Effect of 4-H Time Restricted Feeding on Body Weight, Leptin Concentration and Lipid Profile in Healthy Non-Obese Male Wistar Rats

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Abstract
Background and Aims: The present study investigates the effect of 4-h time restricted feeding on body weight, leptin concentration and lipid profile in healthy non-obese male Wistar rats.

Methods and Results: Rats placed on time-restricted feeding (TRF) regimen had freely access to food for 4 hour per day at designated periods. Twenty four rats divided into four groups (n=6) were used. Group I animals were placed on a 4 hours per day TRF between 8 am-12 pm. Group II rats were also placed on a 4 hours per day TRF between 12 pm - 4 pm. Group III rats also placed on a 4 hours per day TRF between 8 pm- 12 am while Group IV rats had access to food and water on ad libitum. This diet strategy resembles taking only breakfast, lunch or dinner once a day. The study lasted for a period of 4 weeks with daily food intake and weekly body weight determined throughout the period. At the end of the experimental period, blood glucose, lipid profile and leptin concentration were assessed.

In this study, the body weights and leptin concentrations of 8 pm-12 am and ad libitum groups significantly increased compared with the 8 am-12 pm and 12 pm-4 pm groups. Dyslipidemia was observed in the ad libitum group when compared with the 8 am-12 pm and 12 pm-4 pm groups.

Conclusion: 4-hr time restricted feeding for a period of 4 weeks has beneficial effects on body weight, blood glucose, lipid profile and leptin concentration in non-obese male Wistar rats.

Keywords: Time restricted feeding; Leptin; Body weight; Lipid profile

Introduction
Obesity affects one-third of the general population [1]. In 2014, the World Health Organization estimated that there were more than 1.9 billion adult overweight people of whom more than 600 million were obese [2]. More than 115 million people are estimated to have obesity-associated diseases such as cardiovascular disease, stroke, diabetes, dyslipidaemia, obstructive sleep apnea, metabolic syndrome, and breast, colon, and liver cancers [3].

Changes in diet and exercise are the main preventive and management approaches for obesity. However, the majority of obese patients are unable to accomplish or sustain intentional weight reduction by diet and exercise alone [4]. Over the last two decades, human and animal studies have shown that timing of meal intake is as important as the composition of the diet and caloric quantity to prevent obesity and its complications [5-8]. Mealtime and cultural eating habits, the quantity, and type of foods ingested can adversely affect health status and increase the likelihood of developing obesity and related complications. It has been suggested that time-restricted food intake might be a successful intervention to prevent and manage obesity, metabolic syndrome, and its complications [9-11].

Behavioral weight loss approaches are effective at helping individuals reduce weight by 5-10% of their initial body weight [12,13], but a primary public health goal remains prevention of obesity, along with identifying behavioral strategies to optimize weight loss and maintenance. Of the many factors contributing to the obesity epidemic, the timing of food consumption has become the subject of much recent attention for its significant contribution to body weight regulation [14,15]. Notably, food timing is a potentially modifiable behavioral target, and as such, deserves critical examination for its possible role in achieving weight loss or weight maintenance.

Intermittent fasting (IF) is an increasingly popular dietary approach used for weight loss and overall health. While there is an increasing body of evidence demonstrating beneficial effects of IF on blood lipids and other health outcomes in the overweight and obese, limited data are available about the effect of IF in healthy non-obese animals or humans. This study therefore aimed at investigating the effect of 4 hour time-restricted feeding on body weight, leptin concentration and lipid profile in healthy non-obese male Wistar rats.

Methods
Animals
Male Wistar rats (110-140 g) were purchased from animal house, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. All animals were housed in ventilated cages on wood chip bedding with a 12 h light/dark cycle, room temperature of 22°C and 40-60% relative humidity. Rats were acclimatized for a period of 2 weeks before the commencement of the experiment. They also had free access to tap water and were kept on normal diet during the entire study. Body weight was measured every week throughout the experiment. Animal experiments were performed according to the institutional guidelines for the design and statistical analysis of experiments using laboratory animals.

Time-restricted feeding
The rats placed on time-restricted feeding (TRF) regimen had freely access to food for 4 hours per day at designated periods as follows

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– 8 am - 12 pm; 12 pm - 4 pm; 8 pm -12 am for a period of 4 weeks. This diet strategy resembles taking only breakfast, lunch or dinner once a day for a period of 4 weeks.

**Experimental design**

Twenty four rats divided into four groups (n=6) were used. Group I animals were placed on a 4 hour per day TRF between 8 am-12 pm. Group II rats were also placed on a 4 hour per day TRF between 12 pm - 4 pm. Group III rats also placed on a 4 hour per day TRF between 8 pm -12 am while Group IV rats had access food and water ad libitum. The study lasted for a period of 4 weeks.

