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Improvements in body fat distribution and circulating adiponectin by alternate-day fasting versus calorie restriction☆

Krista A. Varady*, Candice A. Allister, Donald J. Roohk, Marc K. Hellerstein

Department of Nutritional Sciences and Toxicology, University of California at Berkeley, Berkeley, CA 94720, USA

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Abstract

Calorie restriction (CR) and alternate-day fasting (ADF) beneficially affect several aspects of adipose tissue physiology, but direct comparisons between regimens have yet to be performed. The present study evaluated the effects of ADF versus CR on body fat distribution and circulating adiponectin levels and examined the kinetic mechanisms that underlie changes in fat distribution. Thirty female C57BL/6J mice were randomized to one of five groups for 4 weeks: (a) CR-25% (25% energy restriction daily), (b) ADF-75% (75% restriction on fast day), (c) ADF-85% (85% restriction on fast day), (d) ADF-100% (100% restriction on fast day) and (e) control (ad libitum fed). Body weights of the CR mice were lower than that of the ADF and control groups posttreatment. After 4 weeks of diet, the proportion of visceral fat decreased ($P<.001$) and the proportion of subcutaneous fat increased ($P<.001$) similarly in ADF and CR animals. Adiponectin increased ($P<.05$) by 62–86% in the ADF groups and by 69% in the CR group. Triglyceride (TG) synthesis and de novo lipogenesis were augmented ($P<.05$) in the subcutaneous fat pad of ADF and CR animals, relative to control. No differences in net lipolysis were observed, resulting in greater TG accumulation in the subcutaneous fat pad, with a shift in the ratio of TG between depots. These findings indicate that ADF (both modified and true) produces similar beneficial modulations in body fat distribution and adiponectin levels as daily CR.

Keywords: Alternate-day fasting; Calorie restriction; Adipose tissue; Visceral fat; Subcutaneous fat; Triglyceride metabolism; Adiponectin; Leptin

1. Introduction

The role of regional fat distribution in the development of certain obesity-related disorders, such as type 2 diabetes and cardiovascular disease, has been firmly established [1]. Visceral obesity, characterized in humans by increased intra-abdominal fat mass at the lumbosacral level, is associated with a higher incidence of insulin resistance, cardiovascular events and premature death [2,3]. In contrast, individuals with increased adipose mass in subcutaneous gluteofemoral depots exhibit lower risk of developing these adverse outcomes than those with comparable amounts of adipose tissue in visceral depots [4]. A mechanism that may link fat distribution to disease risk is adipokine secretion profile. Adiponectin, a hormone mainly expressed by adipose tissue, exhibits both antiatherogenic and insulin-sensitizing effects [5]. Circulating levels of this adipokine have been shown to be inversely correlated with visceral fat mass [6]. Leptin, another adipocyte-derived protein, plays a key role in glucose and lipid metabolism and, hence, may modulate risk of chronic disease [7]. Leptin levels are related to body fat distribution, as mRNA levels and secretion rates are higher in subcutaneous adipocytes, when compared to those from visceral compartments [7].

Dietary interventions that reduce daily energy intake, also known as calorie restriction (CR), have been shown to cause numerous physiological benefits in both animals and humans [8]. Recent evidence with these regimens suggests that CR reduces visceral adipose mass even in nonobese individuals [9]. These CR-induced decreases in visceral fat are accompanied by significant increases in adiponectin [10]. Alternate-day fasting (ADF) represents another form of dietary restriction. ADF consists of alternating 24-h periods of ad libitum feeding and fasting and reproduces several of the physiological benefits of CR [11]. Recently, we demonstrated that true ADF, that is, complete energy deprivation on the fast day, reduced fat cell size by ~35% to 55% in both visceral and subcutaneous adipose tissue depots after 4 weeks in mice [12]. Since smaller fat cells are associated with cardioprotection and insulin sensitivity compared to larger fat cells [13,14], these preliminary results suggest that ADF may have beneficial effects on the qualitative features of adipose tissue. The effect of ADF versus CR on fat distribution and resultant adiponectin and leptin release has yet to be clarified, however. Moreover, if redistribution of adipose tissue from visceral to subcutaneous sites occurs, the kinetic and metabolic basis has yet to be elucidated.

Accordingly, the primary objective of this study was to compare the effects of ADF versus CR on body fat distribution and circulating

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* Corresponding author. Tel.: +1 510 642 0646; fax: +1 510 642 3099.
E-mail address: kvarady@nature.berkeley.edu (K.A. Varady).

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adiponectin levels and to uncover the dynamic metabolic mechanisms that underlie these changes. Additionally, we examined the day-to-day variation in adipose tissue triglyceride (TG) metabolism in response to alternating days of feeding and fasting. We hypothesize that ADF will be equally as effective as CR in reducing visceral adiposity and increasing subcutaneous adiposity. These changes in fat distribution will be associated with higher circulating adiponectin and leptin concentrations in both the ADF and CR groups.

