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

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Abbreviations

ad lib; ad libitum

MCP-1; monocyte chemoattractant protein-1

RER; respiratory exchange ratio

TRF; Time-restricted feeding

TIMP-1; tissue inhibitor metalloproteinase-1

VO_2; rate O_2 consumption

VCO_2; rate of CO_2 production

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 TRF 12-hour group was not different from, while that of TRF 8-hour group was slightly but significantly lower than, that of the high-fat *ad lib* group. Restricted feeding of the high-fat diet reduced body fat mass and body weight compared to 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 were lower than 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 to 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 pro-inflammatory cytokines. These results indicate that circadian timing of food intake may prevent obesity and abate obesity-related metabolic disturbance.

Key words: time-restricted feeding, ad libitum, high-fat diet, adiposity, mice

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 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 pro-inflammatory 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 successes 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 to mice fed a low-fat diet *ad lib*, although they consumed the same amount of calories [5]. Other studies showed that restricted feeding of a high-fat diet in non-obese wild-type mice during the dark cycle did not affect caloric 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) 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 1). All diets were powder diets; they were stored at -20°C until being provided to mice. Gross energy of each diet (Table 1) was analyzed by oxygen bomb calorimetry (Model 6200, Oxygen Bomb Calorimeter, Parr Instrument, Moline, IL).

2.2. Experimental design

This study was approved by the Animal Care and Use Committee of the U.S. 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 $\alpha = 0.05$, 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: 1) mice fed *ad lib* (free access to diet); 2) mice fed for 12 hours between zeitgeber time 12 and 24 (12-hour restricted feeding during the dark cycle); 3) mice fed for 8 hours between zeitgeber time 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 time 12 and 24. The two 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). At termination, mice in the *ad lib* feeding groups were fasted for 8 hours while mice on the 12-hour and 8-hour restriction were euthanized at zeitgeber time 12 (the restricted feeding times served as fasting). Mice were euthanized by an intra-peritoneal 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) 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].

134

135 2.4 Quantification of adipokines and related biomarkers in plasma

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

142

143 2.5. Statistical analyses

144 One-way analysis of variance (ANOVA) and Tukey contrasts were used to compare differences
145 among the groups. Pearson correlations were performed between body weight and body fat mass
146 and between body weight and plasma concentrations of ghrelin, insulin and inflammatory and
147 angiogenic markers. All data are presented as means ± standard error of the mean (SEM).
148 Differences with a p value of 0.05 or less are considered significant. All statistical analyses were
149 performed using SAS software (version 9.4, SAS Institute, Cary, NC).

150

151 3. Results

152 3.1. Body weight, body composition and energy intake

153 Compared to the AIN93G control diet, the high-fat diet increased body weight 2 weeks after the
154 initiation of experimental feeding in *ad lib* fed groups ($p < 0.05$; Fig. 1); the increase in body
155 weight was maintained throughout the experiment (Fig. 1). Compared to mice fed the high-fat
156 diet *ad lib*, mice on 12-hour and 8-hour TRF exhibited lower body weights ($p < 0.05$) at 7 and 3

157 weeks, respectively, after the initiation of restricted feeding (Fig. 1); the lower body weight was
158 maintained throughout the experiment (Fig.1).
159
160 The high-fat *ad lib* feeding increased percent body fat mass ($p < 0.05$, Fig. 2A) and
161 correspondingly reduced percent lean body mass ($p < 0.05$, Fig. 2B), compared to the AIN93G
162 *ad lib* feeding. Restricted feedings reduced percent body fat mass ($p < 0.05$, Fig. 2A) and
163 correspondingly increased percent body lean mass ($p < 0.05$, Fig. 2B) compared to the high-fat
164 *ad lib* feeding. Pearson correlation analysis showed that body fat mass weight was positively
165 correlated with body weight ($r = 0.92$, $p < 0.01$). There were no differences in absolute lean
166 mass weight among the groups (Fig. 2C). There were no differences in energy intake between
167 groups fed the AIN93G and the high-fat diets *ad lib* (Fig. 2D). Energy intake of the 12-hour
168 TRF group did not differ from that of the high-fat *ad lib* group (Fig. 2D). Energy intake of the 8-
169 hour TRF group was lower, 8% and 11%, respectively, than that of the high-fat *ad lib* group ($p <$
170 0.05) and the 12-hour TRF group ($p < 0.05$), but not different from the AIN93G *ad lib* group
171 (Fig. 2D).
172
173 3.2. Metabolic measurements
174 Changes in RER across the dark and light cycles of mice with different feeding regimen are
175 presented in Fig. 3A. The mean RER of mice fed the AIN93G diet *ad lib* was 0.92 (Fig. 3B) and
176 was higher than all other groups, indicating a greater proportional contribution of carbohydrate
177 oxidation. The mean RER of mice fed the high-fat diet, regardless of the feeding regimen, was
178 approximately 10% lower than that of mice fed the AIN93G diet ($p < 0.05$, Fig. 3B), indicating a
179 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 to the AIN93G diet ($p < 0.05$, 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 ($p < 0.05$, Fig. 4A). Similarly, the high-fat diet reduced VCO_2 compared to the AIN93G diet in *ad lib* fed mice ($p < 0.05$, Fig. 4B). Restricted feedings of the high-fat diet elevated VCO_2 slightly compared to the high-fat *ad lib* group, but the differences were not significant (Fig. 4B). There were no significant differences in average ambulatory activity (over 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 to the high-fat *ad lib* feeding ($p < 0.05$, 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 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 to mice fed the high-fat diet *ad lib* ($p < 0.05$, Fig. 5A). Pearson correlation analysis

