Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats

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Running title: Glycine intake and sucrose-induced hypertension

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ABSTRACT

The study investigated the mechanism by which glycine protects against increased circulating nonesterified fatty acids (NEFA), fat cell size, intra-abdominal fat accumulation, and blood pressure (BP), induced in male Wistar rats by sucrose-ingestion. The addition of 1% glycine to the drinking water containing 30% sucrose, for 4 weeks, markedly reduced high BP in sucrose-fed rats (SFR) (122.3±5.6 vs. 147.6±5.4 mmHg in SFR without glycine, p<0.05). Decreases in plasma TG levels (0.9±0.2 vs. 1.4±0.3 mM, p<0.001), intra-abdominal fat (8.9±3.6 vs 21.2±4.9 g, p<0.005), and adipose cell size were observed in SFR treated with glycine, as compared to SFR without treatment. Total NEFA concentration in the plasma of SFR was significantly decreased by glycine intake (0.68±0.08 vs. 1.11±0.09 mM in SFR without glycine p<0.001). In control animals, glycine decreased glucose, TGs, and total NEFA but without reaching significance.

In SFR treated with glycine, mitochondrial respiration, as an indicator of the rate of fat oxidation, showed an increase in the state IV oxidation rate of the β-oxidation substrates, octanoic acid and palmitoyl-carnitine. This suggests an enhancement of hepatic fatty acids metabolism, i.e., in their transport, activation, or β-oxidation. These findings imply that the protection by glycine against elevated BP might be attributed to its effect in increasing fatty acid oxidation, reducing intra-abdominal fat accumulation and circulating NEFA, which have been proposed as links between obesity and hypertension.

Key words: Obesity; Hypertension; Mitochondrial oxygen uptake; fatty acid oxidation.
INTRODUCTION

Obesity is a significant human health problem; its incidence is reaching epidemic proportions in many Western countries (2, 31). Obesity with fat accumulation predominantly in the abdominal cavity is more frequently associated with disorders of glucose and lipid metabolism than is subcutaneous fat obesity (6, 17). It is well known that increased intra-abdominal fat accumulation is also associated with elevated circulating NEFA (33), resulting from the increased lipolytic activity in adipose tissue. High levels of circulating NEFA have been assumed to be a possible link between intra-abdominal fat accumulation and elevated blood pressure (9, 34, 49).

Several factors are known to be involved in the development of intra-abdominal adiposity in both humans and animals, including genetic and environmental factors, such as excessive fat or carbohydrates intake and lack of physical exercise (8, 49). Thus, several animal models of obesity have been developed to investigate the mechanism by which obesity induces hypertension, hyperinsulinemia, and insulin resistance.

In our laboratory, we developed an animal model of intra-abdominal fat accumulation, induced by the addition of sucrose to the drinking water of Wistar rats (1, 18), a variant of the fructose-induced hypertriglyceridemia and hypertension animal model (26, 41). Intra-abdominal fat accumulation in this model is associated with the development of high blood pressure, as described elsewhere (29).

On the other hand, glycine, a non-essential amino acid, when administered in the diet, has been shown to be protective against the non-enzymatic hemoglobin glycation found in diabetic humans and in streptozotocin-induced diabetic rat models (12, 13). Another group has attributed the lowering effect on plasma cholesterol and triglycerides (TGs) of gelatin, as compared to casein, to its high glycine content (41). This group showed that gelatin, which contains 12-times
more glycine than casein, decreases plasma cholesterol and TGs when administered in the diet of hypercholesterolemic rats.

The purpose of this study was to investigate the mechanism by which glycine protects against fat accumulation, hypertriglyceridemia, and high NEFA levels induced by a high sucrose concentration in the drinking water of rats.

**EXPERIMENTAL PROCEDURES**

*Animals and their treatment.* Experiments in animals were approved by the Laboratory Animal Care Committee of our institution and were conducted in compliance with our institution’s ethical guidelines for animal research.