2. Materials and methods

2.1. Study 1: effects of ADF versus CR on body fat distribution and adipokines

Seven-week-old C57BL/6J female mice (n=30, Charles River Breeding Laboratories, Wilmington, MA) were housed individually and maintained under temperature- and light-controlled conditions (12 h light/dark cycle: lights on at 0700 h and lights off at 1900 h). Mice were acclimatized for 1 week and allowed free access to water and a semipurified AIN-93M diet (Bio-Serv, Frenchtown, NJ) prior to initiation of studies. Mice were then randomized into one of five intervention groups: (a) CR-25% (food restricted daily by 25% of baseline dietary needs), (b) ADF-75% (75% CR on fast day, ad libitum fed on feed day), (c) ADF-85% (85% CR on fast day, ad libitum fed on feed day), (d) ADF-100% (100% CR on fast day, ad libitum fed on feed day) and (e) control (ad libitum fed everyday). Mice in all intervention groups were fed the AIN-93M diet. The degree of CR was calculated based on mean daily food consumption during the acclimation period for each mouse. Body weight was assessed weekly on the same day and time, and food was provided or taken away at 1300 h each day and weighed daily. Mice were sacrificed at 12 weeks of age by cardiac puncture under isoflurane anesthesia, followed by cervical dislocation. All procedures and protocols received approval from the University of California Berkeley Animal Use Committee.

2.2. Study 2: acute effects of ADF on adipose tissue TG metabolism

Seven-week-old C57BL/6J male mice (n=30, Charles River Breeding Laboratories) were used. Animals were maintained under temperature- and light-controlled conditions and acclimatized for 1 week, as outlined above. Following acclimation, 6 mice were sacrificed, to measure baseline weights of adipose tissue depots (Group 0). The other 24 mice were randomized into one of two groups: (a) ADF-100% (100% CR on fast day, ad libitum fed on feed day) and (b) control (ad libitum fed everyday). All mice were fed the AIN-93M diet. Food was given or taken away at 1000 h each day, and the amount of food consumed by each mouse was weighed daily. Body weight was measured at the same time of day at the beginning of each week. After 2 weeks of treatment, n=6 mice per day (n=4 ADF-100%; n=2 control) were sacrificed at 1000 h on four consecutive days (Days 15–18). Accordingly, mice sacrificed on Days 15 and 17 had been fed ad libitum for the 24-h period before sacrifice (Groups 1 and 3: fed state mice), while those sacrificed on Days 16 and 18 had been fasted for the 24-h period before sacrifice (Groups 2 and 4: fast state mice). Animals were sacrificed using the same procedure outline above.

2.3. Blood collection and ^2H_2O labeling protocol

In Study 1, fasting blood samples were collected on the last day of the trial (Day 28), the morning after a feed day. A priming dose of 99.9% heavy water (^2H_2O) (0.18 ml/10 g body weight) was administered by intraperitoneal injection on Day 14, to bring the ^2H_2O content of body water up to ~5%. Animals then received drinking water containing 8% ^2H_2O ad libitum for the last 2 weeks of the study (Days 14–28).

In Study 2, a different ^2H_2O labeling protocol was implemented to allow for the acute effects of ADF on adipose tissue TG metabolism to be tested. Two priming doses of 99.9% ^2H_2O (0.18 ml/10 g body weight) were administered via intraperitoneal injection the day before sacrifice (at 1000 and 1200 h). Animals were dosed to bring ^2H_2O content of body water up to ~8%. Drinking water containing 10% ^2H_2O was then provided ad libitum for the 24-h period following the initial injection.

2.4. Isolation of TG–glycerol and FA from adipose tissue

In both Study 1 and Study 2, inguinal (subcutaneous) and intra-abdominal (visceral) fat pads were carefully dissected immediately after sacrifice. The same person performed all dissections to alleviate interinvestigator bias. Each fad pad was then weighed (for the assessment of adipose tissue mass) and placed in glass tubes containing 1 ml of methanol–chloroform (2:1). Chloroform and water were then used to extract the solution. The aqueous fraction was disposed, and the remaining lipid phase was transesterified by incubation with 3 N methanolic HCl (Sigma-Aldrich, St. Louis, MO) for 60 min at 55°C. The Folch technique was employed to separate glycerol from fatty acid (FA) methyl esters. As described elsewhere [15], the aqueous phase containing glycerol was lyophilized by incubation with acetic anhydride–pyridine (2:1), thereby converting glycerol to glycerol triacetate.

2.5. Measurement of ^2H_2O enrichments in body water

^2H_2O enrichments in body water were measured from plasma as described previously [15]. In brief, 100 μl of plasma was reacted with calcium carbide to produce acetylene in an evacuated GC vial. A syringe was used to remove the acetylene gas, and the gas was then injected into a GC vial containing 10% bromine in carbon tetrachloride. The reaction was then left to incubate at room temperature for 2 h to produce tetrabromoethane. Excess bromine was neutralized with 25 μl of 10% cyclohexene, and the sample was suspended in ethyl acetate.

2.6. GC–MS analyses of TG–glycerol, FA and body water

For all analysis, a model 6890 GC with 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) fitted with a DB-225 fused silica column (J&W, Folsom, CA) was used. Glycerol triacetate was analyzed under chemical ionization conditions by selected ion monitoring of mass-to-charge ratios (m/z) 159–161 (representing M0–M2). FA methyl esters were analyzed as described elsewhere [16], with selected ion monitoring of m/z 256–258 (representing M0–M2) of palmitate methyl ester. Body ^2H_2O enrichments were analyzed as tetrabromoethane by monitoring m/z 265 and 266 (representing M0 and M1) of the 79Br79Br81Br (parent minus Br-) isotopomer [15].

