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Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats

Ruiqian Wan, Simonetta Camandola, and Mark P. Mattson

Laboratory of Neurosciences, National Institute on Aging Gerontology Research Center, Baltimore, Maryland

Corresponding author: Mark P. Mattson, National Institute on Aging, GRC 4F01, 5600 Nathan Shock Drive, Baltimore, MD 21224. E-mail: mattsonm@grc.nia.nih.gov

ABSTRACT

Hypertension and insulin resistance syndrome are risk factors for cardiovascular disease, and it is therefore important to identify interventions that can reduce blood pressure and improve glucose metabolism. We performed experiments aimed at determining whether intermittent fasting (IF) can improve cardiovascular health an

rats in groups IF and 2DG exhibited increased levels of plasma adrenocorticotropin and corticosterone, indicating that these diets induced a stress response. We conclude that reductions in blood pressure, heart rate, and insulin levels, similar to or greater than those obtained with regular physical exercise programs, can be achieved by IF and by dietary supplementation with 2DG by a mechanism involving stress responses.

Key words: caloric restriction ! cardiovascular disease ! glucose metabolism ! stress response

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resistance syndrome are not fully established. However, two interventions that reliably reduce blood pressure and improve glucose metabolism in humans and in laboratory animals are low-calorie diets and physical exercise; both interventions improve cardiovascular risk profile not only in obese subjects, but also in individuals with normal body weights (4, 5). Regular exercise enhances cardiovascular fitness and glucose metabolism by subjecting the heart, blood vessels, and skeletal muscle cells to mild metabolic stress; this stress stimulates angiogenesis and signaling pathways that promote relaxation of blood vessels, and increases the sensitivity of skeletal muscle cells to insulin. The mechanism(s) by which caloric restriction improves cardiovascular risk factors are less clear.

Studies of rodents and non-human primates have shown that dietary restriction can increase lifespan and reduce the incidence of various age-related diseases (6). Two general mechanisms have been proposed, namely, decreased free radical production (7) and induction of a cellular stress response, which enhances the ability of cells to cope with more severe stress (8). Previous studies have documented beneficial effects of reduced-calorie diets on cardiovascular function and glucose metabolism. For example, low-calorie diets can reduce blood pressure in humans and monkeys (9) and in rodents (10, 11). It is also well-established that caloric restriction can increase insulin sensitivity in humans, monkeys, and rodents, which manifests as reduced levels of circulating glucose and insulin (12, 13, 14). It has been presumed, but not established, that the beneficial effects of dietary restriction on the cardiovascular and glucose-regulating systems are the result of a reduction in calories. However, emerging findings suggest that an increase in the inter-meal interval can exert beneficial effects on multiple organ systems that are not necessarily a consequence of an overall reduction in calories. Intermittent fasting (IF) feeding regimens, such as every-other-day feeding, can increase the lifespans of rats and mice (15, 16). IF can increase the resistance of neurons in the brains of rats and mice to dysfunction and degeneration in experimental models of stroke, Alzheimer s and Parkinson s diseases (17, 18, 19), and can stimulate stem cells to produce new neurons (20). The amount of food consumed by rodents maintained on an IF regimen varies depending on the strain and, in some cases (e.g., C57BL/6 mice), the animals gorge during the non-fasting time period to an extent that overall food intake is essentially equivalent to that of animals fed ad libitum Surprisingly, such IF without an overall reduction in calorie intake exerts several beneficial effects (lifespan extension, improved glucose regulation, and neuroprotection) equivalent to those achieved by reducing calorie intake by 30 40% (16, 21).

In the present study we tested the hypothesis that IF can improve cardiovascular functional parameters and glucose metabolism by a stress-related mechanism. We show that blood pressure and heart rate, and levels of circulating glucose and insulin, are decreased in rats maintained on an IF regimen. We further demonstrate that similar beneficial effects of IF can be achieved without a reduction in food intake by feeding rats a diet supplemented with 2-deoxy-D-glucose (2DG), which may mimic the effects of IF by inducing a metabolic stress in cells.