showed that body weight was negatively correlated with concentration of ghrelin in plasma (r = -0.45, $p < 0.01$).

In *ad lib* fed groups, the high-fat diet significantly increased fasting plasma concentrations of insulin by 39% compared to the AIN93G diet ($p < 0.05$, Fig. 5B). Restricted feeding for 12 and 8 hours significantly reduced plasma insulin by 18% and 24%, respectively, compared to mice fed the high-fat diet *ad lib* ($p < 0.05$, Fig. 5B). Pearson correlation analysis showed that body weight was positively correlated with plasma concentration of insulin (r = 0.57, $p < 0.01$).

Leptin concentrations in plasma were elevated by 2.4-fold by the high-fat diet compared to the AIN93G diet in *ad lib* fed groups ($p < 0.05$, Fig. 5C). Restricted feeding for 12 and 8 hours reduced plasma leptin by 53% and 63%, respectively, compared to mice fed the high-fat diet *ad lib* ($p < 0.05$, Fig. 5C). Pearson correlation analysis showed that body weight was positively correlated with leptin in plasma (r = 0.89, $p < 0.01$).

Plasma concentrations of adiponectin in mice fed the high-fat diet *ad lib* were 24% lower than those fed the AIN93G diet *ad lib* ($p < 0.05$, 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 ($p < 0.05$, Fig. 5D). Pearson correlation analysis showed that body weight was not correlated with adiponectin in plasma.

Monocyte chemoattractant protein-1 is a potent pro-inflammatory 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 to the AIN93G diet ($p < 0.05$, Fig. 5E). Restricted feeding for 12 and 8 hours reduced plasma MCP-1 by 31% and 35%, respectively, compared to the high-fat diet *ad lib* feeding ($p < 0.05$, Fig. 5E). Pearson correlation analysis showed that body weight was positively correlated with MCP-1 in plasma ($r = 0.59$, $p < 0.01$).

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 to 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 to mice fed the high-fat diet *ad lib* ($p < 0.05$, Fig. 5F). Pearson correlation analysis showed that body weight was positively correlated with TIMP-1 in plasma ($r = 0.43$, $p < 0.01$).

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 non-obese wild-type mice [7, 10]. Most importantly, our results demonstrated that restricted feeding reduced body adiposity following significant increases in