Male Wistar rats aged 28 days and weighing approximately 45 ± 2 g were housed in individual metabolic cages (Nalgene, NY, USA), under controlled temperature and a 12 hour light-dark cycle. They were randomly separated into two groups of twenty animals each: control and experimental. The control group (C) received tap water. The experimental group (SFR) received 30% commercially refined sucrose in their drinking water during a 20-weeks period. All animals were fed Purina 5001 rat chow (Richmond, IN. U.S.A.) *ad-libitum*, which provides 14.63 kJ/g, with 23% protein, 12% fat, and 65% carbohydrate.

After 20 weeks, the period necessary for the SFR to develop a significantly higher blood pressure than control animals, each group was divided into two subgroups. The first subgroup (C) continued to drink water (n=10), the second subgroup (CG) received water supplemented with 1% glycine (n=10). In parallel, the group that had received sucrose in its drinking water was also divided into two subgroups: one subgroup (SFR) continued to receive 30% sucrose in its drinking water (n=10) and the other subgroup (SFRG) received sucrose supplemented with 1% glycine (n=10). Hence, glycine and sucrose treatments were continued for 4 more weeks. The food (g
day
superscript(-1) rat
superscript(-1)) and water (ml day
superscript(-1) rat
superscript(-1)) intake was monitored every 2 days during the experimental period. The individual caloric intake (kJ day
superscript(-1) rat
superscript(-1)) was assessed from the amount of food and sucrose ingested.

At the end of each week, during glycine treatment, systolic arterial pressure (BP) was measured by connecting the tail-cuff to a pneumatic pulse transducer and a programmed electrosphygmonanometer (Narco Bio-systems, TX) as described previously (39). Recordings were made in triplicate by means of a Grass polygraph (Grass Medical Instruments, Quincy, MA).

**Plasma and tissue sampling.** After overnight fasting, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (63 mg/kg body weight). Blood was collected from the aorta in a tube containing EDTA (0.1%) and centrifuged immediately at 600 $x$ g during 20 min at 4°C. The obtained plasma, to which 0.005% butylated hydroxy toluene (BHT) had been added as antioxidant, was stored at -70°C until needed for lipid analysis.

The liver was perfused with 50 ml of cold buffer containing 250 mM sucrose, 10 mM Tris, 1 mM EGTA (pH 7.4, adjusted with KOH), homogenized in the same buffer, and kept on ice. The homogenate was centrifuged at 600 $x$ g for 5 min at 4°C and the supernatant was stored at -70°C until required for lipid analysis.

Plasma triglycerides (TGs) concentration was measured according to the method described by Nägele et al. (37). Plasma insulin was determined through a radioimmunoassay (Coat-a-Count, Diagnostic Products, Los Angeles, CA). Intra-abdominal fat was dissected according to the method described by Belzung et al. (3).

**Adipose tissue histology.** A portion of omental fat pad was rinsed in 0.9% NaCl solution and then placed in a solution of 10% phosphate-buffered formalin. The adipose tissue samples were prepared for sectioning and staining with hematoxylin-eosin and Masson trichrome as described
elsewhere (4). Sections were sliced at a thickness of 3 µm and fixed on slides. The samples were then examined under a light microscope (Olympus BX51), equipped with a digital camera CoolSNAP-Pro to obtain images to determine cell size. From the images taken from the slides, 100 cells per µm² were used to calculate the mean cell area by means of the Image-Pro-plus (version 4.0) software. Fat cells volume and number were calculated using the relationship between diameter and volume as described in detail by Commerford et al. (15).

**Lipid extraction and analysis of nonesterified fatty acids (NEFA) composition.** NEFA were extracted from 100 µl plasma or from 10 mg protein of liver homogenate, in the presence of 10 µg of heptadecanoic acid (internal standard) as described by Folch et al. (22). The obtained lipid residue was dissolved in 1 ml methanol and NEFA were esterified to their corresponding methyl esters as described by Tserng et al. (50) and modified by McClelland et al. (37). The concentration and composition of NEFA were evaluated by gas liquid chromatography (GLC) as described previously (19).

