Neural control of hepatic lipid metabolism: A (patho)physiological perspective

Bruinstroop, E.

Citation for published version (APA):
THE AUTONOMIC NERVOUS SYSTEM REGULATES POSTPRANDIAL HEPATIC LIPID METABOLISM

Eveline Bruinstroop, Susanne la Fleur, Mariette T. Ackermans, Ewout Foppen, Joke Wortel, Sander Kooijman, Jimmy F.P. Berbée, Patrick C.N. Rensen, Eric Fliers & Andries Kalsbeek

ABSTRACT
The liver is a key organ in controlling glucose and lipid metabolism during feeding and fasting. In addition to hormones and nutrients, also inputs from the autonomic nervous system are involved in fine tuning hepatic metabolic regulation. We have previously 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 surgical hepatic denervations on triglyceride metabolism after a meal in male Wistar rats. We report that postprandial plasma triglyceride concentrations were significantly elevated in parasympathetically denervated rats as compared to 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.
INTRODUCTION

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, the liver increases fuel availability for immediate use. These processes are controlled by circulating hormones, such as insulin, and by the availability of different nutrients. It has long been known that also the autonomic nervous input to the liver can alter hepatic function, especially glucose metabolism (27,28). Next to being a key player in glucose metabolism, the liver is also important for the regulation of lipid metabolism, as it synthesizes and degrades lipids. Recently, various investigators have revealed a role for the autonomic nervous system in the regulation of hepatic lipid metabolism as well (6,18,32).

The autonomic nervous system consists of a sympathetic and a parasympathetic branch. Tracing studies have shown that both branches are anatomically connected to the liver and originate from distinct neuronal populations within the hypothalamus (7,17,35). The two autonomic branches are hypothesized to have opposing or complimentary effects on target organs, which has indeed been shown for hepatic glucose metabolism. Studies involving electrical stimulation of the hepatic nerves in vivo or pharmacological interventions in perfused livers show that sympathetic hepatic activation increases hepatic glucose output by stimulating glycogen breakdown, while parasympathetic hepatic activation decreases blood glucose levels by promoting glycogen synthesis (25). Nevertheless, the role of hepatic autonomic nerves under physiological conditions has often been debated as no gross abnormality of metabolic homeostasis was observed after interruption of the nervous supply to the liver. However, effects of a selective parasympathetic denervation on hepatic glucose production, glycogen synthesis and hepatic insulin sensitivity were reported pointing to a possible fast route to fine-tune metabolism in addition to the direct regulation by nutrients and hormones (19,22,37,38). Interestingly, the effects of parasympathetic hepatic denervation were most obvious in the postprandial state, indicating that feeding activates the parasympathetic nervous system. This is in line with earlier studies from Niijima (23) who showed that, after intravenous administration of D-glucose at levels observed postprandially, the firing rate in efferent vagal nerves innervating the liver was increased.

Previously, we showed that the sympathetic nervous input to the liver is necessary to maintain VLDL-triglyceride (VLDL-TG) secretion during fasting, whereas we found no evidence for the involvement of the parasympathetic nervous input (6). We now hypothesized that the parasympathetic nervous system is important in the postprandial state to attenuate VLDL-TG secretion and store TG in the liver. We therefore determined the effect of selective hepatic denervations on TG metabolism acutely after a meal, in a basal and VLDL-TG secretion experiment, and chronically in animals adapted to a six-meals-a-day feeding schedule.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats weighing 280-310 g (Charles River Breeding Laboratories, Sulzfeld, Germany / Harlan Nederland, Horst, The Netherlands) were housed in individual cages...
with a 12 h/12 h light/dark schedule (lights on at 7.00 A.M.). 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 Hypnorm i.m. (Janssen, High Wycombe, Buckinghamshire, UK) and 0.4 ml/kg Dormicum s.c. (Roche, Almere, The Netherlands). After one week 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, eliminating 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 was included in the analysis only if noradrenaline concentration of the liver was below 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 pre-surgery body weight for at least 10 days. The day before the experiment the food was removed at 5.00 P.M. and the rats were attached to a metal collar for adaptation. The next day at 9.00 A.M. 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 the animals. At 1.00 P.M. a 20 hour 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, 8 rats that did not eat between 2.0-4.5 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 15% tyloxapol (Sigma-Aldrich, Germany) directly after the baseline postprandial sample (t = 0), which immediately inhibits lipoprotein lipase (LPL)-mediated TG hydrolysis. After 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 ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22 (ZT0, *i.e.*, Zeitgeber Time = 0, being defined as the “lights on” time point (07.00 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 two weeks to adapt to the feeding schedule and subsequently underwent surgery. A sympathetic 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 two weeks of recovery, 0.2 ml of blood once every hour during 12 consecutive hours was taken on two different occasions, starting at either ZT 6.5 or ZT 18.5. Between both 12hr sampling experiments the rats were allowed to recover for at least ten 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 radioimmunoassay kit, plasma insulin (LINCO Research, St. Charles, MO, USA) 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, Germany) and TG were measured using the “Trig/GB” kit (Roche, Mannheim, Germany).