247 body weights in high-fat diet-fed mice. We accept the hypothesis that TRF reduces high-fat diet-
248 induced adiposity and the concept that disruption of circadian rhythm contributes to obesity.
249
250 Respiratory exchange ratio is a measure of the proportional contribution of carbohydrates and
251 fatty acids as energy sources during feeding [24]. An RER close to 1 indicates that the energy
252 source is mainly carbohydrates and that close to 0.7 indicates that it is mainly fatty acids [24]
253 with some oscillation between the two values throughout a feeding cycle. In the present study,
254 the RER of mice fed the high-fat diet *ad lib* was similar to the reported values from obese mice
255 (around 0.8 to 0.85) with dampened oscillation [25]. Restricted feeding improved the diurnal
256 rhythms of RER oscillation, ranging from approximately 0.9 to 0.75, between the feeding (dark)
257 and fasting (light) cycles. Furthermore, reduced energy expenditure in mice with TRF suggests
258 that these mice conserve energy in response to fasting during the light period. This may be
259 explained by metabolic adaptation [26], in which energy expenditure is reduced in response to
260 low energy intake. Taken together, these results suggest that TRF resets the metabolic rhythms.
261
262 Ghrelin, a peptide hormone produced by ghrelinergic cells in gastrointestinal tract, regulates
263 appetite and food intake and maintains energy balance [20, 21]. Elevations in plasma
264 concentrations of ghrelin in mice with TRF indicate that the circadian timing of food intake
265 results in a state of fasting and chronic negative energy balance. Our results do not agree with a
266 previous report [5] that TRF resulted in a satiated state in mice, as there were no differences in
267 serum concentrations of ghrelin between groups with restricted and *ad lib* feeding of a high-fat
268 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 pro-inflammatory and anti-inflammatory cytokines (e.g., 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 was reversed by TRF to the levels of mice fed the AIN93G control diet supports the concept of the 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 pre-adipocytes increases lipid accumulation during adipocyte differentiation *in vitro* [37]. Significant reductions in plasma concentrations of TIMP-1 by TRF, compared to 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 regimen 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-hour and 8-hour TRF groups, indicating that both regimen are tolerable and adapted by mice. However, an approximately 11% lower energy intake in the 8-hour TRF group compared to 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 even more percent 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 is comprised of 57% linoleic acid (18:2n6) but only 1% alpha-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 pro-inflammatory cytokines and angiogenic factors that are associated with adipogenesis. Our results support the concept that disruption of circadian rhythms 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.

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Disclosure of Potential Conflicts of Interest

The authors have declared that no competing interests exist.

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501 Table – Composition of experimental diets

Ingredient	AIN93G g/kg	High-fat 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 kcal/g [a]	4.4 ± 0.1	5.3 ± 0.1

502

503 [a] Values are means ± SEM, n = 3 per diet.

Legends to figures

Figure 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 ($p < 0.05$). The 12-hour and 8-hour time-restricted feeding of the high-fat diet reduced body weight ($p < 0.05$) compared to the high-fat *ad lib* feeding 7 and 3 weeks, respectively, after initiation of restricted feeding. RF: restricted feeding.

Figure 2. Effects of time-restricted feeding on fat mass: body mass ratio (A), lean mass: body mass ratio (B), absolute lean mass weight (C) and caloric 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 $p < 0.05$ (n = 12 per group; n = 6 per group for caloric intake). ☐ AIN93G *ad lib*, ■ high-fat *ad lib*, ☐ high-fat 12h RF, ☐ high-fat 8h RF. RF: restricted feeding.

Figure 3. Time-restricted feeding improves respiratory exchange ratio (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 $p < 0.05$ (n = 12 per group). ☐ AIN93G *ad lib*, ■ high-fat *ad lib*, ☐ high-fat 12h RF, ☐ high-fat 8h RF. RF: restricted feeding.

Figure 4. Effects of time-restricted feeding 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 $p < 0.05$ (n = 12 per group). ☐ AIN93G *ad lib*, ■ high-fat *ad lib*, ☐ high-fat 12h RF, ■ high-fat 8h RF. RF: restricted feeding.

Figure 5. Effects of time-restricted feeding 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 $p < 0.05$ (n = 12 per group). ☐ AIN93G *ad lib*, ■ high-fat *ad lib*, ☐ high-fat 12h RF, ■ high-fat 8h RF. RF: restricted feeding.