**Mitochondria isolation.** Mitochondria were isolated from the liver. The tissue was homogenized in 20-35 ml cold buffer (250 mM sucrose, 10 mM Tris, 1 mM EGTA, pH 7.4, adjusted with KOH) and kept on ice. The homogenate was centrifuged at 600 x g for 5 min at 4°C. The pellet was discarded, and the supernatant was centrifuged at 8000 x g for 10 min at 4°C. The mitochondrial pellet was washed with a buffer containing 0.1% fatty acid-free bovine serum albumin (BSA) and finally re-suspended in the buffer without BSA to contain 40-60 mg/ml protein.

Protein determination was performed using the method of Lowry et al. (32) and BSA as a standard for the calibration curve.

**Mitochondrial respiration.** Mitochondrial oxygen uptake was measured polarographically at
37°C with a Clark-type oxygen electrode (Yellow Springs, OH, U.S.A.). The medium (1.5 ml) contained 130 mM KCl, 25 mM Hepes, 0.1 mM EGTA, 3 mM MgCl₂, and 10 mM KH₂PO₄ (pH 7.4, adjusted with KOH). Respiratory rate was measured in the presence of 2 mM malate (to measure rates of flux through β-oxidation and the citric acid cycle) and 2 mM ADP. Oxygen uptake was monitored with 1 mM glutamate, 20 µM octanoate, or 20 µM L-palmitoyl-carnitine. State III respiration was always initiated by adding ADP 2 min after pre-incubating mitochondria. State IV oxygen consumption was determined in the presence of the ATP synthase specific inhibitor, oligomycin (8 µg/mg protein).

Statistical analysis. Statistical analysis was performed with the SPSS statistical software. Data are expressed as mean ± SD. Statistical significance was assessed by using one-way ANOVA test. Differences were considered statistically significant at p<0.05. Pearson correlation analysis was used to examine the relationship between NEFA and BP.

**RESULTS**

*Energy intake and liquid and food consumption.* During glycine treatment no difference was found in the amount of liquid consumption among groups (Fig 1A). In contrast, SFR ingested less solid food than control animals and the difference remained significant throughout the remainder of the study (Fig 1B). Food intake was not affected by glycine treatment in either controls or SFR. No significant difference in energy intake was observed among groups (Fig 1C). At the end of the 20 weeks of sucrose ingestion (time 0 of the glycine treatment period) the body weight did not differ between SFR and C and remained unaffected during the 4 weeks of glycine treatment (Fig 1D).

*Blood pressure.* At time 0, BP was significantly higher in SFR as compared with control
animals and this increase was maintained in SFR during the additional four weeks of the experimental period (Fig 2). In contrast, SFRG showed a significant decrease in BP starting at the second week and continuing during the rest of the experimental period. On the fourth week, SFRG had normal BP as compared with control animals with and without glycine treatment.

**Fasting plasma glucose, TGs, and insulin.** Fasting plasma glucose levels were not significantly different between the groups. Fasting plasma TGs and insulin were significantly higher in SFR (p<0.001 and p<0.01, respectively) than in control animals, as described previously (19). Glycine treatment of SFR significantly reduced insulin and TGs to the normal levels found in control animals (Table 1). In control animals, glycine decreased plasma TGs significantly (P<0.01) and the decrease of insulin levels did not reach a significant difference.

**Intra-abdominal fat accumulation and morphology.** A significant increase of intra-abdominal fat accumulation (p<0.01) was observed in SFR as compared with control groups. Intra-abdominal fat accumulation significantly decreased (p<0.01) in the SFRG (Table 1). Histological analysis of the fat pads revealed that adipose cells from SFR were significantly larger than those of control animals (504 ± 54 vs. 210 ± 67 pL, P<0.001). The mean adipocyte volume increased 140% as compared with control animals (Fig 3A). In contrast, SFRG showed a significant decrease (48%) in cell volume (264 ± 56 vs. 504 ± 54 pL, p<0.01). The mean cell number per total fat pad from SFR was not statistically different from control animals (29 ± 5 vs. 22 ± 8 x10^6 cells per total fat pad) and was not significantly affected by glycine treatment (Fig. 3B).

