



Sympathetic Denervation of the Common Hepatic Artery Lessens Glucose Intolerance in the Fat- and Fructose-Fed Dog

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This study assessed the effectiveness of surgical sympathetic denervation of the common hepatic artery (CHADN) in improving glucose tolerance. CHADN eliminated norepinephrine content in the liver and partially decreased it in the pancreas and the upper gut. We assessed oral glucose tolerance at baseline and after 4 weeks of high-fat high-fructose (HFHF) feeding. Dogs were then randomized to sham surgery (SHAM) ($n = 9$) or CHADN surgery ($n = 11$) and retested 2.5 or 3.5 weeks later while still on the HFHF diet. CHADN improved glucose tolerance by ~60% in part because of enhanced insulin secretion, as indicated by an increase in the insulinogenic index. In a subset of dogs (SHAM, $n = 5$; CHADN, $n = 6$), a hyperinsulinemic-hyperglycemic clamp was used to assess whether CHADN could improve hepatic glucose metabolism independent of a change in insulin release. CHADN reduced the diet-induced defect in net hepatic glucose balance by 37%. In another subset of dogs (SHAM, $n = 4$; CHADN, $n = 5$) the HFHF diet was continued for 3 months postsurgery and the improvement in glucose tolerance caused by CHADN continued. In conclusion, CHADN has the potential to enhance postprandial glucose clearance in states of diet-induced glucose intolerance.

The autonomic nervous system helps maintain energy homeostasis by connecting effector organs to the brain through sympathetic and parasympathetic nerves. As a result, the autonomic nervous system is involved in regulating hepatic glucose production (HGP) and uptake, which was demonstrated as early as 1965 when Shimazu and

Fukuda (1) established that stimulation of the splanchnic nerve induced an activation of the glycogenolytic machinery, part of the “fight and flight” reaction. The presence of parasympathetic and sympathetic nerve connections between the hypothalamus and the liver has been further elucidated by numerous studies (1–4). Afferent signals from the splanchnic bed to the brain are essential for the central nervous system (CNS) to be able to regulate the transition between the fasted and fed state (2). Likewise, efferent signals from the brain to the liver impact hepatic glucose metabolism. At the same time, however, total denervation of the liver has been proven to have only minor effects on the maintenance of normal liver function as seen after liver transplantation (5). It is likely that the consequences of the interruption of both sympathetic and parasympathetic signaling are to some extent offsetting (6).

Our previous work described the effects of hepatic sympathetic denervation, by targeting the common hepatic artery (CHA) as the exclusive conduit of sympathetic nerves to the liver. These studies assessed hepatic glucose metabolism and concluded that the sympathetic nervous system exerts a restraining effect on hepatic glucose uptake (HGU) that can be reversed by the entry of glucose into the portal vein (2). In another study (7), we showed that cooling of the vagus nerve reflexively decreased efferent sympathetic outflow to the liver causing a decrease in fasting HGP. Anatomical studies in humans, as well as multiple other species, have confirmed that the nerves surrounding the CHA are sympathetic in nature and not parasympathetic. The sympathetic nerves branch from the

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sympathetic chain and follow the arteries, surrounding the common hepatic and proper hepatic arteries; in contrast, the parasympathetic nerves are anatomically distinct, emanating from the vagus nerve and surrounding the biliary system and portal vein, rather than the CHA (8,9).

The effect of a Western diet on insulin sensitivity has been described in numerous models and is clearly associated with type 2 diabetes (10,11). Chronic hyperglycemia is associated with the inability of the liver to suppress glucose production and is characteristic of an overall impaired ability to switch from the fasted state to the fed state (12,13). The consumption of a high-fat high-fructose (HFHF) diet has adverse effects on the regulation of whole-body metabolism *in vivo*. Our laboratory (among others) has characterized the effect of an HFHF diet, approximating a Western diet, on liver metabolism in the dog. It was demonstrated that an HFHF diet led to an inability of the liver to switch from a normal production of glucose during fasting to an uptake of glucose during meal feeding (simulated by a hyperinsulinemic-hyperglycemic [HIHG] clamp) (14–16). Elevated sympathetic nerve activity has been hypothesized to be the cornerstone linking obesity and the metabolic syndrome (17,18), but it is not clear whether sympathetic nerve overactivity is the consequence or the cause of metabolic abnormalities. Indeed, chronic elevation of plasma insulin or leptin has been hypothesized to induce a sympathoexcitatory response (19–21). Pharmaceutical and surgical approaches have targeted this sympathetic nervous system overactivity to combat the metabolic

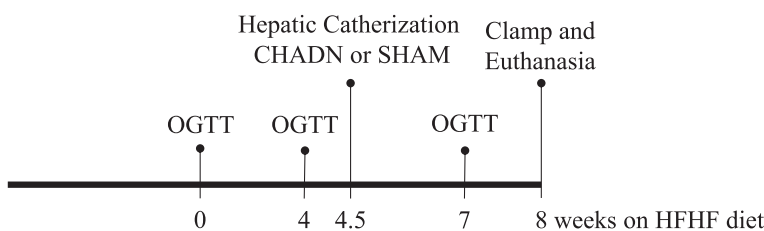
syndrome. Studies using device-based or surgical procedures to reduce tone in renal nerves have been encouraging as a means to treat drug-resistant hypertension (22–24). Renal denervation has also been shown to improve glucose metabolism, with a proposed mechanism involving increased blood flow and glucose uptake in skeletal muscle (25), an elevation of glycosuria (26), or an impact on liver glucose metabolism (27). Studies to date, however, have not focused on denervation of the liver as a therapeutic tool. Our data suggest that targeting the liver with selective sympathetic denervation may provide a more direct and effective approach to improve postprandial glucose metabolism in glucose-intolerant individuals.

As a result, we hypothesized that hepatic sympathetic denervation in the glucose-intolerant dog would reduce sympathetic nerve activity to the liver resulting from HFHF consumption and thus improve hepatic glucose metabolism leading to improved whole-body glucose metabolism. To test this hypothesis, we examined the ability of surgical sympathetic denervation of the CHA (CHADN) to improve oral glucose tolerance in HFHF-fed dogs. We also carried out an HIHG clamp experiment to directly assess the ability of insulin and glucose to trigger HGU in denervated and sham-denervated dogs.

RESEARCH DESIGN AND METHODS

These studies were conducted using 20 male mongrel dogs weighing between 20 and 25 kg on entry into the study.

