Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 881–890

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Glucose and lipid metabolism after liver transplantation in inbred rats: consequences of hepatic denervation

Hermann J. Kissler[a,b,*], Jürgen Hauffen[a], René Hennig[a], Heike Gepp[a], Paul O. Schwille[a]

[a]*Division of Experimental Surgery and Endocrine Research Laboratory, Friedrich Alexander University, Maximiliansplatz 1, 91054 Erlangen, Germany*
[b]*Department of Surgery, Friedrich Alexander University, Maximiliansplatz 1, 91054 Erlangen, Germany*

Received 11 August 2004; accepted 14 January 2005

Abstract

The liver plays a central role in glucose and lipid homeostasis. Because liver transplantation severs the hepatic nerves which influence this function, we hypothesized that insulin resistance and hyperlipidemia develop after liver transplantation, thus increasing the atherosclerotic risk. Therefore, we studied inbred rats 8 months after orthotopic liver transplantation (Tx, n = 39) or laparotomy (sham, n = 37) by either oral glucose tolerance test (Tx, n = 13; sham, n = 8), meal tolerance test (Tx, n = 9; sham, n = 13), or euglycemic hyperinsulinemic clamp with tritiated glucose infusion (Tx, n = 17; sham, n = 16). We found that liver transplantation significantly increased basal hepatic glucose production (HGP) in the clamp study by 20% (37.3 ± 2.2 vs 31.0 ± 2.1 μmol $kg^{-1} \cdot min^{-1}$, $P < .05$) and fasting plasma low-density lipoprotein (LDL) cholesterol by 36% (0.79 ± 0.06 vs 0.58 ± 0.05 mmol/L, $P < .05$). However, it did not affect HGP, total glucose uptake, metabolic clearance rate of insulin, and suppression of plasma nonesterified fatty acids, which were all normal in response to rising plasma insulin concentrations in the dose-response clamp studies. The oral glucose tolerance test and meal tolerance test also showed normal glucose and nonesterified fatty acids homeostasis with adequate pancreatic insulin secretion and hepatic insulin clearance after liver transplantation. The only consequences of liver transplantation are increased basal HGP and plasma LDL cholesterol, which may be caused by persistent vagal denervation of the liver. Although insulin resistance is absent, elevated plasma LDL cholesterol increases the atherosclerotic risk.

1. Introduction

The liver plays an important role in glucose and lipid homeostasis, in which efferent and afferent nerves influence the hepatic regulation of glucose and cholesterol [1,2]. Efferent sympathetic and parasympathetic nerves have been shown to control endogenous hepatic glucose production (HGP) [3] and cholesterol biosynthesis [4,5]. As the hepatic parasympathetic nerves also modulate hepatic insulin extraction [6], pancreatic insulin secretion [6], and insulin sensitivity of skeletal muscle [7], they determine the blood levels and effects of insulin. Thus, by influencing circulating glucose, cholesterol, and insulin levels as well as peripheral insulin sensitivity, hepatic nerves constitute a powerful regulator of glucose and lipid metabolism. This gets lost after liver transplantation which results in persisting intrinsic hepatic denervation [8,9].

Although impaired glucose metabolism and hyperlipidemia have been well documented after liver transplantation, they are mainly attributed to the immunosuppressive treatment [10]. Thus, only a few studies qualify for interpreting the effect of hepatic denervation on glucose and lipid metabolism. Severe postprandial hyperglycemia of liver-transplanted versus kidney-transplanted patients has been reported, which was attributed to insulinopenia resulting from increased insulin clearance secondary to hepatic denervation [11]. Increased hepatic insulin clearance was also confirmed after liver transplantation of patients with chronic liver disease in several other studies [12-14]. However, only minor other metabolic changes have been reported. These comprised increased β-cell secretion [13-15], decreased fasting HGP despite hyperglucagonemia, as well as defective counter-regulation during hypoglycemia [12]. However, β-cell hypersecretion and elevated hepatic insulin clearance could have also been

* Corresponding author. Division of Organ Transplantation, Department of Surgery, Northwestern University Medical School, Chicago, IL 60611, USA. Tel.: +1 312 503 3473; fax: +1 312 503 3366.
E-mail address: h-kissler@northwestern.edu (H.J. Kissler).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.01.036

caused by persistence of hemodynamic abnormalities of chronic liver disease. This has been suggested, because the kinetic behavior of C-peptide, which is an indirect measure of prehepatic insulin clearance, was normal in the frequently sampled intravenous glucose tolerance test after liver transplantation of patients with acute liver failure [16]. Insulin-dependent glucose metabolism has been found to be essentially normal as evidenced by insulin-clamp studies showing normal inhibition of endogenous glucose production and normal peripheral glucose disposal [12,15,17] and by studies with the frequently sampled intravenous glucose tolerance test documenting normal insulin sensitivity [13,14,16]. The finding of seemingly normal insulin sensitivity after clinical liver transplantation is at odds with the observation in animal studies, in which complete and vagal hepatic denervations result in insulin resistance by disrupting a neurogenic insulin-sensitizing mechanism [18-23]. As the latter data strongly suggest that the cholinergic liver nerves are mandatory for full insulin sensitivity, a defect may have been missed in clinical studies. This could have simply been caused by persisting metabolic alterations of the liver disease and immunosuppressive and other medications. However, it could have also been caused by the tests applied, because the dose-response characteristics for the effects of insulin on HGP and total glucose uptake have not been determined [24].

Although it is not yet definitely clarified how severe hepatic denervation affects metabolism after liver transplantation, it has been proven that insulin resistance is a major risk factor for atherosclerosis development and that hyperlipidemia increases cardiovascular morbidity and mortality after liver transplantation [25,26]. The present study was therefore designed to investigate the long-term consequences of hepatic denervation on glucose and lipid metabolism after liver transplantation to establish whether insulin resistance and hyperlipidemia develop, which would increase the atherosclerotic risk. Use of an inbred rat model made it possible to establish the sole effect of graft denervation on glucose and lipid metabolism independently of immunosuppression and liver disease. Liver-transplanted and sham-operated groups were studied 8 months after surgery. Oral glucose tolerance test (OGTT) and meal tolerance test (MTT) served to assess insulin response to oral glucose and to normal diet challenge. Insulin dose-response clamp studies were used to establish for the first time to the best of our knowledge the dose-response characteristics for insulin action on total glucose uptake.

2. Materials and methods

The animal studies described in this work complied with the current German law on the protection of animals, and all were approved by the Governmental Animal Care and Use Committees.