MATERIALS AND METHODS

Animals and surgical procedures

Sprague-Dawley rats (Harlan Teklad, Madison, WI) were maintained under temperature- and light-controlled conditions. The photoperiod in colony and testing room was maintained on a 12-

h light/12-h dark cycle with lights on from 6:00 am to 6:00 pm daily. Rats were individually housed after surgical implantation with telemetric transmitter and were provided food and water ad libitum until experimental diets were initiated. A telemetry system (Data Sciences International, St. Paul, MN) was used to monitor behavioral and physiological parameters. Two types of signal transmitters were used: TA11PA-C40 (C40) for monitoring general activity, heart rate (HR), blood pressure (BP), including diastolic, systolic, and mean blood pressure; and TA10ETA-F20 (F20) for monitoring ambulatory activiy, heart rate (HR), and core body temperature (BT). Surgical implantation of transmitters was performed in 3-month-old rats under isofluorane anesthesia by using a six-station Anesthesia System (SurgiVet, Waukesha, WI). For a C40 implant, the catheter tip was inserted upstream into the descending aorta between the renal arteries and iliac bifurcation. The catheter was secured with tissue adhesive at the insertion point. The body of the implant was inserted into the peritoneal cavity and sutured to the abdominal musculature at the incision site. For a F20 implant, the body of the implant was inserted into the peritoneal cavity and secured to the abdominal musculature at the incision site. The two biopotential leads were routed subcutaneously to the desired placement sites located lateral to midline of the chest. The tips of leads were placed within muscle tissue and secured with a suture. After the implantation procedure was finished, the rat was injected subcutaneously with buprenorphine at the dose of 0.022 mg/kg and was returned to its cage. Rats were allowed to recover at least for 1 month before initiation of the experimental diets.

Diets and experimental procedures

A total of 24 rats were divided into 3 groups (8 rats per group). One group (AL) was fed with regular rat chow (NIH-07) ad libitum; a second group (IF) was maintained on an alternate-day fasting regimen (24 h without food, 24 h with food); and the third group (2DG) was maintained on a regimen in which they were provided regular rat chow and rat chow supplemented with 0.4% 2DG (Harlan Teklad) ad libitum on alternating days, that is, one day on 2DG chow and one day on regular chow. Food containers were taken out, placed in, or exchanged at 5:00 pm daily. All rats had continuous access to water. The rationale for using alternate-day supplementation with 2DG was that the rats would be subjected to a metabolic stress every-other-day (as with IF), but without an overall reduction in food intake. In addition, in preliminary studies we found that continuous feeding of rats the 0.4% 2DG-supplemented food reduced life span, and therefore chose to use the less stressful every-other-day 2DG feeding regimen. Prior to initiating the experimental diets, physiological parameters were recorded during a 72-h period. The rats were then randomly assigned to one of the three diet groups. Four of the rats in each of the groups were implanted with C40 transmitters, and the other four were implanted with F20 transmitters. Rats were maintained on the diets for 6 months, and physiological parameters were recorded at the 1, 3, and 6 month time points. During each recording session all parameters were continuously monitored for at least 72 h. At designated time points, blood samples (2 ml) were taken from the tail vein, and plasma was isolated and stored at 80°C.

Blood chemistry

Rats were lightly anesthetized with a mixture of isofluorane and oxygen during blood collection from the tail vein; blood was collected within 2 min of removal of the rat from its cage. Plasma insulin levels were measured by using a commercially available ultra-sensitive rat insulin ELISA kit (ALPCO Diagnostics, Windham, NH). The level of IGF-1 in plasma was assessed by using a

commercially available ELISA kit (Diagnostic Systems Laboratories, Webster, TX). Plasma glucose levels were measured by using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA). The levels of adrenocorticoreopin (ACTH) and corticosterone (CORT) in plasma were measured by using commercially available radioimmunoassay kits (ICN Diagnostics, Costa Mesa, CA). The correlation coefficients for each of the assays were as follows: ACTH, $r^2 = 0.99$; corticosterone, $r^2 = 0.999$; insulin, $r^2 = 0.9977$; IGF-1, $r^2 = 0.9614$. For the glucose concentration assay the intra- and inter-assay coefficients of variation were each less than 1%.

