Dietary supplementation with 2-deoxy-d-glucose improves cardiovascular and neuroendocrine stress adaptation in rats

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Dietary supplementation with 2-deoxy-d-glucose improves cardiovascular and neuroendocrine stress adaptation in rats. Am J Physiol Heart Circ Physiol 287: H1186–H1193, 2004; 10.1152/ajpheart.00932.2003.—Dietary restriction and physical exercise can enhance stress resistance and reduce the risk of cardiovascular disease. We investigated the effects of dietary supplementation with 2-deoxy-d-glucose (2-DG), a glucose analog that limits glucose availability at the cellular level, on cardiovascular and neuroendocrine responses to stress in rats. Young adult male Sprague-Dawley rats were implanted with telemetry probes to monitor blood pressure (BP), heart rate, body temperature, and body movements. These variables were measured at designated times during a 6-mo period in rats fed control and 2-DG-supplemented (0.4% 2-DG, fed ad libitum on a schedule of 2 days on the diet and 1 day off the diet) diets during unperturbed conditions and during and after immobilization stress or cold-water swim stress. Rats fed the 2-DG diet exhibited significant reductions in resting BP, attenuated BP responses during stress, and accelerated recovery to baseline after stress. Plasma concentrations of ACTH and corticosterone were elevated under nonstress conditions in rats fed the 2-DG diet and exhibited differential responses to single (enhanced response) and multiple (reduced response) stress sessions compared with rats fed control rat chow ad libitum. The 2-DG diet improved glucose metabolism, as indicated by decreased concentrations of blood glucose and insulin under nonstress conditions, but glucose and insulin responses to stress were maintained. We conclude that improvements in some cardiovascular risk factors and stress adaptation in rats maintained on a 2-DG-supplemented diet are associated with reduced neuroendocrine responses to the stressors.

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MO), and rat chow supplemented with 0.4% 2-DG was made by Harlan Teklad. The rats in the 2-DG diet group were fed the 2-DG-supplemented diet and the control diet ad libitum in a repeating pattern as follows: 2 days of 2-DG, 1 day of control, 2 days of 2-DG, 1 day of control, etc. Food containers were exchanged at 1700 daily. All rats had continuous access to water regardless of the type of food supplied. The design of the study is shown in Fig. 1A. Before initiation of the experimental diets, physiological parameters were recorded during a 72-h period. Rats were then randomly assigned to one of the two diet groups. Rats were maintained on the diets for 6 mo, and physiological parameters were recorded under nonstress conditions and during and after stress sessions (Fig. 1A). The physiological and biochemical variables were assessed under nonstress conditions at 3 and 6 mo after the 2-DG diet was initiated (Fig. 1A). For recording under nonstress conditions, all parameters were continuously monitored for ≥72 h. Blood samples were taken (between 0900 and 1200) from rats that had been fasted overnight; in the 2-DG group, samples were taken on the morning after a day when the animals had eaten the 2-DG-supplemented food. Under isoflurane anesthesia, blood samples (2 ml) were collected from the tail vein of each rat into a tube containing K₃ EDTA as an anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The plasma was isolated and stored at −80°C. Glutathione was added to plasma samples used to measure epinephrine and norepinephrine to prevent oxidation of these catecholamines. To examine responses to a stressor, the physiological parameters were recorded overnight before the rat was subjected to the stressor. On the day of the stress test, the rat was subjected to a restraint stress or cold-water swim stress at designated times before and after diet initiation (Fig. 1A). All stress treatments were performed between 0900 and 1500. Blood samples were obtained immediately after the stress session. When five consecutive daily immobilization stress sessions were administered, blood samples were taken only immediately after the fifth daily stress session.

Immobilization stress and cold-water swim stress protocols were performed as described previously (31). Before each stress session, physiological and biochemical variables were recorded from each rat for 1 h after immobilization stress session for 5 consecutive days, which was ~4 mo after initiation of the 2-DG diet. The cold-water swim stress was conducted 5 mo after the 2-DG diet was initiated. No physiological variables were recorded during the 15-min swim period; all variables were recorded for 2 h immediately after the swim period.

