Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

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

*Sneha Sundaram, Lin Yan**

US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202, USA

ARTICLE INFO

Article history:
Received 5 December 2015
Revised 8 February 2016
Accepted 16 February 2016

Keywords:
Time-restricted feeding
Ad libitum
High-fat diet
Adiposity
Mice

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.

Published by Elsevier Inc.

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.

* *Corresponding author at:* USDA, ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, Grand Forks, ND 58202, USA. Tel.: +1 701 795 8499; fax: +1 701 795 8220.

E-mail addresses: sneha.sundaram@ars.usda.gov (S. Sundaram), lin.yan@ars.usda.gov (L. Yan).

http://dx.doi.org/10.1016/j.nutres.2016.02.005
0271-5317/Published by Elsevier Inc.

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

consecutive weeks starting 1 week after the initiation of restricted feeding. Body composition analysis of fat and lean mass from conscious, immobilized mice was performed 1 week before the termination of the experiment by quantitative magnetic resonance imaging (Echo whole-body composition analyzer, Model 100; Echo Medical System, Houston, TX, USA). At termination, mice in the ad lib feeding groups were fasted for 8 hours, whereas mice on the 12- and 8-hour restriction were euthanized at zeitgeber time 12 (the restricted feeding times served as fasting). Mice were euthanized by an intraperitoneal injection of ketamine (100 mg/kg)/xylazine (10 mg/kg). Plasma was collected and stored at –80°C for further analysis.

2.3. *Metabolic study*

Whole-body metabolic status of mice was evaluated by indirect calorimetry in a comprehensive laboratory animal monitoring system (CLAMS; Columbus Instruments, Columbus, OH, USA) 8 weeks after the initiation of restricted feeding. After a 24-hour acclimation period, activity level, rate of O_2 consumption (VO_2), and rate of CO_2 production (VCO_2) were measured for 1 minute every 16 minutes over a 48-hour period. The respiratory exchange ratio (RER = VCO_2/VO_2) was calculated for dark and light cycles. Energy expenditure was calculated using the equations of Weir [19].

2.4. *Quantification of adipokines and related biomarkers in plasma*

Sandwich enzyme-linked immunosorbent assay kits were used to quantify plasma concentrations of ghrelin (R&D Systems, Minneapolis, MN, USA), insulin (Mercodia, Inc, Winston Salem, NC, USA), adipokines (leptin, adiponectin, and monocyte chemoattractant protein-1 [MCP-1]), and angiogenic factor tissue inhibitor of metalloproteinase-1 (TIMP-1; R&D Systems) following manufacturers' protocols. Samples were read within the linear range of the assay, and accuracy of the analysis was confirmed with the controls provided in each kit.

2.5. *Statistical analyses*

One-way analysis of variance (ANOVA) and Tukey contrasts were used to compare differences among the groups. Pearson correlations were performed between body weight and body fat mass and between body weight and plasma concentrations of ghrelin, insulin, and inflammatory and angiogenic markers. All data are presented as means ± SEM. Differences with a P value of .05 or less are considered significant. All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

3. Results

3.1. *Body weight, body composition, and energy intake*

Compared with the AIN93G control diet, the high-fat diet increased body weight 2 weeks after the initiation of experimental feeding in the ad lib–fed groups ($P < .05$; Fig. 1); the increase in body weight was maintained throughout the experiment (Fig. 1). Compared with mice fed the high-fat diet ad lib, mice on 12- and 8-hour TRF exhibited lower body weights ($P < .05$) at 7 and 3 weeks, respectively, after the initiation of restricted feeding (Fig. 1); the lower body weight was maintained throughout the experiment (Fig. 1).

