed, freely moving rats (52). Additionally, the animals were provided with a silicon catheter in the portal vein for intraportal infusion (54). Surgery was performed under ether anesthesia.

For the experiments concerning the determination of blood pressure and HR responses (*experiment 1*), the animals were implanted with a blood pressure transmitter (TA 11PA-C40; Dataquest), which, in combination with a receiver and a data acquisition system, allows continuous registration of blood pressure and HR. The tip of the blood transmitter was inserted in the descending aorta, and the body of the blood pressure transmitter was positioned in the abdominal cavity.

The experiments started when the animals had regained their preoperative body weight, but at least 10 days of recovery after surgery were allowed. To avoid novelty stress, the rats had been accustomed to blood sampling procedures and experimental conditions before the first actual experiment (8, 47).

All procedures were approved by the animal welfare committee of the University of Groningen.

Experimental Protocols

Experiment 1: Hemodynamic and sympathoadrenal effects of intraportal fatty acid infusion. On the day of an experiment, food was removed 3 h before the start of intraportal infusion. At the same time, polyethylene tubing (length 400 mm, 1.25 mm ID, 1.75 mm OD) was connected to the outlets of the catheters at the top of the skull of the animal. After 2 h, when the animals were calm and resting, either blood sampling or blood pressure and HR registration were started. The animals received the infusions at a rate of 1.2 ml/h. Sodium oleate (Sigma, St. Louis, MO) and sodium caprylate (Sigma), dissolved in slightly alkaline saline, were infused at a rate of 250 nmol/min (20, 21). The infusion solutions were protected from light by wrapping the infusion syringes and tubing. The solutions remained clear throughout the experi-

ments. Blood samples were withdrawn at time (t) = -15, -1, 30, 60, 90, 120, 150, and 180 min relative to the start of the infusion at $t = 0$. The infusion was terminated after sampling at $t = 120$ min. Blood pressure and HR were registered for 30 min before the start of the infusion, during infusion, and for 60 min after the infusion was terminated.

The animals received the intraportal oleate, caprylate, or saline infusion or the jugular vein oleate infusion in a random order, with at least 4 days between consecutive experiments. The experiments were performed between 1100 and 1700, i.e., in the light period. All animals participated in multiple experiments.

Experiment 2: Effects of intraportal fatty acid infusion on insulin sensitivity. Polyethylene tubing (length 400 mm, 1.25 mm ID, 1.75 mm OD) was connected to the outlets of the catheters at the top of the animal's skull. Two baseline samples were withdrawn when the animals were calm and resting, usually 1–1.5 h after connection of the sample lines. Thereafter, the animals were connected to an infusion pump for intraportal infusion of either sodium oleate (150 nmol/min), sodium caprylate (150 nmol/min), or the solvent (saline; 1.2 ml/h). In the 24th hour of infusion, two baseline samples were taken again ($t = -15$ and -1 min). At $t = 0$, an intravenous glucose infusion (16 mg/min for 20 min) was started for an IVGTT. Blood samples were withdrawn at $t = 5, 10, 15,$ and 20 min. The infusion was terminated after sampling at $t = 20$ min, and additional blood samples were taken at $t = 25, 30, 35,$ and 40 min. All of the IVGTTs were performed between 1200 and 1400, i.e., in the light period.

After the IVGTT, two to three animals per group were killed for histological examination of the liver. For this purpose, parts of the livers were fixed by immersion in 4% phosphate-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin for routine histology.

The animals that received an intraportal oleate infusion had free access to food and water during the infusion period, but food was taken away 2 h before the start of the IVGTT. To control for feeding-related changes in nutritional status and hormonal status and subsequently insulin sensitivity, the animals that received either intraportal caprylate or saline infusions were pair-fed (10 g/24 h) to the oleate-infused group.