Protocol #1: CHADN n=6; SHAM n=5 (No Baseline OGTT for 3 animals)



Protocol #2: CHADN n=5; SHAM n=4 (No Baseline OGTT)

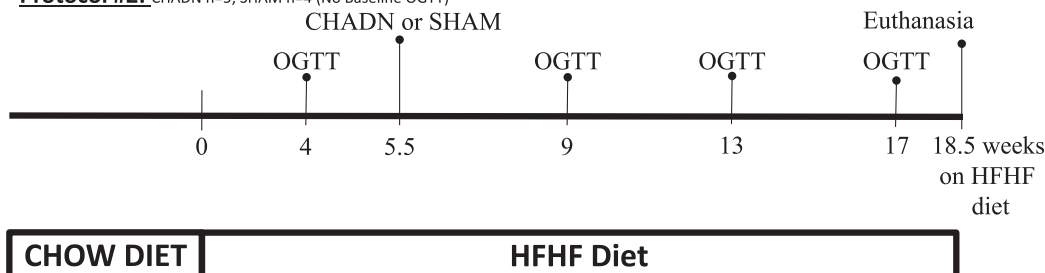


Figure 1—Experimental timeline. Week 0 corresponds to the initiation of experimental diet (HFHF).

The animals were fed a standard chow diet daily for at least 2 weeks prior to being switched to an HFHF diet, as described previously (16). This diet has been shown to rapidly bring about hepatic insulin resistance and reduced glucose effectiveness along with impaired β -cell biology (16,28). The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. Two different protocols were performed as described in Fig. 1.

Experimental Design

Protocol 1

The first protocol included 11 dogs. After an acclimation period of 2 weeks, during which the animals were fed a standard chow diet, oral glucose tolerance tests (OGTTs) were performed on 8 of the 11 dogs (the other three dogs were not given OGTTs) to provide baseline data. All 11 animals were then fed an HFHF diet in which 22% of the energy was derived from protein, 52% from fat, and 26% from carbohydrate, the majority of which (17% of the total energy in the diet) was derived from fructose (Test-Diet; PMI Nutrition, St. Louis, MO). After 4 weeks on this diet, a second OGTT was performed to assess the impact of the HFHF diet on the glucose excursion. The following week, animals underwent a laparotomy for hepatic blood vessel catheterization and were randomly assigned to a surgical CHA sympathectomy group (CHADN, $n = 6$) or a sham surgery group (SHAM, $n = 5$). Briefly, the hepatic catheterization consisted of implanting sampling catheters into the femoral artery, the hepatic portal vein, and a hepatic vein for blood sampling and into a splenic and jejunal vein for infusion, as well as flow probes around the hepatic artery and the hepatic portal vein, as described previously (16). See below for details on the surgical denervation. After an additional 2.5 weeks on the HFHF diet the animals underwent a third OGTT. Finally, a week later, an HIHG clamp was carried out to assess the impact of insulin and glucose on the liver. This test was thus performed 3.5 weeks after denervation and after 8 weeks on the HFHF diet. Each animal was then euthanized for tissue harvest.

Protocol 2

The second protocol included nine dogs with the same characteristics as in Protocol 1. No baseline OGTT was performed before starting the HFHF diet. After 4 weeks of eating the HFHF diet, an OGTT was performed to assess the defect in the glucose excursion induced by the HFHF diet. A laparotomy was performed after 5.5 weeks of eating the diet, and animals were randomly assigned to an hepatic surgical sympathectomized group (CHADN group, $n = 5$) or SHAM group ($n = 4$). Follow-up OGTTs were performed after CHADN or SHAM surgery at 3.5, 7.5, and 11.5 weeks postsurgery. The dogs were euthanized at 13 weeks postsurgery (18.5 weeks on diet) for tissue harvest.

Experiments

OGTT

Following a 10-min basal control period, Polycose (0.9 g/kg body wt; Abbott Nutrition, Columbus, OH) was administered orally, and plasma glucose, insulin, C-peptide, and glucagon concentrations were monitored over the following 180 min. The amount of Polycose administered was determined by the weight of the dog when it started on protocol (including when no baseline OGTT was performed). The baseline OGTTs from eight animals in Protocol 1 were used as a reference for all OGTT data and are representative of previously obtained results in chow-fed control animals (16). Similarly, the OGTT results after 4 weeks of eating an HFHF diet were representative of previous data (16). The OGTTs administered either 2.5 weeks (Protocol 1) or 3.5 weeks (Protocol 2) postsurgery were pooled (CHADN, $n = 11$; SHAM, $n = 9$) since the changes in each protocol were similar at that point.

HIHG

On the day of the study, the catheters and Doppler leads were exteriorized from their subcutaneous pockets using local anesthesia. HIHG clamps consisted of a 100-min equilibration period (-120 to -20 min), a 20-min basal control period (-20 to 0 min), and a 180-min experimental period (0 – 180 min). At -120 min, a priming dose of [3 - 3 H]glucose (38 μ Ci) was given, followed by a constant infusion of [3 - 3 H] glucose (0.38 μ Ci/min). At time 0, a constant infusion of somatostatin (0.8 μ g/kg/min; Bachem) was started in a peripheral vein, and insulin and glucagon were then replaced intraportally at $4\times$ basal (1.2 mU/kg/min) and basal (0.55 ng/kg/min) rates, respectively. At time 0, a variable infusion of 50% dextrose was started in a leg vein to double the hepatic glucose load by clamping the plasma glucose level at ~ 220 mg/dL. Hematocrit and plasma glucose, insulin, and glucagon concentrations as well as blood lactate, alanine, and glycerol concentrations were determined using standard procedures, as previously described (29–31). Hepatic glycogen levels were determined using the amyloglucosidase method described by Keppler and Decker (32). The net hepatic glycogen flux rate was determined as described previously (33). A set of historic control data for chow-fed animals (14) is included in the RESULTS for reference purposes.

Surgical Denervation

The hepatic sympathetic denervation of the CHA was performed by stripping a 5-cm-long area around the CHA and removing the visible nerve fibers encasing the vessel. All nerves surrounding the portal vein and its branches, as well as the vagus nerve, were left intact. Proper denervation was confirmed through the measurement of tissue norepinephrine (NE). At the end of each protocol when the animals were euthanized, ~ 1 – 2 g of tissue was harvested from each lobe of the liver, following which it was freeze clamped and stored at -80°C until subsequent NE analysis. In Protocol 2,

in addition to the liver, the head, body, and tail of the pancreas and a 2-cm segment of the duodenum immediately distal to the major duodenal papilla, as well as a piece of the pylorus, were also harvested and snap frozen in liquid nitrogen for later NE content analysis. Briefly, prior to analysis, 300 mg of tissue was homogenized in a perchloric acid buffer containing glutathione. The homogenate was analyzed for NE by high-performance liquid chromatography with electrochemical detection, as described previously (34).

Animals

During the first week of feeding the HFHF diet ad libitum, the dogs ate ~3,500 kcal/day, and caloric consumption decreased by ~15% after 1 week on the diet. Eventually, food consumption stabilized at ~2,300 kcal/day and was not significantly different between the SHAM and the CHADN groups.

