ASSESSMENT OF ENHANCED ENDOTHELIUM – DEPENDENT VASODILATION BY INTERMITTENT FASTING IN WISTAR ALBINO RATS

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Abstract: Intermittent fasting (IF), a type of feeding regimen where the frequency of eating is reduced enhances cardiovascular stress adaptation and improves cardiovascular risk factors in rats. Data on the effect of IF on the endothelium is not common, so we examined whether IF showed similarity to documented beneficial effects of caloric restriction on endothelium-dependent vasodilatory responses of rat aortic rings. 25 young male Wistar rats had ad libitum (AL) access to food and 25 others were provided with food every other day for 2 months, during which their weight was measured every 2 weeks. Vascular reactivity of abdominal aorta was simultaneously evaluated using dual wire myographs. Weight gain was greater in the AL group (P<0.001) at all weighing intervals. Acetylcholine (ACh; 10⁻¹⁰–10⁻⁵ M) produced greater (P<0.05) vasorelaxation in IF rats at the two highest concentrations. IF reduces weight gain in young male rats and improves their aortic endothelium-dependent vasorelaxation.

Key words: Aorta Acetylcholine Intermittent Fasting Vasodilation

INTRODUCTION

The vascular endothelium is an important regulator of vascular function. The endothelium secretes in a paracrine fashion vasoactive substances that mediate antithrombotic functions (1), and regulate vascular tone and blood pressure (2). Endothelial dysfunction is characterized by prothrombic tendency, a proinflammatory state, and mainly by reduced vasodilatory responses due to decrease in nitric oxide (NO) bioavailability. It also has been implicated in the pathophysiology of different cardiovascular diseases and has been associated with increased risk factors for...
atherosclerosis. In laboratory studies, endothelial dysfunction is evaluated by impairment of endothelium-dependent vasorelaxation by using acetylcholine as the ideal stimulus for endothelium-dependent NO release (3, 4).

Intermittent fasting (IF) is a form of dietary regimen in addition to the common caloric restriction (CR) regimen, which relies on daily reduction of energy intake. IF, however, depends on altering the frequency of food consumption and generally involves alternating feast and fast periods of 24 hours each. Regarding cardiovascular disease risk, comparable improvements of functional and metabolic cardiovascular factors by both types of feeding regimen have been confirmed in rodents (5, 6). These include lower total cholesterol and triacylglycerol concentration (7), decreased heart rate and blood pressure (8). In man, CR can influence endothelial function of the vasculature in obese hypertensive subjects (9) and obese healthy subjects when CR is combined with physical activity (10). In aorta of lean rats, short-term moderate CR was shown to induce coordinated increments of endothelium-dependent vasodilation, eNOS expression and function (11).

The literature is very sparse in data and information about the influence of IF regimen on the endothelium. In a relatively recent study, evidence of increased NO production and decreased arterial blood pressure (ABP) was presented in Wistar rats subjected to a 4 week IF regimen (12). Our aim was to investigate whether an IF regimen would also enhance endothelium-dependent vasodilation in Wistar rats aortic rings.

METHODS

Experimental animals

50 Male Wistar albino rats (175-250 g) were obtained from Al-Hassa Breeding Laboratories, King Faisal University. The animals were housed in a temperature-controlled (22±2°C) room with a 12:12-h light-dark cycle in the animal house at Dammam medical college, K.F.U.

Feeding regimen and weight measurement

All rats had free access to water and were fed standard laboratory rat chow. 25 rats were fed ad-libitum (AL) and the other 25 rats were maintained on an intermittent fasting diet (IF), where they were fed every other day for a period of 8 weeks. Early in the day of fasting, any food pellets remaining from the previous day were removed to ensure none were hidden by the rats in the husk in the cage. Body weight of all rats was determined at the beginning of the feeding regimen and then measured every two weeks.

All animal care and experimental procedures were approved by the Ethics committee on Animal Care and Use in King Faisal University and followed the American Physiological Society Animal Care Guidelines.

