HEPATIC GLUTATHIONE AND NITRIC OXIDE ARE CRITICAL FOR
HEPATIC INSULIN SENSITIZING SUBSTANCE (HISS) ACTION

by

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ABSTRACT

We tested the hypothesis that both hepatic nitric oxide (NO) and glutathione (GSH) are involved in the synthesis of a putative hormone referred to as hepatic insulin sensitizing substance (HISS). Insulin action was assessed in Wistar rats using the Rapid Insulin Sensitivity Test (RIST). Blockade of hepatic NO synthesis with L-NAME (1.0mg/kg, ipv) decreased insulin sensitivity by 45.1±2.1% (from 287.3±18.1 mg glucose/kg in control to 155.3±10.1 mg glucose/kg; p<0.05). Insulin sensitivity was restored to 321.7±44.7 mg glucose/kg after administration of a NO donor, SIN-1 (5mg/kg, ipv), which promotes GSH nitrosation, but not after sodium nitroprusside (20nmol/kg/min, ipv) which does not nitrosate GSH. We depleted hepatic GSH using the GSH synthesis inhibitor BSO (2mmol/kg bw for 20 days, ip), which reduced insulin sensitivity by 39.1%. Insulin sensitivity after L-NAME was not significantly different between BSO and sham-treated animals. SIN-1 did not reverse the insulin resistance induced by L-NAME in the BSO group. These results support our hypothesis that both, NO and GSH, are essential for insulin action.

Keywords: nitric oxide, glutathione, insulin resistance, liver, hepatic insulin sensitizing substance (HISS)
INTRODUCTION

Insulin sensitivity is mediated by a neurohumoral mechanism through which the liver releases a putative hormone referred to as hepatic insulin sensitizing substance (HISS) [36,37]. HISS enters the blood and sensitizes skeletal muscle to insulin action, accounting for approximately 55% of total insulin action [14,15]. Therefore, insulin action consists of two components, a HISS-dependent and a HISS-independent component. HISS release is dependent on the activation of hepatic muscarinic receptors and is mediated by nitric oxide (NO) produced in the liver [35,27,7]. HISS synthesis is also controlled by the prandial status, being maximal in the post-prandial state and minimal in the fasted state [10]. Because hepatic glutathione (GSH) levels are also dependent on prandial status [31] we tested the hypothesis that both GSH and NO control HISS release.

Decreased levels of the thiol GSH in several tissues are a common finding in experimental and human diabetes, in which increased oxidative stress appears to occur [38,29]. Various studies suggest that increased oxidative stress is the major cause for insulin resistance observed in diabetes, either by impairing insulin secretion or insulin hemodynamic effects at the skeletal muscle [6,20,22,23]. Recent publications contradict this hypothesis, by stating that GSH depletion in rats results in impaired glucose tolerance but preserved adipocyte and skeletal muscle insulin responsiveness, as well as insulin secretion, without an increase in oxidative stress markers [10]. Khamaisi et al. [10] suggest that the impaired glucose tolerance observed after GSH depletion is a direct effect of the lack of GSH in the liver, and not an indirect consequence of increased oxidative stress.
In the present study we investigate a possible interaction between hepatic NO and GSH levels in the liver on HISS dependent insulin action. Insulin sensitivity was assessed using the Rapid Insulin Sensitivity Test (RIST), which is a modified euglycemic clamp. Herein, we compared the capacity of the two NO donors 3-morpholinosidnonimine (SIN-1) and sodium nitroprusside (SNP) to restore insulin response after hepatic nitric oxide synthase (NOS) blockade with N\textsuperscript{G}-nitro-L-arginine methyl ester hydrochloride (L-NAME). We also determined the effect of the \(\gamma\)-glutamylcysteine synthetase inhibitor L-buthionine- [S, R]- sulfoximine (BSO), which depletes hepatic GSH levels, on insulin sensitivity. The capacity of the NO donor SIN-1 to reverse insulin resistance induced by L-NAME in hepatic GSH depleted rats was assessed. Our results were consistent with the hypothesis that HISS synthesis in the liver requires NO as well as GSH.
METHODS