Statistical analyses

Physiological and behavioral parameters were recorded with a scheduled sampling of every 5 min, and the results of were presented as an average value for every 30 min throughout a 24-h recording period. Data were analyzed by using ANOVA repeated measure followed by post hoc assessments with Student Newman Keuls test. One way ANOVA followed by SNK test or Student s t-test was used for comparisons of values of hormonal levels.

RESULTS

Effects of IF and 2DG supplementation on body weight and food intake

Body weights of the rats in the three diet groups were measured each week throughout the 6-month period of this study. Body weights of rats maintained on the IF regimen were significantly reduced compared with the AL group and to the 2DG group within one month of diet initiation and were maintained at significantly lower levels throughout the 6-month study period (Fig. 1a). The body weights of rats on the 2DG diet were not significantly different than those of rats fed AL. Rats on the IF regimen consumed ~30% less food compared with rats fed AL, whereas food intake of rats on the 2DG diet was essentially identical to that of group AL (Fig. 1b).

Effects of IF and 2DG supplementation on physiological parameters

Heart rate (HR); blood pressure (BP), including diastolic, systolic, and mean BPs; and core body temperature (BT), as well as general ambulatory activity, were monitored before the start of diet initiation and at 1, 3, and 6 months thereafter. Within-group comparisons revealed no significant changes in the HR, BT, and activity levels of the rats in the AL group during the 6-month period (Fig. 2). There was an age-related increase in mean BP in AL rats, which was due to a significant increase in diastolic BP without a change in the systolic BP. Rats maintained on the IF regimen exhibited highly significant decreases in HR, BPs, BT, and activity, which were evident within 1 month of diet initiation and were maintained or further decreased through the 6-month time point (Fig. 3). The magnitude of the decreases in all parameters were most pronounced during the night, which resulted in a reduction in the magnitude of the circadian variations in BP and HR.

Mean, diastolic, and systolic BPs were decreased in rats maintained on the 2DG supplemented diet, but the magnitude of the decreases was less than that of rats maintained on the IF regimen (Fig. 3 and 4). In rats maintained on the 2DG-supplemented diet, HR was decreased within 1 month of diet initiation and remained reduced throughout the 6-month time period. The magnitude of the decrease in HR of rats in group 2DG was less than that of rats in group IF (Fig. 4). General activity was not significantly affected by feeding rats a 2DG-supplemented diet. BT

did not change significantly during the 6-month period in rats in group 2DG, although there was a trend toward a decrease.

Fig. 5 shows comparisons of physiological parameters at the 6-month time point among the three diet groups. IF resulted in a highly significant reduction in HR, which was particularly prominent shortly after lights out (100 beats/min reduction compared with group AL) and less pronounced during the light period (40 50 beats/min reduction compared with group AL). 2DG feeding resulted in a significant reduction in HR, which was more pronounced during the dark period than during the light period. The magnitude of the decrease in HR in group 2DG was less than that in group IF. Mean BP was significantly decreased throughout the circadian cycle in the IF and 2DG groups compared with the AL group (Fig. 5). The magnitude of the decrease in BP was less in group 2DG compared with group IF. BT was significantly decreased in group IF compared with group AL, whereas group 2DG did not exhibit a decrease in BT. At the 6-month time point the levels of general activity of the rats was significantly decreased in groups IF and 2DG compared with group AL (Fig. 5).

To rule out the possibility of acute effects of IF on BP, HR, and activity, we analyzed these parameters on both feeding and fasting days in rats in the IF group. HR, BP, and activity were decreased by similar amounts on both feeding and fasting days (Fig. 6). In contrast, BT was decreased on fasting days, but not on feeding days. We also compared physiological parameters on successive days in rats in the 2DG group. BP, HR, and activity were similarly reduced on days in which the rats were fed the regular diet and on the 2DG feeding days, while BT did not differ from that of group AL at any time (data not shown).