**Nonesterified fatty acids composition.** Plasma NEFA concentration was significantly increased (p<0.001) in SFR as compared with the control group (Table 2). SFRG showed a significant decrease in the concentration of total NEFA (p<0.001). In control animals, glycine intake tended to reduce plasma NEFA concentrations but did not reach a statistical difference.

As shown in Table 2, sucrose ingestion also induced a significant alteration in the composition
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of plasma NEFA. Significant increases of palmitic (p<0.05), palmitoleic (p<0.01), and oleic (p<0.001) acids and a decrease in linoleic (p<0.01) and arachidonic acid (P<0.05) amounts were observed in SFR while stearic acid were not significantly changed in SFR. Glycine significantly reduced the amount of palmitic, palmitoleic, and oleic acids in SFR, reaching normal values as those found in control animals. Glycine intake also reduced the concentration of linoleic acid and did not affect the concentration of arachidonic acid in SFRG. In CG a significant decrease was noted only in oleic acid concentration (p<0.05).

Table 3 reveals a significant increase in the concentration of total NEFA (p<0.001) in the liver homogenate from SFR as compared with the control group. In both SFRG and CG a significant diminution in the quantity of NEFA was observed.

Composition of NEFA in the liver homogenate is shown in Table 3. A significant increase in the proportion of palmitoleic (p<0.05) and oleic acids (p<0.01) was found in SFR. An increase in the amount of palmitic acid was observed but did not reach statistical significance, whereas no change was detected in the amounts of polyunsaturated fatty acids. Glycine added to the drinking water of the SFR significantly reduced the amount of all the identified fatty acids. In the livers from control animals, glycine intake significantly decreased the amount of palmitic (p<0.05), linoleic (p<0.01), and arachidonic (p<0.001) acids but did not change the concentration of stearic, palmitoleic, and oleic acids.

Mitochondrial respiration as an indicator of the rate of fatty acid oxidation. As shown in Table 3, hepatic fatty acid metabolism is altered because of NEFA accumulation in the livers from SFR rats, suggesting an alteration in the activation, transport, and β-oxidation of fatty acids or in the activity of the respiratory chain. We therefore investigated the oxidative metabolism of different substrates by using isolated liver mitochondria. State III oxidation rate with glutamate as
substrate was significantly increased in SFR (p<0.005) as compared with control animals, whereas state IV oxidation rate of L-glutamate was no different in SFR from that in control animals (Table 4). In the same way, state III oxidation rate of octanoic acid was significantly increased in SFR (p<0.05) whereas the oxidation rate of palmitoyl-carnitine was significantly decreased in SFR (p<0.01). The O$_2$ uptake by mitochondria during state IV oxidation of both palmitoyl-carnitine and octanoic acid was not affected by the sucrose diet. Nevertheless, treatment of SFR with glycine significantly increased state III oxidation rate of glutamate (p<0.01). Using octanoic acid or palmitoyl-carnitine as a β-oxidation substrates, states III and IV oxidation rates were significantly increased in SFRG. In CG, a significant increase of state IV rate oxidation of both octanoic acid (p<0.05) and L-palmitoyl-carnitine (p<0.05) was observed.

**DISCUSSION**

Glycine added to the diet has been described to protect against some pathological conditions such as oxidative stress and endotoxin-induced inflammation (11, 27, 45). In this work we found that glycine addition to a sucrose-diet reverted the elevation of plasma TG levels and reduced intra-abdominal fat accumulation and high BP induced in rats by the high sucrose ingestion. In control animals, glycine intake did not affect significantly these variables.

The high blood pressure induced by the ingestion of the high sucrose diet is in part associated with the high accumulation of intra-abdominal fat involved in an increased release of NEFA, which in turn is due to the probably increased lipolytic activity in this adipose tissue. The accumulation of intra-abdominal fat in SFR did not induce an increase of body weight. The lack of weight difference between the two groups can be explained based on the lack of difference in their energy intake. In this study, body composition was not examined in detail and we do not
know whether sucrose administration resulted in an increased content of adipose tissue at the expense of other tissues. The SFR consumed about half of the amount of chow consumed by the animals not given sucrose; consequently, the availability of nutrients from the solid food was lower. Thus, a lower energy intake by SFR was compensated by additional calories from the sucrose solution.