Analyses of blood samples. Plasma insulin concentrations were measured using a commercially available ultra-sensitive rat insulin ELISA kit (ALPCO Diagnostics, Windham, NH). Plasma glucose levels were measured using a glucose analyzer (Beckman Instruments, Fullerton, CA). Concentrations of ACTH and corticosterone in plasma were measured using commercially available radioimmunoassay kits (ICN Diagnostics, Costa Mesa, CA). The concentrations of epinephrine and norepinephrine in plasma were measured using commercially available catecholamine enzyme immunoassay kits (ICN Diagnostics, Orangeburg, NY).

Statistical analyses. Physiological variables during and after stress were recorded with continuous sampling; the data were presented and analyzed as mean values for each 10 min of recording. Data were analyzed using repeated-measures ANOVA followed by post hoc assessments with Student-Newman-Keuls test. One way ANOVA followed by Student-Newman-Keuls test or Student’s t-test was used for comparisons of values of glucose and hormone levels.

RESULTS

Effects of dietary 2-DG supplementation on cardiovascular responses to stress. Body weights of the rats fed the 2-DG-supplemented diet were slightly, but not significantly, lower than those of rats fed the control diet during the course of the 25 wk after the diets were initiated (Fig. 1B). Food consumption of rats in each group was measured 2–3 mo after the diets were initiated. Food consumption by rats in each group was similar (26.3 and 27.8 g/day for control and 2-DG diet, respectively). The physiological and behavioral variables of the rats, HR, temperature, diastolic and systolic BP, and general activity, were monitored and recorded by telemetry. These parameters were recorded continuously under nonstress conditions during a 72-h period before diet initiation and at designated times after diet initiation (Fig. 1A). In the 2-DG diet group, there were no differences in any of the physiological variables on the days the rats were fed the 2-DG diet compared with the days they were fed the control diet (data not shown). Table 1 summarizes behavioral and physiological parameters before and 6 mo after diet initiation in each group. The results indicate that the 2-DG diet significantly affected cardiovascular function. Rats fed the 2-DG diet for 6 mo showed significantly lower HR and mean BP than those fed the control diet ($P < 0.05$). In rats fed the control diet, there was a significant increase in mean BP as the rats became older ($P < 0.05$). In
Table 1. Behavioral and physiological parameters before and 6 mo after dietary treatment in rats fed control or 2-DG-supplemented diet

<table>
<thead>
<tr>
<th>Activity, counts/min</th>
<th>Heart Rate, beats/min</th>
<th>Mean Blood Pressure, mmHg</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>5.4±1.5 (8)</td>
<td>348.2±11.4 (8)</td>
<td>190.9±4.4 (4)</td>
</tr>
<tr>
<td>6 mo</td>
<td>4.9±1.1 (8)</td>
<td>353.8±11.3 (8)</td>
<td>119.4±5.9 (4)</td>
</tr>
<tr>
<td>Day</td>
<td>2.6±0.8 (8)</td>
<td>304.9±7.6 (8)</td>
<td>102.9±5.2 (4)</td>
</tr>
<tr>
<td>6 mo</td>
<td>2.1±0.5 (8)</td>
<td>304.9±7.4 (8)</td>
<td>114.8±5.1 (4)</td>
</tr>
<tr>
<td>2-DG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>5.4±1.3 (8)</td>
<td>367.1±11.4 (8)</td>
<td>104.3±5.6 (4)</td>
</tr>
<tr>
<td>6 mo</td>
<td>3.9±0.9 (7)</td>
<td>322.8±11.3* (7)</td>
<td>99.1±5.7* (4)</td>
</tr>
<tr>
<td>Day</td>
<td>2.6±1.6 (8)</td>
<td>318.2±7.6 (8)</td>
<td>101.6±3.6 (4)</td>
</tr>
<tr>
<td>6 mo</td>
<td>1.6±0.4 (7)</td>
<td>284.9±7.4* (7)</td>
<td>96.2±4.6* (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of samples in parentheses. Con, control diet; 2-DG, 2-deoxy-D-glucose-supplemented diet. *P < 0.05 compared with Con during the same time period. †P < 0.05 compared with before dietary treatment within the same group.

