The autonomic nervous system regulates postprandial hepatic lipid metabolism

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Submitted 5 December 2012; accepted in final form 22 March 2013


First published March 26, 2013; doi:10.1152/ajpendo.00614.2012.—

The liver is a key organ in controlling glucose and lipid metabolism during feeding and fasting. In addition to hormones and nutrients, inputs from the autonomic nervous system are also involved in fine-tuning hepatic metabolic regulation. Previously, we have shown in rats that during fasting an intact sympathetic innervation of the liver is essential to maintain the secretion of triglycerides by the liver. In the current study, we hypothesized that in the postprandial condition the parasympathetic input to the liver inhibits hepatic VLDL-TG secretion. To test our hypothesis, we determined the effect of selective parasympathetic denervation on hepatic glucose metabolism in male Wistar rats. We report that postprandial plasma triglyceride concentrations were significantly elevated in parasympathetically denervated rats compared with control rats (P = 0.008), and VLDL-TG production tended to be increased (P = 0.066). Sympathetically denervated rats also showed a small rise in postprandial triglyceride concentrations (P = 0.045). On the other hand, in rats fed on a six-meals-a-day schedule for several weeks, a parasympathetic denervation resulted in >70% higher plasma triglycerides during the day (P = 0.001), whereas a sympathetic denervation had no effect. Our results show that abolishing the parasympathetic input to the liver results in increased plasma triglyceride levels during postprandial conditions.

parasympathetic; denervation; liver; triglycerides; feeding

The liver is essential for maintaining metabolic equilibrium in the body by storing and releasing large quantities of energy according to changing demands. In the postprandial state, the liver plays a central role in storing energy for later use, whereas in the fasted state, fuel availability is reduced

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EXPERIMENTAL PROCEDURES

Animals. Male Wistar rats weighing 280–310 g (Charles River Breeding Laboratories, Sulzfeld, Germany, and Harlan Nederland, Horst, The Netherlands) were housed in individual cages with a 12:12-h light-dark schedule (lights on at 7 AM). Chow (Teklad Global 18% protein rodent diet; Harlan) and water were available ad libitum, unless stated otherwise. All procedures were approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

Surgery. All surgeries were performed under anaesthesia with 0.8 ml/kg i.m. Hypnorm (Janssen, High Wycombe, Buckinghamshire, UK) and 0.4 ml/kg sc Dormicum (Roche, Almere, The Netherlands). After 1 wk in the facility, rats underwent denervation surgery according to previous reports (14). In short, a laparotomy was performed in the midline in all groups. For the hepatic sympathectomy (Sx), nerve bundles running along the hepatic artery proper were removed using microsurgical instruments. Any connective tissue attachments between the hepatic artery and portal vein were also dissected without nailing any possible nerve crossings. For the hepatic parasympathectomy (Px) the neural tissue was transected between the ventral vagus trunk and liver. A total liver denervation (Tx) was achieved by cutting the sympathetic and parasympathetic branches to the liver. Rats with sham denervation surgery, as described above except for cutting the nerve, served as the control group. The effectiveness of the hepatic sympathetic denervation was checked by measurement of hepatic noradrenaline concentration using an in-house HPLC method with fluorescent detection (15). A sympathetically or totally denervated rat noradrenaline concentration was determined by measurement of hepatic sham denervation surgery, as described above except for cutting the nerve, served as the control group. The effectiveness of the hepatic sympathetic denervation was checked by measurement of hepatic noradrenaline concentration using an in-house HPLC method with fluorescent detection (15). A sympathetically or totally denervated rat was included in the analysis only if noradrenaline concentration of the liver was <10% of the sham denervated noradrenaline concentration. We have previously validated our method for selective hepatic parasympathectomy by using retrograde viral tracing (14). During the same surgical procedure, an intra-arterial silicone catheter was implanted in the jugular vein according to the method of Steffens (31).

Basal experiment. After surgery, rats were allowed to recover to presurgery body weight for ≥10 days. On the day before the experiment, the food was removed at 5 PM, and the rats were attached to a metal collar for adaptation. The next day, at 9 AM, the rats were connected to the blood sampling line, which was attached to the metal collar and kept out of reach of the animals by means of a counterbalanced beam. This allowed all blood sampling to be performed outside the cages without further handling of the animals. At 1 PM a 20-h-fasted blood sample was taken before the meal, and subsequently 8–10 g of laboratory chow was presented. During a time frame of 20 min, the rats were allowed to eat. The remaining food was removed for the further duration of the experiment and weighed. Immediately after the meal, a baseline postprandial sample was taken (t = 0). After this baseline sample, four more blood samples were taken 20 min apart to create a postprandial curve. Out of the total of 34 rats tested, eight rats that did not eat between 2.0 and 4.5 g during the 20-min meal were excluded from the final analysis of this experiment.