2.7. Calculation of TG–glycerol synthesis and lipolysis

The measurement of all-source TG synthesis is based on the incorporation of deuterium from ^2H_2O into the C–H bonds of the glycerol moiety of TG–glycerol [15]. Deuterium in cellular H_2O exchanges with specific C–H bonds during glycolytic and glyceroneogenic reactions leading to α-glycerol phosphate, the biosynthetic precursor of TG [15]. Accordingly, TG molecules synthesized from α-glycerol phosphate during the period ^2H_2O administration will exhibit ^2H labeling whereas the TG molecules that already existed will remain unlabeled in the glycerol moiety. The fraction of newly synthesized TG–glycerol (f) was measured as described [15]:

$$f_{\mathrm{TG}} = \mathrm{EM1}_{\mathrm{TG-glycerol}} / {A_1}^{\infty}{}_{\mathrm{TG-glycerol}}$$

where f is the fraction of newly synthesized TG molecules present, EM1 is the measured excess mass isotopomer abundance for M1 glycerol at time t and A_1^{∞} is the asymptotic mass isotopomer abundance for M1 glycerol possible at the measured body water enrichment. The calculation of A_1^{∞} utilizes the number (n)=4 (number of C–H bonds in glycerol that are labile and exchange with ^2H_2O in body water in intermediary metabolic pathways leading to α-glycerol phosphate), as shown previously to be the case under these conditions [15]. Absolute synthesis rates of adipose TG were then calculated from fractional TG synthesis multiplied by adipose TG mass [15]. Net lipolysis was calculated based on the absolute TG synthesis rate combined with change in pool size [15]:

$$\text{Absolute synthesis}(\mathrm{g/day}) = f_{\mathrm{TG}} \times \text{adipose TG mass}(\mathrm{g})$$

$$\text{Net lipolysis}(\mathrm{g/day}) = [f_{\mathrm{TG}} \times \text{adipose TG mass}(\mathrm{g})] - \text{change in TG mass}(\mathrm{g})$$

where change in adipose mass (expressed as gain in mass) is calculated by comparison of final mass to the measured baseline mass of each adipose depot. This parameter represents net lipolysis because any TG molecules that were synthesized and then broken down during the labeling period will not be included in the measurement.

2.8. Calculation of de novo lipogenesis (DNL)

The measurement of newly synthesized FA that is formed during the ^2H_2O labeling period (DNL) was assessed using a combinatorial model of polymerization biosynthesis, as described previously [17]. Briefly, mass isotopomer distribution analysis (MIDA) is used to determine the number (n) of hydrogen atoms in C–H bonds of FA that was derived from cellular H_2O during de novo synthesis of FA, using body ^2H_2O to represent the precursor pool enrichment (p), as described previously [17]. Fractional and absolute contributions from DNL are then calculated [17]:

$$f_{\mathrm{DNL}} = \mathrm{EM1}_{\mathrm{FA}} / {A_1}^{\infty}{}_{\mathrm{FA}}$$

$$\text{Absolute DNL}(\mathrm{g/day}) = f_{\mathrm{DNL}} \times \text{adipose TG mass}(\mathrm{g}) \times \text{fraction TG palmitate}$$

where ${A_1}^{\infty}{}_{\mathrm{FA}}$ is calculated from MIDA lookup tables (based on the calculated values of n and p in FA). The value for f DNL represents the fraction of total TG palmitate in the depot derived from DNL during the labeling period, and absolute DNL represents grams of palmitate synthesized by the DNL pathway.

2.9. Plasma adiponectin and leptin

Plasma adiponectin and leptin concentrations were quantified using high-sensitivity ELISA kits (Linco Research, St. Charles, MO). Intra-assay precision of the adiponectin and leptin ELISA kits was 1.2% and 1.3%, respectively.

2.10. Lipogenic and lipolytic gene expression

In Study 2, 0.1 g of subcutaneous and visceral adipose tissue was flash frozen in liquid nitrogen immediately following sacrifice. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) and purified using an RNeasy kit (Qiagen, Valencia, CA). Five hundred nanograms of total RNA from each sample and 2 μg of total RNA pooled from each sample from the control group were reverse transcribed with random hexamers using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). Real-time PCR was performed in 96-well format using the ABI Prism 7900 HT sequence detection system and analyzed using SDS 2.0 software (Applied Biosystems). For each gene transcript, 10 ng of cDNA from each sample was analyzed as an unknown against a standard curve derived from a fivefold dilution series of cDNA reverse transcribed from RNA pooled from the control group. Each 25-μl PCR reaction was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems) and primer and probe sets from Applied Biosystems Assays on demand. Relative mRNA levels for each gene were measured in arbitrary units and normalized to GAPDH levels.

2.11. Estrus cycle status

In Study 1, estrus cycle was determined for female mice via vaginal smear (taken during the last eight consecutive days of the study) and analysis of cell morphology. Samples were fixed and stained on slides with Giemsa blood stain (Medical Chemical Corp., Los Angeles, CA), as previously described [18].