Before the start of the diet, the body weights of animals were 22.8 ± 1.4 and 23.1 ± 1.2 kg, respectively, in the 9 SHAM and 11 CHADN dogs. After 1 week eating the HFHF diet, the animals had gained 1.7 and 1.8 kg, respectively. At the time of the first postsurgery OGTT (2.5 or 3.5 weeks postsurgery, 7–9 weeks of HFHF diet), the weights of the animals were 26.0 ± 1.8 and 26.4 ± 1.6 kg in the SHAM and CHADN groups, respectively. The weight gain was maintained in the nine animals included in Protocol 2 to 19 weeks, and no differences were observed between the groups (25.8 ± 1.9 and 26.1 ± 0.6 kg in the SHAM and CHADN groups, respectively).

During the HIHG clamp, arterial blood pressure and heart rate were monitored during the basal control period by Bridge Amp (ADInstruments, Colorado Springs, CO). The blood pressure (108 ± 4 and 106 ± 4 mmHg) and the heart rate (85 ± 8 and 86 ± 12 beats per minute) were not significantly different in the SHAM and CHADN groups, respectively. The hepatic artery and the portal vein blood

flows were also not significantly different in the SHAM and CHADN groups. The small decrease in portal vein blood flow that occurred when the clamp started can be attributed to the infusion of somatostatin and was observed in both the SHAM and CHADN groups, as shown in Table 4.

Calculations

OGTT

The results from the OGTT led to the calculation of the area under the curve (AUC) for glucose, insulin, and C-peptide. The Δ AUC is the increase in the AUC over the fasting value between 0 and 120 min after the gavage.

The ratio of the incremental insulin concentration to the incremental glucose concentration at the 30-min time point has been termed the insulinogenic index, a parameter that assesses β -cell function from OGTT data (35,36). The AUC data for both glucose and insulin (over 120 min) allowed us to calculate the same ratio for what we believe to be a better indicator of insulin secretion during the OGTT because it does not rely on a discrete difference at one time point but accounts for the entire 2-h response. The results are presented as a scatter point plot comparing this parameter before and after the surgery (SHAM and CHADN) for each animal. If the change in value after the procedure was not different by >1 SD for the parameter in question, it was not considered to have changed in that animal.

HIHG

Net hepatic substrate balances (NHBs) were calculated with the arteriovenous difference method using the formula $NHB = Load_{out} - Load_{in}$, where $Load_{out} = [H] \times HF$ and $Load_{in} = [A] \times AF + [P] \times PF$. [A], [P], and [H] represent substrate concentrations in femoral artery, portal vein, and hepatic vein blood or plasma, respectively, and AF, PF, and HF represent blood or plasma flow (as measured using ultrasonic flow probes) through the

Table 1—Tissue NE content at the end of each protocol in animals that underwent a CHADN or a SHAM procedure

		NE content, ng/g tissue			
Tissue	Lobe	Protocol 1 (3.5 weeks postsurgery)		Protocol 2 (13 weeks postsurgery)	
		SHAM (n = 5)	CHADN (n = 6)	SHAM (n = 4)	CHADN (n = 5)
Liver	Caudate	289 ± 50	$1 \pm 0.2^*$	Not measured	
	Left central	295 ± 70	$1 \pm 0.3^*$	393 ± 75	$7 \pm 4^*$
	Left lateral	180 ± 75	$2 \pm 1.6^*$	316 ± 68	$3 \pm 1^*$
	Left posterior	435 ± 126	$3 \pm 2.5^*$	547 ± 56	$4 \pm 2^*$
	Quadrate	786 ± 88	$17 \pm 8.5^*$	510 ± 148	$26 \pm 14^*$
	Right lateral	522 ± 48	$4 \pm 2.1^*$	658 ± 211	$15 \pm 5^*$
	Right central	129 ± 23	$1 \pm 0.3^*$	373 ± 67	$3 \pm 1^*$
Pancreas	Head	Not measured		496 ± 60	$157 \pm 55^*$
	Body			588 ± 114	$85 \pm 53^*$
	Tail			692 ± 103	756 ± 170
Duodenum		Not measured		243 ± 60	$66 \pm 25^*$
Pylorus		Not measured		284 ± 69	$133 \pm 50^\dagger$

Values are the mean $\pm$ SD in ng/g tissue. Statistical analysis (t test) was performed. *P value <0.05 . † P value between 0.05 and 0.10.

hepatic artery, the portal vein, and the hepatic vein, respectively. With this calculation, positive values reflect net hepatic production and negative values represent net hepatic uptake. Hepatic sinusoidal insulin and glucagon concentrations were calculated as $\text{Loadin} \div \text{HF}$.

Data Analysis

Data were expressed as the mean $\pm$ SD. Individual data and median values were shown after the calculation of ΔAUC , where possible. Two-way ANOVA with a repeated-measures design was used (SigmaStat; Systat Software, Inc., San Jose, CA), with post hoc analysis performed using the Student-Newman-Keuls multiple comparisons model. A P value <0.05 was considered significant.

RESULTS

The extent of sympathetic denervation of the liver at the end of the study (3.5 and 13 weeks postsurgery in Protocols 1 and 2, respectively) can be seen in Table 1. Liver tissue NE content was not different from zero, even up to 13 weeks following surgical denervation, so the overall sympathetic denervation of the liver in the CHADN group can be considered complete. It should be noted that following CHADN in Protocol 2, NE in the pancreas was also measured, and it was found to be decreased by 68% and 86%, respectively, in the head and body, although it did not drop in the tail of the pancreas. Likewise, NE levels in the duodenum and pylorus were reduced by 73% and 53%, respectively. These data indicate that there was substantial denervation of the pancreas and upper gut as well as the liver when the nerves along the CHA were carefully and completely stripped.

Figure 2A and B shows the baseline OGTT data and the pooled data from Protocols 1 and 2 after 4 weeks of an HFHF diet, and the combined 2.5 weeks (Protocol 1) and 3.5 weeks (Protocol 2) postsurgery (7 and 9 weeks of an HFHF diet, respectively). Eight of the animals in Protocol 1 were given an OGTT prior to starting the HFHF diet to provide baseline data. All 11 animals were then fed an HFHF diet for 4 weeks, after which an OGTT was again carried out. The ΔAUC for plasma glucose (0–120 min) increased by 97% and 136% in the SHAM and CHADN dogs, respectively, relative to baseline (Fig. 2A and B and Table 2). The ΔAUC for glucose was markedly (57%, $P = 0.007$) improved by CHADN but was not altered by SHAM denervation ($P = 0.84$) (Fig. 2C and D and Table 2). Nine of 11 dogs showed an improvement in the ΔAUC for glucose following CHADN, 1 showed no change, and 1 showed worsening (Fig. 2C). In the SHAM dogs, the ΔAUC for glucose improved in three, worsened in three, and did not change appreciably in three (Fig. 2D).