Sucrose added to the drinking water of the animals increased the level of circulating NEFA, which were reduced to their normal levels by glycine addition. This decrease in circulating NEFA was associated with a decrease in adipocyte cell volume. Enlarged adipocyte cells are associated with an increased rate of lipolysis (4) and the resulting increased plasma NEFA have been postulated to be responsible for the development of high BP (23, 28). Indeed, a significant correlation between NEFA and BP was found in this study (Fig 4) as described for men in the Paris Prospective Study (20).

At present no data are available showing direct evidences that increasing NEFA increases BP chronically. Nevertheless, experimental studies in humans and in animals support a relationship between NEFA and hypertension. In humans, the acute action of intralipid/heparin infusion to raise BP suggests the involvement of NEFA in enhancing $\alpha_1$-adrenoreceptor-mediated pressor sensitivity (25). In pigs, BP rises when plasma NEFA are elevated during an acute infusion of intralipid/heparin (10). Portal venous infusions of oleate in rats induce a pressor response mediated by $\alpha_1$-adrenoceptors (24). Obesity-induced hypertension in dogs is blocked by the centrally acting sympatholytic clonidine, and combined $\alpha$- and $\beta$-adrenoceptor blockade (42). In vitro, oleic acid impairs endothelial cell nitric oxide synthase and contributes to impaired endothelium-dependent vasodilation (16). The lowering effect of glycine on the concentration of mono-unsaturated fatty acids, such as oleic acid, may be related to the lowering effect on BP in
the SFRG. The increased palmitoleic and oleic acid concentrations in plasma and liver from SFR reflect an increase of the Δ9-desaturase activity as described previously (19). The sucrose diet reduced the amount of linoleic acid, a precursor of arachidonic acid. The latter has been found to be decreased in membrane phospholipids of vascular cells of SFR (18) and in spontaneously hypertensive rats (38). It could be a determining factor that may account for an altered synthesis of the derived eicosanoids involved in the regulation of BP in the SFR.

Lipid abnormalities associated with insulin resistance also contribute to elevated BP (47). Our SFR presented a high concentration of fasting insulin that was reduced to a normal level by glycine treatment. Hyperinsulinemia may reflect insulin resistance, which was not investigated in this study. Indeed, lipid infusion in humans (7) and rats (30) gives rise to insulin resistance in muscle. Furthermore, genetically obese Zucker rats have elevated plasma levels of free fatty acids and show marked muscle insulin resistance (5); thus, the combination of hyperinsulinemia and elevated NEFA may contribute to the development of high BP in SFR.

As in plasma, NEFA were found increased in liver homogenate, probably due to the higher mobilization of NEFA from adipose tissue to the liver, where fatty acid oxidation is probably repressed. The accumulation of NEFA in the liver from SFR rats suggests alteration in hepatic fatty acid metabolism pathways, such as activation, transport, or β-oxidation of fatty acids.