2.12. Statistical analysis

Values are expressed as means±S.E.M. A one-way ANOVA was used to test for differences between group means. Within-group differences from the beginning to the end of the study were analyzed by repeated-measures ANOVA. Pearson correlation coefficients were calculated to test the association between changes in adipose tissue mass, adipokines, lipid kinetic parameters and lipogenic/lipolytic gene expression. A *P* value of .05 was used to represent statistical significance in all analyses. Data were analyzed by SPSS software (version 11 for Mac OS X, SPSS Inc., Chicago, IL).

3. Results

3.1. Study 1: effects of ADF versus CR on body fat distribution and adipokines

3.1.1. Body weight in ADF versus CR mice

Changes in body weight over the course of the study are portrayed in Table 1. Body weights of each intervention group were similar during the first week of treatment. Mice in the CR-25% group weighed less (*P*<.05) than those in the ADF and control groups throughout the last 3 weeks of the study. Body weights of the ADF mice were similar to that of controls at all time points. Mice in the ADF and control groups gained weight (*P*<.05) over the 4-week study period.

3.1.2. Food intake in ADF versus CR mice

Mean weekly food intake in the CR and ADF groups was less (*P*<.0001) than that of the control group at Weeks 1, 2 and 4. During Week 3, only the CR group was eating less (*P*<.01) than the control group. Mean daily food intake over the course of the study was as follows: CR-25%, 2.04±0.06 g/day; ADF-75%, 2.23±0.07 g/day; ADF-85%, 2.21±0.10 g/day; ADF-100%, 2.21±0.06 g/day; control, 2.57 ±0.08 g/day.

Table 1
Body weight after 4 weeks of treatment (Study 1)

Body weight (g)	Day 1	Day 7	Day 14	Day 21	Day 28
Control	16.8±0.2	17.2±0.3	18.0±0.3	18.6±0.3	18.7±0.3
CR-25%	16.1±0.4	15.3±0.5 *	15.6±0.5 *	15.8±0.5 *	16.3±0.5 *
ADF-75%	16.3±0.6	16.6±0.7	16.2±0.6	17.8±0.6	18.1±0.6
ADF-85%	16.1±0.6	16.6±0.5	16.4±0.5	17.5±0.6	17.7±0.6
ADF-100%	16.7±0.4	17.4±0.3	17.5±0.4	18.4±0.4	18.3±0.4

Values are expressed as mean±S.E.M.; n=6 mice per intervention group.

* Mean body weight of CR mice was significantly lower (*P*<.05) than that of the control and ADF mice on Days 7, 14, 21 and 28 (one-way ANOVA with Tukey post hoc test).

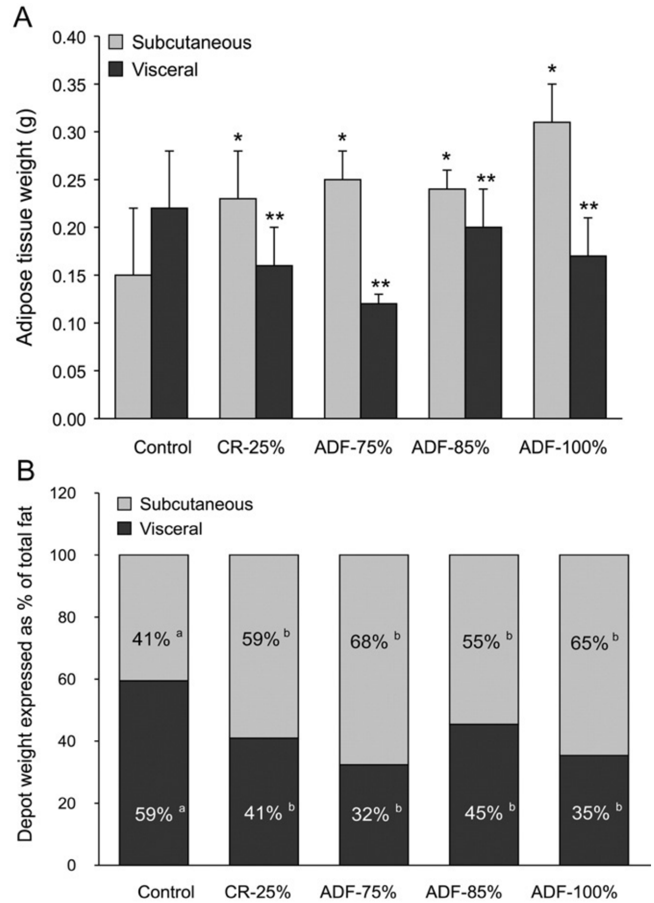

Fig. 1. Relative proportion of subcutaneous and visceral fat in ADF versus CR animals (Study 1). (A) Adipose tissue weight in grams. (B) Relative proportion of subcutaneous versus visceral adipose tissue. Values are expressed as mean±S.E.M. *Subcutaneous fat pad weight was higher (*P*<.05) in the intervention groups compared to controls. **Visceral fat pad weight was lower (*P*<.05) in the intervention groups compared to controls. The proportion of subcutaneous fat was higher (*P*<.001) and the proportion of visceral fat was lower (*P*<.001) in CR and ADF groups compared to controls. Total fat: sum of subcutaneous and visceral body fat compartments. Means with different superscript letters are significantly different between groups within one fat pad (one-way ANOVA with Tukey post hoc test).