VLDL-TG secretion experiment. After the basal experiment, the rats were allowed to recover for 3–5 days before the experiment was repeated to measure VLDL-TG secretion. VLDL-TG secretion was measured by blocking VLDL-TG clearance through an intravenous bolus of 0.7 ml of 15% tyloxapol (Sigma-Aldrich) directly after the baseline postprandial sample (t = 0), which immediately inhibits lipoprotein lipase-mediated TG hydrolysis. LGI this, blood samples were taken once every 20 min to determine the slope in the rise of plasma TG levels, indicating VLDL-TG secretion.

Six-meals-a-day feeding schedule. Rats were entrained to a feeding schedule of six 10-min meals spaced equally over the light-dark cycle. Food pellets were available in metal food hoppers at Zeitgeber time (ZT)2, ZT6, ZT10, ZT14, ZT18, and ZT22 [ZTO is defined as the “lights-on” time point (7 AM)]. Access to the food could be prevented by a sliding door situated in front of the food hopper activated by an electrical motor and controlled by a clock. Water was available ad libitum. Rats were given 2 wk to adapt to the feeding schedule and subsequently underwent surgery. A sympathectic or parasympathetic liver denervation was performed, and a jugular vein catheter was placed. Rats with an intact hepatic innervation, but on the same feeding schedule, served as the control group. After 2 wk of recovery, 0.2 ml of blood once every hour for 12 consecutive hours was taken on two different occasions, starting at either ZT6.5 or ZT18.5. Between both 12-h sampling experiments, the rats were allowed to recover for ≥10 days. All experiments were performed in the rat’s home cage.

Analysis. Plasma TG levels were assayed using a kit from Roche Mannheim, Germany). Blood glucose concentrations were determined during the experiment in blood spots using a glucose meter (Freestyle, Abbott, The Netherlands). By using a radioimmunossay kit, plasma insulin (Linco Research, St. Charles, MO) was measured. TG concentration in liver was measured after a single-step lipid extraction with methanol and chloroform (12). The pellets were finally dissolved in 2% Triton X-100 (Sigma-Aldrich), and TGS were measured using the “Trig/GB” kit (Roche).

RNA isolation and real-time PCR. After the acute experiments, the left lateral liver lobe was removed after an overdose of pentobarbital IV. In addition to the livers from the acute meal experiment, livers from a control experiment were also included to determine whether genes are regulated by the meal. The control experiment contained four intact rats that continued overnight fasting and four intact rats that received the meal using the same method as the final experiment. RNA isolation and real-time PCR were performed as described previously (6). The expression of the reference gene Hprt was not regulated by the different conditions in the control experiment (P = 0.751) or by the different denervations (P = 0.516).

Statistical analysis. Data are presented as means ± SE. When a curve was plotted for the different groups, a general linear model (GLM) analysis with repeated measurements was used, with denervation as between-animal factor and time as within-animal factor. Data were analyzed by one-way ANOVA when single outcome measurements or separate time points between the groups were compared. A significant (P ≤ 0.05) global effect of the repeated-measurements GLM or one-way ANOVA was followed by post hoc tests for individual group differences (Fisher’s protected least significant difference). To investigate the separate effects of time in the basal experiment, a repeated-measurements analysis was done for all groups separately, followed by a paired t-test to plot the significance compared with baseline. Significance was defined at P < 0.05.