In contrast, the BP of rats fed the 2-DG diet did not increase with advancing age (Table 1). The effect of the 2-DG diet on cardiovascular variables occurred long before 6 mo after the diet was initiated. The resting systolic, diastolic, and mean BP were significantly lower in rats fed the 2-DG diet than in those fed the control diet 2 mo after diet initiation (see PreStress in Fig. 2; P < 0.05). The 2-DG diet had no significant effect on levels of activity and core body temperature under nonstress conditions throughout the study (Table 1).

We first examined and compared the responses of rats to a single 1-h immobilization stress that was first conducted before diet initiation with the responses to the same stressor 2 mo after diet initiation. In response to immobilization stress, HR and diastolic and systolic BP increased rapidly and then gradually and significantly declined during the course of the 1-h restraint period in all rats (Fig. 2). There was no significant difference in any of the variables between the control and 2-DG groups before the diet treatment. However, after 2 mo on the 2-DG diet, diastolic and systolic BP declined during and after restraint (Fig. 2; P < 0.05). The HR was lower in the 2-DG than in the control rats during and after restraint, although the reduction was less significant (P = 0.08). The attenuated cardiovascular responses during and after restraint were likely attributed to the reduced resting activities (Fig. 2). In addition, rats that had been maintained on the 2-DG diet exhibited a trend of a rapid recovery of systolic and diastolic BP after release from immobilization stress compared with rats maintained on the control diet (Fig. 2). Interestingly, the body temperatures during stress were significantly lower in the control and 2-DG groups 2 mo after than before diet initiation (Fig. 2). However, there were no significant differences in body temperature between the 2-DG and control groups during or after stress.

We next determined the effects of dietary supplementation with 2-DG on the responses of rats to repeated stress exposure by subjecting control and 2-DG-treated rats to immobilization stress once each day for 5 consecutive days. Rats fed the 2-DG diet had significantly lower diastolic and systolic BP during and after immobilization stress on each daily session of the five consecutive daily stress tests than rats fed the control diet. Figure 3 shows physiological variables in response to the first daily stress compared with those in response to the fifth daily stress. Systolic and diastolic BP showed consistently lower responses during and after stress in the 2-DG than in control group (P < 0.05). The diastolic BP of 2-DG rats displayed a trend of fast decline during 1 h of restraint of the fifth daily stress.
stress (Fig. 3; \( P = 0.06 \)). The HR during immobilization stress showed a trend of attenuated response and recovered to baseline more rapidly after release from immobilization in rats fed the 2-DG diet than in those fed the control diet (Fig. 3). The 2-DG diet had no significant effect on body temperature during and after immobilization stress or on the activity of the rats after release from immobilization (Fig. 3).

To determine whether the improved cardiovascular stress adaptation in rats maintained on the 2-DG-supplemented diet occurred with other kinds of stress, we subjected all rats to a cold-water swim stress. During the 15-min period in the cold water, the rats in both groups had similar behavioral responses to the stressor as measured by total swim time, swim distance, and mean swim speed:mean swim time (14.5 ± 0.3 and 12.6 ± 1.0 min in control and 2-DG, respectively), mean swim distance (11,228 ± 952 and 9,431 ± 1,605 cm in control and 2-DG, respectively), and mean swim speed (770 ± 55 and 725 ± 78 cm/min in control and 2-DG, respectively). Statistical comparisons of swim speed, time, and distance for the control and 2-DG groups revealed no significant effect of diet on any of the three measurements. Immediately after the swim stress, all physiological variables were recorded. Immediately after removal from the cold water, body temperature was decreased markedly by 8–9°C in all rats and then recovered toward the normal level during a 90-min post-swim-stress period; there was no significant effect of diet on body temperature (Fig. 4). BP was elevated after swim stress in rats in both diet groups. However, the magnitude of the stress-induced increase in BP was significantly less in rats fed the 2-DG diet than in those fed the control diet (Fig. 4; \( P < 0.05 \)). There was no significant effect of diet on poststress HR. Rats fed the 2-DG diet showed reduced activity during the post-swim-stress period compared with rats fed the control diet (Fig. 4).