The lowering effect of glycine on plasma TGs and liver NEFA in SFRG suggests that glycine may act at the level of either TGs biosynthesis or fatty acids degradation. As a first step, we investigated oxidative metabolism of different substrates by using isolated liver mitochondria. Thus, O2 uptake through glutamate oxidation in state III was increased in mitochondria from SFR as compared with control animals, indicating a probable activation of the substrate transport or increased activity of the enzymes of the Krebs cycle, as well as enhanced oxidative
phosphorylation. In contrast, $O_2$ uptake through the oxidation of L-palmitoyl carnitine in state III was lower in mitochondria from SFR and larger through octanoic acid oxidation in both states III and IV. This indicates that the diffusion of octanoic acid through the inner membrane and its activation by CoA within the matrix, before entering the $\beta$-oxidation cycle, was not affected by sucrose feeding. In contrast, diminution of $O_2$ uptake through the oxidation of palmitoyl-carnitine (which is acyl-carnitine translocase-dependent for crossing the inner membrane) may be due to altered activity of this enzyme, responsible for the transport of acyl moieties, or of the carnitine-palmitoyl transferase II (CPT II). The rate of palmitoyl-carnitine oxidation was lower than that of octanoic acid, suggesting that CPTI and II activities might constitute a limiting step in the course of fatty acid oxidation in mitochondria from SFR. It is well known that CPTI is an enzyme regulated by malonyl-CoA, which might be increased in the SFR, and is a specific inhibitor of the enzyme and an intermediate substrate of fatty acid biosynthesis. Although CPTI activity was not directly measured in this study, an increased level of circulating TGs reflects a higher activity of fatty acid biosynthesis that could be associated with the inhibition of CPT I (36).

In the presence of glutamate, ADP-stimulated respiration (state III) of mitochondria obtained from SFRG was higher as compared with mitochondria from SFR, suggesting that glycine intake probably induced changes in the transport activity of the substrate or altered the activity of the mitochondrial respiratory chain. In addition, an increase in the rate of $O_2$ uptake through the oxidation of both octanoic and palmitoyl-carnitine in state III was observed in SFRG.

Our data on liver mitochondria oxygen consumption in the presence of $\beta$-oxidation substrates, such as palmitoyl-carnitine and octanoic acid, suggest that one of the possible mechanisms involved in the lowering effect of glycine on circulating NEFA and fat accumulation is the increased rate of oxidation of fatty acids by liver mitochondria. The increased fatty acids
oxidation rate may therefore account for the reduced TG formation, and can ultimately explain the anti-adiposity effect observed after glycine administration through the diet.

The mechanism by which glycine stimulates O$_2$ uptake during fatty acid oxidation in the liver mitochondria may implicate several fatty acid metabolizing enzymes, such as CPT-I and II, mitochondrial β-oxidation enzymes, ketogenesis enzymes, and ω-oxidation enzymes, which can be induced by glycine intake and increase the capacity for fatty acid oxidation. These pathways are under investigation in our laboratory.

In conclusion, this work attempts to describe the possible mechanism by which glycine decreased BP, i.e., reducing adipose tissue accumulation by stimulating fatty acids oxidation. This in turn decreased NEFA concentration, which has been postulated to be a link between obesity and hypertension. However, other possible mechanisms exist by which glycine could reduce BP in SFR. Glycine, as a prominent neurotransmitter in the reflex control of cardiovascular activity, can act at the level of the sympathetic nervous system to modulate heart rate and blood pressure as described by Talman et al. (48). These authors showed that the microinjection of glycine in the nucleus tractus solitarii increases acetylcholine release reducing high blood pressure and heart rate (14). Thus, glycine could also decrease blood pressure in SFR by affecting the sympathetic nervous system, modulating lipid mobilization from adipose tissue that is regulated by cathecolamines, the most potent regulators of lipolysis in human fat cells (21). On the other hand, glycine added to the diet decreases oxidative stress (49) and might increase nitric oxide availability.

Finally, glycine has several beneficial effects that would justify its clinical use: it can be administered in the diet without apparent side-effects, as suggested by Rosse et al. (43) and Carvajal-Sandoval et al. (13).
ACKNOWLEDGMENTS

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48. Talman WT and SC Robertson. Glycine, like glutamate, microinjected into the nucleus


FIGURE LEGENDS

Fig 1. Values correspond to the mean ± SD from ten different animals (n=10). SFR (●), SFRG (○), C (■), CG (□).

A. Liquid consumption by SFR and control rats, during a period of treatment with glycine. Results correspond to the value of liquid consumption in ml day⁻¹ rats⁻¹.

B. Food intake by rats, during experimental period. Results corresponds to the value of solid ingested by animals in mg day⁻¹ rats⁻¹.