Figure 1

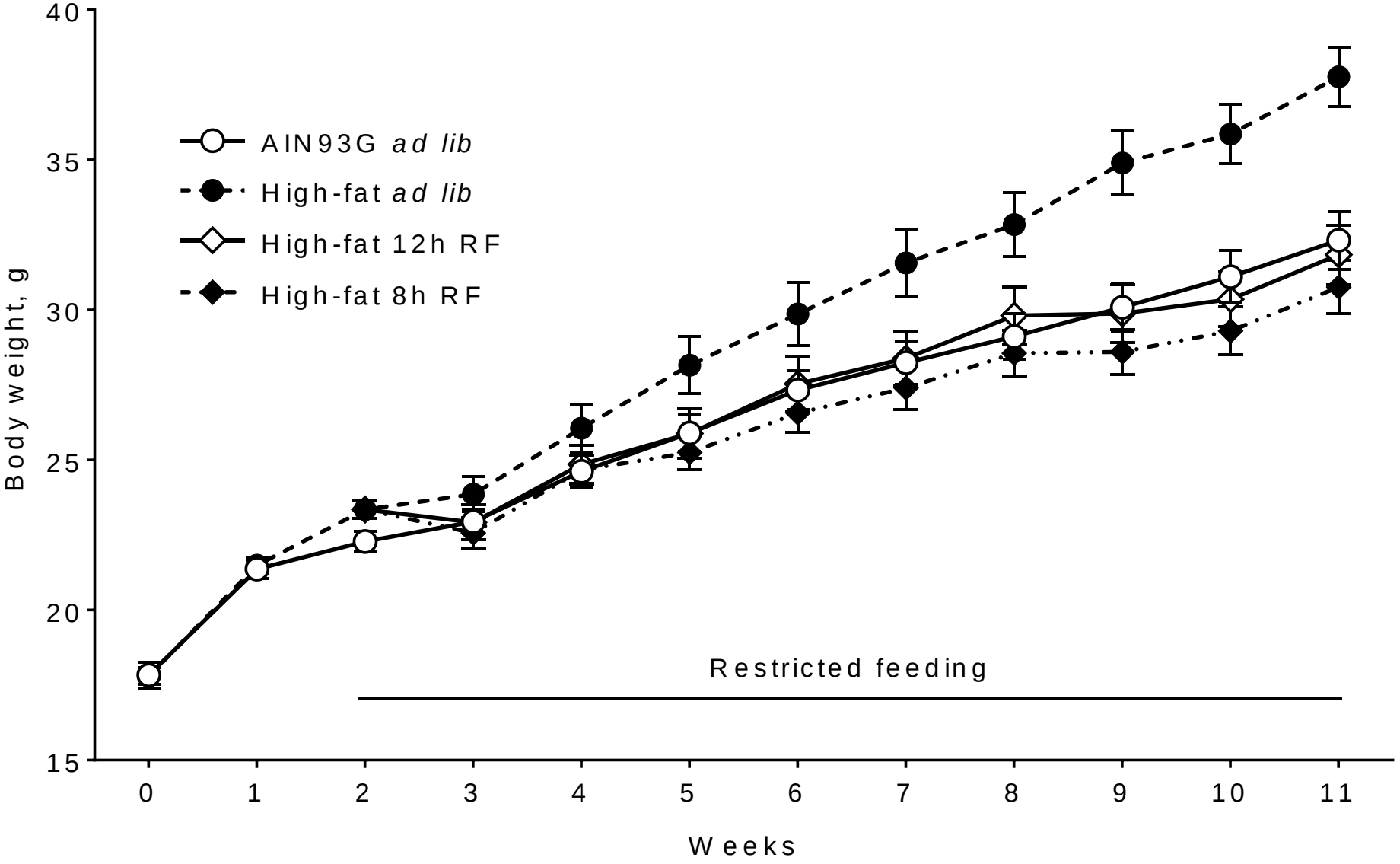

AIN93G *ad lib*
High-fat *ad lib*
High-fat 12h RF
High-fat 8h RF
Restricted feeding
Body weight, g
Weeks
40
35
30
25
20
15
0
1
2
3
4
5
6
7
8
9
10
11