RESULTS

Acute meal experiments. In the acute meal experiments, all groups received a meal after an overnight fast. In the basal experiment, where we looked at the effects of the meal on plasma TG concentrations in the different denervation groups, all animals consumed on average 3.55 ± 0.14 g of chow (means ± SE), with no differences between the groups (P = 0.765) (Table 1). In the VLDL-TG secretion experiment, where we used the same experimental setup and in addition injected tyloxapol after the meal, all animals consumed on average 3.85 ± 0.15 g of chow (means ± SE), with no significant differences between the groups (P = 0.912) (Table 1). Table 1 shows the absolute concentrations of TG and glucose in the fasted sample taken directly before the meal and the baseline postprandial sample (t = 0) collected directly after the meal of the basal and VLDL-TG secretion experiment. In both experiments, consumption of the meal significantly decreased plasma TG concentrations and significantly increased blood glucose concentrations compared with fasted levels in all groups, but no significant differences between groups were observed. In the
observed a significant increase in all groups after the meal, with no basal experiment, we measured plasma insulin concentrations and observed a significant increase in all groups after the meal, with no difference between the groups (Table 1).

**Basal experiment.** The postprandial curves show the difference in plasma TGs from the baseline postprandial sample taken directly after the meal (t = 0) and the subsequent samples taken at t = 20, t = 40, t = 60, and t = 80 in the control, parasympathetically, sympathetically, and total denervated rats (Fig. 1, A–C). After the initial decrease in TG observed in all groups (Table 1), in the control group the postprandial curve showed a further significant decline in plasma TG 20 min after the end of the meal (Fig. 1, A–C). Subsequently, a slow and steady increase was observed. In both the parasympathetically (Fig. 1A) and sympathetically denervated rats (Fig. 1B), we observed a significant postprandial rise in plasma TG levels, reaching significance at t = 40 and t = 60 min, respectively. When all postprandial curves were compared, the curves of both the parasympathetically

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### Table 1. The effects of the meal in the basal and VLDL-TG secretion experiment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Px</th>
<th>Sx</th>
<th>Tx</th>
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<td><strong>Basal experiment</strong></td>
<td></td>
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<tr>
<td>Food intake, g</td>
<td>3.58 ± 0.24</td>
<td>3.34 ± 0.20</td>
<td>3.57 ± 0.35</td>
<td>3.79 ± 0.33</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>3.5 ± 0.1</td>
<td>3.8 ± 0.2</td>
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<tr>
<td>Fasted</td>
<td>5.6 ± 0.1*</td>
<td>5.6 ± 0.2*</td>
<td>5.6 ± 0.2*</td>
<td>5.8 ± 0.3*</td>
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<tr>
<td>Postprandial</td>
<td>0.33 ± 0.04</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.32 ± 0.04</td>
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<tr>
<td>TG, mmol/l</td>
<td>0.26 ± 0.03*</td>
<td>0.20 ± 0.02*</td>
<td>0.20 ± 0.01*</td>
<td>0.23 ± 0.03</td>
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<td>Fasted</td>
<td>0.64 ± 0.19</td>
<td>0.39 ± 0.10</td>
<td>0.43 ± 0.07</td>
<td>0.35 ± 0.10</td>
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<td>Postprandial</td>
<td>4.97 ± 0.55*</td>
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<td>Food intake, g</td>
<td>3.71 ± 0.16</td>
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<td>3.92 ± 0.34</td>
<td>4.02 ± 0.53</td>
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<td>Glucose, mmol/l</td>
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<tr>
<td>Fasted</td>
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<td>5.4 ± 0.1*</td>
<td>5.7 ± 0.5*</td>
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<tr>
<td>Postprandial</td>
<td>0.28 ± 0.05</td>
<td>0.28 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>0.21 ± 0.03*</td>
<td>0.21 ± 0.02</td>
<td>0.19 ± 0.05*</td>
<td>0.20 ± 0.04</td>
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Values are means ± SE. TG, triglyceride; Px, parasympathectomy; Sx, sympathectomy; Tx, total liver denervation. Food intake and fasted and baseline postprandial (t = 0) concentrations of blood glucose, plasma TGs, and plasma insulin are presented for both acute meal experiments. No significant differences between groups were observed in amount of food consumed during the 20-min meal in either experiment. The overall repeated-measures analysis of the 4 groups, including the effect of the meal and the denervation on plasma concentrations of TG, glucose, and insulin, showed a significant effect of the meal on all 3 parameters (post hoc paired-sample t-test: *P < 0.05) but no effect of the denervation or interaction effect.