Effects of IF and 2DG supplementation on glucose metabolism and neuroendocrine stress responses

The levels of glucose, insulin, and IGF-1 in plasma were assessed at 3 and 6 months after diet initiation. Levels of glucose and insulin in plasma were significantly decreased in rats that had been maintained on either the IF or 2DG-supplemented diets compared with rats fed ad libitum at both the 3 and 6 month time points (Table 1). In contrast, there were no significant differences in plasma IGF-1 levels among the three groups at either time point, although IGF-1 levels in group IF were lower after 6 months on the diet (Table 1).

To determine whether IF and the 2DG-supplemented diet exerted a stress on the rats, we measured the levels of adrenocorticotropin (ACTH) and corticosterone in plasma at 3 months after diet treatments. Levels of both ACTH and corticosterone were significantly elevated in the rats maintained on IF and in rats receiving the 2DG-supplemented diet compared with the rats fed ad libitum(Table 2).

DISCUSSION

The present findings show that IF can induce prolonged decreases in BP, HR, glucose, and insulin levels in adult rats. Because hypertension and insulin resistance syndrome are established risk factors for cardiovascular disease, our data suggest that a reduction in meal frequency may reduce the risk of cardiovascular disease. Although previous studies have shown that low-calorie diets can reduce BP and enhance insulin sensitivity (9, 12, 13), the present data are the first to

document quite large and sustained reductions in BP, HR, insulin, and glucose levels in animals maintained on an IF regimen. The magnitude of the reductions in HR and BP that occurred in rats maintained on the IF regimen in the present study is similar to that achieved in rats maintained on a daily diet with a 40% reduction in calories (11). Rats maintained on the IF diet in the present study exhibited 25% and 40% reductions in plasma glucose and insulin levels, respectively, decreases similar to those achieved with a 40% reduction in calories (22). The overall reduction in food intake of the IF rats in the present study was ~30% with body weights that averaged 16% lower than body weights of control AL rats after 6 months on the diet. Thus, IF with relatively modest reductions in food intake and body weight is very effective in reducing blood pressure and improving glucose metabolism.

The mechanism(s) whereby IF reduces BP and HR and improves insulin sensitivity are unknown. Several lines of evidence show that IF induces a stress response, and suggest that such a cellular stress response contributes to many of the beneficial effects of IF (and caloric restriction) on various organ systems. We found that levels of circulating ACTH and corticosterone were increased in rats maintained on the IF regimen. The effects of IF and 2DG on circulating ACTH and corticosterone levels likely reflect changes in basal levels rather than changes in a stress response associated with handling the animals during blood collection. Our data are consistent with a neuroendocrine stress response similar to that previously reported in rats maintained on caloric restriction in which blood was collected under non-stress conditions (23). Previous studies have shown that rats and mice maintained on a similar IF regimen exhibit increase levels of cellular stress proteins, including heat-shock protein-70 and glucose-regulated protein-78 in several different tissues, including liver, stomach, and brain (18, 19, 24). In addition, IF was reported to increase the production of neurotrophic factors in the brain, consistent with a cellular stress response (25). In the present study we tested the hypothesis that an intermittent metabolic stress, every-other-day dietary supplementation with 2DG, would be sufficient to induce cardiovascular and metabolic changes similar to those that occur in animals subjected to caloric restriction or IF. Rats on the 2DG dietary regimen did indeed exhibit reductions in BP, glucose, and insulin levels. Interestingly, the rats fed 2DG did not consume less food than rats fed ad libitum, nor did they lose body weight. Therefore, improved cardiovascular and metabolic parameters were achieved without caloric restriction. These are the first data to document an effect of a dietary supplement that inhibits glycolysis on HR and BP and on circulating glucose and insulin levels. The results with the 2DG diet suggest that a periodic metabolic stress is sufficient to induce changes in cardiovascular function and glucose metabolism similar to those that occur with caloric restriction, IF, and physical exercise.