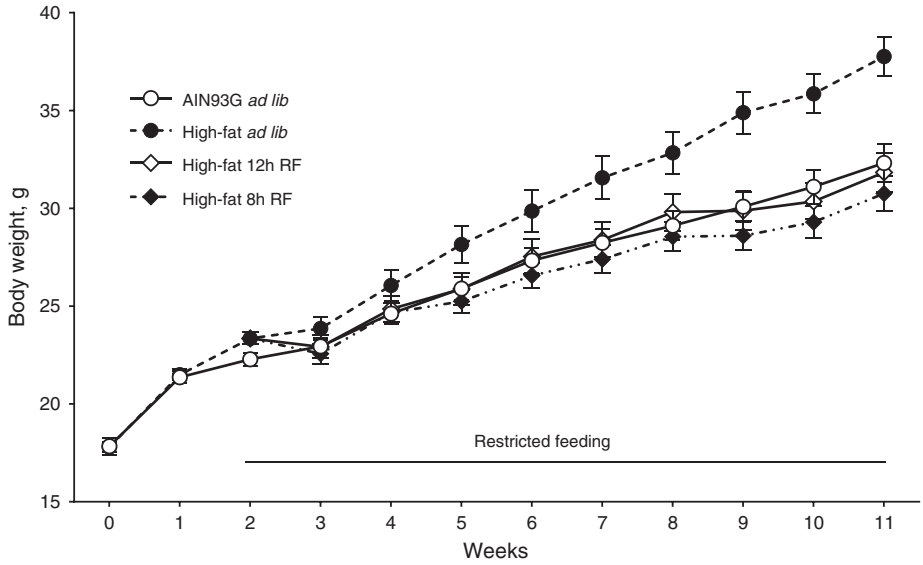

Fig. 1 – Time-restricted feeding reduces body weight in mice fed a high-fat diet. One-way ANOVA and Tukey contrasts were performed to test for differences among the groups. Values are means ± SEM (n = 12 per group). Mice fed the high-fat diet were heavier than those fed the AIN93G diet; the difference was significant 2 weeks after the initiation of experimental feeding ($P < .05$). The 12- and 8-hour TRF of the high-fat diet reduced body weight ($P < .05$) compared with the high-fat ad lib feeding 7 and 3 weeks, respectively, after the initiation of restricted feeding. RF, restricted feeding.

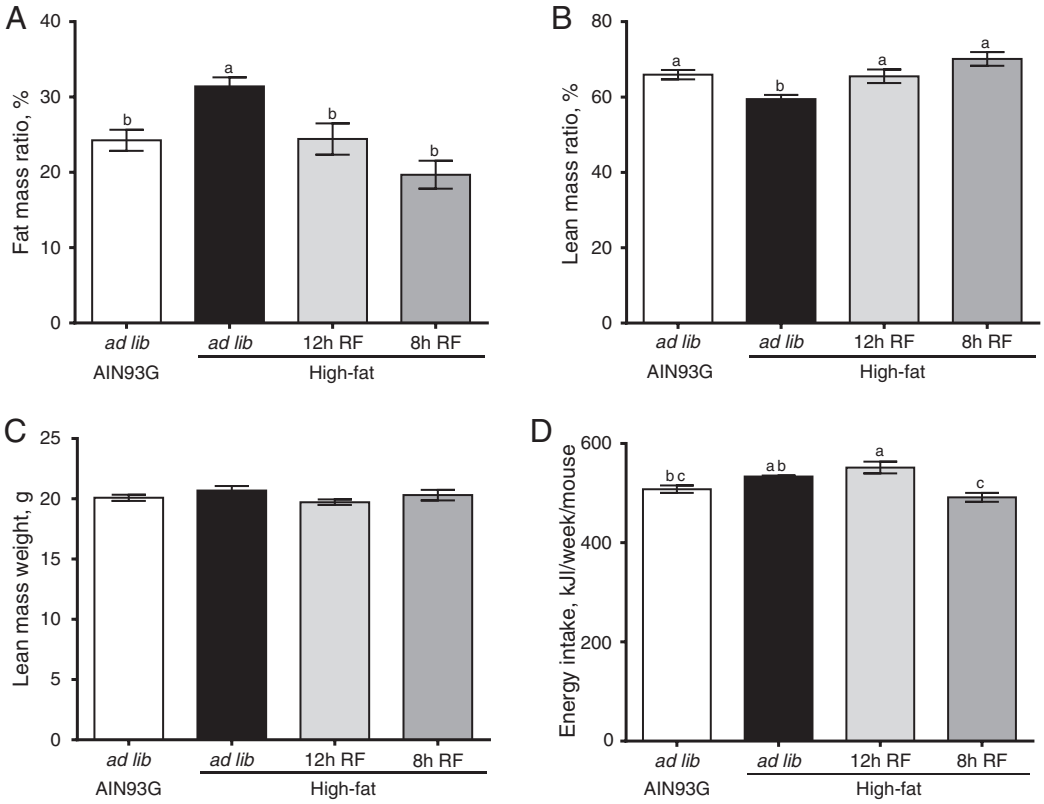

Fig. 2 – Effects of TRF on fat mass: body mass ratio (A), lean mass/body mass ratio (B), absolute lean mass weight (C), and energy intake (D) of mice fed a high-fat diet. One-way ANOVA and Tukey contrasts were performed to test for differences among the groups. Values (means ± SEM) with different letters (a, b, c) are significantly different at $P < .05$ (n = 12 per group; n = 6 per group for energy intake). □, AIN93G ad lib; ■, high-fat ad lib; ▭, high-fat 12-hour RF; ▬, high-fat 8-hour RF. RF, restricted feeding.

The high-fat ad lib feeding increased percent body fat mass ($P < .05$; Fig. 2A) and correspondingly reduced percent lean body mass ($P < .05$; Fig. 2B), compared with the AIN93G ad lib feeding. Restricted feedings reduced percent body fat mass ($P < .05$; Fig. 2A) and correspondingly increased percent body lean mass ($P < .05$; Fig. 2B) compared with the high-fat ad lib feeding. Pearson correlation analysis showed that body fat mass weight was positively correlated with body weight ($r = 0.92$, $P < .01$). There were no differences in absolute lean mass weight among the groups (Fig. 2C). There were no differences in energy intake between groups fed the AIN93G and the high-fat diets ad lib (Fig. 2D). Energy intake of the 12-hour TRF group did not differ from that of the high-fat ad lib group (Fig. 2D). Energy intake of the 8-hour TRF group was lower, 8% and 11%, respectively, than that of the high-fat ad lib group ($P < .05$) and the 12-hour TRF group ($P < .05$), but not different from the AIN93G ad lib group (Fig. 2D).

