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

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El Hafidi, Mohammed, Israel Pérez, Jose Zamora, Virgilia Soto, Guillermo Carvajal-Sandoval, and Guadalupe Baños. Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. Am J Physiol Regul Integr Comp Physiol 287: R1387–R1393, 2004. First published August 26, 2004; doi:10.1152/ajpregu.00159.2004.—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 wk, markedly reduced high BP in sucrose-fed rats (SFR) (122.3 ± 5.6 vs. 147.6 ± 5.4 mmHg in SFR without glycine, P < 0.001). Decreases in plasma triglyceride (TG) levels (0.9 ± 0.3 vs. 1.4 ± 0.3 mM, P < 0.001), intra-abdominal fat (6.8 ± 2.16 vs. 14.8 ± 4.0 g, P < 0.01), and adipose cell size were observed in SFR treated with glycine compared with SFR without treatment. Total NEFA concentration in the plasma of SFR was significantly decreased by glycine intake (0.64 ± 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 acid 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.

Obesity; mitochondrial oxygen uptake; fatty acid oxidation

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 carbohydrate intake and lack of physical exercise (8, 44). 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, 19), 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 BP, as described elsewhere (29).

On the other hand, glycine, a nonessential amino acid, when administered in the diet, has been shown to be protective against the nonenzymatic 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, compared with casein, to its high glycine content (40). 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 ~45 ± 2 g were housed in individual metabolic cages (Nalgene, NY) under controlled temperature and a 12:12-h light-dark cycle. They were randomly separated into two groups of 20 animals each: control and experimental. The control group (C) received tap water. The experimental group [sucrose-fed rats (SFR)] received 30% commercially refined sucrose in their drinking water during a 20-wk period. All animals were fed Purina 5001 rat chow (Richmond, IN) ad libitum, which provides 14.63 kJ/g; with 23% protein, 12% fat, and 65% carbohydrate.

After 20 wk, the period necessary for the SFR to develop a significantly higher BP 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⁻¹·rat⁻¹) and water (ml·day⁻¹·rat⁻¹) intake was monitored every 2 days during the experimental period. The individual caloric intake (kJ·day⁻¹·rat⁻¹) was assessed from the amount of food and sucrose ingested.

At the end of each week, during glycine treatment, systolic arterial BP was measured by connecting the tail cuff to a pneumatic pulse transducer and a programmed electroesphygmonanometer (Narco Biosystems) 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 pentobarbital sodium (63 mg/kg body wt). Blood was collected from the aorta in a tube containing EDTA (0.1%) and centrifuged immediately at 600 g during 20 min at 4°C. The obtained plasma, to which 0.005% butylated hydroxytoluene (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 g for 5 min at 4°C, and the supernatant was stored at −70°C until required for lipid analysis.

Plasma TG 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/μm² were used to calculate the mean cell area by means of the Image-Pro-plus (version 4.0) software. Fat cell 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 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 Bolch 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 Tseng et al. (50) and modified by McClelland et al. (35). The concentration and composition of NEFA were evaluated by gas-liquid chromatography as described previously (18).

**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 g for 5 min at 4°C. The pellet was discarded, and the supernatant was centrifuged at 8,000 g for 10 min at 4°C. The mitochondrial pellet was washed with a buffer containing 0.1% fatty acid-free BSA and finally resuspended 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). 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 preincubating mitochondria. State IV oxygen consumption was determined in the presence of the ATP synthase-specific inhibitor oligomycin (8 μg/ml protein).

**Statistical analysis.** Statistical analysis was performed with the SPSS statistical software. Data are expressed as means ± 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 wk of sucrose ingestion (time 0 of the glycine treatment period), the body weight did not differ between SFR and C rats and remained unaffected during the 4 wk of glycine treatment (Fig. 1D).

**BP.** At time 0, BP was significantly higher in SFR compared with control animals, and this increase was maintained in SFR during the additional 4 wk 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 compared with control animals with and without glycine treatment.

**Fasting plasma glucose, TGs, and insulin.** Fasting plasma levels were not significantly different between the groups. Fasting plasma TGs and insulin were significantly higher in SFR (P < 0.001 and P < 0.001, respectively) than in control animals, as described previously (18). 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 < 0.05) and decreased insulin levels without reaching a significant difference.

**Intra-abdominal fat accumulation and morphology.** A significant increase of intra-abdominal fat accumulation (P < 0.01) was observed in SFR 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% compared with control animals (Fig. 3A). In contrast, SFRG showed a significant decrease (48%) in cell volume (264 ± 56 vs. 504 ± 54 pl, P < 0.001). The mean cell number per total fat pad from SFR was not statistically different from control animals (29 ± 5 vs. 22 ± 8 × 10⁶ cells per total fat pad) and was not significantly affected by glycine treatment (Fig. 3B).
NEFA composition. Plasma NEFA concentration was significantly increased \((P < 0.001)\) in SFR 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 of plasma NEFA. Significant increases of palmitic \((P < 0.001)\), 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, whereas stearic acid was 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 rats, 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 compared with the control group. In both SFRG and CG rats, 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 \((P < 0.01)\) and oleic acids \((P < 0.001)\) 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 except for palmitoleic acid. In the livers from control animals, glycine intake significantly decreased the amount of stearic \((P < 0.05)\) and arachidonic \((P < 0.01)\) acids. Glycine also reduced the amount of arachidonic acid.