3.1.3. Body fat distribution in ADF versus CR mice

After 4 weeks of treatment, visceral fat pad weight was lower (*P*<.05) in the intervention groups relative to controls (Fig. 1). Likewise, subcutaneous fat pad weight was higher (*P*<.05) in the ADF and CR groups, when compared to that of controls. In line with these findings, the relative proportion of visceral fat was lower (*P*<.001), while the proportion of subcutaneous fat was higher (*P*<.001) in each intervention group, compared to controls (Fig. 1). These decreases in the proportion of visceral fat and increases in the proportion of subcutaneous fat were similar between CR and ADF groups. Thus, adipose tissue was beneficially redistributed from the visceral to the subcutaneous depot, in the absence of weight loss, in these female mice. Total percentage of body fat was not different between intervention groups (CR-25%, 2.4±0.5%; ADF-75%, 2.1±0.2%; ADF-85%, 2.5±0.3%; ADF-100%, 2.6±0.4%) and controls (2.0±0.5%).

3.1.4. TG–glycerol synthesis in ADF versus CR mice

Fractional and absolute TG–glycerol synthesis in the CR and ADF animals was higher (*P*<.05) in subcutaneous fat, compared to controls (Fig. 2). These increases in fractional and absolute synthesis were similar between ADF and CR animals. In the visceral fat pad,

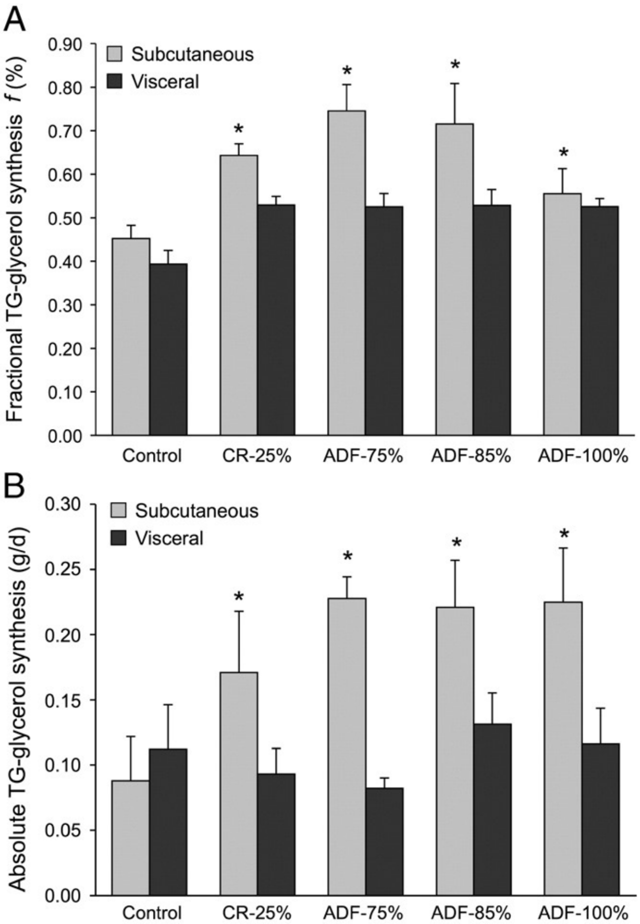

Fig. 2. Fractional and absolute TG synthesis in subcutaneous and visceral fat in ADF versus CR animals (Study 1). (A) Fractional TG synthesis. (B) Absolute TG synthesis. Values are expressed as mean±S.E.M. *CR-25%, ADF-75%, ADF-85% and ADF-100% regimens each increased (P<.05) fractional and absolute TG synthesis in the subcutaneous fat pad relative to controls (one-way ANOVA with Tukey post hoc test).

fractional and absolute synthesis did not differ between CR, ADF and control groups.

3.1.5. Net lipolysis in ADF versus CR mice

Net lipolysis in subcutaneous fat was not significantly different between treatment and control groups. In subcutaneous fat, net lipolysis values were as follows: CR-25%, 0.18±0.05 g/day; ADF-75%, 0.19±0.02 g/day; ADF-85%, 0.19±0.03 g/day; ADF-100%, 0.19±0.04 g/day; control, 0.06±0.02 g/day. Net lipolysis in visceral fat also did not differ between groups: CR-25%, 0.10±0.02 g/day; ADF-75%, 0.07±0.01 g/day; ADF-85%, 0.11±0.02 g/day; ADF-100%, 0.10±0.02 g/day; control, 0.08±0.02 g/day.

3.1.6. DNL in ADF versus CR mice

In subcutaneous fat, fractional and absolute DNL in CR and ADF animals was higher (P<.05), compared to that of controls (Fig. 3). These increases in fractional and absolute DNL were comparable between ADF and CR animals. Fractional and absolute DNL did not differ between treatment and control groups in the visceral fat pad.

3.1.7. Circulating adiponectin and leptin in ADF versus CR mice

Plasma adiponectin levels in the CR-25%, ADF-75%, ADF-85% and ADF-100% groups were higher (P<.05) than that of the control group posttreatment. These increases in adiponectin were similar between CR and ADF groups (Fig. 4). Circulating adiponectin concentrations were inversely related (r=−.37, P=.04) to the proportion of visceral fat. Plasma leptin levels were not different between the CR-25% (2.69±0.39 ng/ml), ADF-75% (3.73±0.36 ng/ml), ADF-85% (3.55±0.59 ng/ml) and ADF-100% (4.98±0.95 ng/ml) groups versus controls (3.65±1.54 ng/ml) at the end of the study. Additionally, there was no relationship between leptin levels and changes in body fat distribution posttreatment.