Experiment 3: Effects of oleate on hepatic TG production rate. In the third experiment, hepatic TG production was tested in either oleate- or caprylate-treated animals. To that end, animals received an intravenous dose of Triton WR-1339 (12% wt/wt in saline, 0.5 ml/100 g body wt; Sigma) after 24 h of intraportal infusion with either sodium oleate or sodium caprylate (150 nmol/min at 1.2 ml/h). Triton WR-1339 is a nonionic detergent that blocks lipoprotein lipase action, thus preventing metabolism of lipoproteins, and traps lipoproteins in the plasma. This allows the calculation of the hepatic very low density lipoprotein (VLDL) TG production rate from the linear increase in plasma TG concentration after intravenous administration of the compound (17). Blood samples (300 μ l) were withdrawn at $t = 0, 1, 2, 3, 4,$ and 5 h relative to the injection of Triton WR-1339.

Blood Sampling and Chemical Determinations

Blood samples were withdrawn for determination of glucose in whole blood and for determination of Cort, Epi, NE, immunoreactive insulin (IRI), FFA, cholesterol (Chol), TG, alanine aminotransferase (ALT), alkaline phosphatase (AP), and total bilirubin (tBi) in plasma. After each sample, an equivoluminal amount of citrated (0.6% citrate) blood, obtained from permanently cannulated donor rats, was rein-

fused. Between successive blood samples, the tip of the catheter was filled with 6% citrate solution as an anticoagulant. Citrate was used instead of heparin to avoid activation of endothelial lipase.

After withdrawal, blood samples were transferred to chilled (0°C) centrifuge tubes that contained 10 μ l EDTA solution (70 g/l) as anticoagulant and antioxidant. Glucose was measured in 50 μ l of blood by the ferricyanide method of Hoffman (Technicon Auto Analyzer TM II). The remaining blood was centrifuged for 15 min at 5,000 rpm at 4°C. One-hundred microliters were stored at -70°C until handling for catecholamine determination. Catecholamines were measured by HPLC with electrochemical detection (46). The remaining plasma was stored at -20°C until handling for IRI and Cort determination. IRI was determined by means of an RIA (Linco). Plasma Cort was determined by means of reversed-phase HPLC with ultraviolet detection (11, 13). Plasma FFA, Chol, TG, ALT, tBi, and AP concentrations were measured by standard laboratory procedures.

Data Analysis and Statistics

Data are expressed as means \pm SE. Data for MAP and HR are expressed as the mean changes over 15-min periods, taking the values of period $t = -15-0$ min before infusion as the baseline. Within an experiment, Wilcoxon's matched-pairs signed rank test was used to compare the data obtained at each moment relative to the baseline level. Two-way ANOVA with repeated measures followed by the Mann-Whitney U -test was used to determine differences between the experimental groups. The level of significance was set at $P < 0.05$.

RESULTS

Experiment 1

Table 1 summarizes the central venous plasma concentrations for NE and Epi and for MAP and HR frequency as determined just before the start of intraportal fatty acid infusion. No significant initial differences between the four conditions were observed.

Figure 1 presents the changes in central venous NE and Epi concentrations as determined during 2 h of fatty acid infusion. The results show a significant increase in central venous plasma NE and Epi concentrations during intraportal oleate infusion (NE: $P < 0.05$ at $t = 60-180$ min; Epi: $P < 0.05$ at $t = 90-180$ min), whereas jugular vein infusion of oleate or intraportal infusion of caprylate or saline had no effect.

Figure 2 presents the changes in MAP and HR as

Table 1. Baseline catecholamine, blood pressure, and heart rate values for the experiments with short-term fatty acid infusion

	PA oleate (n = 7)	PA caprylate (n = 7)	PA saline (n = 7)	Jug oleate (n = 7)
NE, ng/l	142 \pm 25	192 \pm 45	210 \pm 39	184 \pm 36
Epi, ng/l	4 \pm 1	14 \pm 9	19 \pm 13	10 \pm 4
MAP, mmHg	97 \pm 4	97 \pm 3	98 \pm 3	100 \pm 4
HR, beats/min	326 \pm 8	331 \pm 11	327 \pm 6	325 \pm 9

Values for norepinephrine (NE) and epinephrine (Epi) are averages \pm SE at time ($t = -1$ min, i.e., before the start of infusion). Values for mean arterial pressure (MAP) and heart rate (HR) are averages over a 15-min period before the start of infusion; $n = 7$ animals in each group. PA, portal artery; Jug, jugular vein.