The insulin responses (Fig. 3A and B) were greater following 4 weeks of HFHF diet than at baseline in both groups ($P = 0.01$ and 0.004 , respectively, in CHADN and SHAM groups), likely as a result of the increased glycemia. Neither CHADN nor SHAM treatment altered the ΔAUC for insulin seen in response to the oral glucose

load in animals on the HFHF diet ($P = 0.59$ and 0.43 , respectively). The ΔAUC for glucose was significantly reduced following CHADN, and this suggests an improved β -cell response. The ΔAUC for insulin divided by the ΔAUC for glucose (Fig. 3C and D) gives an insulinogenic index, which reflects β -cell function. This index improved in 10 of 11 dogs post-CHADN (average of 0.32 ± 0.2 to 0.48 ± 0.25 , $P = 0.001$) but in only 3 dogs post-SHAM denervation (Fig. 3C and D) (0.40 ± 0.12 to 0.35 ± 0.20 , $P = 0.30$). The plasma C-peptide data (Fig. 3E and F) were consistent with the changes in plasma insulin, indicating that alterations in insulin clearance were not responsible for any of the changes in the plasma insulin concentration. Considering that the circulating plasma glucose level was lower post-CHADN than post-SHAM denervation even though the insulin and C-peptide levels in both groups were equivalently elevated, it seems like the sympathetic nerve resection on the CHA resulted in a new glucose set point for the β -cell, which improved insulin secretion. At the same time, since the circulating insulin concentrations post-CHADN and post-SHAM denervation were similar but the glucose concentration was lower following denervation, it can be concluded that the disposition of glucose by the whole body was improved postdenervation. This suggests that insulin action and/or glucose effectiveness were enhanced. It is difficult to separate the effect of CHADN on insulin secretion from its effect on insulin action, but the data clearly indicate that the improvement in glucose tolerance resulted from some combination of these effects.

In Protocol 2, four SHAM dogs and five CHADN dogs were continued on the HFHF diet beyond the 3.5-week period, with OGTTs being carried out at 7.5 and 11.5 weeks postdenervation to see whether the improvements brought about by the CHADN persisted. The ΔAUC for plasma glucose in the CHADN group remained improved to the same extent at 7.5 and 11.5 weeks as at 3.5 weeks (Table 2). Likewise, the ΔAUC for plasma glucose failed to improve over time in the SHAM animals, thus indicating that the diet-induced defect was stable over time. The insulin and C-peptide responses seen at 2.5–3.5 weeks post-CHADN persisted at 7.5 and 11.5 weeks post-CHADN surgery. The insulinogenic index was 0.31 ± 0.2 pre-CHADN, was significantly reduced from baseline (0.52 ± 0.12), and returned toward normal (0.48 ± 0.25 , 0.42 ± 0.15 , and 0.43 ± 0.19 at 3.5, 7.5, and 11.5 weeks postsurgery, respectively; not significantly different from baseline). In the SHAM animals, it was 0.40 ± 0.12 presurgery and 0.30 ± 0.20 , 0.34 ± 0.20 , and 0.36 ± 0.10 , respectively, at 3.5, 7.5, and 11.5 weeks postsurgery (Table 2). The C-peptide data confirm the drop in the ΔAUC ratio caused by the HFHF diet (0.015 ± 0.001 to 0.010 ± 0.004) and the improvement resulting from CHADN (0.014 ± 0.004 , 0.015 ± 0.003 , and 0.015 ± 0.006 , respectively, at 3.5, 7.5, and 11.5 weeks).

Subgroups of dogs from each group in Protocol 1 were subjected to an HIHG clamp 3.5 weeks after surgery (after 8 weeks on the HFHF diet). Somatostatin was given to disable

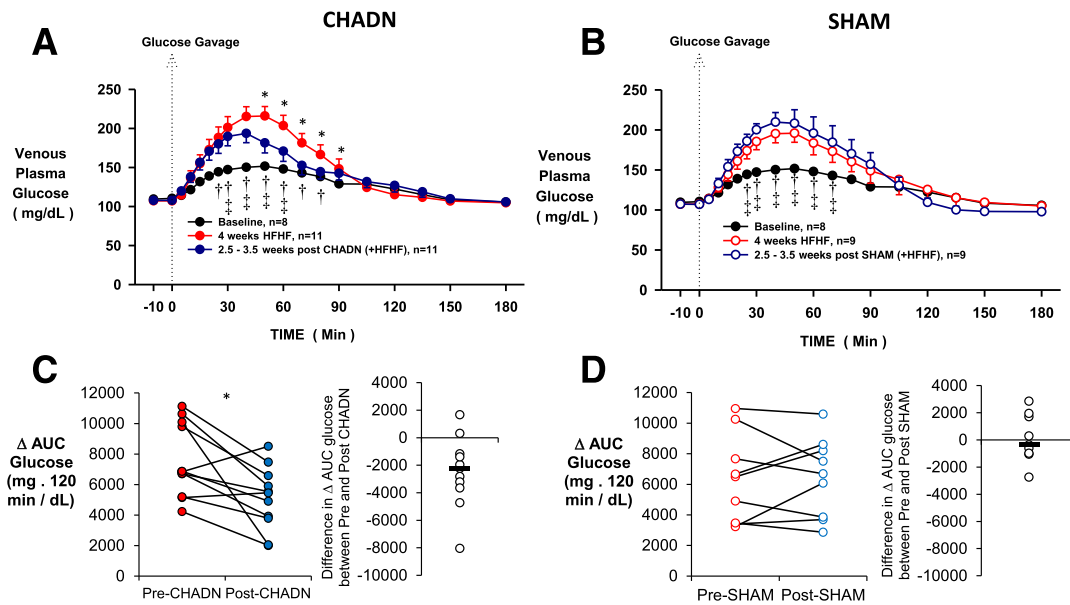


Figure 2—Venous plasma glucose levels during the consecutive OGTTs (A and B) as well as the Δ AUC for the CHADN (C) and SHAM (D) animals. A shows the baseline OGTT in black, the OGTT after 4 weeks of HFHF diet in red, and the same animals postdenervation in blue (closed circles). B shows the same baseline OGTT in black, the OGTT after 4 weeks of HFHF in red, and the same animals post-SHAM surgery in blue (open circles). The Δ AUC was calculated by subtracting the baseline AUC from the AUC for each condition; individual data are shown pretreatment and posttreatment as well as the difference due to treatment. The horizontal bar represents the median of change before and after the treatment (CHADN in C; SHAM in D). In A and B, † shows a difference between baseline OGTT and 4-week OGTT, ‡ shows a difference between baseline OGTT and posttreatment OGTT, and * shows a difference between pretreatment and posttreatment OGTT, as analyzed by repeated values two-way ANOVA with post hoc analysis by the Student-Newman-Keuls model. In C and D, * shows a difference between pretreatment and posttreatment OGTT as analyzed by a paired *t* test ($P = 0.01$). The difference due to treatment was significantly different between the CHADN group and the SHAM group ($P = 0.02$), as analyzed by two-way ANOVA with post hoc analysis by the Student-Newman-Keuls model.