Pre-surgical protocols

Animals were treated according to the Laboratory Animal Care Guidelines of the European Union (86/609/CEE) and U.S National Institutes of Health (NIH). Male Wistar rats (8-9 weeks) were kept on a 12 h light/dark cycle housed one per cage under temperature control. Rats were fed ad libitum with standard rat chow (Panlab A04, Charles River, Spain), except for the day before surgery when the rats were fasted overnight and then allowed access to food for 1 h. Experiments began between 9:00 and 10:00 a.m. Rats were anesthetized with an intraperitoneal injection (ip) of sodium pentobarbital (65mg/kg) and anesthesia was maintained throughout the experiment by continuous infusion of sodium pentobarbital (1.0 mg/ml, 1.0ml/100 g body wt\(^{-1}\).h\(^{-1}\)) through a cannula inserted in the internal jugular vein. The temperature was maintained at 37.0±0.5°C by means of a heating pad (Homeothermic Blanket Control Unit 50-7061, Harvard Apparatus,USA) and monitored with a rectal probe thermometer. Rats were heparinized with 100 IU/kg heparin.

Glutathione depletion

Five-week-old male Wistar rats either received BSO (BSO group, n=5) or saline (sham group, n=6). BSO was dissolved in saline and 2mmol/kg BSO was administered daily for 20 days through ip injection between 10-12 a.m., following previously described protocols [10]. The sham group received ip saline for the same period of time. On day 20, the day
before surgery, the rats were fasted overnight and then allowed access to food for 1 h. Rats were anesthetized and the pre-surgical procedure described above was followed.

**Surgical Preparation**

The trachea was cannulated (polyethylene tubing, PE 240, Becton Dickinson, USA) to allow spontaneous respiration. The arteriovenous (a-v) loop previously described [13] was primed with a saline-heparin solution (200IU/ml). The a-v loop forms a vascular shunt connected by cannulation (polyethylene tubing, PE 50, Becton Dickinson, USA) of the carotid artery with the arterial side of the loop and of the left jugular vein with the venous side of the loop. Arterial blood samples (25µL) were taken by puncture of the loop sleeve. Arterial blood pressure was monitored by briefly clamping the venous outlet of the loop. The patency of flow in the shunt was also monitored by recording pressure from the non-occluded loop. Insulin, glucose and anesthetic were administered intravenously (iv) by puncturing (infusion line PE50 with a cut 23g needle at the delivery end) the loop on the venous side. The portal vein was cannulated with a 24G iv catheter (Jelco; Johnson & Johnson Medical, Italy), after laparotomy.

Rats were allowed to stabilize from the surgical intervention for 50 min before any procedures were carried out. Arterial blood samples were collected every 5 min after stabilization and glucose concentrations were immediately analysed by the oxidase method with a glucose analyser (1500 YSI SPORT, Yellow Springs Instruments, USA) until three successive stable glucose concentrations were obtained. The mean of these three values is referred to as the basal glucose level.
RIST

The RIST, which is a modified euglycemic clamp reproducible for four consecutive tests in the same animal has previously been described [13].

Insulin infusion was started using an infusion pump (Perfusor fm, Bbraun, Germany) to administer the dose of insulin 50mU/kg, iv over 5 min. After 1 minute of insulin infusion, arterial blood glucose was measured and glucose infusion (D-Glucose/saline, 100mg/ml, iv) was started at a rate of 5mg/kg/min. According to arterial glucose concentrations measured at 2 min intervals, the infusion rate of the glucose pump was readjusted to maintain euglycemia. When no further glucose infusion was required, usually within 35 min, the test period was concluded. The amount of glucose infused after insulin administration quantifies insulin sensitivity, and is referred to as the RIST index (mg glucose/kg) [13].