Previous findings suggest that caloric restriction-induced decreases in HR and BP are mediated, in part, through autonomic and peripheral cardiovascular regulatory mechanisms. Caloric restriction increased the baroreflex sensitivity in rodents (26) humans (27), and improves several hemodynamic parameters (11). Caloric restriction also affects the contractile machinery of the heart resulting in a prolongation of both contraction and relaxation (28). Studies of rodents (29) and humans (30) suggest that an overall reduction in sympathetic activity contributes to the lowering of BP during caloric restriction and that, conversely, increased sympathetic activity may promote hypertension in individuals who overeat. Changes in the hypothalamus might also contribute to the effects of caloric restriction and IF on cardiovascular function and glucose metabolism. As evidence, administration of a NPY antagonist into the paraventricular nucleus

(PVN) of the hypothalamus abolished caloric restriction-induced reduction of BP, demonstrating a requirement for NPY activity in the PVN (31).

Regular physical exercise, which induces metabolic stress on the cardiovascular system and skeletal muscles, can also reduce BP, HR, glucose, and insulin levels (32). Human athletes who maintain regular aerobic exercise regimens typically reduce their resting HR and BP by 2 10% below those who do not maintain a regular exercise schedule (33 35). When physical exercise of rats is increased by providing them with access to a running wheel, their resting HR is significantly decreased and their BP is reduced by 5 10% (36). Levels of plasma glucose and insulin are decreased in humans and rodents maintained on regular exercise programs; insulin levels typically decrease by 15 25%, while glucose levels decrease by 5 15% (37, 38). The magnitudes of decreases in BP, HR, and insulin levels in rats maintained on the IF diet and the 2DG-supplemented diet in the present study are therefore equal to or greater than those achieved by regular aerobic exercise.

The abilities of IF and dietary supplementation with 2DG to improve cardiovascular risk factors in rats suggest novel approaches for reducing several major diseases associated with increased BP and insulin resistance, including atherosclerotic cardiovascular disease, stroke, and type 2 diabetes. One implication of our findings is that a reduction in meal frequency may be beneficial to the cardiovascular system. It will be of considerable interest to determine whether long-term dietary supplementation with 2DG is beneficial in animal models of atherosclerosis and diabetes. Although the possible effects of dietary supplementation with 2DG on specific diseases and life span remain to be determined, emerging data suggest that 2DG supplementation can mimic several different beneficial effects of caloric restriction. For example, daily administration of 2DG to rats or mice for 2 weeks protected neurons and improved functional outcome in models of stroke (19) and Parkinson s disease (18). IF and 2DG have each been shown to induce a cellular stress response which has been proposed to be central to their cytoprotective actions in the nervous system (18, 19). A well-known beneficial effect of caloric restriction is to increase the resistance of animals to various environmental stressors, including high temperature (39) and to a number of different toxins (40). Daily administration of 2DG to rats increased amounts of heat-shock protein-70 and glucose-regulated protein-78 in brain cells, consistent with 2DG inducing a cellular stress response similar to that observed in rats or mice maintained on a dietary restriction regimen (12, 16). Several clinical uses of 2DG have been suggested based on studies of rodents, including treatment of tumors (41, 42) and neuroprotection in stroke and Parkinson s diseases (18, 19). Our findings suggest the possibility that IF and dietary supplementation with 2DG can reduce the risk of cardiovascular disease and diabetes. Studies of the effects of IF and 2DG supplementation in humans will be required to determine the extent to which the improvements in cardiovascular and metabolic risk factors can be achieved in humans.

Although the present findings demonstrate beneficial effects of IF and 2DG supplementation on cardiovascular parameters (BP and HR) and glucose metabolism (decreased glucose and insulin levels), it remains to be established whether these effects are key to life span extension by dietary

