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Time-restricted feeding reduces adiposity in mice fed a high-fat diet

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ABSTRACT

Disruption of the circadian rhythm contributes to obesity. This study tested the hypothesis that time-restricted feeding (TRF) reduces high-fat diet–induced increase in adiposity. Male C57BL/6 mice were fed the AIN93G or the high-fat diet ad libitum (ad lib); TRF of the high-fat diet for 12 or 8 hours during the dark cycle was initiated when high-fat diet–fed mice exhibited significant increases in body weight. Energy intake of the TRF 12-hour group was not different from that of the high-fat ad lib group, although that of the TRF 8-hour group was slightly but significantly lower. Restricted feeding of the high-fat diet reduced body fat mass and body weight compared with mice fed the high-fat diet ad lib. There were no differences in respiratory exchange ratio (RER) among TRF and high-fat ad lib groups, but the RER of these groups was lower than that of the AIN93G group. Energy expenditure of the TRF groups was slightly but significantly lower than that of the high-fat ad lib group. Plasma concentrations of ghrelin were increased in TRF groups compared with both AIN93G and high-fat ad lib groups. Elevations of plasma concentrations of insulin, leptin, monocyte chemoattractant protein-1, and tissue inhibitor metalloproteinase-1 by high-fat ad lib feeding were reduced by TRF to the levels of mice fed the AIN93G diet. In conclusion, TRF during the dark cycle reduces high-fat diet–induced increases in adiposity and proinflammatory cytokines. These results indicate that circadian timing of food intake may prevent obesity and abate obesity-related metabolic disturbance.

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1. Introduction

All mammals exhibit circadian rhythms in daily functions. An important component of energy homeostasis is the coordination of daily rhythms in rest and activity, feeding behavior, energy utilization, and energy storage over the light/dark cycle [1]. Disruption of the circadian rhythm by eating at the "wrong" time may lead to disruption of energy homeostasis and obesity [2,3]. Chronic overeating [4] during the "wrong" times of the day is often observed in obese humans due to unremitting hunger without satiation leading to exacerbation of metabolic syndrome [5]. Laboratory rodents fed energy-rich high-fat diets exhibit loss of the circadian rhythm, increase food intake, and have greater body fat mass and body weight [6].

Regulation of energy homeostasis involves adipose tissue and brain [7]. Adipose tissue mediates long-term energy storage and

Abbreviations: ad lib, ad libitum; MCP-1, monocyte chemoattractant protein-1; RER, respiratory exchange ratio; TIMP-1, tissue inhibitor of metalloproteinase-1; TRF, time-restricted feeding; VCO_2, rate of CO_2 production; VO_2, rate O_2 consumption.

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signals the brain regarding whole-body energy homeostasis and thermoregulation [8]. Disruption of this rhythm further enhances the development of obesity and metabolic syndrome [9]. Adipokines (leptin, adiponectin, etc) and nutrient-sensitive hormones (ghrelin and insulin) exhibit a circadian rhythm–dependent secretory pattern [9]. The temporal disruption in cellular metabolic processes controlled by adipokines predisposes the organism to obesity and obesity-related diseases [10]. Obesity in turn exacerbates adipose tissue dysfunction and modulates the secretion of proinflammatory cytokines leading to chronic low-grade inflammation and angiogenesis, which enhances obesity-related systemic metabolic disorders such as cardiovascular diseases, diabetes, and cancer [11].

Prevention of obesity can attenuate health problems, including those associated with metabolic syndrome [12]. Current intervention strategies to alleviate obesity and its associated complications focus on lifestyle interventions including reducing energy intake and increasing energy expenditure by physical exercise [13,14]. Successful initial and long-term maintenance of weight loss by dietary changes is hampered by the need for behavioral adherence to food choices, portion sizes, and participation in physical exercise. Another behavioral weight control strategy is intermittent fasting, involving either complete or partial restriction of energy intake several days a week [15]. In many cases, however, the success of weight loss and behavioral strategies are limited [12] because of lack of compliance and long-term adherence.