2.1. Experimental groups

Highly inbred male Wistar-Lewis rats (Charles River Breeding Laboratories, Sulzfeld, Germany), 12 weeks old on entry into the study, were used. They were housed in an environmentally controlled room with a 12-hour light/dark cycle and had free access to tap water and standard rat food (1320, pelleted rat maintenance diet, Altromin, Lage, Germany). The rats were randomly assigned to 1 of 2 groups: orthotopically liver-transplanted rats (Tx , n = 39) and laparotomized rats (sham, n = 37).

2.2. Orthotopic liver transplantation

Using ether anesthesia, the liver was transplanted according to the technique described by Engemann [27]. In short, the liver was harvested from an age-matched donor rat together with a segment of the inferior vena cava, portal vein, common bile duct, and aorta including the celiac and hepatic artery. After removal of the recipient's liver, the graft was transplanted orthotopically by anastomosing in an end-to-end fashion suprahepatic inferior vena cava, portal vein, and infrahepatic inferior vena cava, in that order. The graft aorta was then anastomosed to the recipient's infrarenal aorta in an end-to-side fashion. Finally, the bile duct was joined with a stent (Vasofix Braunüle, 1.0 mm/20 G; Braun, Melsungen, Germany).

2.3. Implantation of indwelling catheters

Eight months after the first operation, the rats were again anesthetized with ether, and indwelling catheters were inserted so that the animals could be studied in the awake unstressed state [28]. Rats later on subjected to OGTT or MTT received indwelling silastic catheters (Thomafluid-Silikon-Hochtemperatur-Chemieschlauch High Flexible, 0.5-mm inner diameter × 0.9-mm outer diameter and 1.0-mm inner diameter × 1.4-mm outer diameter for vascular and oral insertion, respectively) in the left carotid artery, left jugular vein, and in the mouth [29]; in those rats subjected to undergo the euglycemic hyperinsulinemic clamp study, silastic catheters were implanted in the left carotid artery and both jugular veins.

2.4. Oral glucose tolerance test

One week after insertion of the catheters, when the rats returned to a normal body weight gain, the first OGTT was done in 8 animals of the sham and 13 animals of the Tx group as previously described [29]. Briefly, at 8 PM, after 12 hours of food deprivation, glucose 2 g/kg body weight was infused as 50% solution via the intraoral catheter over 1.5 minute by pump (model 5003; Precidor Infors, Basel, Switzerland). Blood samples were obtained from the left carotid catheter at 30 and 10 minutes before the start of the glucose infusion (0 minute) and after 30, 60, 90, 120, 150, and 180 minutes. One week later, when animals had fully recovered, the test was repeated to determine glucose, nonesterified fatty acids (NEFA), insulin, C-peptide, and

fasting glucagon concentrations in the pooled plasma of the same rat.

2.5. Meal tolerance test

One week after catheter insertion, 13 rats of the sham and 9 rats of the Tx group were deprived of food during daytime and received 3 g of the standard rat diet (1320, powdered rat maintenance diet, Altromin) per kilogram of body weight as 60% solution via the intraoral catheter over 30 minutes using the Precidor pump. Blood samples were taken at the same time points and for the same variables as in the OGTT. The test was repeated after 1 week to measure variables in the pooled plasma of the same rat.

2.6. Euglycemic clamp study

Insulin-mediated whole body glucose uptake was measured 1 week after catheter placement in the evening after 12 hours of food deprivation using the euglycemic clamp in combination with tritiated glucose infusion, as previously described [30]. Briefly, 60 minutes before starting the insulin clamp, a primed (111 kBq) continuous (3.7 kBq/min) infusion of tritiated glucose (Amersham Buchler, Braunschweig, Germany; specific activity 488 GBq/mmol, high-performance liquid chromatography-purified approximately 99.2%) was started and continued throughout the study. At time 0, a continuous infusion of porcine insulin (Insulin S; Hoechst, Frankfurt am Main, Germany) was administered at time 0 to 130 minutes. The insulin infusion rate varied over a 10-fold range (14, 22, 29, 86, and 129 pmol $kg^{-1} \cdot min^{-1}$) to increase the plasma insulin concentration to approximately 574, 718, 1292, 5023, and 10763 pmol/L in the respective clamp studies. A variable infusion of 25% glucose was started at time 0 and adjusted to clamp the plasma glucose concentration at 5.8 mmol/L. Plasma samples for the determination of tritiated glucose specific activity were obtained from the arterial catheter at 5- to 10-minute intervals from −30 to 0 minutes and at 10-minute intervals after starting the insulin infusion. Arterial plasma samples for measurement of insulin, C-peptide, and NEFA concentrations were taken at −10, −5, 110, 120, and 130 minutes during the study. As in the OGTT and MTT, after removal of the plasma, the packed blood cells were resuspended in heparinized saline, and the equivalent volume reinfused after each blood sampling to prevent blood volume depletion and anemia. Initial and final hematocrit values were similar in all studies (data not shown). Studies were repeated at 7-day intervals as established by Smith et al [28]. No radioactivity was detected in plasma samples from restudied rats before the start of the repeat clamp study. Sixteen sham and 17 Tx rats were used in 32 studies, respectively, in which a steady state was reached during the insulin infusion, and in 3 and 5 studies in which glucose specific activity only attained a steady state in the basal state. Hereby, 2 sham and 5 Tx rats were studied once, 9 sham and 5 Tx rats twice, 5 sham and 6 Tx rats thrice, and 1 Tx rat 4 times.

2.7. Collection of blood and tissue samples

Nine months after liver transplantation or sham operation, food was withheld for 12 hours overnight, and the animals were laparotomized under ether anesthesia and exsanguinated from the infrarenal aorta. Blood samples were collected into prechilled tubes containing either no additive or 2.5 mg EDTA and 500 U Trasylol (aprotinin; Bayer, Leverkusen, Germany) per 1 mL of blood to obtain serum and plasma. The liver was quickly removed, immediately frozen in liquid nitrogen, and stored at −80°C, whereas serum and plasma were stored at −30°C.