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Fig. 3

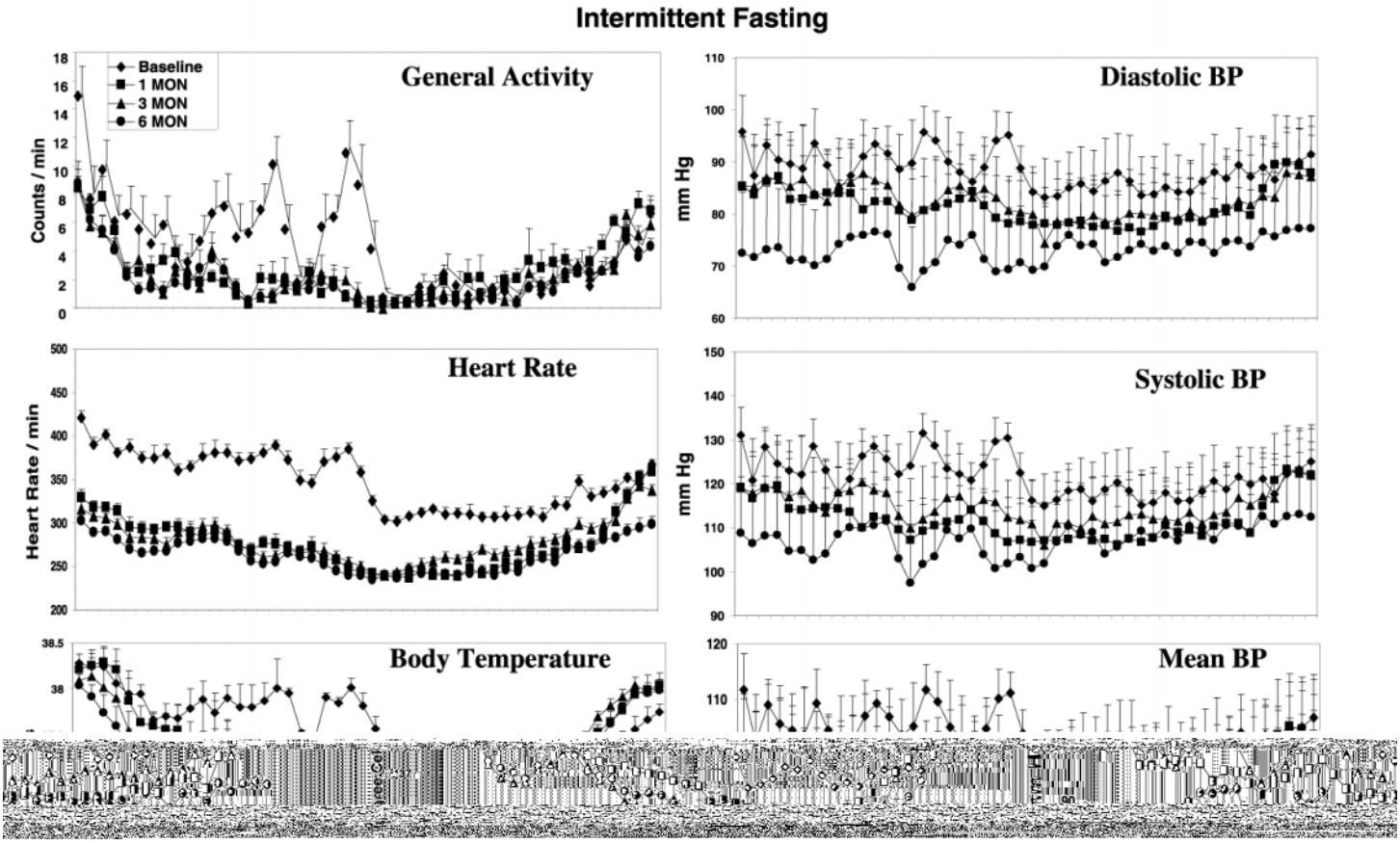

Figure 3. Circadian changes of physiological parameters prior to and during a 6-month period in rats maintained on an IF regimen. Values are the mean and SD of measurements taken from eight rats. There were significant decreases with time on diet for each of the physiological parameters: general activity baseline versus 1, 3, and 6 months ($P<0.05$ for each diet time point); HR baseline versus 1, 3, and 6 months ($P<0.01$ for each diet time point); BT baseline versus 1, 3, and 6 months ($P<0.05$ for the 1-month time point, $P<0.01$ for the 3 and 6 month time points); diastolic BP baseline versus 1, 3, and 6 months ($P<0.05$ for the 1- and 3-month time points, and $P<0.01$ for the 6-month time point); systolic BP baseline versus 1, 3, and 6 months ($P<0.05$ for the 1- and 3-month time points, and $P<0.01$ for the 6-month time point); mean BP baseline versus 1, 3, and 6 months ($P<0.05$ for the 1- and 3-month time points, and $P<0.01$ for the 6-month time point).