the endocrine pancreas, and insulin was infused intraportally at 1.2 mU/kg/min ($4 \times$ basal secretion) (Fig. 4A), while glucagon was infused intraportally at 0.55 ng/kg/min to maintain basal glucagon levels (Fig. 4B). The results from these two groups can be compared with control data obtained in earlier identical experiments (14), which are provided for reference purposes. The hepatic sinusoidal insulin level rose approximately fourfold in response to insulin infusion and was similar ($\sim 70 \pm 8 \mu\text{U/mL}$) in all three groups (control, CHADN, and SHAM). Glucagon remained at basal levels and equal in all groups. Glucose was infused into a leg vein to clamp the glucose at ~ 200 mg/dL in each group (Fig. 4C). In the control dogs, net hepatic glucose balance (NHGB) switched from an output of 1.4 ± 0.2 mg/kg/min to an uptake approaching 2.0 mg/kg/min within ~ 75 min, after which it increased slightly over time. Consuming the HFHF diet for 8 weeks completely abolished the activation of net HGU (NHGU) in the SHAM dogs such that NHGB fell from 1.6 ± 0.3 to 0.2 ± 0.2 mg/kg/min (3 h), but the animals did not switch to NHGU. The dogs that consumed an HFHF diet for 4.5 weeks and then underwent CHADN, followed by 3.5 more weeks of HFHF diet, on the other hand, switched from a net glucose output of 1.3 ± 0.3 mg/kg/min to an uptake of 0.8 ± 0.2 mg/kg/min (3 h). Thus, in the presence of identical insulin, glucagon, and glucose levels, sympathetic CHADN significantly improved the response of the liver to the glucose

load. The tracer-determined unidirectional HGU (Table 3) rose from a baseline of 0.3 ± 0.2 to 1.6 ± 0.2 mg/kg/min in the last 60 min of the experimental period in the chow-fed controls, from 0.3 ± 0.2 to 0.4 ± 0.3 mg/kg/min in the SHAMs, and from 0.3 ± 0.2 to 0.8 ± 0.4 mg/kg/min in the CHADN group (Table 3). HGP, on the other hand, fell from 1.9 ± 0.4 to 0.1 ± 0.5 mg/kg/min in the control group, from 1.8 ± 0.6 to 0.5 ± 0.7 mg/kg/min in the SHAM group, and from 1.9 ± 0.8 to 0.2 ± 0.6 mg/kg/min in the CHADN group (Table 3).

Glucose taken up by the liver can be converted to lactate via glycolysis, oxidized to CO_2 , or stored as glycogen. In controls, the liver switched from lactate uptake to output as a result of the switch from glucose output to uptake. In contrast, in the SHAM dogs hepatic lactate uptake continued throughout the experimental period consistent with the lack of HGU. Sympathetic CHADN did not alter hepatic lactate kinetics in the HFHF-fed dogs even though their livers actually took up glucose. In control animals, the glycogen synthetic rate during the last hour was 1.9 ± 0.2 mg/kg/min, while it reached only 0.4 ± 0.2 mg/kg/min in the SHAM dogs. It was partially restored in the CHADN dogs (1.1 ± 0.2 mg/kg/min), thereby providing an explanation for why net lactate release from the liver did not occur (Table 4).

The glucose infusion rates (GIRs) required to clamp glucose at ~ 200 mg/dL during the last hour of the study

Table 2—Glucose, insulin, and C-peptide Δ AUC over the first 120 min after gavage of a 0.9 g/kg glucose load in conscious dogs fasted for 18 h at baseline (chow fed, $n = 8$) after 4 weeks of HFHF diet (pre-SHAM, $n = 9$; pre-CHADN, $n = 11$), 2.5 and 3.5 weeks postsurgery (post-SHAM, $n = 9$; post-CHADN, $n = 11$), 7.5 weeks postsurgery (post-SHAM, $n = 4$; post-CHADN, $n = 5$), and 11.5 weeks postsurgery (post-SHAM, $n = 4$; post-CHADN, $n = 5$)

	Group	ΔAUC		Ratio of ΔAUC	
		Glucose (mg/120 min/dL)	Insulin (μU/120 min/mL)	C-Peptide (mg/120 min/mL)	Insulin/glucose C-Peptide/glucose
Chow	Baseline (n = 8)	3,215 ± 352	1,671 ± 191	47 ± 4	0.52 ± 0.12 0.015 ± 0.001
4 weeks on HFHF diet	Pre-SHAM (n = 9)	6,341 ± 289†	2,586 ± 756†	78 ± 29†	0.40 ± 0.12 0.012 ± 0.004†
	Pre-CHADN (n = 11)	7,580 ± 2,418†	2,185 ± 1,062†	73 ± 20†	0.31 ± 0.2† 0.010 ± 0.004†
2.5–3.5 weeks postsurgery	Post-SHAM (n = 9)	6,458 ± 2,583†	2,295 ± 475†	66 ± 15†	0.30 ± 0.20† 0.012 ± 0.005†
	Post-CHADN (n = 11)	5,104 ± 2,059	2,309 ± 1,322†	69 ± 29†	0.48 ± 0.25 0.014 ± 0.004
7.5 weeks postsurgery	Post-SHAM (n = 4)	6,413 ± 2,568†	2,222 ± 333†	81 ± 13†	0.34 ± 0.20† 0.012 ± 0.005†
	Post-CHADN (n = 5)	4,720 ± 629	1,996 ± 977†	70 ± 13†	0.42 ± 0.15 0.015 ± 0.003
11.5 weeks postsurgery	Post-SHAM (n = 4)	5,657 ± 1,063†	2,088 ± 812†	68 ± 16†	0.36 ± 0.10 0.012 ± 0.005†
	Post-CHADN (n = 5)	4,940 ± 1,509	2,139 ± 662†	67 ± 12†	0.43 ± 0.19 0.015 ± 0.006

Values are the mean $\pm$ SD. Ratios of the Δ AUC for insulin over the Δ AUC for glucose and of the Δ AUC for C-peptide over the Δ AUC for glucose represent the amount of insulin and C-peptide secreted in response to glucose appearing in the circulation. Δ AUC values were calculated by subtracting the baseline AUC from the AUC for each condition between 0 and 120 min. † $P < 0.05$ SHAM vs. baseline. ‡ $P < 0.05$ CHADN vs. baseline (two-way ANOVA with post hoc analysis by Student-Newman-Keuls model).