Preparation of aortic rings

Eight weeks after the initiation of the feeding regimen, one rat from each group (one AL and one IF) was used daily. The rat was anaesthetized with chloroform, and the abdominal aorta was excised and trimmed of connective tissue and fat around it. The
vessel was then immersed in a Petri dish filled with Krebs solution of the composition (mmole/L): NaCl, 118.4; KCl, 4.7; CaCl₂, 2.52; MgSO₄, 1.18; KH₂PO₄, 1.18; D-glucose, 11.1 and NaHCO₃, 25.0. The dissected aorta was then cut into 1.8 mm long ring segments.

Measurement of vascular reactivity

Two ring segments (one from AL rat and the other form IF rat) were mounted on dual wire-type myographs (ADInstruments). The dual wire myograph is suitable for simultaneous testing of two vessels exposed to identical conditions. Two stainless steel wires (o.d. = 40 µm) were inserted through the lumen of the vessel and then secured to two jaws. One jaw is attached to a micropositioner to control vessel circumference and pretension. The other jaw is attached to a sensitive built-in force transducer for the measurement of tension. The aortic rings were immersed in a 10 ml bath containing oxygenated (95% O₂ and 5% CO₂) Krebs solution (pH = 7.4) maintained at 37°C. The rings were stretched gradually to an optimal resting tension of 2 g and equilibrated for 60 minutes (13).

All drugs were purchased from Sigma Pharmaceutical Group. To evaluate endothelium-dependent vasodilation, acetylcholine was used as an agonist. The rings were precontracted submaximally by adding 2×10⁻⁷ M epinephrine (4), and acetylcholine was then added cumulatively (10⁻¹⁰ to 10⁻⁵ M) to the myograph bath to obtain concentration-relaxation curves. Vasoconstriction due to epinephrine was considered as an increase in tension from the resting value after equilibration. The relaxation response to acetylcholine was expressed as a percentage decrease of the maximum contractile response induced by epinephrine. The integrity of the endothelium was initially tested with 10⁻⁵ M acetylcholine, and rings that produced less than 50% relaxation were discarded (14). The EC₅₀, the concentration of acetylcholine that produces 50% of its maximum response were determined graphically from the concentration-response curve for each aortic ring (IF and AL). The mean EC₅₀ for the IF group was then compared to the mean EC₅₀ of the AL group.

To clarify whether the vasorelaxation due to acetylcholine in our study is NO mediated and endothelium-dependent, the nitric oxide synthase (NOS) inhibitor, N⁶-nitro-L-arginine methyl ester (L-NAME, 2×10⁻⁴ M) was added to the organ bath before adding the highest concentration of acetylcholine (10⁻⁵ M) (15).

Statistics

Data are shown as means±SEM. Statistical analysis of the data was carried out by using independent Student's t-test for unpaired observations. Differences were considered to be statistically significant when P<0.05. The Statistical Package for Social Sciences (SPSS: version 11) program was used for data analysis.

RESULTS

Effect of intermittent fasting (IF) on body weight

Body weight recorded every other week showed a progressive increase in both ad libitum (AL) and intermittent fasting (IF)
was always lower in the IF group at any interval than the AL group. Interestingly, the ratio of percent weight gain in the IF to the AL group was maintained almost constant at a value close to 0.6 throughout the feeding regimen. This represents about 40% reduction in weight gain in the IF rats compared to the AL rats at all weighing intervals (Table II).

There was no significant difference in the mean basal body weight between the two groups at the beginning of the study (P = 0.966), however the mean weight of the IF group was significantly lower than the AL group at any weighing interval (Table I, Fig. 1). As for the net weight gain from the initial weight, AL rats gained about 145 g by the end of the 8 week feeding period, while in the IF group, the weight gain was only 83 g. The weight gain every two weeks in relation to the initial weight was expressed as a percentage. The percentage weight gain was always lower in the IF group at any interval than the AL group. Interestingly, the ratio of percent weight gain in the IF to the AL group was maintained almost constant at a value close to 0.6 throughout the feeding regimen. This represents about 40% reduction in weight gain in the IF rats compared to the AL rats at all weighing intervals (Table II).