3.1.8. Estrus cycle

Results from the morphological analysis of vaginal cytology indicate that CR mice were anestrus (not cycling), whereas the ADF-75%, ADF-85%, ADF-100% and control mice were actively cycling [11]. The effect of CR on circulating adiponectin levels might, therefore, be influenced by a reduction in reproductive hormone levels [19].

3.2. Study 2: acute effects of ADF on adipose tissue TG metabolism

3.2.1. Body weight and body fat distribution

The study timeline is displayed in Fig. 5. Mean body weight of the ADF-100% groups (Groups 1–4) did not differ from their respective controls at acclimation and throughout the study (Fig. 6). Mean subcutaneous fat pad weights at sacrifice were as follows: Group 0, 0.21±0.01 g; Groups 1 and 3 (fed state mice), 0.37±0.02 g; Groups 2 and 4 (fast state mice), 0.32±0.04 g; control mice, 0.24±0.04 g. Mean

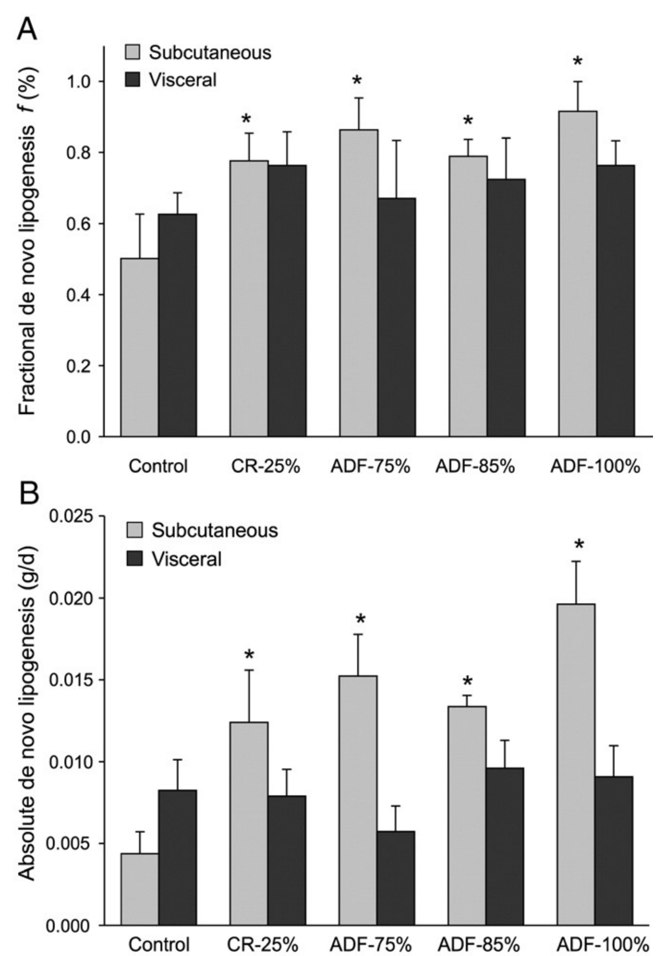

Fig. 3. Fractional and absolute DNL in subcutaneous and visceral fat in ADF versus CR animals (Study 1). (A) Fractional DNL. (B) Absolute DNL. Values are expressed as mean ±S.E.M. *CR-25%, ADF-75%, ADF-85% and ADF-100% regimens each increased (P<.05) fractional and absolute DNL in the subcutaneous fat pad relative to controls (one-way ANOVA with Tukey post hoc test).

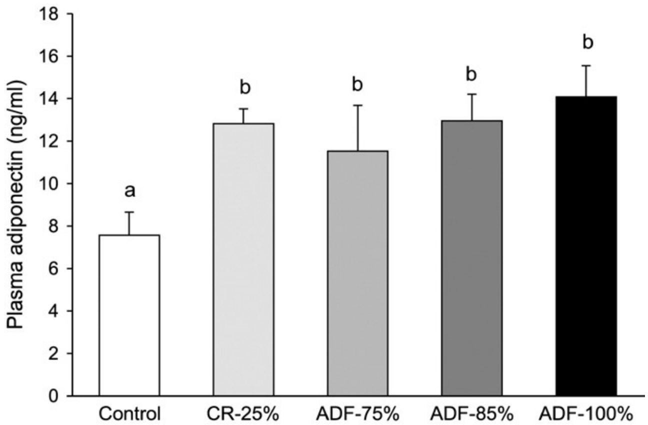

Fig. 4. Plasma adiponectin levels in ADF versus CR animals (Study 1). Values are expressed as mean±S.E.M. CR-25%, ADF-75%, ADF-85% and ADF-100% regimens each increased (P<.05) plasma adiponectin levels similarly, relative to control. Means with different superscript letters are significantly different between groups (one-way ANOVA with Tukey post hoc test).

visceral fat pad weights on the day of sacrifice were as follows: Group 0, 0.31±0.03 g; Groups 1 and 3 (fed state mice), 0.35±0.03 g; Groups 2 and 4 (fast state mice), 0.33±0.03 g; control mice, 0.49±0.05 g.