2.8. Analytical procedures

The plasma glucose level was measured using the glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Munich, Germany). Established laboratory methods were used for the determination of total bilirubin and transaminases in serum, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and NEFA in plasma. Plasma immunoreactive insulin was measured by radioimmunoassay (radioimmunoassay kit, Linco Research Inc, St Charles, Mo), using an antibody cross-reacting equally with rat and porcine insulin. Radioimmunoassays were also used to determine plasma C-peptide and glucagon [31,32]. Plasma tritiated glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulfate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate ^3H_2O. Two separate aliquots of the tracer infusate underwent the Somogyi procedure and were counted together with the plasma samples for 1 minute in a liquid scintillation spectrophotometer. After thawing at 4°C, tissue specimens were taken periportally at random from one of the liver lobes in 6 Tx and 6 sham rats. Then, samples were immersed in ice-cold 6 mol/L perchloric acid and homogenized. After incubation and centrifugation (20 000*g*) for 15 minutes at 4°C, respectively, the supernatant was stored at −80°C after mixture with sodium phosphate buffer (0.5 mol/L, pH 6.5) until determination of the norepinephrine content by high-performance liquid chromatography [33].

2.9. Calculations and statistics

Low-density lipoprotein (LDL) cholesterol was calculated as follows: LDL cholesterol (mmol/L) = plasma

Table 1
Triglycerides, total cholesterol, and cholesterol lipoprotein concentrations in fasting plasma of sham-operated (Sham) or orthotopically liver-transplanted (Tx) rats

	Sham (n = 35)	Tx (n = 35)
Triglycerides (mmol/L)	0.92 ± 0.06	0.84 ± 0.07
Total cholesterol (mmol/L)	2.35 ± 0.06	2.39 ± 0.05
HDL cholesterol (mmol/L)	1.40 ± 0.07	1.25 ± 0.06
LDL cholesterol (mmol/L)	0.58 ± 0.05	0.79 ± 0.06*
LDL cholesterol/HDL cholesterol	0.47 ± 0.04	0.77 ± 0.10*
Total cholesterol/HDL cholesterol	1.75 ± 0.05	2.04 ± 0.10

Data are means ± SEM.
* $P < .05$ vs sham.

total cholesterol − (plasma triglyceride/2.2) − plasma HDL cholesterol.

For plasma glucose, NEFA, insulin, and C-peptide, the incremental area under the respective curves achieved in the period from 0 to 180 minutes during OGTT and MTT was assessed by the trapezoidal method. The hepatic insulin clearance was calculated from the molar ratio of the integrated responses of C-peptide and insulin. The ratio of the integrated incremental response of insulin and of glucose was taken as insulinogenic index.

Data for total body glucose uptake and suppression of HGP represent the mean values during the last 20 minutes (110- to 130-minute period) of the euglycemic clamp studies when tritiated glucose specific activity had reached

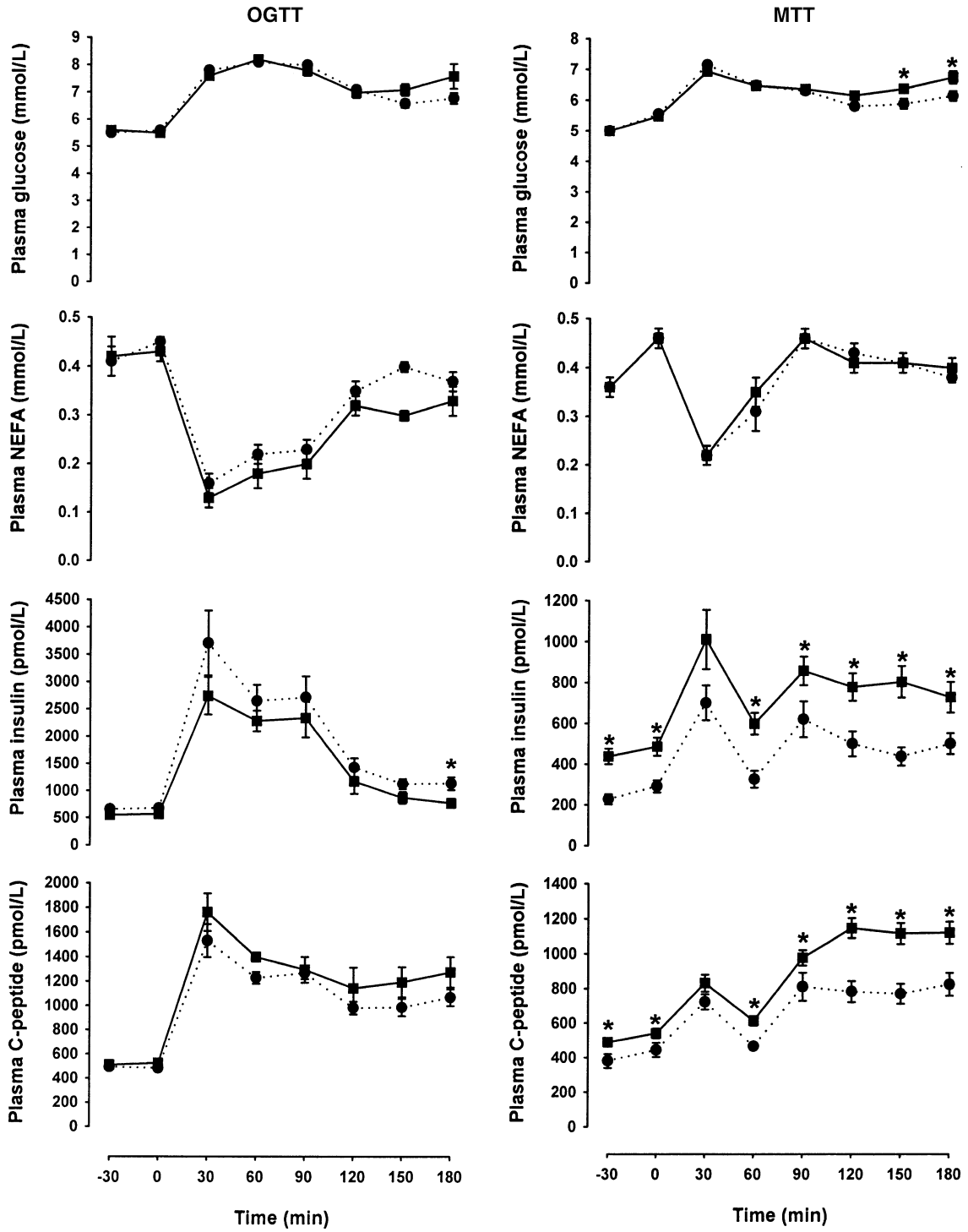

Fig. 1. Arterial plasma levels of glucose, NEFA, insulin, and C-peptide in 13 orthotopically liver-transplanted (Tx) and 8 sham rats after 2 g/kg oral glucose (OGTT), and in 9 Tx and 13 sham rats after 3g/kg standard rat diet (MTT) (Tx, •; sham, ■). Data are means ± SEM; *$P < .05$, Tx versus sham.