3.2. *Metabolic measurements*

Changes in RER across the dark and light cycles of mice with different feeding regimen are presented in Fig. 3A. The mean RER of mice fed the AIN93G diet ad lib was 0.92 (Fig. 3B) and was higher than all other groups, indicating a greater proportional contribution of carbohydrate oxidation. The mean RER of mice fed the high-fat diet, regardless of the feeding regimen, was approximately 10% lower than that of mice fed the AIN93G diet ($P < .05$; Fig. 3B), indicating a greater proportional contribution of fatty acid metabolism for energy expenditure.

In ad lib–fed mice, consumption of the high-fat diet reduced VO_2 by 15% compared with the AIN93G diet ($P < .05$; Fig. 4A). Restricted feedings of the high-fat diet, regardless of the feeding regimen, elevated VO_2, but only the difference between the 12-hour TRF group and the high-fat ad lib group was significant ($P < .05$; Fig. 4A). Similarly, the high-fat diet reduced VCO_2 compared with the AIN93G diet in ad lib–fed mice ($P < .05$; Fig. 4B). Restricted feedings of the high-fat diet elevated VCO_2 slightly compared with the high-fat ad lib group, but the differences were not significant (Fig. 4B). There were no significant differences in average ambulatory activity (for 48 hours) among all 4 groups, regardless of the type of diet or the feeding regimen (Fig. 4C). There was no significant difference in energy expenditure between the AIN93G and the high-fat diet in ad lib–fed mice (Fig. 4D). Restricted feedings, regardless of the feeding regimen, reduced energy expenditure by approximately 10% compared with the high-fat ad lib feeding ($P < .05$; Fig. 4D).

3.3. *Plasma concentrations of ghrelin, insulin, inflammatory cytokines, and angiogenic factors*

In order to further characterize TRF-induced metabolic responses, we measured multiple plasma hormones and cytokines that are modified by obesity. Ghrelin is a peptide hormone that