3.2.2. Food intake

Group 0, ADF-100% and control mice consumed similar amounts of food during acclimation (3.62±0.08, 3.50±0.05 and 3.56±0.05 g/day, respectively). Food intake of the ADF-100% groups (Groups 1–4) did not differ from their respective controls at any time point. Mean food intake throughout the 2-week study was as follows: 2.71±0.19 g/day in Groups 1 and 3 (fed state mice), 2.83±0.20 g/day in Groups 2 and 4 (fast state mice) and 3.06±0.12 g/day in control mice.

3.2.3. TG synthesis rates in response to 24 h of feeding or fasting

The day-to-day variability of adipose tissue TG dynamics was evaluated. In subcutaneous fat, fractional TG–glycerol synthesis did not change in response to 24 h of feeding (f=0.21±0.01 in Group 1 and f=0.21±0.02 in Group 3) or fasting (f=0.21±0.01 in Group 2 and 0.22±0.01 in Group 4). Similarly, in visceral fat, fractional TG synthesis was not altered by 24-h periods of either feeding (f=0.21±0.02 in Group 1 and f=0.18±0.01 in Group 3) or fasting (f=0.20±0.04 in Group 2 and 0.22±0.02 in Group 4).

No differences in absolute synthesis rates in subcutaneous fat were observed in response to 24 h of feeding (0.08±0.007 g/day in Group 1 and 0.07±0.006 g/day in Group 3) or fasting (0.08±0.006 g/day in Group 2 and 0.05±0.005 g/day in Group 4) or in visceral fat in response to feeding (0.07±0.01 g/day in Group 1 and 0.06±0.006 g/day in Group 3) or fasting (0.08±0.01 g/day in Group 2 and 0.07±0.005 g/day in Group 4). Thus, alternating 24-h periods of feeding or fasting had no effect on TG synthesis in either subcutaneous or visceral fat.

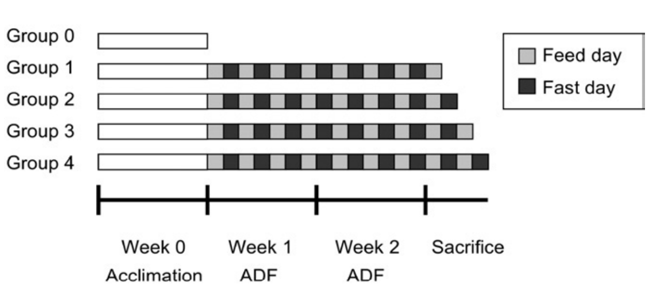

Fig. 5. Study timeline and sacrifice days (Study 2). After 1 week of acclimation, Group 0 mice were sacrificed, to measure baseline mass of adipose tissue depots (n=6). Groups 1 and 3 (fed state mice) were sacrificed on Days 15 and 17 after being fed ad libitum for the preceding 24-h period. Groups 2 and 4 (fast state mice) were sacrificed on Days 14 and 16 after being fasted for the preceding 24-h period. Each group consisted of n=6 mice (n=4 ADF-100%; n=2 control).

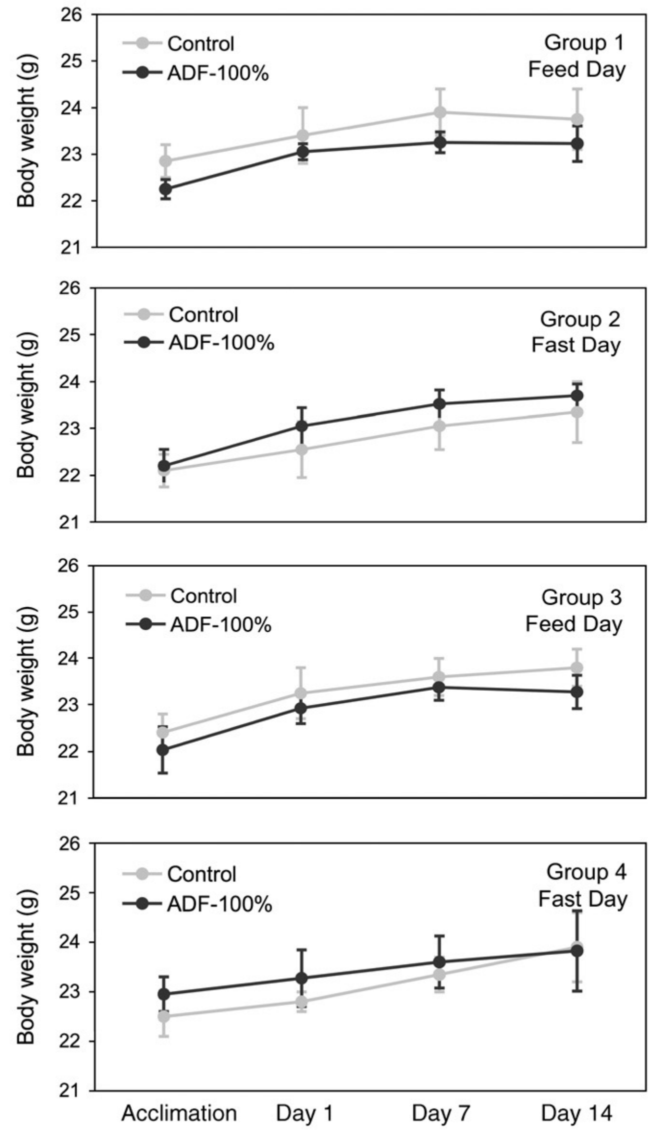

Fig. 6. Body weight after 2 weeks of treatment (Study 2). Values are expressed as mean ±S.E.M. Mean body weight in the feeding and fasting ADF groups was not significantly different from their respective controls throughout the study (one-way ANOVA).