Table 2
Data of OGTT and MTT

	Sham (n = 8 and 13 in OGTT and MTT, respectively)	Tx (n = 13 and 9 in OGTT and MTT, respectively)
Body weight (g)		
OGTT	485 ± 4	496 ± 4
MTT	458 ± 5	450 ± 6
Glucose (mmol/L per min)		
OGTT	332 ± 19	311 ± 16
MTT	167 ± 14	125 ± 11*
NEFA (mmol/L per min)		
OGTT	−32 ± 3	−27 ± 2
MTT	−15 ± 2	−15 ± 3
Insulin (nmol/L per min)		
OGTT	210 ± 18	249 ± 29
MTT	52 ± 7	37 ± 6
C-peptide (nmol/L per min)		
OGTT	139 ± 14	115 ± 8
MTT	68 ± 5	46 ± 5*
Hepatic insulin clearance		
OGTT	0.7 ± 0.1	0.5 ± 0.1
MTT	0.6 ± 0.9	1.0 ± 0.4
Insulinogenic index (pmol/mmol)		
OGTT	651 ± 58	822 ± 89
MTT	350 ± 52	319 ± 77

Data are means ± SEM.
* $P < .05$ vs sham.

a steady-state plateau. During this steady state, when the rate of glucose appearance is equal to the rate of glucose disappearance, the total body glucose uptake was calculated by dividing the tritiated glucose infusion rate by the steady-state value of glucose specific activity. Under these conditions, total body glucose uptake is equal to the sum of the rates of exogenous glucose infusion and HGP. From this equation, the rate of HGP can be calculated. In the basal state, HGP was determined by dividing the tritiated glucose infusion rate by the steady-state plateau of glucose specific activity achieved during the last 10 minutes of the basal period. The validity of the measurement of HGP by tritiated glucose infusion and the reproducibility of the euglycemic clamp studies in the rat have been shown previously [28,34]. The metabolic clearance rate (MCR) of insulin measured by the constant infusion technique was calculated by dividing the infusion rate of insulin by the mean steady-state insulin concentration [35]. This calculation is based on the assumption that endogenous insulin production is suppressed during insulin infusion, as evidenced by a decrease of C-peptide to values near the detection limit.

All values were represented as arithmetic means ± SEM; the pooled mean data for each animal were used in the basal state of the clamp studies. The significance ($P < .05$) of group differences was examined using the Student t test or the Mann-Whitney rank sum test for unpaired observations, as appropriate.

3. Results

3.1. Body weight, and liver function and innervation

The body weight of liver-transplanted and sham rats was comparable preoperatively (313 ± 3 g in Tx vs 320 ± 4 g in sham) and 9 months after surgery at the end of the study period (506 ± 11 g in Tx vs 498 ± 7 g in sham). Therefore, they gained weight similarly (194 ± 9 g in Tx vs 183 ± 8 g in sham). During the OGTT, MTT, and clamp study, both groups also had similar body weights (Tables 2 and 3).

Table 3
Body weight, specific activity of glucose, glucose, NEFA, insulin, C-peptide in plasma, and HGP during the euglycemic clamp studies

Group	Insulin infusion rate (pmol $kg^{-1} \cdot min^{-1}$)					
	0 (Basal)	14	22	29	86	129
Sham						
n	16	5	6	7	7	7
BW (g)	461 ± 10	481 ± 11	462 ± 11	485 ± 12	450 ± 30	465 ± 14
Glucose (mmol/L)	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.2	6.0 ± 0.2	5.7 ± 0.1	5.8 ± 0.1
SA (cpm/mmol)	59.7 ± 8.0	38.0 ± 2.2	29.2 ± 3.5	23.1 ± 4.5	–	–
NEFA (μmol/L)	894 ± 55	730 ± 60	487 ± 34	473 ± 69	317 ± 48	295 ± 29
Insulin (pmol/L)	205 ± 26	658 ± 103	797 ± 172	1292 ± 167	4626 ± 571	11 247 ± 729
C-peptide (pmol/L)	311 ± 26	8 ± 3	3 ± 0	3 ± 0	5 ± 1	5 ± 1
HGP (μmol $kg^{-1} \cdot min^{-1}$)	31.0 ± 2.1	18.6 ± 8.1	25.8 ± 9.1	8.9 ± 10.5	–	–
Tx						
n	17	6	5	5	7	10
BW (g)	462 ± 7	451 ± 13	468 ± 12	457 ± 19	473 ± 12	468 ± 12
Glucose (mmol/L)	5.8 ± 0.1	5.8 ± 0.2	5.8 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	5.6 ± 0.2
SA (cpm/mmol)	62.1 ± 6.7	42.2 ± 3.7	35.5 ± 1.1	30.7 ± 3.8	–	–
NEFA (μmol/L)	854 ± 47	457 ± 102	565 ± 48	367 ± 96	245 ± 45	268 ± 27
Insulin (pmol/L)	206 ± 20	582 ± 86	871 ± 63	1304 ± 139	5977 ± 616	11 237 ± 993
C-peptide (pmol/L)	284 ± 28	11 ± 4	6 ± 2	4 ± 0	5 ± 1	6 ± 2
HGP (μmol $kg^{-1} \cdot min^{-1}$)	37.3 ± 2.2*	18.1 ± 8.5	29.3 ± 11.7	3.9 ± 8.3	–	–

Data are means ± SEM; n Indicates, number of rats; BW, body weight; glucose, steady-state plasma glucose; SA, steady-state glucose specific activity; NEFA, steady-state NEFA; insulin, steady-state plasma insulin; C-peptide, steady-state plasma C-peptide.
* $P < .05$ vs sham.

Liver function was normal after transplantation as evidenced by unchanged serum levels of aspartate aminotransferase (25.9 ± 1.0 U/L in Tx vs 31.1 ± 2.0 U/L in sham), alanine transferase (11.9 ± 0.5 U/L in Tx vs 13.5 ± 0.6 U/L in sham), and total bilirubin (1.1 ± 0.1 μmol/L in Tx vs 1.1 ± 0.1 μmol/L in sham). The significantly decreased norepinephrine content of the liver (2 ± 1 ng per 100 mg in Tx vs 14 ± 2 ng per 100 mg in sham; $P < .01$, n = 6, respectively) indicated persisting sympathetic denervation 9 months after transplantation.