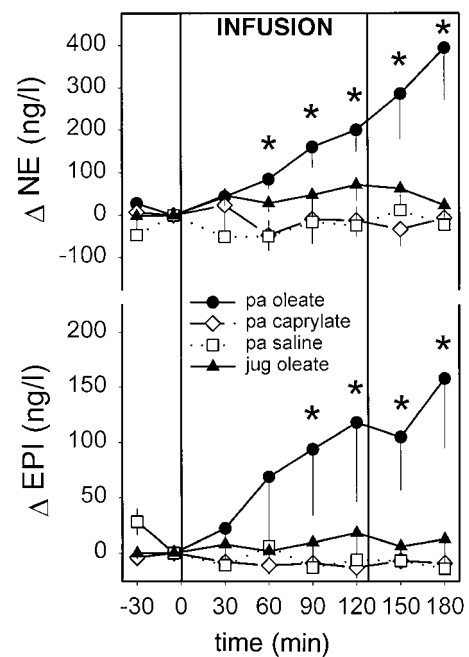


Fig. 1. Sympathoadrenal responses to 2 h of intraportal fatty acid infusion (250 nmol/min). Data are expressed as average change ($\Delta \pm$ SE) from baseline, i.e., just before the start of infusion. Δ , Change; NE, norepinephrine; Epi, epinephrine; pa, portal artery; jug, jugular vein. *Significant differences between the experiment with intraportal oleate infusion and the experiments with caprylate or saline infusion ($P < 0.05$).

determined during intraportal fatty acid infusion. Both MAP and HR showed a significant increase during intraportal oleate infusion (Δ MAP: $P < 0.05$ at $t = 22.5-172.5$ min; Δ HR: $P < 0.05$ at $t = 37.5-172.5$ min).

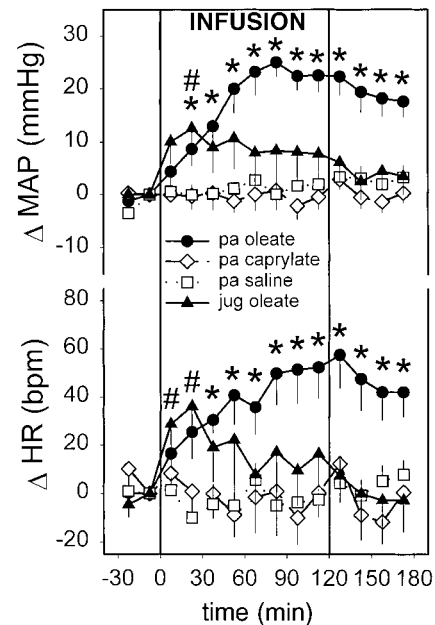


Fig. 2. Mean arterial pressure (MAP) and heart rate (HR) frequency responses to 2 h of intraportal fatty acid infusion (250 nmol/min). Data are expressed as average change ($\Delta \pm$ SE) from baseline, i.e., just before the start of infusion. *Significant differences between the experiment with oleate infusion and the experiments with caprylate and saline infusion ($P < 0.05$). #Significant increases in MAP and HR in the experiment with jugular vein oleate infusion ($P < 0.05$).