![Figure 1](http://ajpregu.physiology.org/)

**Values correspond to means ± SD from 10 different animals \((n = 10)\).** SFR: sucrose-fed rats; SFRG: sucrose-fed rats treated with glycine; CG: control rats treated with sucrose; C: control rats treated with glycine.

**Significantly different from SFR: \((P < 0.05)\);** significantly different from C: \((P < 0.05)\); **Significantly different from SFRG: \((P < 0.01)\);** significantly different from CG: \((P < 0.01)\).
of palmitic, oleic, and linoleic acids without reaching a statistically significant difference and did not affect the concentration of palmitoleic acid.

**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 < 0.001) 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 O2 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 < 0.001). Using octanoic acid or palmitoyl carnitine as a β-oxidation substrate, states III and IV oxidation rates were significantly increased in SFRG. In CG rats, a significant increase of state IV oxidation rate of both octanoic acid (P < 0.01) and 1-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, 52). 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 BP 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 rats, suggesting an alteration in the activation, metabolism is altered because of NEFA accumulation in the livers from SFR rats, indicating 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 < 0.001) 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 O2 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 < 0.001). Using octanoic acid or palmitoyl carnitine as a β-oxidation substrate, states III and IV oxidation rates were significantly increased in SFRG. In CG rats, a significant increase of state IV oxidation rate of both octanoic acid (P < 0.01) and 1-palmitoyl carnitine (P < 0.05) was observed.

**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>
</tbody>
</table>

Data represent the concentration in µmol/mg protein (means ± SD; n = 8). Significantly different, CG vs. C: *P < 0.05, †P < 0.01. Significantly different, SFR vs. C: ‡P < 0.01, §P < 0.001. Significantly different, SFRG vs. SFR: *P < 0.01, †P < 0.001.

**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 carnitine state III</td>
<td>89±10</td>
<td>87±9</td>
<td>75±7‡</td>
<td>104±17‡</td>
</tr>
<tr>
<td>Malate + palmitoyl carnitine 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±2b</td>
<td>14±2</td>
<td>20±3‡</td>
</tr>
<tr>
<td>Malate + glucose state III</td>
<td>87±19</td>
<td>73±27</td>
<td>123±13a</td>
<td>138±22b</td>
</tr>
<tr>
<td>Malate + glucose state IV</td>
<td>15±2</td>
<td>14±3</td>
<td>14±2</td>
<td>19±3‡</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD of n = 5–7 different mitochondrial preparations. State III and IV are expressed in nmol. O2 uptake by mitochondria during state III oxidation rate of glutamate was significantly increased in SFRG. In CG rats, a significant increase of state IV oxidation rate of both octanoic acid (P < 0.01) and 1-palmitoyl carnitine (P < 0.05) was observed.

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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, 52). 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 BP 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 rats, suggesting an alteration in the activation, metabolism is altered because of NEFA accumulation in the livers from SFR rats, indicating 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 < 0.001) 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 O2 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 < 0.001). Using octanoic acid or palmitoyl carnitine as a β-oxidation substrate, states III and IV oxidation rates were significantly increased in SFRG. In CG rats, a significant increase of state IV oxidation rate of both octanoic acid (P < 0.01) and 1-palmitoyl carnitine (P < 0.05) was observed.

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The high BP 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 high BP (23, 28). Indeed, a significant correlation between NEFA and BP was found in this study (Fig. 4) as described for high BP (23, 28).

At present no data are available showing direct evidence 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 acute action of Intralipid/heparin infusion to raise BP. 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 TG biosynthesis or fatty acid 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 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, O2 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 β-oxidation cycle, was not affected by sucrose feeding. In contrast, diminution of O2 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 (CPT) II. The rate of palmitoyl carnitine oxidation was lower than that of octanoic acid, suggesting that CPT I and II activities might constitute a limiting step in the course of fatty acid oxidation in mitochondria from SFR. It is well known that CPT I 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 CPT I 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 compared with mitochondria from SFR, suggesting that glycine intake probably induced changes in the transport activity of the Δ9-desaturase activity as described previously (18). 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 (19) 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.

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In the presence of glutamate, ADP-stimulated respiration (state III) of mitochondria obtained from SFRG was higher compared with mitochondria from SFR, suggesting that glycine intake probably induced changes in the transport activity

Fig. 4. Relationship between nonesterified fatty acids (NEFA) and BP. Data represent 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.
of the substrate or altered the activity of the mitochondrial respiratory chain. In addition, an increase in the rate of O\textsubscript{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 acid oxidation rate may therefore account for the reduced TG formation and ketogenesis, thus increasing fatty acid oxidation and fat accumulation is the increased rate of oxidation of fatty acids in liver mitochondria. 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, i.e., reducing adipose tissue accumulation by stimulating fatty acid oxidation.

In conclusion, this work attempts to describe the possible mechanism by which glycine decreased BP, i.e., reducing adipose tissue accumulation by stimulating fatty acid oxidation. These pathways are under investigation in our laboratory.

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).

**REFERENCES**


3. Belzung F, Raclot T, and Groscolas R. Fish oil n-3 fatty acids selectively impair several fatty acid metabolizing enzymes, such as CPT I and II, mitochondrial \(\beta\)-oxidation enzymes, ketogenesis enzymes, and \(\omega\)-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.


GLYCINE INTAKE AND SUCROSE-INDUCED HYPERTENSION