3.2. Fasting plasma lipids

See Table 1. Plasma triglycerides were similar in Tx and sham rats. Although total cholesterol and HDL cholesterol were similar in both groups, LDL cholesterol was significantly increased by 36% in Tx versus sham rats. Consequently, the ratio of LDL and HDL cholesterol was significantly higher in Tx versus sham rats, and the ratio of total cholesterol and HDL cholesterol showed the same trend, however, without reaching statistical significance.

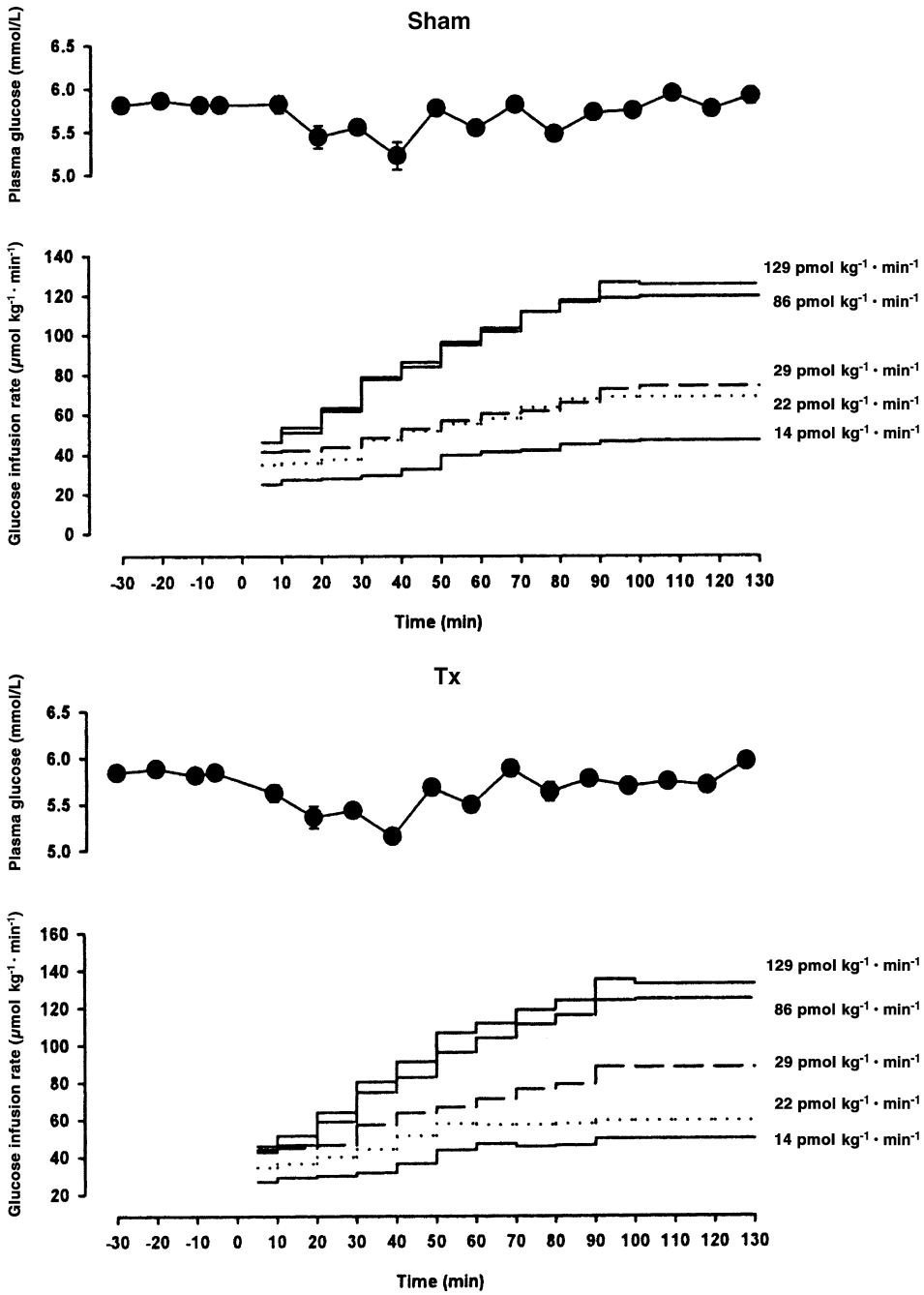

Fig. 2. Plasma glucose and exogenous glucose infusion rate in 17 orthotopically liver-transplanted (Tx) and 16 sham rats during euglycemic hyperinsulinemic clamp. Data are means ± SEM. Insulin infusion rates are indicated.

3.3. Oral glucose tolerance test

In the basal state and after oral glucose, both groups had similar arterial plasma glucose, NEFA, insulin, and C-peptide concentrations (Fig. 1). Fasting plasma glucagon levels were also similar in Tx and sham group (43 ± 5 vs 53 ± 6 ng/L). Consequently, transplantation preserved all integrated responses, the hepatic insulin clearance, and the insulinogenic index (Table 2).

3.4. Meal tolerance test

Despite preserved basal arterial plasma glucose and NEFA levels, basal plasma insulin and C-peptide levels were significantly lower in Tx versus sham rats (Fig. 1). It has to be noted that this difference in basal levels only occurred in this test. After meal ingestion, Tx rats had also lower plasma insulin and C-peptide levels (Fig. 1). However, the normal insulinogenic index of Tx rats indicates adequate insulin release. This was correctly adjusted to the significantly lower integrated incremental response (IIR) of glucose, resulting from an earlier decline of the arterial plasma glucose levels in Tx versus sham rats (Table 2, Fig. 1). Consequently, the IIR of C-peptide was significantly lower after transplantation, and the IIR of insulin tended to be lower because the hepatic insulin clearance of Tx rats remained unchanged (Table 2). It has to be noted, however, that the hepatic insulin clearance had a very large SEM. Taken together, the results of the MTT indicate that the glucose tolerance remained normal after liver transplantation, although it is intriguing that the insulinogenic index went different directions in the MTT and OGTT.