Table 2. Effects of intraportal fatty acid infusion on glucose, hormone, and lipid concentrations

	Oleate (n = 9)		Caprylate (n = 9)		Saline (n = 11)	
	Pre	Post	Pre	Post	Pre	Post
Glucose, mmol/l	5.9 ± 0.3	5.1 ± 0.2	6.7 ± 0.2	5.8 ± 0.2	6.1 ± 0.1	5.6 ± 0.1
IRI, mg/l	3.1 ± 0.5	1.7 ± 0.1	3.0 ± 0.3	1.3 ± 0.2	3.3 ± .3	1.7 ± 0.2
FFA, mmol/l	0.24 ± 0.07	0.49 ± 0.05*	0.21 ± 0.03	0.36 ± 0.03	0.18 ± 0.02	0.48 ± 0.04
Δ FFA, mmol/l		+0.25 ± 0.08		+0.15 ± 0.05		+0.30 ± 0.04
TG, mmol/l	0.86 ± 0.16	0.48 ± 0.07	1.23 ± 1.06	1.06 ± 0.11	0.69 ± 0.10	1.16 ± 0.21
Δ TG, mmol/l		-0.38 ± 0.14		-0.17 ± 0.16		0.50 ± 0.13†
Chol, mmol/l	1.62 ± 0.23	0.69 ± 0.11	2.14 ± 0.20	1.21 ± 0.08	2.04 ± 0.1	2.07 ± 0.04
Δ Chol, mmol/l		-0.93 ± 0.32		-0.93 ± 0.13		0.04 ± 0.09†

Values are means ± SE; n, no. of rats. Pre, blood sample taken at $t = -1$ min before start of infusion; post, blood sample taken in 24th hour of intraportal infusion; IRI, immunoreactive insulin; FFA, free fatty acids; TG, triglycerides; Chol, cholesterol, all measured in central venous plasma samples. Δ, difference in parameter value from pre to post. * $P < 0.05$, oleate vs. caprylate and saline. † $P < 0.05$, saline vs. oleate and caprylate.

Infusion of oleate in the jugular vein induced significant increases in MAP and HR at $t = 22.5$ min and at $t = 7.5$ and 22.5 min, respectively. Neither intraportal caprylate nor saline affected MAP or HR.

Experiment 2

Table 2 summarizes the central venous blood glucose concentrations and plasma concentrations for IRI, FFA, TG, and Chol as determined just before the start of the fatty acid infusion (pre) and in the 24th hour of intraportal fatty acid infusion (post). No significant differences between the groups for glucose and IRI were observed; both glucose and IRI slightly decreased during the infusion period. FFA levels increased in all three groups, whereas TG and Chol declined in oleate- and caprylate-treated animals only ($P < 0.05$).

Figure 3 presents the changes in central venous plasma concentration for Epi, NE, and Cort during infusion. There were no significant differences between the groups at the onset of infusion. In the 24th hour of intraportal infusion, both Cort and NE were significantly increased in the oleate-infused group compared with the baseline and with the control groups. No effects on Epi were observed.

Table 3 shows the central venous plasma concentrations for ALT, AP, and tBi. There are no significant differences between the groups at the onset of the infusion. Intraportal oleate infusion induced a significant increase in plasma ALT concentrations, whereas caprylate and saline infusion had no effect. AP and tBi, on the other hand, were not affected either by oleate, caprylate, or saline infusion.

Figure 4 presents the mean changes from baseline at $t = -1$ min in blood glucose and plasma insulin concentrations during and after intravenous glucose infusion. Intravenous infusion of glucose caused an increase in blood glucose of 6.1 ± 0.5 , 6.7 ± 0.7 , and 5.1 ± 0.5 mmol/l at $t = 20$ min in oleate-, caprylate-, and saline-treated animals, respectively. After termination of glucose infusion, blood glucose declined to reach the baseline at $t = 40$ min in all three groups. There were no significant differences in the areas under the response curves, i.e., 121 ± 13 , 135 ± 20 , and 95 ± 11 for the oleate-, the caprylate-, and the solvent-treated an-

imals, respectively. At the end of the infusion period, i.e., at $t = 20$ min, IRI had increased with 13.6 ± 1.9 ng/ml in the oleate-treated animals. After termination of glucose infusion, plasma IRI declined again to reach the baseline at $t = 40$ min. In the caprylate- and solvent-treated groups, IRI showed an increase of only 6.6 ± 0.9 and 6.4 ± 0.7 ng/ml, respectively, above the baseline at $t = 20$ min ($P < 0.05$ compared with oleate). After termination of glucose infusion, IRI declined to reach the baseline at $t = 35$ min in both control groups. Calculated areas under the IRI response curves were 220 ± 35 ($P < 0.05$), 112 ± 13 , and 106 ± 8 for the oleate-, caprylate-, and solvent-treated groups, respectively.