HISS quantification

The RIST index is composed of two components: the HISS-dependent component and the HISS-independent component. In this study, the HISS-independent component, or insulin action per se, is obtained by inhibition of hepatic NO release through the use of L-NAME administered directly into the portal vein. The HISS-dependent component of insulin action is calculated by subtracting the RIST index obtained after NOS blockade from the control RIST index, as previously reported [37, 27].
Experimental protocols

**Effect of intraportal (ipv) administration of the NOS antagonist, L-NAME, on insulin sensitivity**

After the control RIST was performed, L-NAME at a dose of 1mg/kg was infused ipv over 5 min. A stable basal arterial glucose concentration was determined and a new RIST was performed, 30 min after L-NAME infusion. Following restabilization, consecutive RISTs were performed at 90 min, 140 min and 190 min after L-NAME infusion in order to evaluate the duration of action of L-NAME dose (n=5).

**Effect of iv versus ipv administration of the NO donor, SNP, on L-NAME-induced insulin resistance**

The RIST index was determined before and 60 min after ipv infusion of L-NAME (1mg/kg, 5 min bolus). SNP (20nmol/kg/min) was administered ipv (n=6) or iv (n=5), 90 min after L-NAME. After a basal glucose level was established, a new RIST was performed.

**Effect of ipv administration of the NO donor, SIN-1, on L-NAME-induced insulin resistance**

The RIST index was determined before and 60 min after infusion of L-NAME (1mg/kg, ipv, 5 min bolus). SIN-1 (5mg/kg, 5 min bolus) was infused ipv (n=6), 90 min after L-NAME infusion, and the RIST was repeated.
Effect of glutathione depletion on insulin sensitivity and determination of the HISS-dependent component of insulin action

A control RIST was performed as described in BSO (2mmol/kg bw, 20 days, ip) treated (n=5) and in sham (ip saline) treated rats (n=6) to evaluate insulin sensitivity. Following this, the RIST index was determined 60 min after infusion of L-NAME (1mg/kg, ipv, 5 min bolus), either in BSO treated or sham animals in order to evaluate HISS action.

Effect of the NO donor, SIN-1, on L-NAME-induced insulin resistance in glutathione depleted rats

SIN-1 (5mg/kg, ipv, 5 min bolus) was infused 90 min after L-NAME, and the RIST was repeated in both groups (BSO-treated, n=5; sham, n=6). Rats were allowed to stabilize between each RIST.

Hepatic GSH determination

After performing the RISTs in the BSO-treated and in the sham-treated rats, the liver was rapidly dissected out, immediately frozen on liquid nitrogen and stored at –70°C until further analysis. Liver GSH was determined by the peroxidase-reductase assay following a method described by Marinho et al [16].
Drugs

BSO, L-NAME, SNP, SIN-1 and D-Glucose were purchased from Sigma-Aldrich Chemical, Co; Spain. Human insulin (HUMULIN, Regular) was obtained from Lilly, Portugal. Pentobarbital (EUTASIL) was obtained from Sanofi, Portugal. Heparin was purchased from B-Braun, Portugal. All chemicals were dissolved in saline.

Data Analysis

Data were analysed by using repeated measures ANOVA followed by the Tukey Kramer multiple comparison test in each group. GSH quantification data were compared using the student-t test. The data are expressed as mean ± SEM throughout. Differences were accepted as statistically significant at p<0.05. Whenever p value is not indicated, differences are not statistically significant.
RESULTS

Effect of ipv administration of the NOS antagonist, L-NAME, on insulin sensitivity

The control RIST was 202.4±11.1 mg glucose/kg (n=5). Thirty min after L-NAME (1mg/kg, ipv) administration, the RIST index was reduced to 139.8±26.7 mg glucose/kg, which corresponds to a statistically non significant decrease of 28.9±14.9% in insulin sensitivity. Ninety min after L-NAME administration, the RIST index was 80.2±6.1 mg glucose/kg, which corresponds to a 59.5±4.3% decrease in insulin sensitivity (p<0.001). When 140 min were passed after L-NAME administration, the RIST index was 97.3±24.0 mg glucose/kg, and insulin sensitivity was decreased by 50.7±12.9% (p<0.01). Finally, 190 min after the L-NAME bolus, the RIST index was 112.8±17.7 mg glucose/kg with 43.4 ±9.8% decrease compared to control values (p<0.01). According to these results, the maximal L-NAME effect on insulin sensitivity was achieved between 90 min and 190 min after the drug infusion. The mean arterial blood pressure increased after L-NAME infusion from 119.3±4.0mmHg to 141.4±3.2mmHg, at 20 min (p<0.01) and 190 min after it was 131.7±6.7mmHg.