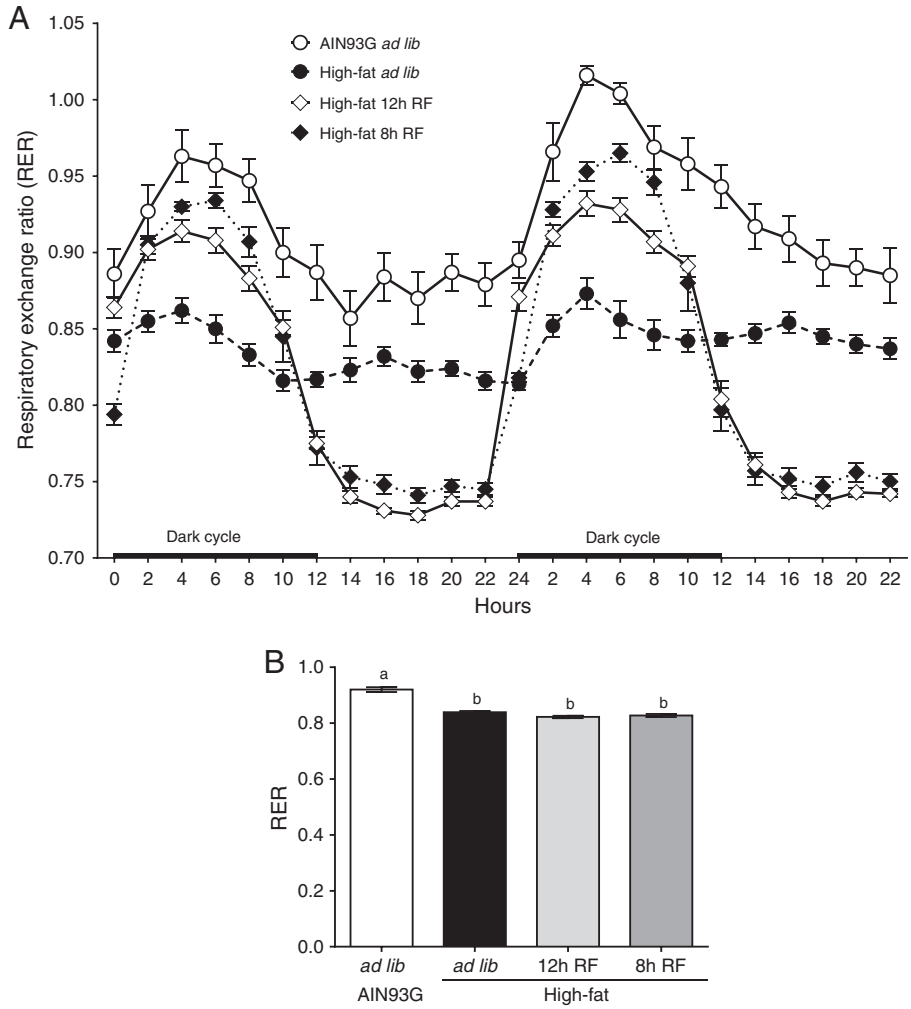

Fig. 3 – Time-restricted feeding improves RER (A) and mean RER (B) in mice fed a high-fat diet. One-way ANOVA and Tukey contrasts were performed to test for differences among the groups. Values (means ± SEM) with different letters are significantly different at $P < .05$ (n = 12 per group). □, AIN93G ad lib; ■, high-fat ad lib; ▫, high-fat 12-hour RF; ▪, high-fat 8-hour RF. RF, restricted feeding.

regulates appetite and food intake [20,21]; concentrations of ghrelin are elevated in hunger and reduced in satiation. There was no significant difference in plasma concentrations of ghrelin between the AIN93G and the high-fat diets in ad lib–fed groups (Fig. 5A). Restricted feeding of the high-fat diet for 12 and 8 hours resulted in dose-dependent increases in plasma ghrelin compared with mice fed the high-fat diet ad lib ($P < .05$; Fig. 5A). Pearson correlation analysis showed that body weight was negatively correlated with concentration of ghrelin in plasma ($r = –0.45$, $P < .01$).

In ad lib–fed groups, the high-fat diet significantly increased fasting plasma concentrations of insulin by 39% compared with the AIN93G diet ($P < .05$; Fig. 5B). Restricted feeding for 12 and 8 hours significantly reduced plasma insulin by 18% and 24%, respectively, compared with mice fed the high-fat diet ad lib ($P < .05$; Fig. 5B). Pearson correlation analysis showed that body weight was positively correlated with plasma concentration of insulin ($r = 0.57$, $P < .01$).

Leptin concentrations in plasma were elevated by 2.4-fold by the high-fat diet compared with the AIN93G diet in ad lib–fed groups ($P < .05$; Fig. 5C). Restricted feeding for 12 and 8 hours reduced plasma leptin by 53% and 63%, respectively, compared with mice fed the high-fat diet ad lib ($P < .05$; Fig. 5C). Pearson correlation analysis showed that body weight was positively correlated with leptin in plasma ($r = 0.89$, $P < .01$).

Plasma concentrations of adiponectin in mice fed the high-fat diet ad lib were 24% lower than those fed the AIN93G diet ad lib ($P < .05$; Fig. 5D). Restricted feeding of the high-fat diet resulted in slight, but dose-dependent increases in plasma adiponectin; the difference between the 8-hour TRF group and the high-fat ad lib group was significantly different ($P < .05$; Fig. 5D). Pearson correlation analysis showed that body weight was not correlated with adiponectin in plasma.

Monocyte chemoattractant protein-1 is a potent proinflammatory cytokine, and its expression is in proportion with body adiposity [22]. In ad lib–fed mice, the high-fat diet significantly increased plasma concentrations of MCP-1 by 58% compared with the AIN93G diet ($P < .05$; Fig. 5E). Restricted feeding for 12 and 8 hours reduced plasma MCP-1 by 31% and 35%, respectively, compared with the high-fat diet ad lib feeding ($P < .05$; Fig. 5E). Pearson correlation analysis showed