Livers of animals killed after the IVGTT did not show gross histological abnormalities or signs of inflammation.

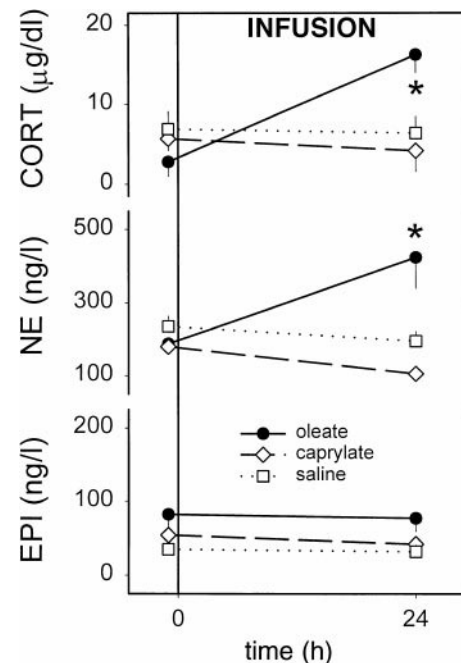


Fig. 3. Plasma corticosterone (Cort), NE, and Epi concentrations as determined at the onset and in the 24th h of intraportal fatty acid infusion (150 nmol/min). Data are expressed as averages ± SE. *Significant differences between groups ($P < 0.05$).

Table 3. Effects of intraportal fatty acid infusion on liver function parameters

	Oleate		Caprylate		Saline	
	Pre	Post	Pre	Post	Pre	Post
ALT, U/l	45 ± 2	277 ± 102*	47 ± 3	46 ± 2	43 ± 2	40 ± 2
AP, U/l	<11	<11	<11	<11	<11	<11
tBi, μmol/l	5.3 ± 0.8	5.3 ± 0.3	5.8 ± 0.8	4.8 ± 0.8	7.0 ± 0.8	5.8 ± 0.6

Values are means ± SE. ALT, alanine aminotransferase; AP, alkaline phosphatase; tBi, total bilirubin; all measured in central venous plasma samples. * $P < 0.05$, oleate vs. caprylate and saline.

Experiment 3

Figure 5 shows the accumulation of TGs in plasma after intravenous Triton WR-1339 administration. In the oleate-treated animals, total plasma TG concentration increased from 0.6 ± 0.21 to 3.9 ± 0.4 mmol/l over the 5-h period after Triton WR-1339 administration. In the caprylate-treated group, total plasma TG concentration increased from 1.0 ± 0.2 to 12.9 ± 0.5 mmol/l in the 5 h after Triton administration. The hepatic VLDL TG production rates calculated from these curves were 11.0 ± 1.2 and 36.8 ± 2.9 μmol/h for oleate- and caprylate-treated animals, respectively ($P < 0.05$). The latter value is similar to our control values in untreated Wistar rats (44.8 ± 6.9 μmol/h, $n = 3$) and representative data from the literature (17).