Effect of iv versus ipv administration of the NO donor, SNP, on L-NAME-induced insulin resistance

Neither ipv (142.5±15.8mg glucose/kg, n=6), nor iv (110.5±34.1mg glucose/kg, n=5) infusion of SNP (20nmol/kg/min) affected the RIST index after L-NAME, as shown in
Figure 1 and Figure 2, respectively. The mean arterial blood pressure increased after L-NAME infusion from 125.3±8.1mmHg to 136.0±6.8mmHg, and it stayed stable, 131.7±6.7mmHg, for approximately 2 hours. The mean arterial blood pressure decreased after ipv SNP (from 124.2±4.2 mmHg to 95.0±2.6mmHg, p<0.01) and after iv SNP (from 123.0±8.0 mmHg to 88.0±3.7mmHg, p<0.01), but it remained constant during the RIST.

Effect of ipv administration of the NO donor, SIN-1, on L-NAME-induced insulin resistance

The control RIST index of 271.3±37.6 mg glucose/kg was significantly reduced to 152.2±21.3 mg glucose/kg (p<0.01), after ipv infusion of L-NAME (1mg/kg) (n=5). Administration of SIN-1 (5mg/kg, ipv) completely reversed the inhibition caused by L-NAME (321.7 ± 44.7 mg glucose/kg)(Fig.3). After L-NAME infusion the mean arterial blood pressure increased from 115.8±8.5mmHg to 127.5±5.3mmHg, and stayed at this level during the RIST. The mean arterial blood pressure decreased (from 123.3±5.4mmHg to 80.8±6.5mmHg, p<0.001) after SIN-1 administration, but remained constant during the RIST.

Effect of glutathione depletion on insulin sensitivity and determination of the HISS-dependent component of insulin action

The RIST index in BSO treated animals (158.4±12.2 mg glucose/kg, n=5) (Fig.4) was significantly lower compared to sham-treated animals, which showed a RIST index of 260.2±15.6 mg glucose/kg (n=6, p<0.001) (Fig.5). Therefore, GSH depletion with BSO
reduced insulin sensitivity by 39.1%. Ipv infusion of L-NAME (1mg/kg) decreased the RIST index both in BSO-treated and in sham-treated animals. In the BSO group, the RIST index after L-NAME was 109.0±9.1 mg glucose/kg corresponding to a change from control of 30.6±4.4% (Fig.4), while in the sham group the RIST index was 121.2±12.8 mg glucose/kg corresponding to a change from control of 52.3±5.8% (p<0.05) (Fig.5). HISS action, quantified by subtracting the post L-NAME RIST from the control RIST, was 138.9±22.8 mg glucose/kg for the sham group and only 49.3±8.6 mg glucose/kg for the BSO group (p<0.01), which corresponds to a 64.4% reduction of HISS action, after BSO treatment.

Effect of the NO donor SIN-1 on L-NAME-induced insulin resistance in BSO treated rats

In the BSO treated group SIN-1 (5mg/kg, ipv) did not reverse the decrease in insulin sensitivity caused by L-NAME (77.8±12.4 mg glucose/kg)(Fig.4). However, in the sham group, SIN-1 completely restored insulin sensitivity (258.1±18.5 mg glucose/kg, p<0.001)(Fig.5).

Hepatic GSH determination

GSH concentration was decreased in BSO treated rats, as expected. The GSH levels obtained were 5.9±0.4 µmol/g fresh liver in the sham group and 3.0±0.4 µmol/g fresh liver in the BSO treated group, which corresponds to a decrease of 49.2% (p<0.001) (Fig.6).
DISCUSSION

A novel neurohumoral regulatory mechanism for insulin action was recently reported [14]. According to this mechanism, insulin action at the skeletal muscle consists of two components, one that is mediated through hepatic NO and another component, which is independent of NO production in the liver [28]. The hepatic-NO-dependent component is responsible for the release of a putative hormone referred to as HISS (hepatic insulin sensitizing substance), which accounts for approximately 55% of the whole body insulin action [15]. Recent studies have demonstrated that HISS release is impaired in the fasted state, being maximal in the immediate post-prandial state [15]. It has also been suggested that lack of HISS release after feeding leads to insulin resistance typical of type 2 diabetes [14]. Hepatic GSH levels are depleted during fasting [31] and serum GSH levels are decreased in type 2 diabetes [34,17,33], suggesting the involvement of GSH in HISS release, as well as in insulin action. Our findings confirm the hypothesis that hepatic GSH, together with NO are determinant to permit full HISS-dependent insulin action.