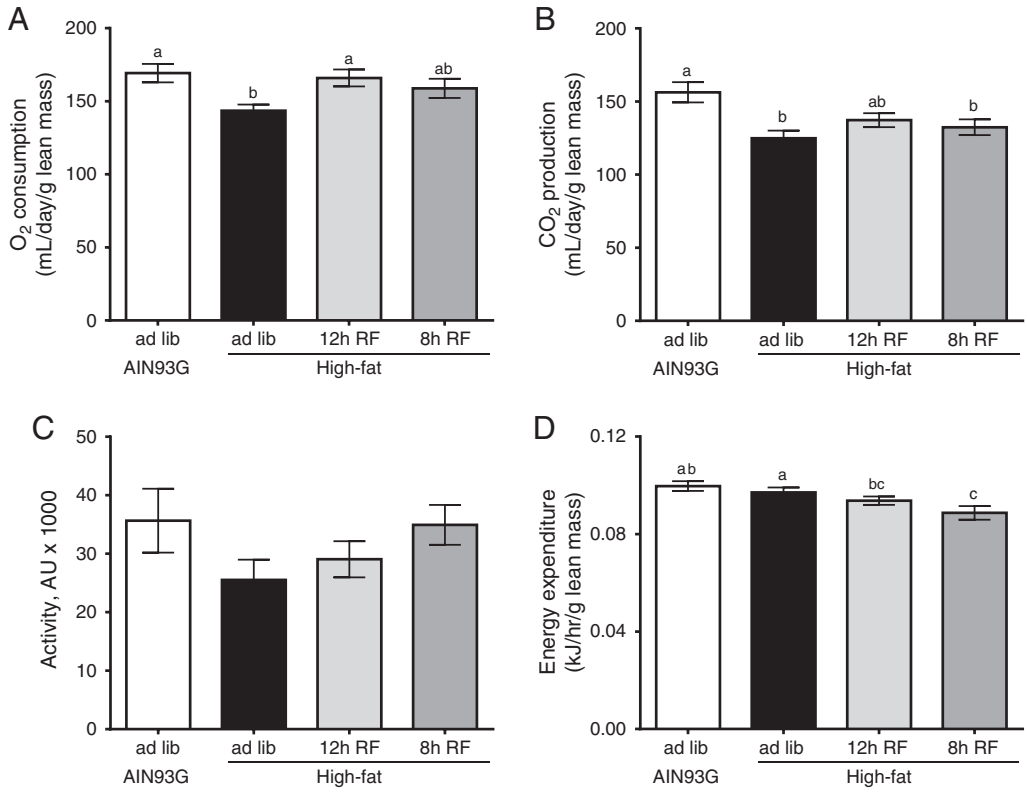

Fig. 4 – Effects of TRF on oxygen consumption (A), CO_2 production (B), physical activity (C), and energy expenditure (D) in mice fed a high-fat diet. One-way ANOVA and Tukey contrasts were performed to test for differences among the groups. Values (means ± SEM) with different letters (a, b, c) are significantly different at $P < .05$ (n = 12 per group). □, AIN93G ad lib; ■, high-fat ad lib; ▨, high-fat 12-hour RF; ▩, high-fat 8-hour RF. RF, restricted feeding.

that body weight was positively correlated with MCP-1 in plasma ($r = 0.59$, $P < .01$).

Tissue inhibitor of metalloproteinase-1 is an angiogenic factor that contributes to obesity; blood concentrations of TIMP-1 are elevated in obese humans [23]. Mice fed the high-fat diet ad lib exhibited a 28% increase in plasma concentrations of TIMP-1 compared with the AIN93G-fed controls, but the difference was not statistically significant (Fig. 5F). Restricted feedings, regardless of the feeding regimen, significantly reduced plasma TIMP-1 by approximately 25% compared with mice fed the high-fat diet ad lib ($P < .05$; Fig. 5F). Pearson correlation analysis showed that body weight was positively correlated with TIMP-1 in plasma ($r = 0.43$, $P < .01$).

4. Discussion

The present study showed that restricted feeding of a high-fat diet to mice during the dark cycle resulted in reductions in body adiposity and body weight but with comparable energy intake to mice fed a high-fat diet ad lib. Our results support previous reports that circadian timing of food intake affects weight gain [3,16] and that restricted feeding of a high-fat diet during dark cycle prevents adipogenesis in nonobese wild-type mice [7,10]. Most importantly, our results demonstrated that restricted feeding reduced body adiposity following significant increases in body weights in high-fat diet–fed mice. We accept the hypothesis that TRF reduces high-fat diet–induced adiposity and the concept that disruption of circadian rhythm contributes to obesity.

Respiratory exchange ratio is a measure of the proportional contribution of carbohydrates and fatty acids as energy sources during feeding [24]. An RER close to 1 indicates that the energy source is mainly carbohydrates and that close to 0.7 indicates that it is mainly fatty acids [24] with some oscillation between the 2 values throughout a feeding cycle. In the present study, the RER of mice fed the high-fat diet ad lib was similar to the reported values from obese mice (around 0.8 to 0.85) with dampened oscillation [25]. Restricted feeding improved the diurnal rhythms of RER oscillation, ranging from approximately 0.9 to 0.75, between the feeding (dark) and fasting (light) cycles. Furthermore, reduced energy expenditure in mice with TRF suggests that these mice conserve energy in response to fasting during the light period. This may be explained by metabolic adaptation [26], in which energy expenditure is reduced in response to low energy intake. Taken together, these results suggest that TRF resets the metabolic rhythms.

Ghrelin, a peptide hormone produced by ghrelinergic cells in gastrointestinal tract, regulates appetite and food intake and maintains energy balance [20,21]. Elevations in plasma concentrations of ghrelin in mice with TRF indicate that the circadian timing of food intake results in a state of fasting and chronic negative energy balance. Our results do not agree