DISCUSSION

This study was designed to investigate whether excessive supply of long-chain fatty acids to the liver could play a role in the induction of the metabolic syndrome X. We hypothesized an increased portal ve-

nous supply of long-chain fatty acids to the liver to induce reflex activation of the HPA axis and the sympathetic system, leading to increased blood pressure and the development of insulin resistance, both major characteristic symptoms of syndrome X. Intraportal infusion of the long-chain fatty acid oleate for 2 h at a rate of 250 nmol/min caused an increase in resting central venous plasma NE and Epi concentrations, indicating increased resting sympathoadrenal activity. MAP and HR frequency showed concomitant increases during oleate infusion. The medium-chain fatty acid caprylate and the solvent saline had no effect on either sympathoadrenal activity or hemodynamic parameters. Jugular vein infusion of oleate at an equimolar rate did not induce significant effects, indicating that the effects on sympathetic activation and consequently MAP and HR are specific for portal venous infusion. These results confirm earlier observations by Grekin and coworkers (20, 21). These investigators showed that the increase in MAP could be abolished by pretreatment with an α-adrenoceptor antagonist, indicating that sympathoadrenally mediated vasoconstriction, besides sympathoadrenergic stimulation of HR frequency, is likely to play part in the increase in MAP (20).

The experiments with long-term oleate infusion showed significantly increased NE and Cort concentrations during the 24th hour of infusion, indicating that the sympathetic system and HPA axis were activated. In the series with oleate infusion, a significantly increased IRI response to intravenous glucose infusion

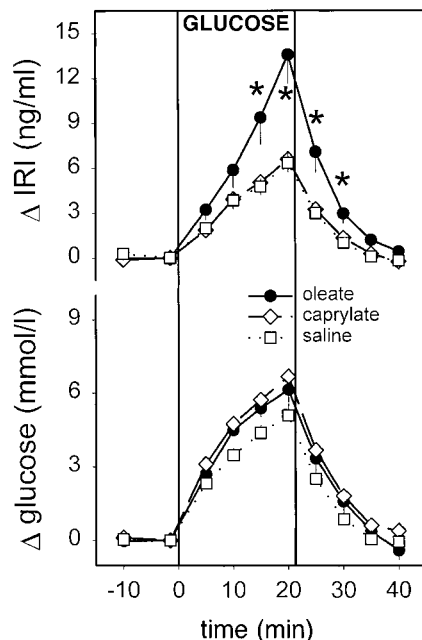


Fig. 4. Plasma immunoreactive insulin (IRI) and blood glucose profiles to the intravenous glucose tolerance tests during intraportal fatty acid infusion. Data are expressed as average change ($\Delta \pm$ SE) from baseline, i.e., just before the start of infusion. *Significant differences between groups ($P < 0.05$).

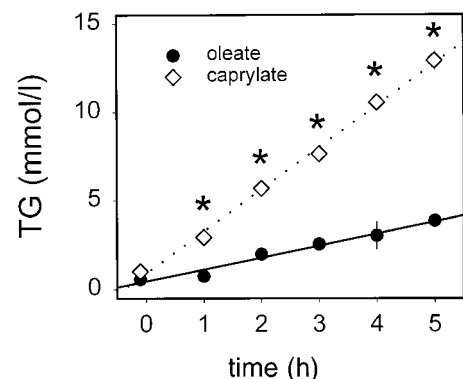


Fig. 5. Effect of intravenous Triton WR-1339 injection on plasma triglyceride (TG) concentrations in rats that had received an intraportal infusion of either oleate ($n = 5$) or caprylate ($n = 5$) for 24 h. Data are expressed as averages ± SE. *Significant differences between the groups ($P < 0.05$).

was observed compared with caprylate or the solvent, whereas the glucose response did not differ between groups. Despite this increased IRI response in the oleate-treated group, there was no difference in glucose disappearance when the glucose infusion was terminated. These observations clearly indicate that intraportal oleate infusion did induce insulin resistance, whereas intraportal caprylate infusion did not. This induction of insulin resistance was independent of the feeding state of the animals, since the control groups were pair-fed to the oleate-treated animals. These findings are consistent with our proposed concept concerning the induction of visceral obesity-related insulin resistance.