C. Animal energy intake in KJ day⁻¹ animal⁻¹.

D. Body weight in g.

* Significantly different from C (P<0.01).

** Significantly different from SFR (P<0.001).

Fig 2. Blood pressure in SFR (●), SFRG (○), C (■), CG (□). Data are expressed as mean ± SD (n = 10 different animals). * significantly different from C (P<0.01). ** significantly different from SFR (P<0.001).

Fig 3. Adipocyte size (3A) and number (3B): Effect of sucrose diet and glycine treatment. Values corresponded to the mean ± SD (n = 6 per group).

* Significantly different from C (P<0.001).

‡ Significantly different from SFR (P< 0.001).

Fig 4. Relationship between NEFA and BP. Data represents the combination of all SFR and control animals with and without glycine treatment. Pearson correlation was applied and it was significant at P<0.01. Correlation coefficient was r=0.74.
Table 1. General Characteristics of Animals

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CG</th>
<th>SFR</th>
<th>SFRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-abdominal fat (g)</td>
<td>4.3 ± 0.8</td>
<td>4.5 ± 0.6</td>
<td>14.8 ± 4.0**</td>
<td>6.8 ± 2.16‡‡‡</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.1§</td>
<td>1.4 ± 0.3***</td>
<td>0.9 ± 0.3‡</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>6.9 ± 2.1</td>
<td>6.2 ± 0.9</td>
<td>6.2 ± 0.9</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>50.36 ± 5.45</td>
<td>42.12 ± 8.9</td>
<td>71.43 ± 6.9***</td>
<td>36.51 ± 9.50‡‡‡</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD (n = 8 different animals). The values for all variables were obtained at the end of the treatment period. Triglycerides and glucose were determined in plasma. C, control. CG control with glycine. SFR, sucrose-fed rats. SFRG, sucrose-fed rat with glycine

** significantly different from C (P<0.01), *** P<0.001.

‡ Significantly different from SFR (P<0.05), ‡‡ P<0.01.

§ Significantly different from C (P<0.05).
Table 2. NEFA concentration in the Plasma From SFR and Control Rats With or Without Glycine

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>C</th>
<th>CG</th>
<th>SFR</th>
<th>SFRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>0.22 ± 0.02</td>
<td>0.21 ± 0.06</td>
<td>0.39 ± 0.06*</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.18 ± 0.06**</td>
<td>0.064 ± 0.002‡</td>
</tr>
<tr>
<td>Stearic</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01‡</td>
</tr>
<tr>
<td>Oleic</td>
<td>0.15 ± 0.02</td>
<td>0.09 ± 0.03 §</td>
<td>0.32 ± 0.06***</td>
<td>0.17 ± 0.04‡‡</td>
</tr>
<tr>
<td>Linoleic</td>
<td>0.14 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.02**</td>
<td>0.06 ± 0.01‡‡</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.002</td>
<td>0.05 ± 0.01*</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.68 ± 0.07</td>
<td>0.57 ± 0.11</td>
<td>1.11 ± 0.09***</td>
<td>0.64 ± 0.08‡‡</td>
</tr>
</tbody>
</table>

Data represent the concentration in mM (mean ± SD; n = 8).

*** Significantly different from C (P<0.001); ** (P<0.01); * (P<0.05)

‡‡ Significantly different from SFR (P<0.001); ‡ (P<0.05).

§ Significantly different from C (P<0.05).
Table 3. NEFA concentration in the Liver From SFR and Control Rats With or Without Glycine