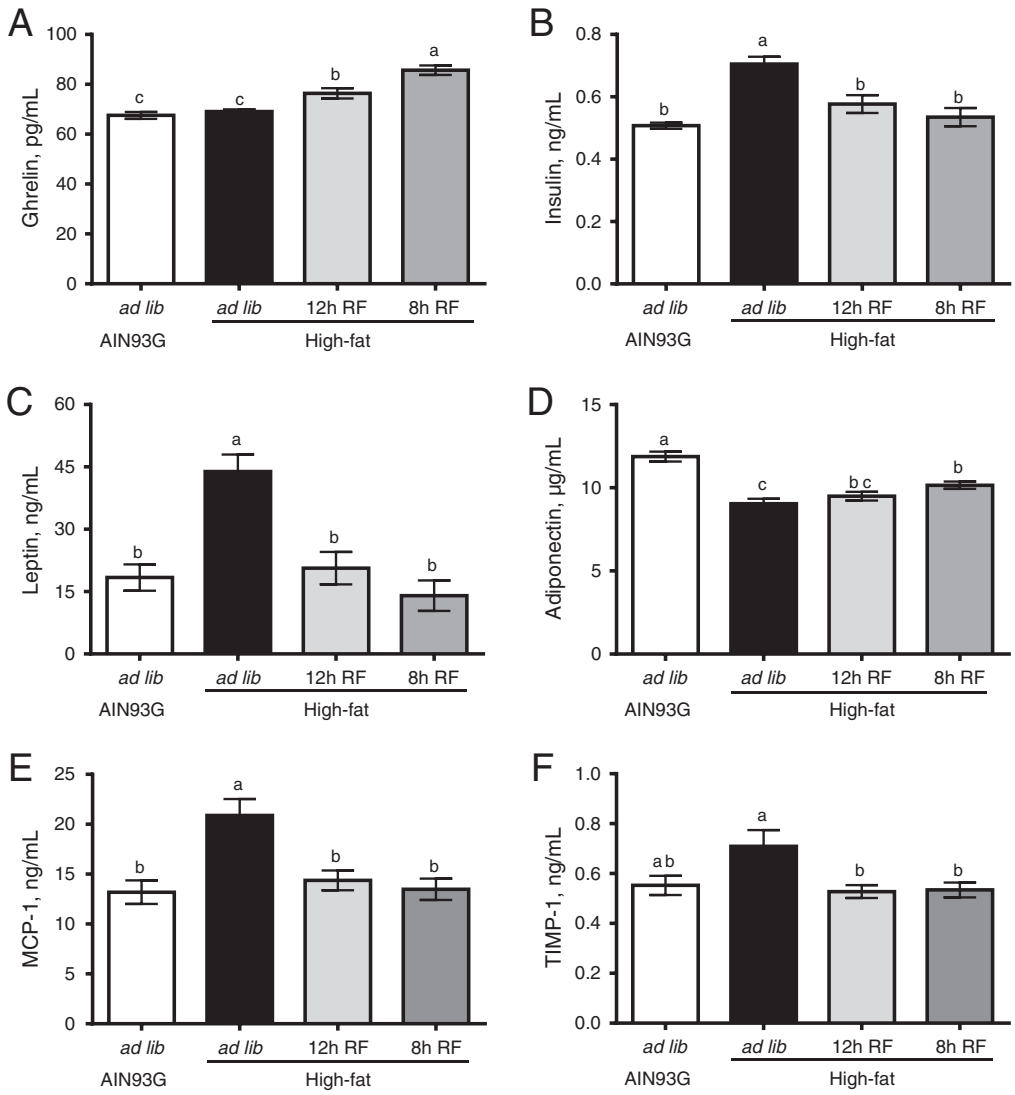

Fig. 5 – Effects of TRF on plasma concentrations of ghrelin (A), insulin (B), leptin (C), adiponectin (D), MCP-1 (E), and TIMP-1 (F) in mice fed a high-fat diet. One-way ANOVA and Tukey contrasts were performed to test for differences among the groups. Values (means ± SEM) with different letters (a, b, c) are significantly different at $P < .05$ (n = 12 per group). □, AIN93G ad lib; ■, high-fat ad lib; ▨, high-fat 12-hour RF; ▩, high-fat 8-hour RF. RF, restricted feeding.

with a previous report [5] that TRF resulted in a satiated state in mice, as there were no differences in serum concentrations of ghrelin between groups with restricted and ad lib feeding of a high-fat diet. This disagreement may be explained by differences in feeding regime. In our study, the diet was available to mice for 12 or 8 hours during the dark cycle, but it was only available for 4 hours during the light cycle in the previous study [5].

Adipose tissue is an endocrine organ that produces and releases proinflammatory and anti-inflammatory cytokines (eg, leptin and adiponectin, respectively) that actively participate in regulation of energy metabolism in physiology and pathophysiology. Leptin regulates satiety and energy intake [27]; elevations in blood concentrations of leptin, which is proportional to that of insulin, correlate with metabolic disturbance in rodent obesity models [28,29]. Adiponectin regulates lipid and glucose metabolism, increases insulin sensitivity, and protects against chronic inflammation [30,31]. Consistent with our previous reports [28], feeding mice the high-fat diet significantly elevated concentrations of leptin and insulin and reduced that of adiponectin in plasma. Restricted feeding of the high-fat diet, regardless of the feeding regimen, significantly reduced concentrations of leptin and insulin and elevated that of adiponectin in plasma. These results indicate that TRF entrains the circadian clock and metabolic regulators to fixed feeding times and attenuates the high-fat diet–induced metabolic disturbance.