**Measurement of body weight and food intake**

Body weight of rats was measured weekly using a weighing balance. The percentage (%) weight gain was calculated as: (body weight on specific week (g) – initial body weight)/initial body weight ×100. Food intake was measured once a week over 24 h based on the weight of leftover feed out of 160 g given.

**Sample preparation**

At the end of treatment period, the rats were anesthetized with sodium pentobarbital. Blood was collected by cardiac puncture into heparinized bottle and was centrifuged at 3000 g for 5 mins. Plasma was stored frozen until needed for biochemical assay.

**Biochemical assays**

Rats were fasted for 12 h before blood glucose measurement. Food was removed from the food dispenser and bedding of the cages was changed to avoid coprophagy. Blood from the tail vein was measured for glucose using the OneTouch Basic glucose monitor (Lifescan) and reported as mg/dl. Blood glucose level was measured during the study and prior to sacrifice. Fasting plasma levels of total cholesterol (TC) and triglyceride (TG) were measured by standardized enzymatic colorimetric methods using assay kit obtained from Fortress Diagnostics Ltd. (Antrim, UK). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daichi Pure Chemicals Co., Ltd., Tokyo, Japan) whereas low-density lipoprotein-cholesterol (LDL-C) was estimated using modified Friedewald’s formula. TC/HDL-C and TG/HDL-C ratios were estimated as marker of atherogenic lipid indices. Plasma leptin was determined using enzyme-linked immunosorbent assay (ELISA) kits following manufacturer’s instruction (leptin: RayBio® Rat Leptin ELISA Kit, Cat # ELR-Leptin-001, Norcross, GA, USA).

**Data analysis and statistics**

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS statistical software (version 21). One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni’s posthoc test was used to identify the significance of pair wise comparisons of mean values among the groups. Statistically significant differences were accepted at p<0.05.

**Results**

**Physiological parameters (Body weight, food intake)**

The body weight of 8 pm -12 am and ad libitum groups significantly increased compared with the 8 am -12 pm and 12 pm - 4 pm groups (Figures 1 and 2).

**Blood glucose**

The fasting blood glucose levels of the normal and the time-restricted fed rats were shown in Figure 3. Fasting glycemia in the 12 pm - 4 pm fed rats increased significantly when compared with the other three groups.

**Circulating lipids**

Figure 4 depicts the lipid profile of the normal and the time-restricted fed rats. There were significant increases (p<0.05) in the total cholesterol (TC), triglyceride (TG) and very low density lipoprotein cholesterol (VLDL-C) levels of group fed ad libitum when compared with the 8 am -12 pm and 12 pm - 4 pm groups. However, there was
no significant difference between the 8 pm -12 am and ad libitum TC, TG and VLDL-C levels. For the LDL-C level, there was a significant increase (p<0.05) in the 4 pm - 8 pm rats when compared with the 8 am - 12 pm and 12 pm - 4 pm rats. There was however a non-significant increase in the ad libitum group compared with the 8 am -12 pm group. HDL-C levels of the 8 pm -12 am and ad libitum groups decreased significantly when compared with the 8 am -12 pm and 12 pm - 4 pm groups.

Leptin concentration

Animals in the groups fed ad libitum had significantly elevated leptin concentration (p<0.001) when compared with the other three groups. There was however a non-significant increase in the leptin concentration of the 8 pm - 12 pm when compared with the 8 am -12 pm and 12 pm - 4 pm groups (Figure 5).

Discussion

Intermittent fasting has gained huge recognition over the past decade. Intermittent fasting can be divided into two major subcategories: 1) fasting 1–4 d per week, i.e. alternate day fasting (ADF) or the 5:2 diet [16]; or 2) fasting every day for a 14 to 20 h period, i.e. time restricted feeding [17,18]. This study examined the impact of time restricted feeding (TRF) on body weight and metabolic disease risk factors in healthy non-obese rats. Time-restricted feeding (TRF) is a type of intermittent fasting that involves having a longer fasting period daily. A key point of the TRF approach made use of in the present study is that total daily calorie intake remained the same while the frequency of meals (i.e. time between meals) was altered. This is unlike many other IF regimens, most of which have the goal of reducing total energy intake.