3.5. Euglycemic hyperinsulinemic clamp study

Basal and steady-state arterial plasma glucose, its specific activity, insulin, and C-peptide concentrations were similar in both groups (Table 3). The coefficients of variation for steady-state plasma glucose and insulin were 6% ± 1% and 33% ± 3%, respectively. The MCR of insulin was unchanged after transplantation (Fig. 3). Plasma NEFA levels were comparable in the basal state and declined similarly after hyperinsulinemia in sham and Tx rats. After a 12-hour fast, the basal HGP was elevated by 20% in Tx versus sham rats, whereas the HGP was similarly suppressed in both groups during the insulin infusions (Table 3). As the calculation of HGP rendered negative values in the 2 higher insulin infusion studies, HGP was thought to bottom out at an insulin infusion rate of 86 pmol $kg^{-1} \cdot min^{-1}$. Therefore, these values were not given (Table 3), and the glucose infusion rate was assumed to adequately represent the total glucose uptake in the 2 higher insulin infusion studies at 86 and 129 pmol $kg^{-1} \cdot min^{-1}$ (Fig. 3). In the basal state, HGP equals total glucose uptake, which thus was also elevated in the Tx versus sham rats (Fig. 3). The mean glucose infusion rate reached a plateau 110 to 130 minutes after the start of the insulin infusion (Fig. 2). Tx and sham rats had similar glucose infusion rates during steady state (final 20 minutes of the clamp) at the respective clamp levels (Fig. 3). This is in accordance with the similar total glucose uptake in both groups after hyperinsulinemia (Fig. 3). The total glucose uptake rose to 68.1 ± 10.0, 82.0 ± 9.9, 92.2 ± 5.4, 124.5 ± 4.3, and 132.6 ± 7.1 μmol $kg^{-1} \cdot min^{-1}$ in Tx , and to 58.5 ± 2.3, 94.4 ± 10.5, 82.8 ± 8.8, 125.5 ± 12.5, and 119.4 ± 6.6 μmol $kg^{-1} \cdot min^{-1}$ in sham rats at a steady-state plasma insulin concentration of approximately 574, 718, 1292, 5023, and 10763 pmol/L. The maximal rate of

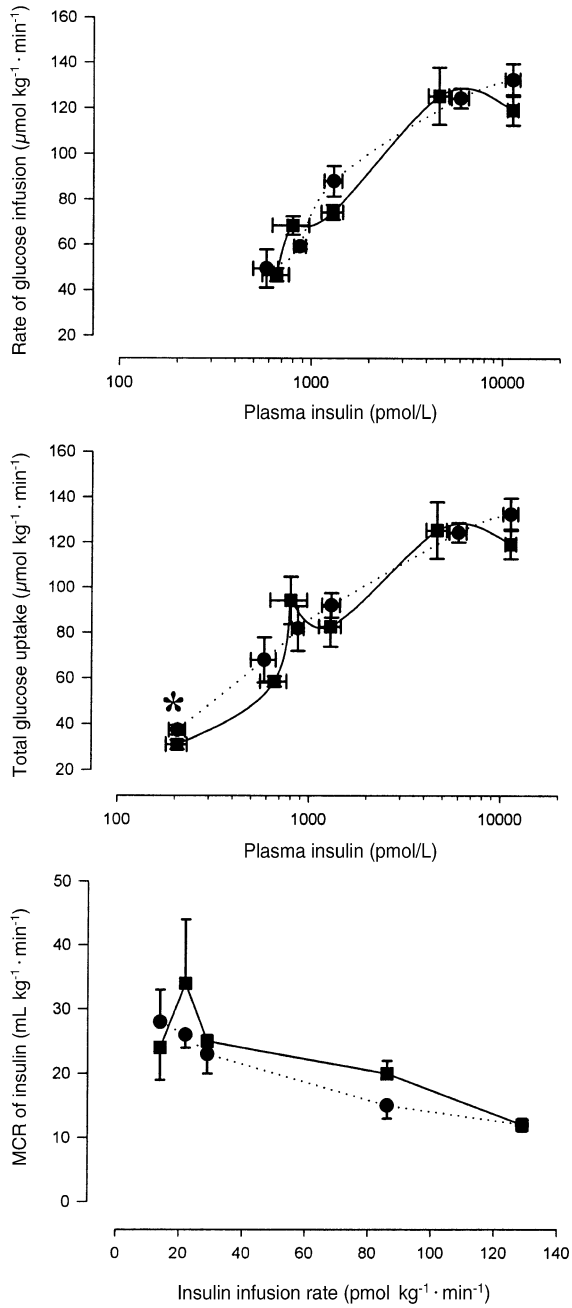

Fig. 3. Dose-response relationships in the steady state of the euglycemic clamp studies between plasma insulin concentration and glucose infusion rate, between plasma insulin concentration and total glucose uptake, and between insulin infusion rate and MCR of insulin in orthotopically liver-transplanted (Tx, •) and sham-operated (sham, ■) rats. Data are means ± SEM; *$P < .05$, Tx vs sham.

total glucose uptake (133 μmol $kg^{-1} \cdot min^{-1}$ in Tx vs 137 μmol $kg^{-1} \cdot min^{-1}$ in sham) and the half-maximally effective plasma insulin concentration for total glucose uptake (533 pmol/L in Tx vs 701 pmol/L in sham), which were calculated from averaged group values, were comparable in Tx and sham rats. This suggests that insulin resistance was absent after liver transplantation.

4. Discussion

The question addressed in the present study in inbred rats was whether and how the graft denervation affects long-term glucose and lipid metabolism after liver transplantation, that is, whether insulin resistance and hyperlipidemia develop and increase the atherosclerotic risk. The only derangements found in our studies after transplantation are a 20% increase of HGP in the euglycemic hyperinsulinemic clamp study and a 36% increase of the fasting plasma LDL cholesterol level. Thus, our findings indicate that in the present work, liver transplantation prevents insulin resistance but causes hyperlipidemia by elevating plasma LDL cholesterol levels, which alone would be sufficient to increase the atherosclerotic risk.