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Fig. 1. Postprandial triglyceride, glucose, and insulin curves during the basal experiment. A–C: postprandial plasma triglyceride curves of control (●) compared with parasympathectomy (Px) (○, A), sympathectomy (Sx) (∆, B), and total liver denervation rats (Tx) (□, C). For clarity, the denervation groups are compared with the control group in separate graphs, plotted as the delta from the first postprandial sample (t = 0). The overall repeated-measures analysis of the 4 groups showed significant effects of time (P < 0.001) and denervation (P = 0.046) on plasma triglycerides. The interaction effect of time × denervation just missed significance (P = 0.062). Post hoc analysis revealed a significant difference between the Px and control groups (P = 0.008; A) as well as between the Sx and control groups (P = 0.045; B). In all groups but the Tx group, plasma triglyceride concentrations changed significantly over time (control, P = 0.019; Px, P < 0.001, Sx, P = 0.020; Tx, P = 0.254; *P < 0.05 compared with t = 0). When comparing the denervation groups at the different time points, only t = 40 min shows a significant difference (1-way ANOVA, P = 0.043), with a significant difference between the control and Px group (#P = 0.006). D: postprandial glucose curves of control (●), Px (○), Sx (∆), and Tx (□) rats plotted as the delta from the first postprandial sample (t = 0). The overall repeated-measures analysis of the 4 groups showed that glucose remained stable throughout the experiment, with no significant effects of time (P = 0.353), denervation (P = 0.858), or time × denervation (P = 0.756). E: postprandial insulin curves of control (●), Px (○), Sx (∆), and Tx (□) rats plotted as the delta from the first postprandial sample (t = 0). The overall repeated-measure analysis showed that postprandial insulin decreased significantly over time, with no significant differences between the groups (time, P < 0.001; denervation, P = 0.645; time × denervation, P = 0.939). Values are means ± SE.
Fig. 2. Plasma triglycerides during VLDL-triglyceride secretion experiment. A–C: plasma triglyceride accumulation after blockade of the triacylglycerol hydrolase activity of lipoprotein lipase in control (•) vs. Px (C: A), Sx (C: B), and Tx rats (C: C). For clarity, the denervation groups are compared with the control group in separate graphs. The overall repeated-measures analysis of the 4 groups showed a significant effect of time (P < 0.001) and a significant interaction effect (time × denervation, P = 0.039). No significant effect of denervation was found (P = 0.244). In a post hoc analysis, only the difference between the para-sympathetic denervated and the control group approached significance (P = 0.066). Values are means ± SE.

(P = 0.008; Fig. 1A) and sympathetically denervated rats (P = 0.045; Fig. 1B) were significantly different from that of the control group. However, the postprandial curve of rats with a total denervation showed no significant difference compared with the control group (P = 0.312; Fig. 1C). We performed gel electrophoresis on the samples at t = 0, t = 40, and t = 80 min to separate chylomicrons and VLDL particles and check whether chylomicrons could contribute to this effect. In the t = 40-min sample, no significant amounts of chylomicrons were detected. After 80 min a small increase was observed, but this was only a fraction of the chylomicrons found in an ad libitum-fed sample. Postprandial blood glucose concentrations showed no significant increase and were not different between the four groups (Fig. 1D). Plasma insulin concentrations were measured at three time points after the meal, starting at t = 0 min. Although a significant postprandial decrease at t = 40 and t = 80 min was observed, no significant differences between the groups were observed (Fig. 1E).

VLDL-TG secretion experiment. In a second experiment, the hepatic VLDL-TG secretion was determined after the meal by blocking the clearance of TG in peripheral tissues using tyloxapol. In Fig. 2, the absolute TG concentrations from the first postprandial sample (t = 0) and the subsequent samples after injection of tyloxapol are shown. TG accumulation in plasma differed significantly between groups, as indicated by the significant interaction effect of time × denervation (P = 0.039; Fig. 2, A–C). Post hoc testing showed that, compared with the control group, only the increased VLDL-TG secretion in the parasympathetically denervated rats approached significance (P = 0.066; Fig. 2A). At the end of the experiment the rats were euthanized, and the stomach was removed and weighed, including its content, to get an indication of whether passage of food was changed by the denervation. The overall wet weight of the stomach, including content, was 5.54 ± 0.32 g (means ± SE), with no significant differences between the groups (1-way ANOVA, P = 0.715).