Figure 2

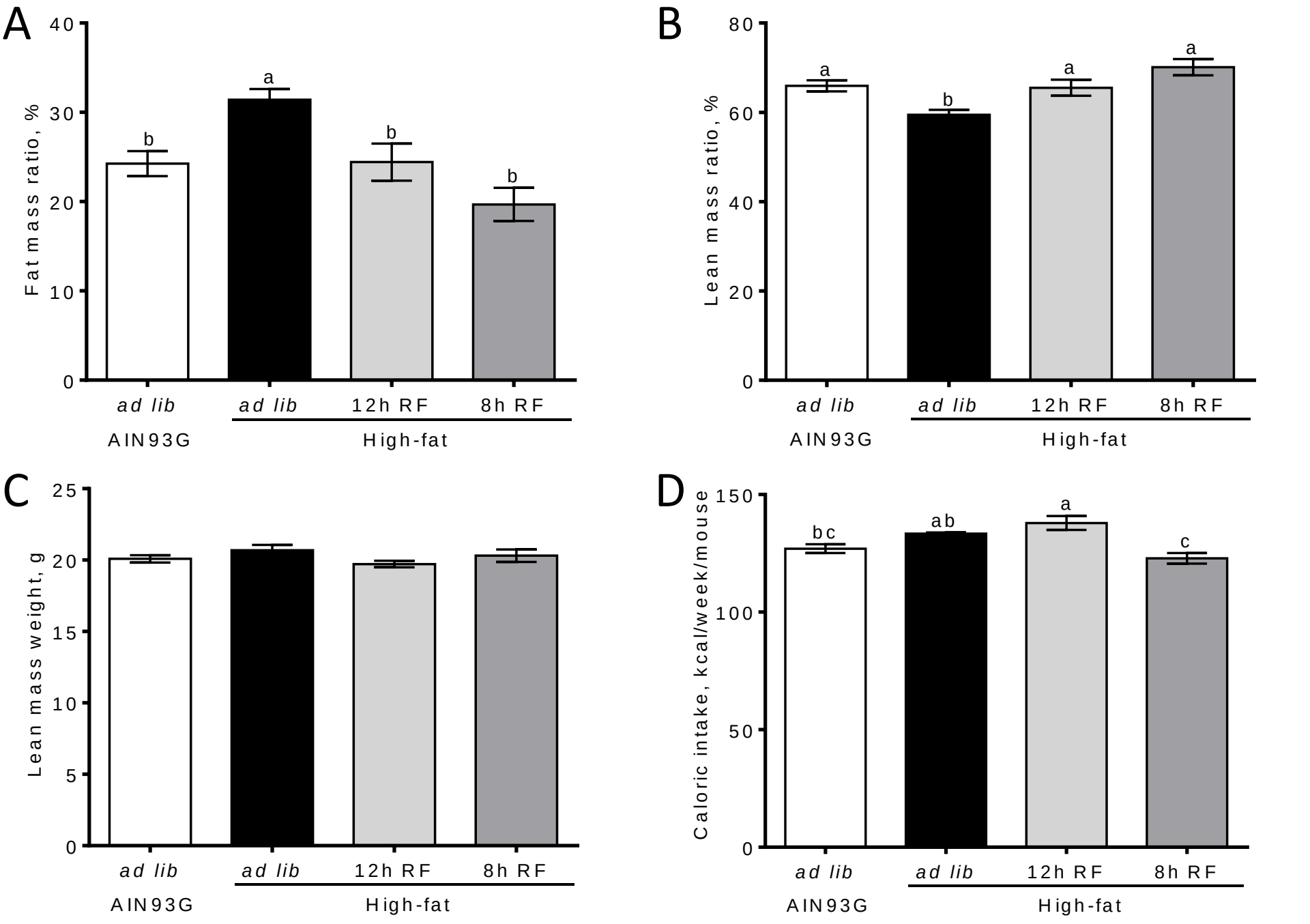
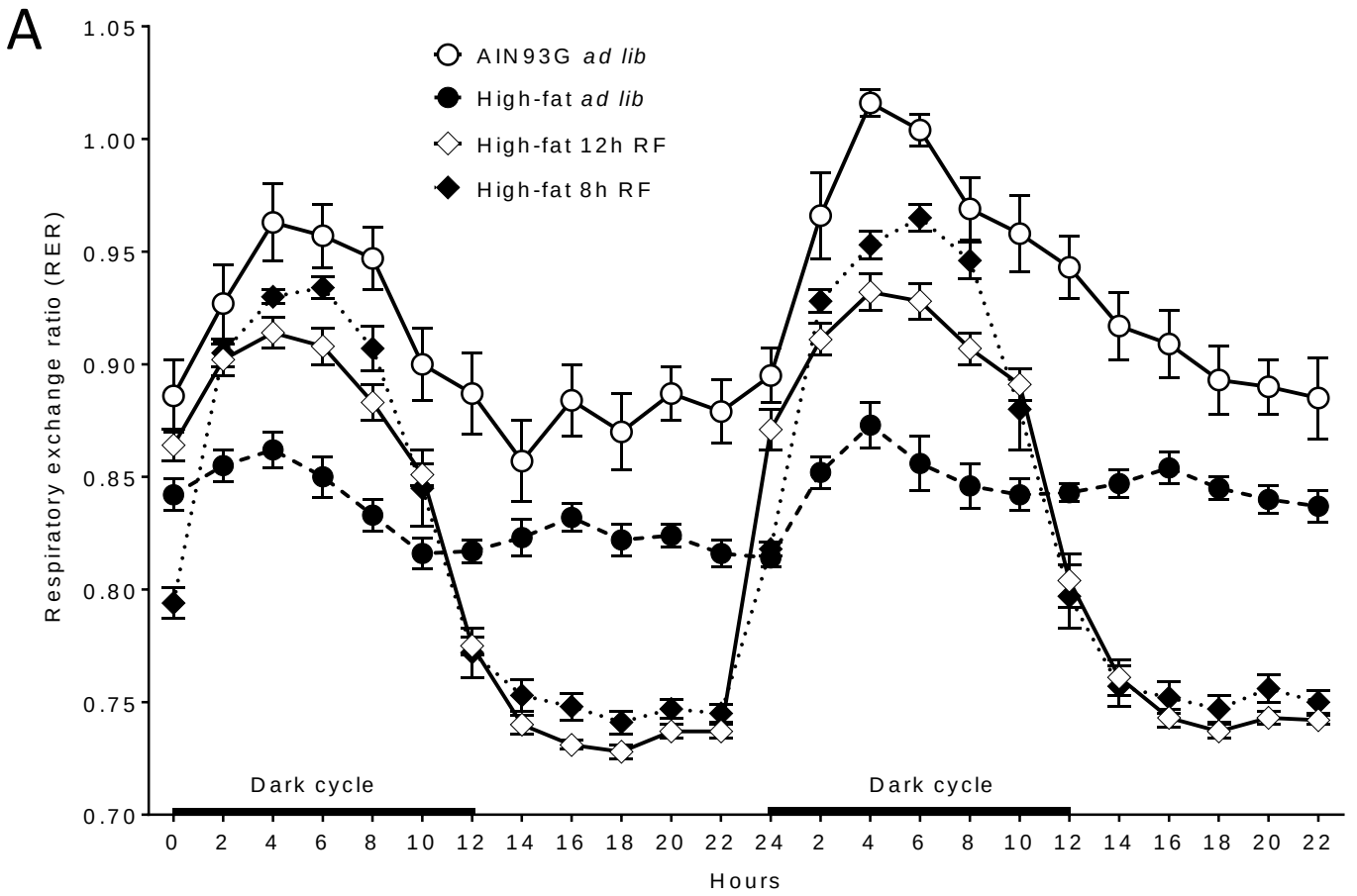