The increased basal HGP and plasma LDL cholesterol after liver transplantation in our study could at least partially be explained by persisting denervation of the liver. Hereby, the loss of the inhibitory effect of the parasympathetic nervous system on basal HGP outweighed the loss of the promoting effect of the sympathetic nervous system. This is supported by studies, reporting that hepatic vagotomy increased basal HGP in rats, whereas parasympathetic activation by electrical stimulation of the hepatic nerve reduced HGP in cats with chemically sympathectomized livers [36,37]. Although hepatic denervation up-regulates HGP in the basal state, it spares the response of HGP to hyperinsulinemia. This is evidenced by our findings in the clamp studies that rising plasma insulin levels normally suppressed HGP after liver transplantation. These results are in line with the correct regulation of HGP by hyperinsulinemia, reported after hepatic vagotomy in rats and after clinical liver transplantation [12,17,36]. Our finding of increased plasma LDL cholesterol in the presence of only marginally higher total plasma cholesterol after liver transplantation is similar to that reported after vagotomy of diabetic rats [4]. The only discrete derangement of the cholesterol metabolism after liver transplantation may be caused by the fact that rats are normally not cholesterolemia-prone. Thus, the rat is not a particularly good model for cholesterol metabolism. However, the disrupted hepatic vagus nerve seems at least partially responsible for the increased LDL cholesterol after liver transplantation.

Although the increased basal HGP and plasma LDL cholesterol and the decreased norepinephrine content of the liver confirm hepatic denervation after transplantation, insulin resistance did not develop in our study in contrast to reports from animal models of hepatic denervation [18-23]. Insulin resistance was attributed in those studies to interruption of a neurogenic insulin-sensitizing mechanism. Using the rapid insulin sensitivity test, which is a rapidly sampled euglycemic clamp in response to a pulse of insulin, Lautt [7] was the first to suggest that postprandial insulin initiates a hepatic parasympathetic- and nitric oxide–mediated reflex resulting in the release of a hormone from the liver, termed hepatic insulin-sensitizing substance (HISS), which stimulates insulin-mediated glucose uptake in skeletal muscle. Although the hyperinsulinemic euglycemic clamp studies of Porszasz et al [23] failed to demonstrate that a parasympathetic reflex was involved in the HISS mechanism, they confirmed its sensitivity to nitric oxide and identified HISS as somatostatin, released from capsaicin-sensitive sensory fibers in the anterior hepatic plexus. Thus, HISS action can readily be detected in rats with the hyperinsulinemic euglycemic clamp, which is the gold standard of measuring insulin sensitivity [38].

Therefore, we used this method in conjunction with tritiated glucose infusion to determine the dose-response relationship between plasma insulin and total body glucose uptake. This parameter is a better index of tissue sensitivity to insulin than the exogenous glucose infusion rate used by Porszasz et al [23], because it takes into account the rate of endogenous glucose production by the liver which is quite significant in the rat and may be impaired in insulin-resistant states. As we have demonstrated that the suppression of HGP was similar in response to rising insulin levels in the Tx and sham groups, the glucose infusion rate can also be used as insulin sensitivity index in our study. The glucose infusion rate can thus be compared between our second lower dose clamp study (22 pmol $kg^{-1} \cdot min^{-1}$) and the higher dose clamp of Porszasz et al (97 pmol $kg^{-1} \cdot min^{-1}$) because both studies had a plasma insulin plateau of approximately 718 pmol/L in common [23]. Despite the difference in body weight, fasting time, and anesthesia, our sham and their control group [23] had similar glucose infusion rates (68.6 vs 72.2 pmol $kg^{-1} \cdot min^{-1}$). On the contrary, the glucose infusion rate was only insignificantly decreased by approximately 14% after liver transplantation in our study, whereas it was significantly lowered by approximately 40% after chronic denervation of the anterior hepatic plexus in the study of Porszasz et al [23]. This suggests that insulin resistance was absent after liver transplantation in our study. That insulin action was in fact normal after liver transplantation in our study is corroborated by the similar findings of the complete dose-response curves for the effects of insulin on glucose uptake in Tx and sham rats. The unchanged maximal rate of insulin-mediated glucose uptake hereby indicates normal insulin responsiveness after liver transplantation, as the also preserved half-maximally effective insulin concentration for glucose uptake also indicates normal insulin sensitivity. That insulin sensitivity remains normal is in line with reports after clinical liver transplantation [12,15,17]. However, these studies evaluated insulin action at only 1 or 2 insulin doses in the physiological

concentration range of insulin [12,15,17], whereas our study determined the complete dose-response characteristics for the effects of insulin on glucose uptake. Furthermore, our findings of comparable glucose tolerance of Tx and sham rats in the OGTT and MTT confirm that liver transplantation actually preserves normal insulin action on glucose metabolism also under physiological conditions of food intake.

Although insulin resistance is generally defined as impaired action of insulin on glucose uptake, it is almost always linked to changes in lipid metabolism. Circulating NEFA released from adipose tissue are usually elevated in insulin resistance and have been suggested to aggravate this state by inhibiting glucose uptake, glycogen synthesis, and glucose oxidation and by increasing HGP [39]. Normal plasma NEFA concentrations after liver transplantation in our OGTT, MTT, and clamp studies thus indicate normal insulin action on lipid metabolism. Furthermore, the NEFA flux to the liver influences hepatic insulin clearance and thus controls circulating insulin levels [40]. This at least partially explains why hepatic insulin clearance, given either as molar ratio of the integrated responses in the OGTT and MTT or as clearance rate in the clamp studies, remained unchanged after liver transplantation in our study. It also confirms the finding that insulin clearance was unaffected by liver transplantation in patients without chronic liver disease [16]. Other alterations in lipid metabolism commonly associated with insulin resistance, that is, elevated plasma triglyceride and decreased plasma HDL cholesterol levels [41], were absent in our Tx animals. Thus, the isolated increase in plasma LDL cholesterol after transplantation in our study is rather an indicator of denervation than of impaired insulin action.

In conclusion, the present study shows that liver transplantation increases basal HGP and fasting LDL cholesterol concentration but avoids insulin resistance. Although the consequences of the elevated basal HGP seem negligible, the elevated LDL cholesterol concentration increases the atherosclerotic risk after liver transplantation.

Acknowledgments

The authors thank K. Schwille, I. Schreiber, and M. Manoharan for excellent technical assistance.