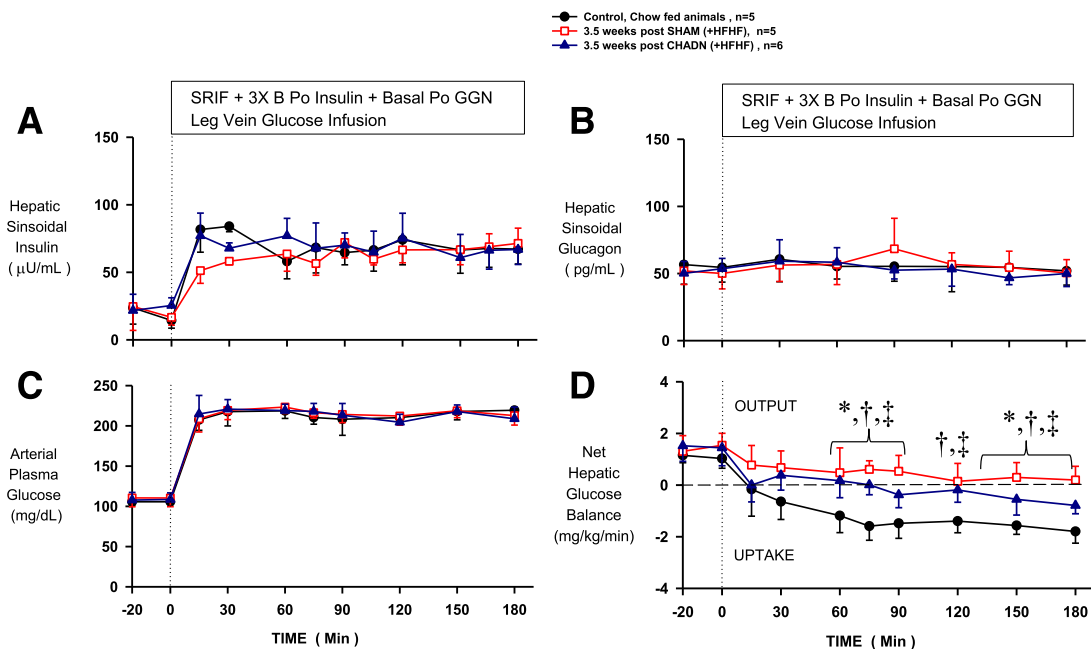


Figure 4—Hepatic sinusoidal insulin (A) and glucagon (B), arterial plasma glucose (C), and NHGB (D) during the basal (−20 to 0 min) and experimental periods (0–180 min) of HIHG clamps conducted on 18-h fasted conscious dogs fed chow (Control chow-fed animals; $n = 5$) or after 8 weeks of an HFHF diet in dogs with surgically sympathetically denervated livers (CHADN, $n = 6$) or SHAM denervated livers (SHAM, $n = 5$) at 3.5 weeks postsurgery. B, basal; Po GGN, portal infusion of glucagon (0.55 ng/kg/min); Po Insulin, portal infusion of insulin (1.2 mU/kg/min). SRIF, somatostatin. Statistical analysis with two-way repeated-measures ANOVA: $*P < 0.05$ for HFHF + SHAM vs. HFHF + CHADN; $\dagger P < 0.05$ for HFHF + SHAM vs. chow-fed controls; $\ddagger P < 0.05$ for HFHF + CHADN vs. chow-fed controls. Data from control chow-fed animals were obtained under identical conditions, were previously published (14), and are included for reference purposes.

were 12.9 ± 2.7 , 10.8 ± 1.6 , and 11.7 ± 3.4 mg/kg/min in the control, SHAM, and CHADN groups (Table 4) ($P > 0.10$), respectively. This suggests that the small reduction in GIR ($\Delta 2.1$ mg/kg/min) caused by the HFHF diet

was primarily attributable to a reduction in net glucose uptake by the liver rather than an effect of diet on the periphery as shown by the absence of a difference in the non-HGU. In line with this, the improvement in GIR caused by CHADN was attributable to a partial correction of the hepatic defect.

The arterial blood glycerol level clearly indicated that lipolysis was markedly inhibited during the clamp in the control dogs. The baseline arterial blood glycerol level in the SHAM HFHF-fed dogs was not different from the level in the control animals, but the suppression of lipolysis during the clamp was reduced significantly (Table 4). Sympathetic CHADN in HFHF-fed dogs did not alter the baseline glycerol levels or modify the suppression of lipolysis seen in the SHAM HFHF-fed dogs (Table 4). Net hepatic glycerol uptake changed in accord with the blood glycerol levels, in line with it being a substrate-driven process. Taken together, these data suggest that the HFHF diet had a mild inhibitory effect on adipose tissues.

DISCUSSION

The liver can both consume and produce glucose. In response to a moderate glucose load, the liver is responsible for the storage of about a third of the ingested glucose (37). The uptake of glucose by the liver is regulated by an interplay among the load of glucose reaching the organ, the plasma insulin level, and the neural input the liver receives. There is now good evidence to support the concept that glucose ingestion triggers the removal of

Table 3—Tracer-determined unidirectional HGU as well as production and NHGB during an HIHG clamp in conscious dogs fasted for 18 h fed a chow or HFHF diet for 8 weeks

	Basal period	Experimental period
HGU		
Chow-CTRL	0.3 ± 0.2	1.6 ± 0.2
HFHF + SHAM	0.3 ± 0.2	$0.4 \pm 0.3\dagger$
HFHF + CHADN	0.3 ± 0.2	$0.8 \pm 0.4^{*,\ddagger}$
HGP		
Chow-CTRL	1.9 ± 0.4	0.1 ± 0.5
HFHF + SHAM	1.8 ± 0.6	0.5 ± 0.7
HFHF + CHADN	1.9 ± 0.8	0.2 ± 0.6
NHGB		
Chow-CTRL	1.6 ± 0.3	-1.5 ± 0.8
HFHF + SHAM	1.5 ± 0.5	$0.1 \pm 0.4\dagger$
HFHF + CHADN	1.6 ± 0.5	$-0.5 \pm 0.3^*$

Values are means of the last 60 min of the experimental period $\pm$ SD in mg/kg/min. Negative values for balance data indicate net hepatic uptake. CHADN animals underwent sympathetic CHADN after 4.5 weeks of diet, while SHAM dogs underwent a sham procedure at the same time. The clamp was carried out 3.5 weeks after surgery. CTRL, control. $*P < 0.05$ for HFHF + SHAM vs. HFHF + CHADN. $\dagger P < 0.05$ for HFHF + SHAM vs. CTRL. $\ddagger P < 0.05$ for HFHF + CHADN vs. CTRL.