A
Fat mass ratio, %
40
30
20
10
0
b
a
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
B
Lean mass ratio, %
80
60
40
20
0
a
b
a
a
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
C
Lean mass weight, g
25
20
15
10
5
0
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
D
Caloric intake, kcal/week/mouse
150
100
50
0
bc
ab
a
c
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat

Figure 3


A
AIN93G ad lib
High-fat ad lib
High-fat 12h RF
High-fat 8h RF
Respiratory exchange ratio (RER)
1.05
1.00
0.95
0.90
0.85
0.80
0.75
0.70
Dark cycle
Dark cycle
0 2 4 6 8 10 12 14 16 18 20 22 24 2 4 6 8 10 12 14 16 18 20 22
Hours

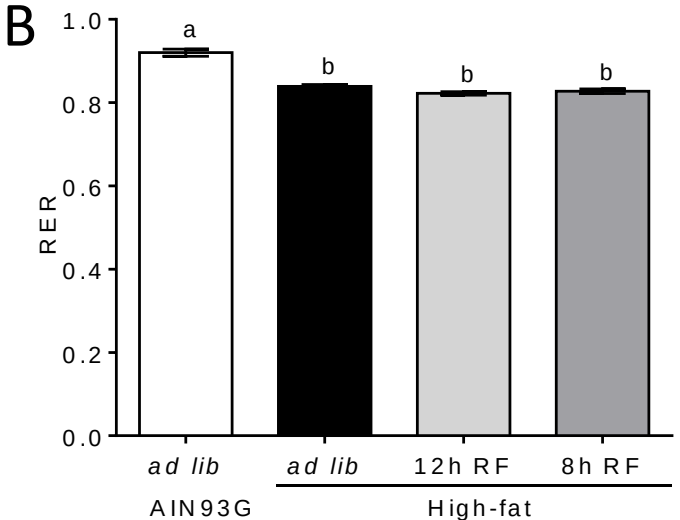

B
RER
1.0
0.8
0.6
0.4
0.2
0.0
a
b
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat

Figure 4

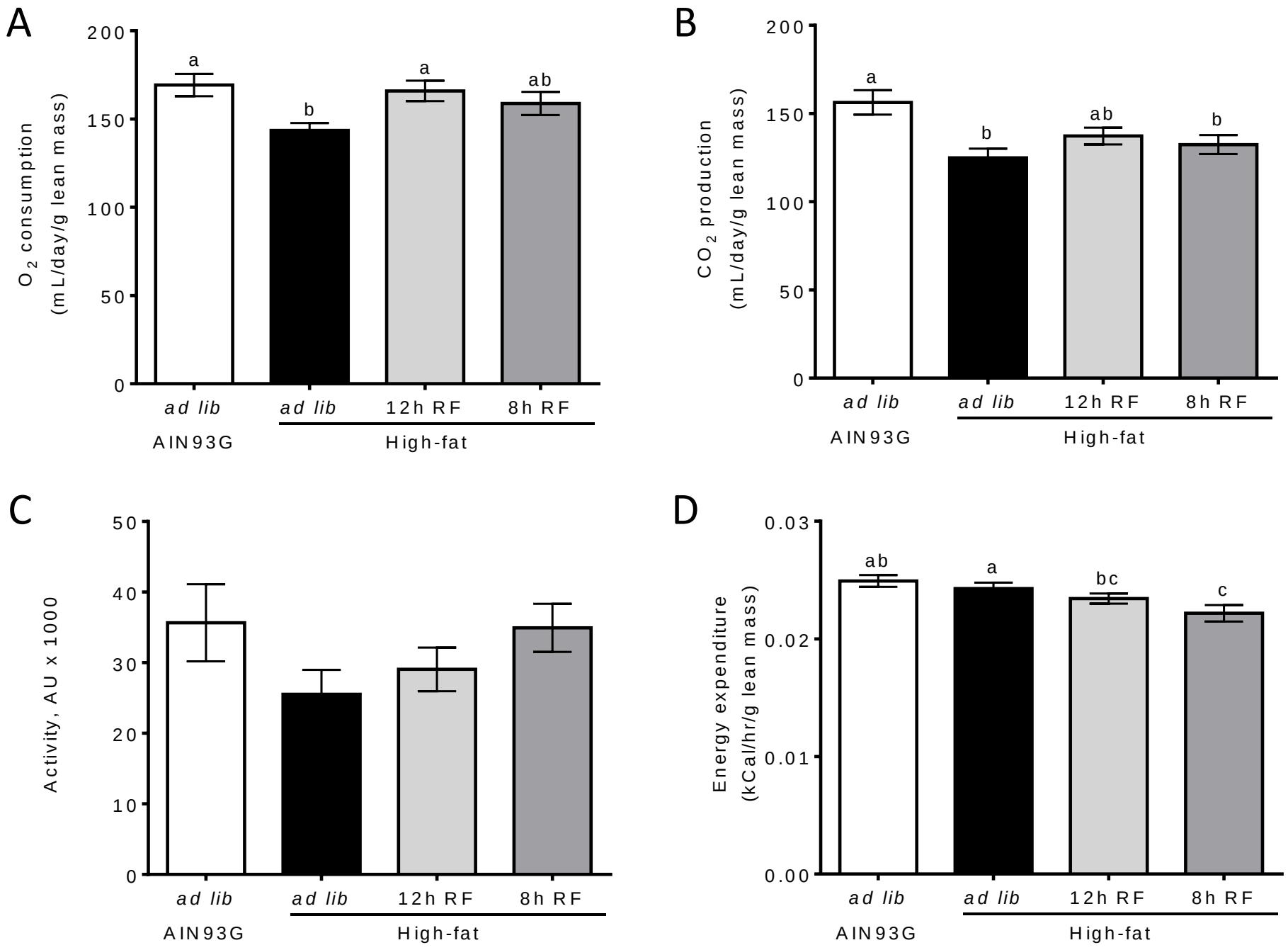
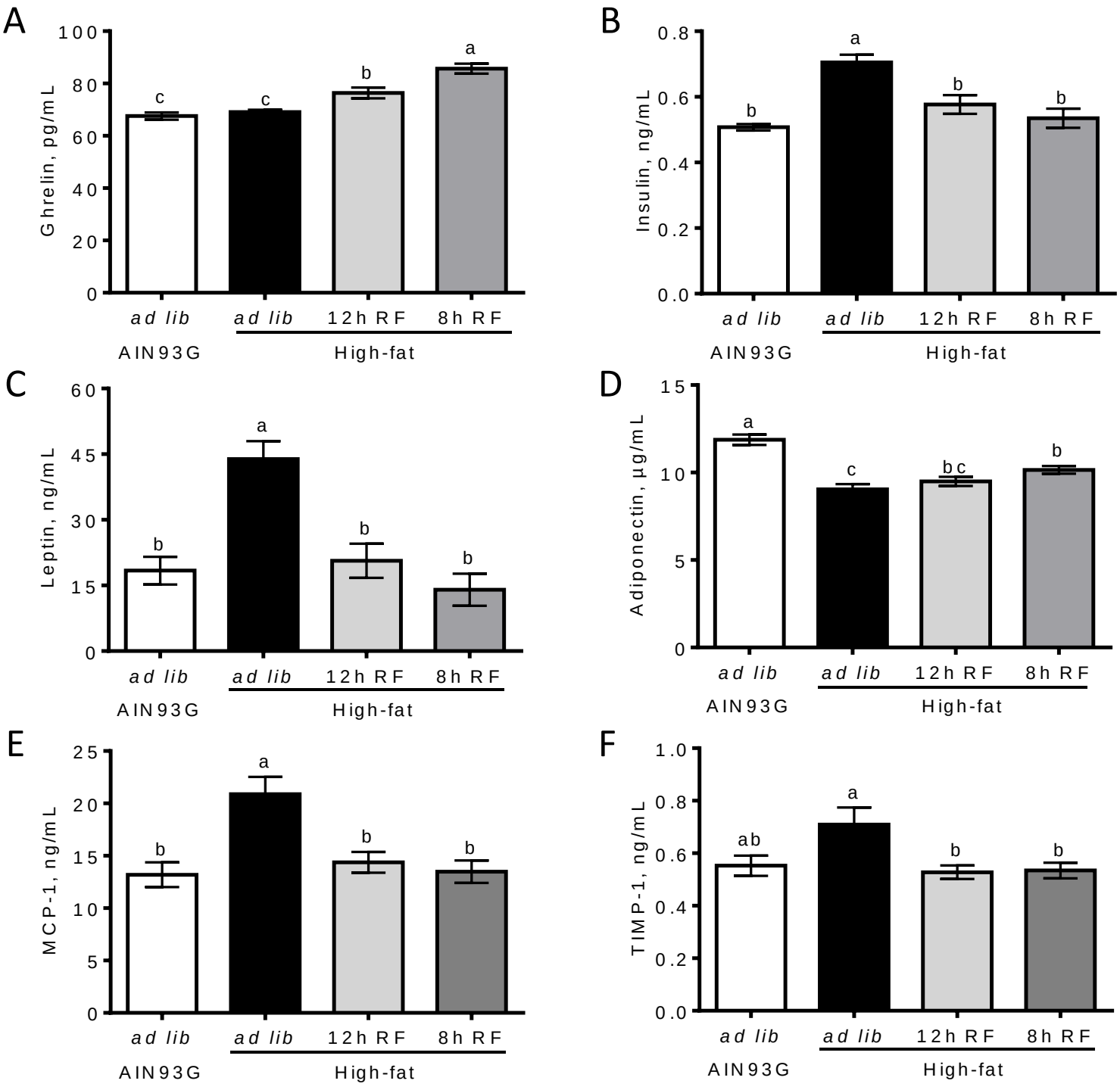

A
200
150
100
50
0
O2 consumption
(mL/day/g lean mass)
a
b
a
ab
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
B
200
150
100
50
0
CO2 production
(mL/day/g lean mass)
a
b
ab
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
C
50
40
30
20
10
0
Activity, AU x 1000
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
D
0.03
0.02
0.01
0.00
Energy expenditure
(kCal/hr/g lean mass)
ab
a
bc
c
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat

Figure 5


A
Ghrelin, pg/mL
100
80
60
40
20
0
c
c
b
a
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
B
Insulin, ng/mL
0.8
0.6
0.4
0.2
0.0
b
a
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
C
Leptin, ng/mL
60
45
30
15
0
b
a
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
D
Adiponectin, µg/mL
15
10
5
0
a
c
bc
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
E
MCP-1, ng/mL
25
20
15
10
5
0
b
a
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat
F
TIMP-1, ng/mL
1.0
0.8
0.6
0.4
0.2
0.0
ab
a
b
b
ad lib
ad lib
12h RF
8h RF
AIN93G
High-fat