Monocyte chemoattractant protein-1, primarily identified as a chemotactic factor for attracting immune cells to the sites of inflammation, is a potent inflammatory cytokine. Adipose MCP-1 expression is in proportion with adiposity and body mass index [22], and circulating MCP-1 is reduced after weight loss in obese subjects [32] or after fasting in obese rodents [33]. That elevations in plasma MCP-1 concentrations in mice fed the high-fat diet were reversed by TRF to the levels of mice fed the AIN93G control diet supports the concept of a

positive correlation between adiposity and MCP-1 secretion [32,33] and suggests that TRF may reduce the production or secretion of adipose-produced MCP-1, at least partly, by reducing adiposity.

Adipogenesis is accompanied with angiogenesis [34]. The formation of blood vessels provides nutrients to the expanding adipose tissue and transports adipokines to the body. Tissue inhibitor of metalloproteinase-1 is a potent angiogenic factor and contributes to obesity. Blood concentrations of TIMP-1 are elevated in obese humans [23]. The expression of TIMP-1 mRNA in adipose tissues is up-regulated in mice with genetic or diet-induced obesity [35,36]. Addition of recombinant TIMP-1 to 3T3-L1 preadipocytes increases lipid accumulation during adipocyte differentiation in vitro [37]. Significant reductions in plasma concentrations of TIMP-1 by TRF, compared with mice fed the high-fat diet ad lib, suggest that the circadian timing of food intake mitigates angiogenic process during adipogenesis. This is further supported by our results that TRF reduced plasma levels of MCP-1, which is angiogenic by directly inducing vascular smooth muscle cell proliferation [38] and migration [39] and by synergistic interactions with vascular endothelial growth factor to enhance angiogenesis [40,41].

Two feeding regimens were compared in the present study to determine a period of restriction that reduces body adiposity without adversely affecting animal growth. We found similar results from both the 12- and 8-hour TRF groups, indicating that both regimens are tolerable and adapted by mice. However, an approximately 11% lower energy intake in the 8-hour TRF group compared with the 12-hour TRF group suggests that fasting beyond 12 hours a day may deprive mice of energy and nutrients necessary for optimal growth and maintenance.

Diets containing 45% or higher percentage of energy from fat are commonly used to induce adiposity in rodent models of obesity. It has been generally accepted that it is the fat content of diet that is responsible for the increases in body adiposity and body weight. The high-fat diet used in the present study contained 45% of energy from corn oil. Our results indicate that it is not the fat content of a diet but the ad lib consumption of the high-fat diet that causes attenuation of the diurnal rhythm of food intake which may be responsible for gaining excessive body fat mass in mice.

A potential limitation of the study is that corn oil was used as the source of dietary fat. Corn oil is a source of dietary fat commonly used in nutrition research. It comprises 57% linoleic acid (18:2n6) but only 1% α-linolenic acid (18:3n3), an essential n3 polyunsaturated fatty acid [42]. This should be considered when comparing our results with other studies using different sources of dietary fat.

In summary, we found that TRF of a high-fat diet during the dark cycle reduced body adiposity and body weight, following significant increases in body weight in high-fat diet–fed mice, to levels similar to AIN93G-fed controls. Furthermore, TRF decreased plasma concentrations of proinflammatory cytokines and angiogenic factors that are associated with adipogenesis. Our results support the concept that disruption of the circadian rhythm contributes to obesity. Most importantly, we conclude that TRF reduces high-fat diet–induced increase in body adiposity. Future translational studies are warranted to investigate the circadian timing of food intake in preventing obesity and obesity-related metabolic disturbance in overweight and obese populations.

Disclosure of Potential Conflicts of Interest

The authors have declared that no competing interests exist.

Acknowledgment

The authors gratefully acknowledge the assistance of the following staff of the Grand Forks Human Nutrition Research Center: Lana DeMars and Kay Keehr for technical support, LuAnn Johnson for statistical analysis, and James Lindlauf for preparing experimental diets and vivarium staff for providing high-quality animal care. The US Department of Agriculture (USDA), Agricultural Research Service, Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of trade names or commercial products in this article is solely for providing specific information and does not imply recommendation or endorsement by the USDA. Funding for this work was provided by the USDA, ARS, Research Project 3062-51000-050-00D.

REFERENCES

[1] Froy O. Circadian rhythms and obesity in mammals. ISRN Obes 2012;2012, 437198.
[2] Mattson MP, Allison DB, Fontana L, Harvie M, Longo VD, Malaisse WJ, et al. Meal frequency and timing in health and disease. Proc Natl Acad Sci U S A 2014;111:16647–53.
[3] Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. Obesity 2009;17:2100–2.
[4] Jaakkola J, Hakala P, Isolauri E, Poussa T, Laitinen K. Eating behavior influences diet, weight, and central obesity in women after pregnancy. Nutrition 2013;29:1209–13.
[5] Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. Timed high-fat diet resets circadian metabolism and prevents obesity. Faseb J 2012;26:3493–502.
[6] Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, et al. Obesity and metabolic syndrome in circadian clock mutant mice. Science 2005;308:1043–5.
[7] Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 2014;20:991–1005.
[8] Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, et al. Characterization of peripheral circadian clocks in adipose tissues. Diabetes 2006;55:962–70.
[9] Wardlaw SL, Burant CF, Klein S, Meece K, White A, Kasten T, et al. Continuous 24-hour leptin, proopiomelanocortin, and amino acid measurements in human cerebrospinal fluid: correlations with plasma leptin, soluble leptin receptor, and amino acid levels. J Clin Endocrinol Metab 2014;99:2540–8.
[10] Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 2012;15:848–60.