Obesity-related insulin resistance has been suggested to result from hyperinsulinemia as a consequence of reduced insulin uptake by the liver, caused by overexposure of the liver to FFAs (30, 39). However, *in vitro* and *in vivo* studies by Boden and coworkers (6, 7, 29) have failed to support this concept. Alternatively, it has been hypothesized that FFAs induce insulin resistance, either directly or indirectly, and thereby provoke a compensatory increase in insulin secretion (5). The results of the present study support this concept, since insulin resistance did develop in oleate-treated rats, despite decreasing baseline IRI concentrations. The latter is probably due to the feeding condition of the animals, because semistarvation (~10 g/24 h), with subsequently decreased blood glucose concentrations, induced a lowering of IRI in all three groups.

The results therefore strongly support a role for the HPA axis and sympathetic activation in mediating the oleate-induced development of insulin resistance. Increased HPA axis activity has been reported to be a potent mediator of insulin resistance (26, 27). Humans (44) as well as animals (57) treated with glucocorticoids develop insulin resistance. Moreover, insulin resistance is commonly observed in people suffering from Cushing's syndrome (36). Corticosteroids have been suggested to induce insulin resistance via an increase in circulating FFAs. However, dexamethasone-induced impairment in skeletal muscle glucose transport is not reversed by inhibition of FFA oxidation (58), indicating that corticosteroids may have a direct effect on insulin sensitivity. This is confirmed in an animal model for high-fat feeding-induced insulin resistance in skeletal muscle, where treatment with the antigluocorticoid RU-486 resulted in an amelioration of insulin resistance (31). As such, this finding is consistent with the hypothesis that HPA axis hyperreactivity may be involved in the development of obesity-related insulin resistance. In addition to increased HPA axis activity, increased sympathetic activity, as seen with oleate infusion, may also act as a causal factor in the development of insulin resistance (12, 15, 26, 41).

We do observe an increase in HPA axis activity, as reflected by a significant increase in plasma Cort, and sympathetic activity, as reflected by an increase in plasma NE, after 24 h of intraportal oleate infusion. Although these increases are rather acute, they are

consistent with the increase in HPA axis and sympathetic activity observed in obesity (1, 26, 55, 56). The origin of this increased HPA axis and sympathetic activity is still unclear. Reflex activation originating from the liver is a possible source. There is abundant evidence from animal studies that afferent sympathetic and parasympathetic nerves from the liver are responsive to metabolic changes in hepatic cells (19, 34). In a study directly relevant to the basic hypothesis of this study, Orbach and Andrews (37) reported increases in vagal afferent activity after infusion of the long-chain fatty acid palmitate into the hepatic artery of rabbits, whereas infusion of short-chain fatty acids had no effect.

Hepatic vagal afferent activity in obesity could originate from changes in hepatic functioning. Intraportal infusion of oleate, caprylate, or the solvent saline did not lead to increases in either AP or tBi, indicating that the animals were not hampered by cholestasis. If the oleate infusion had had detergent-like properties, then hemolysis would have occurred and tBi would have increased. We did not observe any increase in tBi, thus excluding hemolysis. We did observe moderate increases in plasma ALT, a marker for hepatic leakage, after 24 h of intraportal oleate infusion. This increase in plasma ALT could be seen as a marker for hepatic steatosis; however, no changes in morphology were observed in paraffin-embedded liver coupes. We therefore conclude that the unspecific detergent-like properties of the fatty acid solutions that we infused were minimal at the most. Alternatively, the increase in ALT can very well be due to oleate-induced activation of hepatic peroxisome proliferator-activated receptor (PPAR)- α . In Hep G2 cells, PPAR α stimulation with fenofibrate induces markedly increased ALT-mRNA levels (14). Long-chain fatty acids are, like fenofibrate, potent stimulants for PPAR α , indicating that transaminase activity can be altered independently of a toxic phenomenon (14).

Although the increase in circulating hepatic enzymes that we observe is rather acute, it is remarkable that analog increases of serum transaminase activity have been documented in obese patients (16, 23, 25, 35). The increases in serum transaminase activity in obesity can be observed before overt fatty liver or steatosis. It is therefore unclear whether increased transaminase activity results from fatty liver or steatosis or whether these symptoms have one common source. In the present case, hepatic transaminase leakage could be an early symptom of obesity-induced alterations in hepatic functioning, leading to fat deposition and ultimately steatosis.