Fig. 4

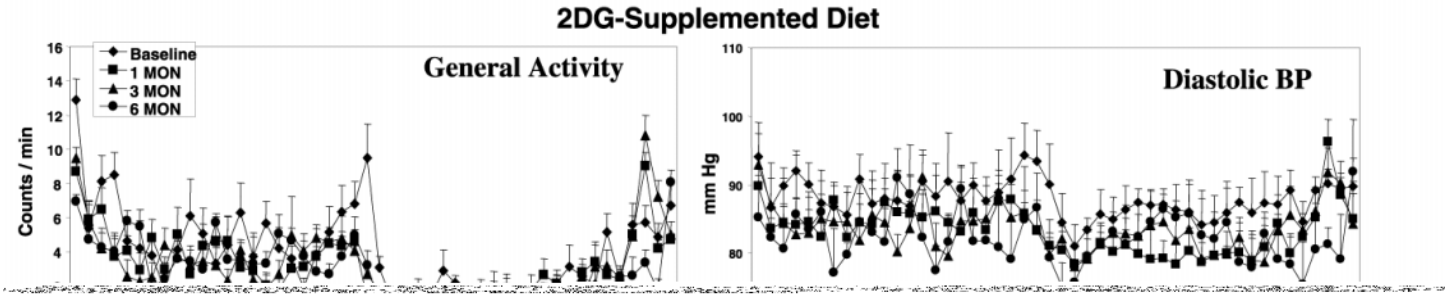

Figure 4. Circadian changes of physiological parameters prior to and during a 6-month period in rats maintained on a 2DG-supplemented diet. Values are the mean and SD of measurements made in eight rats. There was no significant effect of time on general activity or BT. HR showed a significant decrease with time ($P<0.05$ at each time point, compared with baseline). Diastolic, systolic and mean BPs showed an overall time effect ($P<0.05$ in each case).

Fig. 5

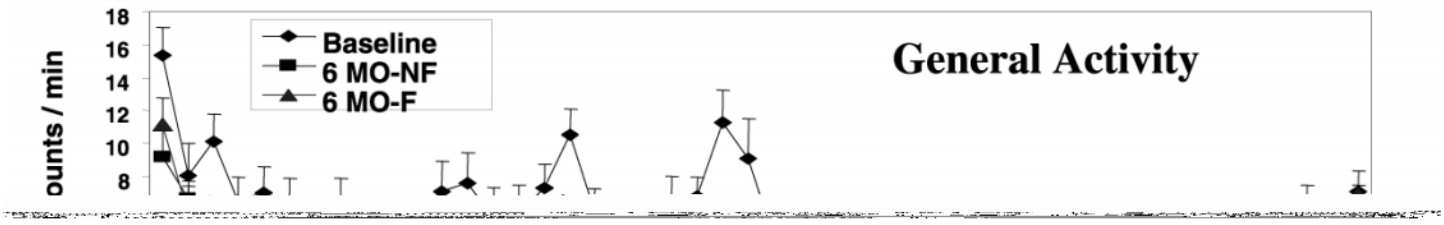

Figure 5. IF and dietary supplementation with 2DG decrease BP and HR in rats. Values are the mean and SE of determinations made in five to eight rats. Compared with rats fed ad libitum, IF significantly reduced HR ($P<0.01$), mean BP ($P<0.01$), general activity ($P<0.05$), and body temperature ($P<0.01$). Compared with rats fed ad libitum, 2DG supplementation significantly reduced HR ($P<0.05$), mean BP ($P<0.01$), but had no significant effects on general activity or body temperature.