Table 4—Hepatic artery and portal vein blood flow, total GIR, arterial blood lactate and glycerol concentrations, net hepatic balances, and net hepatic glycogen flux during HIHG clamps in dogs fasted for 18 h fed a chow or HFHF diet for 8 weeks and 3.5 weeks after undergoing an CHA sympathectomy (HFHF + CHADN) or a SHAM procedure (HFHF + SHAM)

Group	Basal period	Experimental period		
		0–60 min	60–120 min	120–180 min
Hepatic artery blood flow, mL/kg/min				
Chow-CTR	5 ± 1	5 ± 1	7 ± 2	7 ± 2
HFHF + SHAM	5 ± 1	6 ± 1	6 ± 2	7 ± 2
HFHF + CHADN	5 ± 2	6 ± 1	7 ± 2	7 ± 2
Portal vein blood flow, mL/kg/min				
Chow-CTR	25 ± 8	18 ± 5	19 ± 4	19 ± 4
HFHF + SHAM	20 ± 5	17 ± 3	17 ± 3	18 ± 3
HFHF + CHADN	22 ± 6	18 ± 4	19 ± 4	20 ± 4
Total GIR, mg/kg/min				
Chow-CTR	0.0 ± 0.0	7.1 ± 1.0§	10.1 ± 3.5§	12.9 ± 6.1§
HFHF + SHAM	0.0 ± 0.0	6.9 ± 1.8§	8.8 ± 3.2§	10.8 ± 3.9§
HFHF + CHADN	0.0 ± 0.0	6.3 ± 2.4§	9.4 ± 4.1§	11.7 ± 5.7§
Arterial blood lactate concentration, μmol/L				
Chow-CTR	325 ± 97	880 ± 236§	842 ± 145§	803 ± 183§
HFHF + SHAM	327 ± 165	386 ± 142	598 ± 253	644 ± 236§
HFHF + CHADN	284 ± 90	298 ± 103	390 ± 151	539 ± 198§
Net hepatic lactate balance, μmol/kg/min				
Chow-CTR	−6.6 ± 1.6	5.4 ± 3.6§	2.1 ± 3.4§	0.7 ± 2.5§
HFHF + SHAM	−6.1 ± 3.1	−3.8 ± 3.5	−3.1 ± 1.8	−3.8 ± 1.4
HFHF + CHADN	−5.4 ± 1.8	−4.4 ± 2.0	−2.9 ± 0.9	−3.9 ± 1.5
Arterial blood glycerol concentration, μmol/L				
Chow-CTR	81 ± 27	25 ± 14§	21 ± 16§	26 ± 21§
HFHF + SHAM	92 ± 29	54 ± 13§	56 ± 17†,§	44 ± 12§
HFHF + CHADN	92 ± 7	54 ± 20§	55 ± 23§	39 ± 11§
Net hepatic glycerol balance μmol/kg/min				
Chow-CTR	−1.7 ± 0.8	−0.4 ± 0.2§	−0.3 ± 0.2§	−0.5 ± 0.4§
HFHF + SHAM	−1.8 ± 0.8	−0.9 ± 0.5	−1.0 ± 0.4	−0.8 ± 0.3
HFHF + CHADN	−2.0 ± 0.9	−1.1 ± 0.9	−1.2 ± 0.9	−1.0 ± 0.7
Hepatic glycogen flux mg glucose equivalent/kg/min				
Chow-CTR	−0.3 ± 0.2	0.7 ± 0.8§	1.0 ± 0.4§	1.9 ± 0.2§
HFHF + SHAM	−0.7 ± 0.5	0.0 ± 0.5†,§	0.1 ± 0.3†,§	0.4 ± 0.3†,§
HFHF + CHADN	−0.7 ± 0.5	0.3 ± 0.6	0.5 ± 0.7*,§	1.1 ± 0.4§,*

Negative values for balance data indicate net hepatic uptake. CHADN animals underwent sympathetic CHADN after 4.5 weeks of diet, while SHAM dogs underwent a sham procedure at the same time. The clamp was carried out 3.5 weeks after surgery. CTR, control. * $P < 0.05$ for HFHF + SHAM vs. HFHF + CHADN; † $P < 0.05$ for HFHF + SHAM vs. CTR; § $P < 0.05$ for experimental period vs. basal period.

an inhibitory sympathetic tone, which in turn facilitates hepatic glucose entry (2,38). At a mechanistic level, this may reflect the unopposed action of the parasympathetic nervous system. At the cellular level, this involves the movement of glucokinase (GK) from the nucleus to the cytosol as well as an increase in GK transcription. We have shown in the dog that the consumption of a diet rich in fat and fructose for 4 weeks renders the liver incapable of taking up glucose in response to a hyperglycemic and hyperinsulinemic signal (14). The defect persists as long as the dogs remain on the diet (15), resulting in continued glucose intolerance (16). The dogs also exhibit a 10–20% increase in their body weight, mostly in the form of abdominal fat (39). Since obesity is associated with increased sympathetic tone (17), we hypothesized that increased sympathetic neural input to the liver drives at least a part of the defect in HGU. We further proposed that

surgically sympathectomizing the liver would improve HGU. The data presented in this article support these hypotheses. The HFHF diet, as expected, caused a marked increase in the level of plasma glucose seen following an OGTT when compared with that in chow-fed dogs. Surgical resection of the sympathetic nerves on the CHA in the HFHF-fed animals reduced the abnormality in glucose tolerance by ~60%.

This raises the question of the mechanism by which this improvement comes about. The insulinogenic index, which was impaired by the HFHF diet in the SHAM dogs, increased by 80% in the CHADN animals, indicating improved β -cell function. This may be attributed to the reduction in sympathetic innervation of the pancreas, which occurred in parallel with liver denervation. Since NE is inhibitory to insulin secretion (40,41) it stands to reason that in its absence there would be an improvement in