Intermittent fasting (IF) and vasodilation

Vasorelaxation was achievable in the aortic rings before incubation with L-NAME, however incubation with L-NAME caused significant attenuation of acetylcholine induced relaxation of the aortic rings in both groups (data not included). Acetylcholine (ACh) induced a greater relaxation in the IF group than the AL group (Fig. 2). The
percentage relaxation due to cumulative concentrations of acetylcholine (10^{-10}–10^{-5}M) in IF rats exceeds that in AL rats. Although the mean percentage relaxation showed an obvious greater relaxation effect of acetylcholine in IF group when compared to AL group, the existing difference only reached significant levels (P<0.05) with the highest two acetylcholine concentrations (10^{-6} and 10^{-5}M). However, neither of the effective acetylcholine concentrations (10^{-6} and 10^{-5}M) could produce 100% relaxation in IF rat aortic rings (Fig. 3).

Comparison of the mean EC_{50} values for acetylcholine’s effect on vasodilation showed that the IF feeding regimen caused a statistically significant decrease in the mean EC_{50} from 4.21×10^{-7}M in AL fed animals to 0.784×10^{-7}M in IF rats (Table III), representing a higher sensitivity of IF rings to acetylcholine.

### DISCUSSION

The present findings demonstrate the effect of IF on rat body weight and on endothelium-dependent vasodilation. The
issue of the effect of IF regimen on body weight has been debated for long in both humans and rodents, since body weight responses have been variable in both. In mice, weight gain was noted after 8 weeks of IF regimen (8, 16), but in other trials on mice, IF for 12 weeks induced a reduction in body weight (17, 18).

In our study, there was weight gain in both groups rather than weight loss in the IF rats, and this could be due to the young age of the rats in both groups. Studies relating male Wistar rat body weight to age place the rats used at the beginning of our study (175-250 g) at an approximate juvenile age of 6-8 weeks (8, 20). Moreover, the final weight achieved in the AL group at 15-16 weeks (after 8 weeks of feeding) is consistent with values in other studies on male Wistar rats, ensuring appropriate weight gain in the AL group (19, 20). During juvenile years, rats usually undergo a growth spurt, the reason why IF regimen may have caused a reduction in weight gain rather than weight loss: this reduction in weight gain could only have been caused by a reduction in energy intake in the IF rats. Another interesting finding in our study is that reduction in weight gain was noted as early as 2 weeks of IF regimen, whereas other studies on rats have not shown any effect on body weight after 2 weeks of IF (21).

To date, few studies using animal models have shown beneficial effects of IF on cardiovascular disease risk factors (5, 6, 7, 17, 22). Our results demonstrate that IF may enhance endothelial function by improving endothelium-dependent vasodilation to acetylcholine. Our results are similar to the effects of short term, moderate CR which has shown improvement in endothelium-dependent vasodilation in rat aortic rings (11).

The mechanism by which IF improves endothelium-dependent vasodilation to acetylcholine is still unclear. One suggestion is that IF could be increasing the bioavailability of NO. The addition of L-NAME in our study was for the purpose of proving that the acetylcholine-induced vasorelaxation was partly mediated by NO in both the AL and IF groups. Since acetylcholine-induced vasodilation was greater in the IF group, this may provide some evidence to the effectiveness of IF in stimulating some pathways responsible for NO production. In a recent study (12), a shorter term IF regimen (4 weeks) increased NO production as well as reduced arterial blood pressure in Wistar rats. Another hypotheses on the mechanism of how IF can protect the heart is by a significant improvement in the NO-cGMP pathway (23) and enhancement of the relaxation mechanism in aortic smooth muscle cells.

The limitations to this study lie in two factors. First, the amount of food consumed daily by both groups of rats was not measured, but since the weight in the AL group was consistent with another study (20) that showed that male Wistar rats gained around 135 g (220 g at 7 weeks to 355 g at 15 weeks), we felt confident that their daily food intake was adequate. Second, that the mechanisms of action of IF are merely suggested through judgment from the physiological effects observed, however, measuring directly such parameters as eNOS, NO and cGMP levels upon stimulation by acetylcholine in rat aortic rings (4, 24) is required and would offer more detail to IF’s effect on endothelium-dependent vasodilation.

In conclusion, applying a dietary regimen such as IF may help in reducing endothelium-related cardiovascular risk factors. Since IF, not necessarily on an
alternating day basis, may probably be easier for humans to follow as a dietary modification than CR, further studies and research on humans would be very valuable.

REFERENCES