Liver analysis acute meal experiments. Noradrenaline concentration in liver tissue was measured to ensure that the denervation was successful. Two sympathetically denervated rats were excluded from the analysis for the basal and VLDL-TG secretion experiment based on a noradrenaline concentration of >10% of control noradrenaline liver concentration (86 ± 7 ng/g; means ± SE). For all other Sx and Tx liver samples, noradrenaline values were <10% of control noradrenaline concentration (Fig. 3A). The TG concentration of the livers was not different between the groups (Fig. 3B). We performed RT-PCR analysis on the liver tissue to investigate possible pathways in liver lipid metabolism controlled by liver innervation. In a separate set of animals, we first investigated the genes that were changed by the meal by comparing four animals that received a meal in the same experimental setup as above and four animals that did not receive the meal but continued the overnight fast. Rats were euthanized ∼3 h after the start of the meal. We measured the expression of acetyl-coenzyme A carboxylase-α (Acc1), acetyl-coenzyme A carboxylase-β (Acc2), apolipoprotein B (ApoB), ADP ribosylation factor (Arf-1), carnitine palmitoyltransferase 1α (Cpt1α), fatty acid synthase (Fas), microsomal triglyceride transfer protein (Mttp), stearoyl-coenzyme A desaturase 1 (Scd1), sterol regulatory element-binding transcription factor-1c (Srebp1c), and peroxisome proliferator-activated receptor-γ (Pparg). Liver gene expression data showed that the meal increased mainly expression of genes involved in lipogenesis (Fas, P = 0.019; Srebp1c, P = 0.021; Pparg, P = 0.010). Subsequently,
we measured gene expression in the denervation groups after the meal to determine whether these genes are in part regulated by innervation, but we found no significant differences in gene expression between the denervation groups.

Six-meals-a-day feeding schedule. Finally, we investigated the potential physiological relevance of the autonomic nervous system for hepatic lipid metabolism in a chronic experiment by challenging the rats with a six-meals-a-day schedule that consisted of 1 meal every 4 h for ≥4 wk. This model ensures that the rat is maintained in the postprandial state during the entire 24-h cycle. Previous experiments have shown that this model is very suitable to standardize the meals for all rats over the 24-h cycle concerning the timing of meals and amount of chow consumed. All rats readily adapted to the feeding schedule within a couple of days, as evidenced by a continued increase in their body weight, and consumed ~3.5 g during every single meal, comparable with the previous experiments. No significant differences in meal size between time points or groups were detected. After 4 wk, blood samples were taken every hour for 12 consecutive hours on two different occasions to measure plasma TG. All groups showed a significant effect of time on plasma TG during the total 24-h sampling period (Fig. 4A). However, parasympathetically denervated rats had significantly higher plasma TG levels during the day-night cycle compared with control (P = 0.001) and sympathetically denervated rats (P = 0.005) (Fig. 4A). Twenty-four-hour plasma TG profiles of sympathetically denervated rats were not significantly different from those of control rats. The average concentration of TG during the entire 24-h sampling period was also increased for parasympathetically denervated rats (Px: 3.46 ± 0.36 mmol/l; Sx: 2.20 ± 0.09 mmol/l; control: 2.01 ± 0.13 mmol/l; means ± SE). Further analysis of the difference between the premeal samples and the first sample taken 30 min after the meal revealed again that sympathetic and parasympathetic denervation tended to increase the meal-induced rise in plasma TG (Fig. 4B). However, in these non-fasting-induced feeding conditions the effect of the parasympathetic denervation reached significance only when meals were consumed during the normal time of feeding, i.e., the dark phase (Fig. 4C).

**DISCUSSION**

This study revealed a physiologically relevant role for the parasympathetic nervous innervation of the liver in lipid metabolism after a meal. Denervation of the parasympathetic nerves innervating the liver resulted in higher postprandial plasma TG responses, indicating that after a meal the parasympathetic nervous system is necessary to inhibit the release of plasma TGs from the liver. Since this effect is rather small compared with the initial decrease of plasma TG levels during the meal (Table 1), we postulate that the autonomic nervous system has a role in fine-tuning postprandial liver lipid metabolism as opposed to the more robust and direct effects of hormones and nutrients. However, when challenged chronically with a six-meals-a-day feeding schedule, these subtle effects became more apparent, and the disinhibitory effect of the parasympathetic denervation resulted in higher plasma TG levels during the entire day. These data complement our previous findings in which we showed that during fasting the sympathetic nervous system is necessary to stimulate TG secretion, whereas the parasympathetic nervous system did not play a role (6). Together, these data indicate that the traditional concept, i.e., that parasympathetic nerves favor fuel storage postprandially and sympathetic nerves stimulate fuel availability during fasting, also holds true for hepatic lipid metabolism.