the amount of insulin secreted in response to a given glucose level. In order to determine whether the sensitivity of the liver to insulin/glucose was also altered, we carried out an HIRG clamp 3.5 weeks after a CHA sympathectomy or SHAM sympathectomy. In SHAM animals fed the HFHF diet for 8 weeks, the liver failed to switch to NHGU, replicating earlier findings (16). Following CHADN, however, the liver was able to take up glucose (NHGU during the third hour 0.5 mg/kg/min), albeit at a rate still significantly below the rate seen in normal chow-fed dogs (1.6 mg/kg/min under these conditions). Sympathetic denervation of the CHA increased the flux of glucose into the liver (Table 3) and tended to decrease the flux out (HGP). There are two possible ways in which sympathetic nervous system input to the liver could be deranged by the diet. First, the HFHF diet could increase overall sympathetic signaling to the liver such that the portal glucose signal cannot reduce the sympathetic input adequately to enhance NHGU. When we compared hepatic NE content in chow-fed and HFHF-fed dogs, however, they were not different (346 ± 141 ng/g in chow-fed animals [2] vs. 383 ± 82 ng/g in the HFHF-fed dogs), suggesting that basal sympathetic tone was not increased by the diet. Second, the diet could impair the ability of the portal glucose signal to cause a decrease in sympathetic signaling to the liver in response to oral glucose. Our observation that NHGU increased in HFHF-fed dogs following hepatic denervation supports this conclusion. The fact that hepatic denervation did not restore NHGU to normal despite complete denervation indicates that the diet must have had an additional effect at the liver. The most likely locus for this lesion is GK. HFHF feeding causes a marked reduction in hepatic GK protein and activity in the fasted and fed states (14). In the relative absence of GK, it would be difficult for the removal of sympathetic tone to activate NHGU. Net hepatic lactate balance and glycogen flux data allow assessment of the fate of the glucose taken up by the liver. In chow-fed dogs, the hyperglycemic/hyperinsulinemic stimulus caused HGU of 1.6 ± 0.6 mg/kg/min during the last hour of the experiment. Oxidation of glucose, although not measured, was probably ~ 0.2 mg/kg/min, as shown in our previous studies (42,43). Net hepatic lactate output was trivial such that virtually all of the extracted glucose was deposited in glycogen (Table 4). In the HFHF-fed SHAM dogs, absolute HGU was 0.5 mg/kg/min, but the liver was also taking up lactate (0.4 mg/kg in glucose equivalents). Assuming glucose oxidation of 0.2 mg/kg/min, this suggests that glycogen synthesis was ~ 0.7 mg/kg/min. The measured flux into glycogen (Table 4) was in fact 0.5 mg/kg/min. Hepatic sympathetic denervation did not change lactate metabolism or presumably oxidation relative to that seen in the SHAM dogs, but it increased flux into glycogen by 0.6 mg/kg/min, which is consistent with the observed increase in HGU (0.3 mg/kg/min) and the decrease in HGP (0.3 mg/kg/min). Thus, the impact of hepatic denervation was not only to increase NHGU, but also to direct that glucose to deposition in glycogen. Since hepatic

glycogen levels are low in individuals with type 2 diabetes (44), restoring hepatic glycogen toward normal could be of benefit in defending against low blood glucose levels.

To provide some insight into the durability of the effect, a subgroup of dogs was studied over 3 months, with OGTTs being carried out at 3.5, 7.5, and 11.5 weeks postdenervation or SHAM denervation in Protocol 2. There was no change in the abnormal OGTT findings across time in the SHAM dogs, thus indicating that the defect was stable. Likewise, the improvement in glucose tolerance caused by hepatic sympathectomy continued to be evident. Consistent with this, the enhancement in β -cell function also continued. It is worth pointing out that even at 13 weeks postsurgery the liver appeared to be completely denervated with no sign of reinnervation.

It should be noted that the difference in NHGB in the controls and SHAMs in the last hour of the experiment was 1.6 mg/kg/min, and the difference in the GIR in the two groups was 1.0 mg/kg/min, meaning that non-HGU was unaffected by the diet. In line with this, while hepatic denervation improved hepatic metabolism, it had no effect on nonhepatic glucose metabolism. Given that insulin-independent glucose uptake is driven by the glucose level, and the glucose levels were identical in the three groups during the clamp, one can conclude that muscle glucose uptake was unaffected by CHA denervation in the HFHF-fed dogs. On the other hand, the blood glycerol data suggest that the HFHF diet modestly reduced the ability of insulin/glucose to suppress lipolysis (Table 4), suggesting impaired fat cell metabolism; however, CHA denervation failed to correct the defect.

It should be noted that there was no hepatic portal vein glucose infusion in the clamp study. Thus, a component of the normal homeostatic response to HGU was missing. The rationale for this related to our hypothesis that hepatic sympathectomy would bring about the same response as hepatic portal vein glucose delivery (2). Therefore, it was felt best not to infuse glucose portally so that the impact of surgical denervation would be more evident. It is also worth pointing out that sympathetic denervation of the liver in the HFHF dogs had no impact on food intake, body weight, blood pressure, or hepatic arterial/portal vein blood flow. Furthermore, it did not alter any metabolic parameters in the basal state after an overnight fast; the effects of denervation only became manifest in response to glucose loading.

In summary, surgically stripping the nerve along the CHA in HFHF-fed dogs improved glucose tolerance for at least 3 months. Its effect appears to involve two mechanisms, an enhancement of β -cell function and an improvement in insulin/glucose action on the liver. In line with this, the surgical procedure targeted the CHA and reduced sympathetic input to the liver and the pancreas. This observation has anatomical relevance as the nerves along the CHA originate from the celiac ganglion and go on to innervate part of the pancreas and the splanchnic bed (45). On the other hand, CHA denervation had no effect on

muscle or adipose tissue metabolism. Our data agree with those of others, which show that targeting the sympathetic nervous system, via the renal nerves, can improve glucose kinetics in insulin-resistant states (25,27). It has also been suggested that bariatric surgery can reduce sympathetic tone (46).

Our findings support the concept of metabolic neuro-modulation as a therapeutic opportunity. Hepatic glucose output is normally regulated by the interplay between the direct inhibitory effects of insulin and the stimulatory effects of glucagon on the liver. In times of need (trauma, infection, exercise, hypoglycemia), there is an increase in sympathetic drive to the liver, pancreas, and adrenal glands that serves to increase glucose production. When the liver is in a storage mode, on the other hand, the effects of insulin interact with the CNS to control HGU (47). Insulin stimulates NHGU, whereas sympathetic nervous system input inhibits it. On the consumption of glucose, plasma insulin rises and the inhibitory impact of the CNS is withdrawn, allowing glucose uptake to occur. The consumption of an HFHF diet interferes with the removal of sympathetic tone (48,49), decreases glucose/insulin effectiveness, and reduces β -cell responsiveness. Stripping of the nerves on the CHA lessens both the hepatic and β -cell defects, thereby increasing glucose tolerance. The excellent safety profiles of the procedure demonstrated in our study raise the possibility that removal of the sympathetic nerves on the CHA may be a useful approach, less dependent than medications on patient compliance, for improving glucose tolerance in individuals with metabolic syndrome or type 2 diabetes.

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Author Contributions. G.K. directed all experiments, collected and interpreted data, and drafted and revised the manuscript. A.V., D.S.E., P.E.W., S.B.V., and B.R.A. participated in the design of the experiment and reviewed the manuscript. M.S., E.A., and D.S.E. participated in the experiments and biochemical analysis of the samples. P.E.W. was responsible for surgical preparation and oversight of animal care. B.R.A. and A.D.C. interpreted the results, contributed to the discussion, and edited the manuscript. A.D.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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