Effects of dietary 2-DG supplementation on neuroendocrine responses to stress. The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to stress, resulting in elevations in ACTH and corticosterone concentrations in the blood. At 3 mo after diet initiation, the concentrations of corticosterone (\( P < 0.05 \)) and ACTH under nonstress conditions were 20–40% greater in the rats fed the 2-DG diet than in those fed the control diet (Fig. 5, A and B). The elevation of plasma corticosterone and ACTH concentrations suggests that the 2-DG diet imposed a mild tonic stress on the rats similar to that observed in rats subjected to intermittent fasting (31) and caloric restriction (33). However, after 6 mo on the 2-DG diet, the concentrations of ACTH (\( P < 0.01 \)) and corticosterone (\( P < 0.01 \)) under nonstress conditions were decreased compared with the levels at 3 mo on the 2-DG diet under nonstress conditions (Fig. 5). After 6 mo on the diets, levels of ACTH in rats fed the 2-DG diet were similar to those of rats fed the control diet, whereas levels of corticosterone in rats fed the 2-DG diet were significantly less than levels in rats fed the control diet (\( P < 0.01 \); Fig. 5).

In response to a single immobilization stress, plasma concentrations of corticosterone and ACTH were increased in all rats, with no significant differences between the diet groups (Fig. 5, A and B). In response to repeated immobilization stress, ACTH (\( P < 0.05 \)) and corticosterone (\( P < 0.01 \)) concentrations remained elevated in rats fed the control diet (Fig. 5, A and B). However, rats fed the 2-DG-supplemented diet exhibited a reduced hypothalamic response to the repeatedly applied stressor, as indicated by a significantly lower stress-induced elevation of ACTH than in rats fed the control diet (Fig. 5A; \( P < 0.05 \)). Because the latter result suggested that rats fed the 2-DG diet more rapidly adapted to repeated exposure to the same stressor, we subjected the rats to a novel stressor, cold-water swim stress. Immediately after cold-water swim stress, plasma concentrations of ACTH (\( P < 0.05 \) or \( P < 0.01 \)) and corticosterone (\( P < 0.01 \)) were significantly elevated in rats in the control and 2-DG diet groups relative to their concentrations under nonstress conditions at 3 and 6 mo (Fig. 5, A and B). The concentrations of ACTH and corticosterone were
increased similarly in rats in the 2-DG and control groups. Collectively, these results suggested that the 2-DG diet did not impair the responsiveness of the HPA axis to a novel stressor but did enhance adaptation to repeated exposures to the same type of stress.

Because the sympathetic nervous system plays important roles in stress responses and in the regulation of BP, we measured plasma levels of epinephrine and norepinephrine after repeated immobilization stress and a cold-water swim stress in rats that had been maintained on control and 2-DG diets (Fig. 5, C and D). The concentration of epinephrine after repeated immobilization stress was significantly lower in rats fed the 2-DG diet than in those fed the control diet (Fig. 5, C and D; \( P < 0.05 \)), whereas there was no difference between the two diet groups in norepinephrine concentrations measured in the same samples. There was no significant difference in epinephrine concentrations after cold-water swim stress between rats fed the control diet and those fed the 2-DG diet. However, post-swim-stress norepinephrine concentrations were significantly greater in rats fed the 2-DG diet than in those fed the control diet (Fig. 5, C and D; \( P < 0.05 \)). No measurements of basal levels of catecholamines were made under nonstress conditions.

Effects of dietary 2-DG supplementation on glucose regulation. A profile of glucose and lipid metabolism characterized by increased plasma glucose and insulin levels (insulin resistance syndrome) increases the risk of cardiovascular disease (9). We therefore measured concentrations of glucose and insulin in plasma samples from rats fed the 2-DG and control diets under nonstress and stress conditions. Under nonstress conditions, the concentrations of glucose (\( P < 0.01 \)) and insulin (\( P < 0.05 \)) were significantly lower in rats fed the 2-DG diet than in those fed the control diet 3 and 6 mo after diets were initiated (Fig. 6). In response to stress, glucose level was significantly increased in rats fed the control or 2-DG diet compared with the nonstress levels (Fig. 6A; \( P < 0.01 \)).