In the fed state, insulin resistance induced by NOS antagonism can be restored by providing NO to the liver [27,7]. According to Sadri et al. [27], ipv administration of L-NAME, at a dose of 1mg/kg, significantly reduced the response to insulin, while the administration of the same dose iv did not cause a significant decrease in the insulin response. Insulin sensitivity was restored after administration of ipv, but not iv, SIN-1. These experiments support the hypothesis that hepatic NO mediates the HISS-dependent component of insulin action [27,14].

In the present work we studied the ability of different NO donors to reverse HISS-dependent insulin resistance. Two NO donors with distinct chemistries were used. SIN-1
decomposes nonenzymatically to yield both NO and superoxide (O$_2^-$) [5]. Recent data suggests that simultaneous production of NO and O$_2^-$ is an intrinsic activity of NOS, and that the enzyme does not catalyze production of free NO unless high concentrations of superoxide dismutase (SOD) are present [18]. According to these findings, SIN-1, as a NO/O$_2^-$ provider, might be regarded as the donor that better mimics NOS activity [30]. Schrammel et al. [30] showed that NO/O$_2^-$ rapidly reacts with GSH to produce an intermediate with biological activity, S-nitrosoglutathione (GSNO). The formation of GSNO from NO/O$_2^-$ is clearly different from the nitrosation of GSH by peroxinitrite (ONOO$^-$), with the reaction of GSH with NO/O$_2^-$ being at least 20-fold more efficient than the reaction of GSH with ONOO$^-$ [19]. Besides, ONOO$^-$ formation is partially outcompeted by the rapid reaction of NO/O$_2^-$ with GSH, which seems to occur preferentially even when SOD is present [18].

The other NO donor used in the present work was sodium nitroprusside (SNP), which releases NO promptly and spontaneously, without O$_2^-$ formation [5]. Free NO does not nitrosate thiols at significant rates [9], thus SNP has low capacity to nitrosate hepatic GSH. Therefore, the main difference between the two NO donors used in the present work, is their ability to nitrosate thiols: SIN-1 promotes GSNO formation while SNP does not [9,18,11].

In the light of the facts presented, we suggest that ipv SIN-1 was able to reverse L-NAME-induced insulin resistance because it mimics hepatic NOS activity by releasing NO/O$_2^-$ simultaneously and, likely, inducing GSNO synthesis in the liver which, we hypothesize, is important for HISS secretion. Iv administration of SIN-1 does not reverse HISS inhibition caused by NOS antagonism confirming that the drug is acting through the liver [27]. Neither ipv nor iv administration of SNP reversed insulin resistance induced by L-NAME,
probably due to the lack of ability of free NO to nitrosate thiols [9], which appears to be essential for triggering HISS synthesis. Another alternative hypothesis to explain why SNP did not restore insulin sensitivity is that NO might have been scavenged by hemoglobin [2]. However, administration of SNP, both iv and ipv, promoted a decrease in mean arterial blood pressure, showing that NO was not being inactivated by hemoglobin. Although other investigators suggest that insulin resistance induced by NOS blockade is secondary to a reduction in skeletal muscle perfusion and, consequently, to a reduction in the delivery of insulin and glucose to its target tissues [1], in our testing conditions we observed that the administration of the NO donor SNP did not improve insulin sensitivity, despite its notorious vasodilatory effects. Furthermore, if the insulin resistance observed after NOS blockade was secondary to inhibition of the dilatory responses to insulin in skeletal muscle, iv administration of SNP or SIN-1 should have produced more pronounced effects on insulin action than the ipv dose. Actually, neither we nor Sadri et al. [27] observed any effect of iv NO donors infusion in restoring impaired insulin action, after L-NAME administration. Our results agree with our previously described hypothesis, that insulin resistance in skeletal muscle caused by NOS blockade is related to an hepatic effect of NO, rather than to its vascular effects.