**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, also livers from a control experiment were included to determine which 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 was 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), nor by the different denervations (*p* = 0.516).

**Statistical analysis**

Data are presented as means ± SEM. 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 comparing single outcome measures or separate time points.
between the groups. A significant ($p \leq 0.05$) 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 to 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 triglyceride concentrations in the different denervation groups, all animals consumed on average 3.55 ± 0.14 g of chow (mean ± SEM) 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 (mean ± SEM) 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

<table>
<thead>
<tr>
<th>Table 1. The effects of the meal in the basal and VLDL-TG secretion experiment.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal experiment</strong></td>
</tr>
<tr>
<td><strong>Food intake (g)</strong></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>VLDL-TG secretion experiment</strong></td>
</tr>
<tr>
<td><strong>Glucose (mmol/l)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/l)</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Food intake and fasted and baseline postprandial (t = 0) concentrations of blood glucose, plasma triglycerides and plasma insulin are presented for both acute meal experiments. No significant differences between groups were observed in amount of food consumed during the twenty-minute meal in both experiments. The overall repeated measures analysis of the 4 groups including the effect of the Meal and the Denervation on plasma concentrations of triglycerides, glucose and insulin, showed a significant effect of the Meal on all three parameters (Post-hoc paired samples T-test: * $p < 0.05$), but no effect of the Denervation or Interaction effect. Values are means ± SEM.
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 to fasted levels in all groups, but no significant
differences between groups were observed. In the 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 triglycerides 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 (Figure 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 (Figure 1 A-C). Subsequently, a slow and steady
increase was observed. In both the parasympathetically (Figure 1A) and sympathetically
denervated rats (Figure 1B), we observed a significant postprandial rise in plasma TG
levels reaching significance at t = 40 min and t = 60 min, respectively. When comparing all
postprandial curves, both the curves of the parasympathetically (Figure 1A; p = 0.008) and
the sympathetically (Figure 1B; p = 0.045) denervated rats were significantly different from
that of the control group. However, the postprandial curve of rats with a total denervation
showed no significant difference compared to the control group (Figure 1C; p = 0.312). We
performed gel electrophoresis on the samples at t = 0 min, t = 40 min 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 (Figure 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 min and t = 80 min was observed, no
significant differences between the groups were observed (Figure 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 Figure 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 significantly differed
between groups as indicated by the significant interaction effect of *Time * Denervation
(p = 0.039; Figure 2A-C). Post-hoc testing showed that, compared to the control group, only
the increased VLDL-TG secretion in the parasympathetically denervated rats approached
significance (Figure 2A; p = 0.066). At the end of the experiment the rats were euthanized
and the stomach was removed and weighed including its content to get an indication
whether passage of food was changed by the denervation. The overall wet weight of the
stomach including content was 5.54 ± 0.32 g (Mean ± SEM) with no significant differences between the groups (one-way ANOVA, p = 0.715).

Figure 1. Postprandial triglyceride, glucose and insulin curves during the basal experiment.
A, B, C: Postprandial plasma triglyceride curves of control (closed squares) compared to Px (A; open circles), Sx (B; open triangles) and Tx (C; open squares) rats. For clarity, the denervation groups are compared with the control group in separate figures, 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 (A; p = 0.008) as well as between the Sx and control group (B; p = 0.045). In all groups but the Tx group plasma triglyceride concentrations significantly changed over time (Control, p = 0.019; Px, p < 0.001, Sx p = 0.020, Tx p = 0.254; * p < 0.05 compared to t = 0). When comparing the denervation groups at the different time points only t = 40 min shows a significant difference (one-way ANOVA, p = 0.043) with a significant difference between the control and Tx group (# p = 0.006).

D: Postprandial glucose curves of control (closed squares), Px (open circles), Sx (open triangles) and Tx (open squares) 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 (closed squares), Px (open circles), Sx (open triangles) and Tx (open squares) rats, plotted as the delta from the first postprandial sample (t = 0). The overall repeated measurement 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 ± SEM.
Figure 2. Plasma triglycerides during VLDL-TG secretion experiment. A, B, C: Plasma triglyceride accumulation after blockade of the triacylglycerol hydrolase activity of LPL in control (closed squares) versus Px (A; open circles), Sx (B; open triangles) and Tx (C; open squares) rats. For clarity, the denervation groups are compared with the control group in separate figures. 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 parasympathetic denervated and the control group approached significance (p = 0.066). Values are means ± SEM.