[11] Zhang Y, Daquinag A, Traktuev DO, Amaya-Manzanares F, Simmons PJ, March KL, et al. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. Cancer Res 2009;69:5259–66.
[12] Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001;74:579–84.
[13] Haas MC, Bodner EV, Brown CJ, Bryan D, Buys DR, Keita AD, et al. Calorie restriction in overweight seniors: response of older adults to a dieting study: the CROSSROADS randomized controlled clinical trial. J Nutr Gerontol Geriatr 2014;33: 376–400.
[14] Riebe D, Greene GW, Ruggiero L, Stillwell KM, Blissmer B, Nigg CR, et al. Evaluation of a healthy-lifestyle approach to weight management. Prev Med 2003;36:45–54.
[15] Varady KA, Bhutani S, Church EC, Klempel MC. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. Am J Clin Nutr 2009;90:1138–43.
[16] Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 2007;6: 414–21.
[17] Reeves PG, Nielsen FH, Fahey GCJ. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[18] Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. 8th ed. Washington, D.C.: National Academies Press; 2011
[19] Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109: 1–9.
[20] Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 2000;141:4255–61.
[21] Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50:1714–9.
[22] Christiansen T, Richelsen B, Bruun JM. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. Int J Obes (Lond) 2005;29: 146–50.
[23] Kralisch S, Bluher M, Tonjes A, Lossner U, Paschke R, Stumvoll M, et al. Tissue inhibitor of metalloproteinase-1 predicts adiposity in humans. Eur J Endocrinol 2007;156: 257–61.
[24] Virtue S, Vidal-Puig A. Assessment of brown adipose tissue function. Front Physiol 2013;4:128.
[25] Speakman JR. Measuring energy metabolism in the mouse—theoretical, practical, and analytical considerations. Front Physiol 2013;4:34.
[26] Dulloo AG, Girardier L. Adaptive changes in energy expenditure during refeeding following low-calorie intake: evidence for a specific metabolic component favoring fat storage. Am J Clin Nutr 1990;52:415–20.
[27] Williams KW, Scott MM, Elmquist JK. From observation to experimentation: leptin action in the mediobasal hypothalamus. Am J Clin Nutr 2009;89:985S–90S.
[28] Yan L, Graef GL, Claycombe KJ, Johnson LK. Effects of voluntary running and soy supplementation on diet-induced metabolic disturbances and inflammation in mice. J Agric Food Chem 2013;61:9373–9.
[29] Fenton JI, Nunez NP, Yakar S, Perkins SN, Hord NG, Hursting SD. Diet-induced adiposity alters the serum profile of inflammation in C57BL/6N mice as measured by antibody array. Diabetes Obes Metab 2009;11:343–54.
[30] Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004;279: 12152–62.
[31] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–6.
[32] Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci U S A 2003;100:7265–70.
[33] Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, et al. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. J Biol Chem 2003;278:46654–60.
[34] Wang H, Chen Y, Lu XA, Liu G, Fu Y, Luo Y. Endostatin prevents dietary-induced obesity by inhibiting adipogenesis and angiogenesis. Diabetes 2015;64:2442–56.
[35] Maquoi E, Munaut C, Colige A, Collen D, Lijnen HR. Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. Diabetes 2002;51:1093–101.
[36] Chavey C, Mari B, Monthouel MN, Bonnafous S, Anglard P, Van Obberghen E, et al. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J Biol Chem 2003;278: 11888–96.
[37] Alexander CM, Selvarajan S, Mudgett J, Werb Z. Stromelysin-1 regulates adipogenesis during mammary gland involution. J Cell Biol 2001;152:693–703.
[38] Selzman CH, Miller SA, Zimmerman MA, Gamboni-Robertson F, Harken AH, Banerjee A. Monocyte chemotactic protein-1 directly induces human vascular smooth muscle proliferation. Am J Physiol Heart Circ Physiol 2002;283:H1455–61.
[39] Streblow DN, Soderberg-Naucler C, Vieira J, Smith P, Wakabayashi E, Ruchti F, et al. The human cytomegalovirus chemokine receptor US28 mediates vascular smooth muscle cell migration. Cell 1999;99:511–20.
[40] Hong KH, Ryu J, Han KH. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood 2005;105:1405–7.
[41] Yamada M, Kim S, Egashira K, Takeya M, Ikeda T, Mimura O, et al. Molecular mechanism and role of endothelial monocyte chemoattractant protein-1 induction by vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 2003;23: 1996–2001.
[42] Corn Refiners Association. Corn oil; 2006 1–22[http://www.corn.org/wp-content/uploads/2009/12/CornOil.pdf].