Our data shows that 4 weeks of 4-h time restricted feeding (i.e. between the hours of 8 am – 12 pm, 12 pm - 4 pm) resulted in a significant decrease in body weight of the rats in these groups when compared to the group that had access to food ad libitum. This result is however in agreement with other forms of time restricted feeding which reported significant decreases in the body weight of obese and trained athletes exposed to 8-h and 10-h time restricted feeding respectively (A,B,C). Gill and Panda [19] who also examined the effects of 10-h time restricted feeding in overweight healthy adults, showed a 4% weight loss that was sustained for one year. The significant change observed in body weight of rats exposed to 4-h time restricted feeding between the hours of 8 am – 12 pm, 12 pm – 4 pm for a period of 4 weeks, suggests that non-obese individuals can also not benefit from time restricted feeding in terms of weight loss. Intermittent fasting (IF) of daily fasting for up to 16 hours; or fasting periods of up to 24 hours interspersed with normal feeding days have also been reported to result in weight loss in both humans and laboratory animal models [20].

Dyslipidemia is a condition characterized by abnormal lipid status (such as triglycerides [TG], cholesterol and/or phospholipids) in the blood. It includes elevated blood concentrations of the triad; low density lipoprotein cholesterol (LDL-C), TG, and decreased high-density lipoprotein cholesterol (HDL-C) [21]. Diet is considered to be one of the most important risk factors, which plays a key role in
the development of dyslipidemia [22]. Results from this work showed altered circulating plasma lipid concentrations i.e dyslipidemia. There were significant increases in TC, TG, LDL-C and VLDL-C concentrations with a significant decrease in HDL-C concentration in the 8.00 pm - 12 am and ad libitum rats compared to other regimes of TRF.

Several epidemiological studies have established that dyslipidemia is a major cause of cardiovascular disease [23,24]. Altered circulating lipid concentrations are recognized as risk factors for cardiovascular diseases (CVDs) [25]. CVDs are the leading cause of global premature mortality and disability [26]. It has also been shown that an increased level of TC (hypercholesterolemia), particularly LDL-C promotes the atherosclerosis process, leading to the deposition of cholesterol and fatty acids in the artery wall, whilst HDL-C is usually considered to be protective and returns cholesterol to the liver [27,28]. Epidemiological studies including that of the American Heart Association have also shown that elevated TG levels correlate with elevated CV risk, thus elevated TG levels are an important marker of CV risk [29,30].

This diet strategy of 4 weeks of 4-h TRF (between the hours of 8 am – 12 pm and 12 pm - 4 pm) suggests cardio-protective effects in non-obese rats, by lowering total cholesterol, triglycerides, LDL and VLDL while increasing HDL cholesterol concentrations. These changes in lipid risk factors are in line with what has been reported for obese alternate day fasting (ADF) subjects [31]. Also, In two ADF studies, triacylglycerols decreased by 15% after 8 weeks of treatment in obese men and women [32]. Thus, TRF may improve plasma lipids in non-obese subjects as it does in obese subjects. Our results were also confirmed by previous researches suggesting a positive effect of TRF (between the hours of 8 am -12 pm, 12 pm - 4 pm) on blood lipid profiles [33-35].

Additional vascular benefit including decrease in circulating leptin was also noted in non-obese rats in our diet strategies of TRF compared to the group fed ad libitum. The group fed ad libitum has an outrageous elevated significant leptin level compared to the TRF groups Leptin is a hormone. However, obesity is characterized by hyperleptinemia due to the development of leptin resistance [37]. Scarpone and Zhang (2007) also reported a positive correlation between elevated leptin level and the development of leptin resistance and obesity [38]. Apart from obesity, hyperleptinemia has been also associated with hypertension and insulin resistance [39,40]. The peripheral actions of leptin include stimulation of inflammatory reaction, oxidative stress, atherogenesis and thrombosis, thus promoting endothelial dysfunction, arterial stiffness, development and vulnerability of atherosclerotic plaques [41,42]. Higher leptin levels have also been considered to be the cause of infertility [43].

Results from this study also suggest that of all the TRF groups, the 8 am -12 pm, which is a representation of breakfast consumption was the most beneficial since it prevents dyslipidemia, increase in body weight, blood glucose and leptin concentrations. Regular breakfast consumption has been reported to improve several metabolic parameters, mostly associated with cardiovascular risk, reducing circulating LDL cholesterol levels, LDL oxidation and lowers blood triglycerol concentrations [44-46]. This also brings to mind the quote of the nutritionist Adelle Davis in the (1960s), which says: “Eat breakfast like a king, lunch like a prince and dinner like a pauper” [47].

In conclusion, 4-h time restricted feeding for a period of 4 weeks has beneficial effects on body weight, blood glucose, lipid profile and leptin concentration in healthy non-obese male Wistar rats.

References

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