Fig. 4. Rats maintained on 2-DG supplementation exhibit lower blood pressure during cold-water swim stress than rats fed control diet. Physiological variables were recorded before and for 2 h after cold-water swim stress in rats that had been maintained for 5 mo on control or 2-DG diet. Values are means ± SE. Mean blood pressure during 2-h post cold-water swim stress was significantly lower in rats fed 2-DG than in those fed control diet (\( P < 0.05 \)). Core body temperature was markedly reduced by cold-water swim in all rats initially but recovered gradually during 2 h postswim stress. There were no significant differences in heart rate between control and 2-DG groups after stress.

Fig. 5. Effects of dietary 2-DG supplementation and stress on circulating concentrations of ACTH (A), corticosterone (B), epinephrine (C), and norepinephrine (D). Values are means ± SE. *\( P < 0.05 \); **\( P < 0.01 \) compared with control diet group after the same treatment. +\( P < 0.05 \); ++\( P < 0.01 \) compared with concentration under nonstress conditions within the same diet group.
DIETARY 2-DEOXYGLUCOSE IMPROVES STRESS ADAPTATION

The data obtained in the present study show that intermittent dietary supplementation with 2-DG has significant effects on cardiovascular and neuroendocrine responses to stress in rats that are similar to those previously reported in a study of rats maintained on an intermittent fasting (every-other-day food deprivation) regimen (31). Rats maintained on the 2-DG diet exhibited lower BP and HR under nonstress conditions, lower maximum BP and HR responses to repeated stress, and more rapid recovery of BP and HR after stress than rats fed the control diet. The effects of the 2-DG diet on BP and HR under nonstress conditions are similar to those achieved by caloric restriction and regular physical exercise (1, 22, 24, 28, 29). However, the effects of the 2-DG diet on BP and HR were due neither to increased physical exercise nor reduced energy intake, because activity levels were not increased and food intake was not reduced in rats fed the 2-DG diet. In humans, hypertension is a major risk factor for cardiovascular disease and stroke, and this risk factor can be reduced by regular physical exercise and low-calorie diets. The effects of 2-DG supplementation on the cardiovascular system in humans remain to be established. It is well known that the phosphorylated product of 2-DG, 2-deoxyglucose-6-phosphate, accumulates in tissues in a mathematically definable relation to the rate of glucose utilization by the tissue (26). When cells are continuously exposed to very high concentrations of 2-DG, its poor clearance can result in cytotoxic effects (6, 15). In addition, lower doses of 2-DG may have toxic effects due to disruption of thiol metabolism and glucopenia. However, the relatively low amount of 2-DG in the diet fed in the present study did not result in any overt pathology. Indeed, the results of the present study reveal beneficial effects on the cardiovascular system of relatively small amounts of 2-DG in the diet.

Several findings suggest that the 2-DG diet employed in the present study may exert its effects on the cardiovascular, neuroendocrine, and glucose-regulating systems by imposing a mild stress response in the rats. First, it is well known that 2-DG can cause a metabolic stress in cells by suppressing glycolysis, and we previously showed that daily administration of 2-DG (single intraperitoneal injection) upregulates the expression of stress resistance proteins in neurons (5, 13, 35). Second, nonstress levels of ACTH and corticosterone were increased in rats fed the 2-DG diet, indicating that 2-DG results in a tonic activation of the HPA stress axis, although it is not known whether activation of this stress axis is required for the beneficial effects of 2-DG supplementation on the cardiovascular and/or glucose-regulating systems. Third, 2-DG supplementation mimics some effects of caloric restriction and intermittent fasting, and the latter dietary restriction regimens have been reported to increase the resistance of rodents to several different stressors, including heat and exposure to chemical toxins (7, 13). Intermittent fasting and 2-DG administration have each been shown to increase the resistance of neurons to oxidative and metabolic stress, and it will be of interest to determine whether 2-DG induces similar changes in cells of the cardiovascular system. Beneficial effects of intermittent fasting on the nervous system may result, in part, from increased production of brain-derived neurotrophic factor and stress resistance proteins (e.g., 70-kDa heat shock protein and 78-kDa glucose-regulated protein) (3, 4, 12). Because energy restriction has been shown to induce an increase in levels of heat shock and other stress proteins in heart cells (12) and can protect the heart against ischemic injury (16), it might be expected that similar cytoprotective responses occur in heart and blood vessel cells of rats fed the 2-DG diet. In this view, dietary supplementation with 2-DG induces a preconditioning response that increases the resistance of cells to injury and disease.