In the current experiments, a sympathetic hepatic denervation resulted in a small but significant increase in plasma TGs after a meal. We believe that feeding shifts the autonomic balance toward both increased parasympathetic activity and decreased sympathetic activity. Because of the opposing forces, losing either the parasympathetic or sympathetic innervation yields similar results compared with the control condition. Denervation of the sympathetic hepatic nerve will result in a lesser inhibition of TG secretion after a meal, with a resulting tendency to increase plasma TG. However, the less pronounced effects and the absence of an effect in the six-meals-a-day feeding schedule suggests that there is no major role for the sympathetic nervous system after feeding. Interestingly, the disinhibitory effects of the sympathetic and parasympathetic denervations were lost in rats with a total dener-
vation of the liver. Previous studies have also indicated that the sympathetic and parasympathetic nervous system work together to cause an effect on the liver. Gardemann and Jungermann (13) showed that the effects of parasympathetic stimulation on glucose metabolism occurred only during concurrent α- and β-adrenergic blockade in perfused rat liver. In a previous experiment, we also observed that the effects of a parasympathetic or sympathetic denervation, i.e., a loss of the daily glucose rhythm, were not observed in animals with a total denervation (10). Together, these observations lead us to propose that it is not the absence but rather the disbalance of the autonomic nervous system that induces an abnormal metabolism in the liver. This is in concordance with the observation that no major metabolic abnormalities occur after liver transplantation. When the liver has no autonomic innervation, it responds mainly to important signals from the periphery such as insulin and free fatty acids.

By using a surgical technique that enabled us to selectively cut the autonomic nerves innervating the liver, we can conclude that the changes observed are most likely due to a hepatic mechanism. Because the ingested chow also contains 6% fat (soybean oil), the possibility that the effects observed were caused by a change in the uptake of TG from the gut as a consequence of the surgery could not be ruled out. However, based on the following arguments, we think an indirect effect via the intestinal system is highly unlikely. First of all, the uptake of dietary lipids and the subsequent formation of chylomicrons is a time-consuming process compared with glucose uptake. Based on the literature, we have estimated the contribution of TGs packed in chylomicrons to total plasma TGs to be negligible within the first 80 min after consumption of chow (1, 2, 4). With gel electrophoresis we confirmed that after 80 min only a minor fraction of lipids is packed in chylomicrons, whereas the effects of the denervation were already apparent after 40 min. However, for the VLDL-TG secretion experiment, we had to take samples over a longer period of time (160 min) to be able to estimate VLDL-TG secretion rate. Therefore, in these experiments we cannot exclude a contribution of chylomicrons after 80 min. Because the increase of TG remained linear during the experiment, no major increases in chylomicrons during these 160 min are to be expected. Because of the presence of tyloxapol in the samples, this could not be confirmed by gel electrophoresis. We have no reason to believe that the uptake of TGs between the denervation groups was different. The rats were fasted overnight to ensure that no chylomicrons were present in plasma at the start of the experiment and that all rats were motivated to eat. There were no differences in food intake or wet weight of the stomach between the groups, which was indicative of similar gastric motility after denervation. Therefore, it is highly unlikely that our results are based on a differential intestinal uptake of TG between the groups. Nevertheless, it would be interesting to confirm the origin of TGs after a meal with tracer methods.