Several lines of evidence suggest that increased oxidative stress may play a role in peripheral insulin resistance [4,20,22]. Submitting muscle and fat cells to oxidative stress has been shown to result in a dramatic decrease of both insulin-stimulated glucose transport [12,25,32] and glycogen synthesis [3]. In adipocytes, hydrogen hydroperoxide (H$_2$O$_2$) impairs various metabolic effects of insulin, including the stimulation of glucose uptake activity, through alterations in the expression and translocation capacity of the glucose
transporter GLUT 4 [10,25]. It was also reported that oxidative stress has an inhibitory effect on tyrosine phosphorylation of the insulin receptor β subunit [8].

Decreased GSH levels were found in adipocytes exposed to a H₂O₂ generating system [26] and also in blood and tissues of diabetic rats, [34,17,33], which raises the possibility that decreased GSH is causative in impairing insulin action through enhancement of oxidative stress. Nevertheless, diminished glucose tolerance was observed in GSH depleted rats, although in vitro insulin responsiveness in skeletal muscle and adipocytes was preserved [10]. Khamaisi et al. [10] describe the intriguing observation that GSH depletion by BSO was progressively associated with abnormal glucose tolerance tests, which could not be attributed to impaired insulin secretion or insulin action in skeletal muscle or adipose tissue.

According to these authors, GSH levels in the muscle were reduced by 14±1% in skeletal muscle; nevertheless the muscle responsiveness to insulin, assessed ex vivo by measuring 2-deoxyglucose uptake in the absence and presence of insulin, was not significantly different in BSO treated and control animals although in vivo impaired glucose tolerance was observed [10].

Other studies report beneficial metabolic effects of antioxidants when administered to diabetic subjects [21,24]. De Mattia et al. [4] state that GSH infusion increases total glucose uptake in type 2 diabetes patients.

The exposure to oxidative stress conditions is characterized by decreased GSH/GSSG ratio, decreased GLUT 4 protein and mRNA expression and generation of lipid peroxidation products [10,25,32,3]. However, these oxidative stress markers were not altered in BSO treated compared to control rats, using the 2mmol/kg/day dose of BSO [10]. Regarding the possible effects of oxidative stress induced by GSH depletion on insulin action, the
2mmol/kg/day BSO dose allowed us to assess the isolated effect of GSH depletion, without mimicking the complex reactions associated with oxidative stress [10,12]. GSH depletion by BSO did not significantly affect basal or insulin-stimulated-glucose-uptake in adipocyte and skeletal muscle cell lines, in contrast with H$_2$O$_2$ exposure which significantly impaired glucose uptake activity in the same conditions [10]. This supports the hypothesis that the effects of GSH depletion by BSO on insulin sensitivity are not secondary to oxidative stress.

Our hypothesis is that decreased hepatic GSH levels promote a HISS-dependent impairment of insulin responsiveness in skeletal muscle. In BSO-treated rats, GSH levels decreased by 49.8% and insulin sensitivity decreased by 39.1%. After L-NAME infusion, the RIST index was similar both in the BSO treated and the sham group, indicating that HISS-independent insulin action was normal and that GSH depletion affected HISS action only. Moreover, SIN-1 administration completely restored insulin sensitivity in the sham group but not in the BSO group. We, therefore, suggest that insulin resistance observed in BSO treated rats is due to hepatic GSH depletion and subsequent decreased ability to synthesize GSNO which we hypothesize is involved in HISS release.

We have shown that in the fasted state, when HISS release is suppressed, ipv SIN-1 administration is not able to restore HISS-dependent insulin action (Guarino, M.P.; and Macedo, M.P., unpublished observations), probably because hepatic GSH levels are reduced in the fasted state [31].