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>C</th>
<th>CG</th>
<th>SFR</th>
<th>SFRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>29.8 ± 15.3</td>
<td>18.4 ± 3.7</td>
<td>35.8 ± 6.1</td>
<td>20.3 ± 9.1^‡</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>0.6 ± 0.7</td>
<td>0.5 ± 0.2</td>
<td>6.3 ± 2.1**</td>
<td>7.5 ± 8.7</td>
</tr>
<tr>
<td>Stearic</td>
<td>12.2 ± 3.8</td>
<td>9.3 ± 1.7 §</td>
<td>13.3 ± 2.4</td>
<td>7.4 ± 3.2^§</td>
</tr>
<tr>
<td>Oleic</td>
<td>11.1 ± 6.5</td>
<td>7.6 ± 2.5</td>
<td>32.1 ± 8.5***</td>
<td>16.7 ± 8.7‡‡</td>
</tr>
<tr>
<td>Linoleic</td>
<td>14.3 ± 8.4</td>
<td>9.4 ± 6.2</td>
<td>17.9 ± 6.4</td>
<td>7.9 ± 5.5^‡‡</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>10.2 ± 5.6</td>
<td>6.2 ± 1.6§§</td>
<td>11.9 ± 3.7</td>
<td>3.5 ± 3.7^‡‡‡</td>
</tr>
<tr>
<td>Total</td>
<td>78.5 ± 11.4</td>
<td>51.8 ± 10.9§</td>
<td>117.5 ± 27.6***</td>
<td>62.5 ± 31.7‡</td>
</tr>
</tbody>
</table>

Data represent the concentration in µmol/mg protein (mean ± SD; n = 8).

*** Significantly different from C (P<0.001) and ** (P<0.01), * (P<0.05),

‡‡‡ Significantly different from SFR (P<0.01), ‡‡ (P<0.01), ‡ (P<0.05),

§§ Significantly different from C (P<0.01), § (P<0.05).
### Table 4. *Mitochondrial respiration*

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>CG</th>
<th>SFR</th>
<th>SFRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malate + Palmitoyl State III</td>
<td>89 ± 10</td>
<td>87 ± 9</td>
<td>75 ± 7‡‡</td>
<td>104 ± 17 *</td>
</tr>
<tr>
<td>Malate + Palmitoyl State IV</td>
<td>13 ± 3</td>
<td>26 ± 9</td>
<td>14 ± 2</td>
<td>18 ± 2 *</td>
</tr>
<tr>
<td>Malate + octanoate state III</td>
<td>61 ± 12</td>
<td>66 ± 4</td>
<td>75 ± 11‡</td>
<td>87 ± 14</td>
</tr>
<tr>
<td>Malate + octanoate state IV</td>
<td>13 ± 3</td>
<td>22 ± 2 §§</td>
<td>14 ± 2</td>
<td>20 ± 3 *</td>
</tr>
<tr>
<td>Malate + glutamate state III</td>
<td>87 ± 19</td>
<td>73 ± 27</td>
<td>123 ± 13 ‡‡‡</td>
<td>138 ± 22 **</td>
</tr>
<tr>
<td>Malate + glutamate state IV</td>
<td>15 ± 2</td>
<td>14 ± 3</td>
<td>14 ± 4</td>
<td>19 ± 3 **</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD of n = 5 different mitochondrial preparations. State III and IV are expressed in nmol O₂ min⁻¹ mg⁻¹ of mitochondrial protein. Final substrate concentration used: 20 µM palmitoyl-carnitine, 20 µM octanoate, 2 mM glutamate. Malate (2mM) was also added to all incubation.

*** Significantly different from SFR (P<0.005) and ** (P<0.01), * (P<0.05), ‡‡‡ Significantly different from C (P<0.001). ‡‡ (P<0.01), ‡ (P<0.05), §§ Significantly different from C (P<0.01). § (P<0.02).
GLYCINE INTAKE AND SUCROSE-INDUCED HYPERTENSION

Fig 1

A. Liquid consumption (ml) over Days

B. Food intake (g) over Days

C. Caloric intake (KJ/day/rat) over Days

D. Body weight (g) over Weeks

* indicates significant difference.
Fig 2

Weeks

Blood Pressure (mmHg)

100
110
120
130
140
150
160

0 1 2 3 4 5

Weeks
Fig. 3

A. Cell volume (pL) for different groups: SFRG, SFR, CG, C.

B. Cell number (x10^6/total fat pad) for different groups: SFRG, SFR, CG, C.

* ‡
Fig. 4

![Graph showing the relationship between NEFA (mM) and Blood Pressure (mm Hg).]