In the fed state, the liver and adipose tissue are set to store the newly available nutrients. High levels of insulin acutely inhibit VLDL-TG secretion in humans and human hepatocytes in culture (8, 20). Bülow et al. (8) have measured VLDL-TG secretion across the splanchnic bed in humans receiving a glucose meal and during a hyperinsulinemic euglycemic clamp and concluded that only the latter inhibits VLDL-TG secretion. It seems that high concentrations of glucose in turn stimulate VLDL-TG secretion. In our study, using a mixed meal, glucose levels do not peak but remain stable during the experiment. In the control rats, we do find that plasma TGs decrease after feeding. In our denervated rats, a smaller decrease in TGs is observed, from which we conclude that the autonomic nerves also act to inhibit TG secretion after a meal. We have sought to elucidate the mechanism via which the autonomic nerves exert their fine-tuning on lipid metabolism by measuring VLDL-TG secretion, liver TG concentrations, and expression of various genes involved in hepatic lipid metabolism. We indeed found a trend toward an increase in VLDL-TG secretion in the parasympathetic denervated group. No effects on liver TG were observed, although this study could be underpowered to show the expected small differences when taking into account the variation within the groups. Although we did observe the known effects of feeding on the expression of lipogenic genes, we found no effect of a denervation on the expression level of these genes. Perhaps the major effects of nutrients and hormones on the liver after a meal mask the more subtle effects of a denervation. Next to a direct effect of the autonomic nervous system on lipid metabolism, it is possible that parasympathetic activity increases insulin sensitivity of the liver. We did not find an effect of a parasympathetic denervation on plasma concentrations of glucose or insulin, but previous studies using several methods to measure insulin sensitivity found decreased insulin sensitivity after parasympathetic denervation (19, 22, 37, 38). Despite stimulating hepatic fatty acid and TG synthesis, which are substrates for VLDL-TG secretion, acute elevations of insulin are known to inhibit VLDL-TG secretion (20). By affecting insulin sensitivity, parasympathetic activity could enhance this insulin-induced inhibition of VLDL-TG secretion, and a denervation would then cause the opposite effect. Alternatively, the parasympathetic nervous system may regulate lipid metabolism by other mechanisms, e.g., by altering hepatic blood flow. Clearly, further studies are necessary to investigate how the parasympathetic nervous system fine-tunes lipid metabolism after a meal.

Next to the mechanism within the liver, several previous studies have provided evidence for peripheral signals that could activate the parasympathetic nervous system innervating the liver. Van den Hoek et al. (34) showed that the effect of peripheral hyperinsulinemia on VLDL-TG secretion is prevented by intracerebroventricular (icv) infusion of NPY, suggesting that insulin could possibly act via both a peripheral and central route to inhibit VLDL-TG secretion. In addition to insulin, Lam et al. (18) showed that also an icv infusion of glucose inhibits VLDL-TG secretion. This effect was dependent on an intact vagal nerve, thereby supporting the results from our experiments; i.e., vagal nerve activity inhibits VLDL-TG secretion. In addition and in line with our previous results, they found no effects of a selective parasympathetic denervation in fasted rats, emphasizing that a meal or icv glucose should be given to observe an effect of a parasympathetic denervation.

Finally, the results presented here are of interest for human pathophysiology. Similar to our rats under a six-meals-a-day schedule, many humans spend the entire day and most of the night in the postprandial state (36). Despite this, most studies and clinical guidelines of cardiovascular risk to date have focused on fasting conditions. Interestingly, a few studies have demonstrated that the postprandial TG level is a better inde-
ependent predictor than the fasting TG level for assessing present and future cardiovascular disease events (3, 24). Therefore, the need for routine screening tests of postprandial TG in humans is acknowledged using either nonfasting levels or TGs measured after an oral TG tolerance test (5, 26). In addition, a small number of studies have addressed the possible relations between autonomic nervous activity and the metabolic syndrome in humans. Interestingly, Licht et al. (21) showed that decreased parasympathetic activity correlates with higher TG levels in patients with the metabolic syndrome. Although their study has been performed with cardiac measurements of autonomic activity, and no causal effect could be shown, it is interesting to note that these results are in accord with the data found in our study. One may speculate whether stimulating vagal nerve activity would induce beneficial changes in the lipid spectrum in these patients. Several animal studies found associations between decreased body weight gain and vagal nerve stimulation, although small retrospective studies in humans with a vagal nerve stimulator for the treatment of epilepsy are inconclusive on this matter (9, 16, 29, 33). Larger prospective studies, including different components of the metabolic syndrome, are necessary to show that this could indeed be a novel treatment, as hypothesized previously (11).

ACKNOWLEDGMENTS

We thank Mariem Bouali and Marja Neelaman from the Department of Clinical Chemistry, Laboratory of Endocrinology, Academic Medical Center, University of Amsterdam, for technical assistance. Part of the data has been presented as an abstract at ENDO 2012 (OR22-2).

GRANTS

This work is supported by NWO-ZonMw (TOP 91207036). P. C. N. Rensen is an Established Investigator of the Netherlands Heart Foundation (2007B081, 2009T138).

DISCLOSURES

There are no conflicts of interest, financial or otherwise, for any of the authors.

AUTHOR CONTRIBUTIONS


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