References

[1] Tiniakos DG, Lee JA, Burt AD. Innervation of the liver: morphology and function. Liver 1996;16(3):151-60.
[2] Jungermann K, Stümpel F. Role of hepatic, intrahepatic and hepatoenteral nerves in the regulation of carbohydrate metabolism and hemodynamics of the liver and intestine. Hepatogastroenterology 1999;46(Suppl 2):1414-7.
[3] Shimazu T. Reciprocal innervation of the liver: its significance in metabolic control. Adv Metab Disord 1983;10:355-84.
[4] Scott LM, Tomkin GH. Cholesterol metabolism: regulatory effects of the vagus in the normal and diabetic animal. Diabetes Res 1985; 2(6):313-7.
[5] O'Meara NM, Devery RA, Owens D, et al. Alterations in cellular cholesterol metabolism following administration of 6-hydroxydopamine to rabbits. Br J Pharmacol 1992;105(2):495-9.
[6] Lee KC, Miller RE. The hepatic vagus nerve and the neural regulation of insulin secretion. Endocrinology 1985;117(1):307-14.
[7] Lautt WW. The HISS story overview: a novel hepatic neurohumoral regulation of peripheral insulin sensitivity in health and diabetes. Can J Physiol Pharmacol 1999;77(8):553-62.
[8] Dhillon AP, Sankey EA, Wang JH, et al. Immunohistochemical studies on the innervation of human transplanted liver. J Pathol 1992;167(2):211-6.
[9] Boon AP, Hubscher SG, Lee JA, Hines JE, Burt AD. Hepatic reinnervation following orthotopic liver transplantation in man. J Pathol 1992;167(2):217-22.
[10] Reuben A. Long-term management of the liver transplant patient: diabetes, hyperlipidemia, and obesity. Liver Transpl 2001;7(11 Suppl 1): S13-S21.
[11] Schneiter P, Gillet M, Chioléro R, et al. Mechanisms of postprandial hyperglycemia in liver transplant recipients: comparison of liver transplant patients with kidney transplant patients and healthy controls. Diabetes Metab 2000;26(1):51-6.
[12] Perseghin G, Regalia E, Battezzati A, et al. Regulation of glucose homeostasis in humans with denervated livers. J Clin Invest 1997; 100(4):931-41.
[13] Konrad T, Steinmüller T, Vicini P, et al. Regulation of glucose tolerance in patients after liver transplantation: impact of cyclosporin versus tacrolimus therapy. Transplantation 2000;69(10):2072-8.
[14] Konrad T, Markus B, Allers C, et al. Impact of cyclosporine and low-dose steroid therapy on insulin sensitivity and beta-cell function in patients with long-term liver grafts. Transpl Int 2001;14(1):6-11.
[15] Merli M, Leonetti F, Riggio O, et al. Glucose intolerance and insulin resistance in cirrhosis are normalized after liver transplantation. Hepatology 1999;30(3):649-54.
[16] Konrad T, Golling M, Vicini P, et al. Insulin sensitivity and beta-cell secretion after liver transplantation in patients with acute liver failure. Transplant Proc 2001;33(4):2576-9.
[17] Luzi L, Perseghin G, Regalia E, et al. Metabolic effects of liver transplantation in cirrhotic patients. J Clin Invest 1997;99(4):692-700.
[18] Moore MC, Satake S, Baranowski B, et al. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab 2002;282(2):E286-96.
[19] Xie H, Tsybenko VA, Johnson MV, Lautt WW. Insulin resistance of glucose response produced by hepatic denervations. Can J Physiol Pharmacol 1993;71(2):175-8.
[20] Xie H, Lautt WW. Insulin resistance caused by hepatic cholinergic interruption and reversed acetylcholine administration. Am J Physiol 1996;271(3 Pt 1):E587-92.
[21] Xie H, Lautt WW. Insulin resistance of skeletal muscle produced by hepatic parasympathetic interruption. Am J Physiol 1996;270(5 Pt 1): E858-63.
[22] Latour MG, Lautt WW. The hepatic vagus nerve in the control of insulin sensitivity in the rat. Auton Neurosci 2002;95(1-2):125-30.
[23] Porszasz R, Legvari G, Pataki T, et al. Hepatic insulin sensitizing substance: a novel 'sensocrine' mechanism to increase insulin sensitivity in anaesthetized rats. Br J Pharmacol 2003;139(6):1171-9.
[24] Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 1978;27(12 Suppl 2):1893-902.
[25] Rewers M, Zaccaro D, D'Agostino R, et al. Insulin sensitivity, insulinemia, and coronary artery disease: the insulin resistance atherosclerosis study. Diabetes Care 2004;27(3):781-7.
[26] Johnston SD, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. Transplantation 2002;73(6):901-6.
[27] Engemann R. Technique for orthotopic rat liver transplantation. In: Thiede A, et al, editors. Microsurgical Models in Rats for Transplantation Research. Heidelberg: Springer-Verlag Berlin; 1985. p. 69-75.

[28] Smith D, Rossetti L, Ferrannini E, et al. In vivo glucose metabolism in the awake rat: tracer and insulin clamp studies. Metabolism 1987;36(12):1167-74.
[29] Kissler HJ, Gepp H, Schmiedl A, et al. Preservation of the incretin effect after orthotopic pancreas transplantation in inbred rats. Metabolism 1999;48(5):645-50.
[30] Kissler HJ, Gepp H, Tannapfel A, et al. Effect of venous drainage site on insulin action after pancreas transplantation in the rat—is there insulin resistance and a risk for atherosclerosis. Metabolism 2000; 49(4):458-66.
[31] Akpan JO, Weide LG, Gingerich RL. A specific and sensitive radioimmunoassay for rat C-peptide. Int J Pancreatol 1993;13(2): 87-95.
[32] Aguilar-Parada E, Eisentraut AM, Unger RH. Pancreatic glucagon secretion in normal and diabetic subjects. Am J Med Sci 1969;257(6): 415-9.
[33] Oka K, Kojima K, Togari A, et al. An integrated scheme for the simultaneous determination of biogenic amines, precursor amino acids, and related metabolites by liquid chromatography with electrochemical detection. J Chromatogr 1984;308:43-53.
[34] Rossetti L, Giaccari A. Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. J Clin Invest 1990;85:1785-92.
[35] Katz AI, Rubenstein AH. Metabolism of proinsulin, insulin, and C-peptide in the rat. J Clin Invest 1973;52(5):1113-21.
[36] Matsuhisa M, Yamasaki Y, Shiba Y, et al. Important role of the hepatic vagus nerve in glucose uptake and production by the liver. Metabolism 2000;49(1):11-6.
[37] Lautt WW, Wong C. Hepatic parasympathetic neural effect on glucose balance in the intact liver. Can J Physiol Pharmacol 1978; 56(6):679-82.
[38] Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocr Rev 1985;6(1):45-86.
[39] Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol Metab 2000;11(9):351-6.
[40] Bergman RN. Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein. Diabetologia 2000;43(7):946-52.
[41] Frayn KN. Insulin resistance and lipid metabolism. Curr Opin Lipidol 1993;4:197-204.