Increased serum transaminase activity has been associated with symptoms of syndrome X. Hollmann et al. (25) showed a strong correlation between the waist-to-hip ratio and plasma ALT concentrations and other characteristics of syndrome X. Fagerberg et al. (16) reported a significant correlation of ALT to plasma insulin, independent of body mass index. Moreover, Perry et al. (40) observed a strong, graded, and independent association between the concentration of

γ -glutamyltransferase in serum and the risk of NIDDM during long-term follow-up. Based on these observations and on the results obtained in the present study, we advocate the hypothesis that excessive portal venous supply of long-chain fatty acids alters hepatic functioning, resulting in increased transaminase activity. This altered hepatic functioning is recognized by the central nervous system and results in HPA axis and sympathetic activation, leading to the symptoms of syndrome X. It may therefore serve as the causal factor in the development of syndrome X. It could be the "common soil" that has been referred to in the perspective of the development of syndrome X and NIDDM (53).

It is surprising that oleate infusion at a rate of only 150 nmol/min has the significant effects that are observed. Grekin et al. (21) calculated that exogenous infusion of oleate at a rate of 250 nmol/min (*experiment 1*) increased hepatic oleate delivery only by 12% and total fatty acid delivery by 2%. When extrapolated, this would give increases in hepatic oleate and total fatty acid delivery of only 7 and 1.2%, respectively, for an infusion rate of 150 nmol/min (*experiment 2*) based on values for the postabsorptive state. In the postprandial state, plasma FFA concentrations may decrease by 80–90%, meaning that an infusion rate of 150 nmol/min could have increased hepatic oleate delivery by as much as 100%. After oleate infusion for 24 h, the actual plasma FFA concentration had doubled from 0.24 ± 0.07 to 0.49 ± 0.05 mmol/l. This increase, however, cannot be responsible for the observed effects, since the control groups showed comparable increases in total plasma FFA due to the feeding conditions of the animals and the related decrease in plasma IRI. It may be that the observed effects are due to specific effects of increased supply of unbound long-chain, rather than medium-chain, FFAs to the liver (21), which is estimated to be increased about three times during infusion of oleate at a rate of 150 nmol/min.

Intraportal oleate infusion resulted in decreased hepatic TG secretion compared with caprylate infusion. At first sight, this result seems rather paradoxical. In vitro studies have shown that long-chain fatty acids stimulate hepatic VLDL production (e.g., Ref. 33). Moreover, corticosteroids, which significantly increased in the experiments with oleate infusion, are potent stimulators of hepatic VLDL production in vivo (10). On the contrary, α -adrenoceptor stimulation is known to decrease VLDL production (9). NE, a potent stimulator of α -adrenoceptors, is significantly increased after intraportal oleate infusion. Therefore, it seems likely that an NE-induced, α -adrenoceptor-mediated decrease of VLDL production overrules a potential corticosteroid-mediated stimulation of VLDL TG production, leading to a net decrease in VLDL production. Because insulin resistance and NIDDM are characterized by increased VLDL production (18), it is likely that the decreased VLDL TG production reflects the acute nature of this experiment and, in fact, is a transient phenomenon. In any case, the results of the present study emphasize the strong metabolic actions of portal oleate supply to the liver.

Taken together, the results from the present study in part confirm the data presented by Grekin and colleagues (20, 21). Moreover, the results show that an increase in portal venous supply of long-chain fatty acids to the liver induces HPA axis and sympathetic activation with concomitant development of increased MAP and insulin resistance. These metabolic actions of portal venous fatty acids might contribute to the etiology of obesity-related insulin resistance. These findings expand the original hypothesis for the induction of obesity-related hypertension to the development of obesity-related insulin resistance and probably syndrome X.

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