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; mean ± SEM). For all other Sx and Tx liver samples noradrenaline values were below 10% of control noradrenaline concentration (Figure 3A). The TG concentration of the livers was not different between the groups (Figure 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 4 animals that received a meal in the same experimental setup as above and 4 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 alpha (Acc1), acetyl-coenzyme A carboxylase beta (Acc2), apolipoprotein B (ApoB), ADP-ribosylation factor (Arf-1), carnitine palmitoyltransferase 1 alpha (Cpt1a), 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 gamma (Pparg). Liver gene expression data showed that the meal mainly increased 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 at least 4 weeks. This model ensures the rat is maintained in the postprandial state during the entire 24h cycle. Previous experiments have shown that this model is very suitable to standardize the meals for all rats over the 24h 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 of their body weight, and consumed approximately 3.5 g during every single meal, comparable to the previous experiments. No significant differences in meal size between time points or groups were detected. After 4 weeks, blood samples were taken every hour during 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 24h sampling period (Figure 4A). However, parasympathetically denervated rats had significantly higher plasma TG levels during the day/night cycle as compared to control (p = 0.001) and sympathetically denervated rats (p = 0.005) (Figure 4A). 24h plasma TG profiles of sympathetically denervated rats were not significantly different from those of control rats. The average concentration of TG during the entire 24h 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; mean ± SEM). Further analysis of the difference between the pre-meal samples and the first sample taken 30 min after the meal revealed again that sympathetic and parasympathetic denervation tended to increase the meal-induced raise in plasma TG (Figure 4B). However, in these non-fasting-induced feeding conditions the effect of the parasympathetic denervation only reached significance when meals were consumed during the normal time of feeding, i.e., the dark phase (Figure 4C).
Figure 4. Plasma triglycerides in the six-meals-a-day feeding schedule. A: 24h plasma triglyceride profile of control (closed squares), Px (open circles) and Sx (open triangles) rats on a six-meals-a-day feeding schedule (ZT0, i.e., Zeitgeber Time = 0, being defined as the "lights on" time point (07.00 AM)). The meals are indicated by the dotted lines. There was a significant difference between the groups (Time \( p < 0.001 \); Denervation \( p = 0.002 \); Time*Denervation \( p < 0.001 \)). Post-hoc analysis revealed that parasympathetic denervated rats had higher plasma triglyceride levels over the 24h light/dark-cycle as compared to control rats (\( p = 0.001 \)) and sympathetically denervated rats (\( p = 0.005 \)). All separate time points were analyzed by one-way ANOVA with post-hoc LSD between all groups (* \( p < 0.05 \) compared to control and Sx; # \( p < 0.05 \) compared to control; § \( p < 0.05 \) compared to Sx). B,C: Mean triglyceride increments to a meal in control, Px and Sx rats expressed as the difference between the first sample point after the meal and the last sample point before the meal in the six-meals-a-day experiment. The results are shown for all meals (B) and only the meals consumed during the dark phase (C). Only in the dark phase a significant difference was observed between the groups (one-way ANOVA, \( p = 0.033 \); * \( p < 0.05 \)) with a significant difference between the Control and Px rats. Values are means ± SEM.
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 TG from the liver. Since this effect is rather small when compared to 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 the 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 of plasma TG after a meal. We believe that feeding shifts the autonomic balance towards both increased parasympathetic activity and decreased sympathetic activity. Due to the opposing forces, losing either the parasympathetic or sympathetic innervation yields similar results as compared to 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 denervation 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 only occurred during concurrent alpha and beta-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, was 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. As 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 to glucose uptake. Based on literature, we have estimated the contribution of TG packed in chylomicrons to total plasma TG to be negligible within the first 80 min after consumption of chow (1,2,4). With gel electrophoresis we confirmed that only after 80 min 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. As the increase of TG remained linear during the experiment, no major increases of chylomicrons during these 160 min are to be expected. Due to 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 TG 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, 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 TG 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 triglycerides decrease after feeding. In our denervated rats a smaller decrease of triglycerides is observed, from which we conclude that the autonomic nerves also act to inhibit triglyceride 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 towards 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 after a meal on the liver mask the more subtle effects of a denervation. Next to a direct effect of the autonomic nervous system on lipid metabolism, it is well 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 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 both via a peripheral and a 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 nervous 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 in order 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). In spite of 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 independent predictor than the
fasting TG level for assessing present and future CVD events (3,24). Therefore, the need for
routine screening tests of postprandial TG in humans is acknowledged using either nonfasting
levels or triglycerides measured after an oral triglyceride 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 measures of
autonomic activity, and no causal effect could be shown, it is interesting to note that these
results are in accordance 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 previously hypothesized (11).
ACKNOWLEDGMENTS

This work is supported by NWO-ZonMw (TOP 91207036). PCN Rensen is Established Investigator of the Netherlands Heart Foundation (2007B081; 2009T038). There is no conflict of interest for any of the authors.

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

REFERENCES


15. Klieverik LP, Sauerwein HP, Ackermans MT, Boelen A, Kalsbeek A and Fliers E. Effects of thyrotoxicosis and selective hepatic autonomic denervation on hepatic glucose