Our findings support the hypothesis that both NO and GSH are essential in HISS synthesis/release. Additional studies are required to evaluate the role of GSH/NO in animal models of insulin resistance having in mind further pharmacological manipulations to increase HISS dependent insulin sensitivity.
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REFERENCES


FIGURE LEGENDS

Fig. 1: Rapid Insulin Sensitivity Test (RIST) index (mg glucose/kg) in control, after intraportal (ipv) 1mg/kg N-nitro-L-arginine methyl ester (L-NAME) and after ipv 20nmol/kg/min sodium nitroprusside (SNP) administration (n=6). Values are means ± SEM. *** P<0.001 from control group. Ipv administration of SNP did not reverse the insulin resistance caused by blockade of hepatic nitric oxide synthase.

Fig. 2: Rapid Insulin Sensitivity Test (RIST) index (mg glucose/kg) in control, after intraportal (ipv) 1mg/kg N-nitro-L-arginine methyl ester (L-NAME) and after intravenous (iv) 20nmol/kg/min sodium nitroprusside (SNP) administration (n=5). Values are means ± SEM. *** P<0.05 from control group. The nitric oxide (NO) donor SNP administered iv did not reverse the insulin resistance caused by blockade of hepatic nitric oxide synthase.

Fig. 3: Control Rapid Insulin Sensitivity Test (RIST) followed by a RIST performed after intraportal (ipv) 1mg/kg N-nitro-L-arginine methyl ester (L-NAME) and ipv 5mg/kg 3-morpholinosydnonimine (SIN-1) administration (n=5). Results are means ± SEM. ** P<0.01 from control group. Insulin resistance produced by nitric oxide synthase blockade was completely reversed after ipv administration of the NO/O₂⁻ donor, SIN-1.

Fig. 4: Rapid Insulin Sensitivity Test (RIST) in glutathione (GSH) depleted rats (n=5). GSH depletion was achieved by the glutathione synthesis inhibitor L-buthionine-[S,R]-sulfoximine (BSO), administered intraperitoneally (ip) at 2mmol/kg bw, for 20 days. The
control RIST was followed by a RIST after intraportally (ipv) infused N-nitro-L-arginine methyl ester (L-NAME, 1mg/kg) and this was followed by a RIST after 5mg/kg 3-morpholinosydnonimine (SIN-1) ipv. Results are means ± SEM. * P<0.05 from control.

BSO induced insulin resistance, which was enhanced by blockade of hepatic nitric oxide (NO) synthesis with L-NAME and not reversed by ipv administration of the NO donor, SIN-1.

Fig.5: Control Rapid Insulin Sensitivity Test (RIST) in the sham rats (n=6), followed by RISTs after 1mg/kg N-nitro-L-arginine methyl ester (L-NAME) intraportal (ipv) administration and after 5mg/kg 3-morpholinosydnonimine (SIN-1) ipv. Results are means ± SEM. *** P<0.001 from control. Insulin action is inhibited by L-NAME and restored after administration of the NO/O$_2^\cdot$ donor, SIN-1.

Fig.6: Hepatic reduced glutathione (GSH) concentrations in the sham group (n=6) and in the group treated with the GSH synthesis inhibitor L-buthionine-[S,R]-sulfoximine (BSO), at 2mmol/kg bw, 20 days, intraperitoneally administered (n=5). Values are means ± SEM. *** P<0.001. BSO decreases hepatic GSH levels.
FIGURE 1

![Graph showing RIST INDEX (mg glucose/kg) for different groups: CONTROL, L-NAME, SNP IFV. The graph includes error bars and asterisks indicating statistical significance.](image-url)
FIGURE 4

[Bar chart showing RIST INDEX (mg glucose/kg) for BSO, L-NAME, SIN-1, and IPV. Each bar is labeled with an asterisk (*) indicating a significant difference.]
FIGURE 5

Bar chart showing RIST index (mg glucose/kg) for different conditions: Control, L-NAME, and SIN-1 IFV. The chart indicates a significant difference between the groups, with the L-NAME group having a lower RIST index compared to the Control and SIN-1 IFV groups.
FIGURE 6

GSH (μmol/g fresh liver)

Control  BSO

***