The insulin resistance syndrome is becoming increasingly prevalent in modern societies as food intake increases and exercise decreases. Plasma insulin and glucose concentrations were lower in rats fed the 2-DG diet than in those fed the control diet. Similar changes in insulin and glucose levels occur in rats and mice maintained on dietary restriction regimens and are indicative of increased insulin sensitivity (8, 33). We previously found that intermittent fasting (every-other-day food deprivation) without caloric restriction causes decreases in circulating insulin and glucose concentrations that are as

**DISCUSSION**

Corresponding to the increase in blood glucose after stress, the plasma insulin level was elevated, with the magnitude of the increase being significantly greater in rats fed the 2-DG diet than in those fed the control diet (Fig. 6B; $P < 0.05$). Collectively, these findings suggest that rats maintained on the 2-DG diet are better able to regulate blood glucose levels than rats fed the control diet.

**Fig. 6.** Dietary 2-DG supplementation reduces concentrations of plasma glucose (A) and insulin (B) under nonstress conditions but has no significant effect on glucose and insulin responses to immobilization and cold-water swim stress. Values are means ± SE. *$P < 0.05$; **$P < 0.01$ compared with corresponding condition for rats fed control diet. +$P < 0.05$; ++$P < 0.01$ compared with concentration under nonstress conditions within the same diet group.
great as or greater than a 40% caloric restriction (1). The latter observation suggests that an intermittent energetic stress can have beneficial effects on glucose metabolism that are independent of caloric intake and provide a rationale for the every-other-day 2-DG diet (intermittent energetic stress) employed in the present study. While decreasing plasma glucose and insulin concentrations under nonstress conditions, 2-DG did not compromise the ability of the rats to elevate glucose and insulin levels during stress, indicating that the decreased insulin levels under nonstress conditions did not result from impaired ability of pancreatic cells to release insulin.

The ability to resist or adapt to stress is increasingly recognized as an important factor in resisting various diseases, including cardiovascular disease and diabetes (21, 30). After repeated daily sessions of immobilization stress, rats fed the 2-DG diet exhibited reduced ACTH and corticosterone responses to stress, suggesting that they had adapted to the stress. The mechanism underlying this adaptation remains to be determined and could involve changes in brain, hypothalamus, pituitary, and/or adrenal gland. We recently reported similar effects of an intermittent fasting regimen on neuroendocrine stress responses in rats (31). The responses of the sympathetic nervous system to stress and the ability of rats to mobilize glucose in response to stress were maintained in rats fed the 2-DG-supplemented diet. Thus it appears that 2-DG supplementation greatly improves cardiovascular risk factors (reduced BP and glucose and insulin levels) under nonstress conditions and reduces the hypertensive effects of acute and repeated stressors, while allowing normal activation of sympathetic and energy-mobilizing responses to stress. The magnitude of the effects of dietary supplementation with 2-DG on BP, insulin, and glucose levels documented in the present study is equal to or greater than that previously obtained with exercise-training regimens in rats and humans (2, 10, 25).

The purpose of the present study was to determine the effects of the 2-DG-supplemented diet on cardiovascular and hormonal stress responses in rats. However, this 2-DG diet regimen undoubtedly has effects on cells throughout the body; therefore, it will be important to characterize those effects and to determine which are beneficial and which are detrimental. Our findings in rats cannot establish clinical safety in humans, and further studies are required to identify possible adverse effects of chronic 2-DG dietary supplementation before such a regimen can be applied to humans.

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GRANTS

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