Time-restricted feeding (TRF) is another form of intermittent fasting, wherein energy intake is scheduled to specified hours in a day [15]. Such restriction in energy intake is suggested to be useful in regulation of weight and adiposity [15]. Mice consumed a higher amount of their daily food intake during the light cycle than during the dark cycle [16]. Restricted feeding of a high-fat diet during the light cycle for a short time (4 hours) resulted in lower body weight compared with mice fed a low-fat diet ad libitum (ad lib), although they consumed the same amount of calories [5]. Other studies showed that restricted feeding of a high-fat diet in nonobese wild-type mice during the dark cycle did not affect energy intake but reduced body weight, body fat mass, and markers related to metabolic disturbance [7,10]. However, the potential of TRF to reduce adiposity in obese mice or mice with excessive body fat has not been explored. The objective of this study was to test the hypothesis that TRF reduces high-fat diet–induced increase in body adiposity. We took the approach of applying restricted feeding during the dark cycle to high-fat diet–fed mice showing significant increases in body weight. The dark cycle was chosen for the restricted feeding because it is the active phase of the diurnal rhythm for nocturnal animals [1].

2. Methods and materials

2.1. Animals and diets

Three-week-old male C57BL/6 mice (Harlan, Madison, WI, USA) were maintained in a pathogen-free room on a 12:12-hour light/dark cycle with a temperature of 22 ± 1°C. Two diets were used in this study, the AIN93G diet [17] providing 16% of energy from corn oil and a modified AIN93G diet providing 45% of energy from corn oil (high-fat diet; Table). All diets were powder diets; they were stored at –20°C until being provided to mice. Gross energy of each diet (Table) was analyzed by oxygen bomb calorimetry (Model 6200, Oxygen Bomb Calorimeter; Parr Instrument, Moline, IL, USA).

Table – Composition of experimental diets

	AIN93G	High-fat
Ingredient	g/kg	g/kg
Corn starch	397.5	42.4
Casein	200	239.2
Dextrin	132	239.2
Sucrose	100	119.6
Corn oil	70	239.2
Cellulose	50	59.8
AIN93 mineral mix	35	41.9
AIN93 vitamin mix	10	12
L-Cystine	3	3.6
Choline bitartrate	2.5	3
t-Butylhydroquinone	0.014	0.02
Total	1000	1000
Energy	%	%
Protein	20	20
Fat	16	45
Carbohydrate	64	35
Analyzed gross energy (kJ) [a]	18.41 ± 0.42	22.18 ± 0.42

[a] Values are means ± SEM (n = 3 per diet).

2.2. Experimental design

This study was approved by the Animal Care and Use Committee of the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center. The procedures followed the National Institute of Health guidelines for the care and use of laboratory animals [18]. To determine differences in body weight, assuming a standard deviation of 3 g and $\alpha = .05$, 12 mice per group were needed to have 90% power to detect a difference of 5 g in body weight between any 2 treatment groups. After acclimation with the AIN93G diet for 1 week, mice were randomly assigned into 2 groups and fed the AIN93G (n = 12) or the high-fat diet (n = 36) ad lib. When significant differences in body weights were observed between the groups (2 weeks after the initiation of experimental feeding), the high-fat diet–fed mice were further assigned into 3 groups of 12 animals. The 3 high-fat diet groups were as follows: (1) mice fed ad lib (free access to diet), (2) mice fed for 12 hours between zeitgeber times 12 and 24 (12-hour restricted feeding during the dark cycle), and (3) mice fed for 8 hours between zeitgeber times 13 and 21 (8-hour restricted feeding during the dark cycle). Zeitgeber time 0 is the time of lights on. Food access to TRF groups was regulated by transferring mice daily between cages with diet and water and cages with water alone. To control mouse handling, mice in unrestricted feeding groups were transferred between cages with both diet and water between zeitgeber times 12 and 24. The 2 TRF groups were designed to determine an optimal level of restriction that reduced body fat mass without adversely affecting animal growth. Food intake was recorded 5 days per week for 5