3.2.4. Net lipolytic rates in response to 24 h of feeding or fasting

Net lipolysis in subcutaneous fat was not affected by 24 h of feeding (0.07±0.01 g/day in Group 1 and 0.06±0.01 g/day in Group 3) or fasting (0.07±0.01 g/day in Group 2 and 0.08±0.01 g/day in Group 4). Lipolysis in visceral fat was also not affected by 24 h of feeding (0.07±0.01 g/day in Group 1 and 0.06±0.04 g/day in Group 3) or fasting (0.07±0.02 g/day in Group 2 and 0.08±0.01 g/day in Group 4).

3.2.5. DNL in response to 24 h of feeding or fasting

In contrast to TG synthesis and lipolysis, DNL was shown to be highly responsive to alternating 24-h periods of feeding and fasting (Figs. 7 and 8). In subcutaneous fat, both fractional and absolute DNL

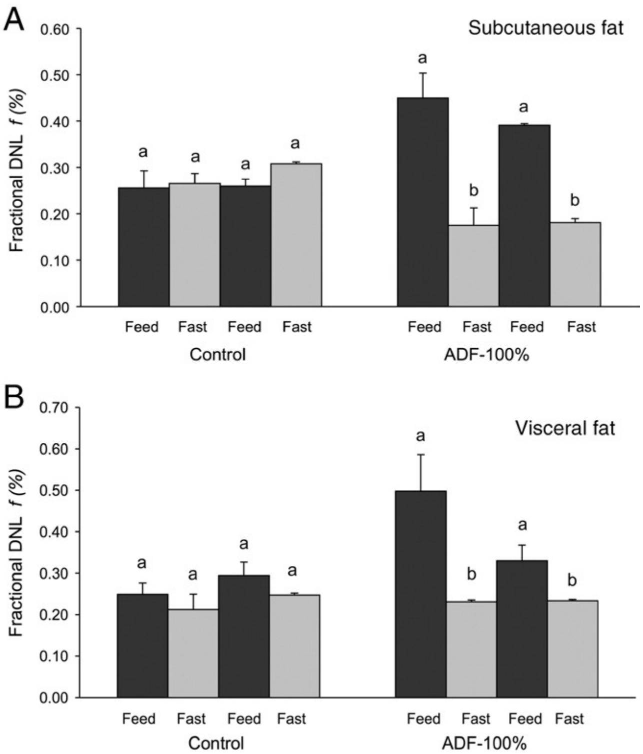

Fig. 7. Response of fractional DNL to alternating 24-h periods of feeding or fasting (Study 2). (A) Fractional DNL in subcutaneous fat. (B) Fractional DNL in visceral fat. Values are expressed as mean±S.E.M. Fractional DNL was augmented (*P*<.001) during the 24-h feeding period and declined (*P*<.001) during the 24-h fasting period in the ADF group in both fat depots. Fractional DNL in the control group remained stable from day to day. Means not sharing a common superscript letter are significantly different (*P*<.001) (one-way ANOVA with Tukey post hoc test).

rates increased (*P*<.001) in response to a preceding 24-h period of feeding and decreased (*P*<.001) in response to a preceding 24-h period of fasting. These feeding-related increases (*P*<.001) and fasting-related decreases (*P*<.001) in fractional and absolute DNL rates were also evident in the visceral fat depot.

3.2.6. Lipogenic gene expression

We also tested the effect of 24-h periods of fasting and feeding on the expression of certain lipogenic genes, that is, FAS, DGAT-1, DGAT-2 and GPAT (Table 2). Gene expression was not altered by 24-h periods of either feeding or fasting in either fat pad. Moreover, there were no associations observed between lipogenic gene expression and any kinetic parameter measured.

3.2.7. Lipolytic gene expression

Alternating 24-h periods of feeding and fasting had no effect on the expression of HSL, CPT-1a and CPT-1b (Table 2). We also observed much higher mRNA levels of CPT-1b when compared to CPT-1a, which may suggest that CPT-1b is more widely expressed in adipose tissue than CPT-1a. Lipolytic gene expression was not significantly correlated to net lipolysis for any gene measured.

4. Discussion

We report here, for the first time, a redistribution of fat from visceral to subcutaneous depots, as a result of true and modified ADF in female C57BL/6J mice. We also show that this redistribution in fat by ADF is comparable to that of CR. The marked decrease in visceral fat was related to an increase in circulating adiponectin levels in all dietary restriction groups. This redistribution in fat was explained kinetically by an increase in TG synthesis and DNL in the subcutaneous fat pad of intervention mice. Since no concomitant changes in net TG lipolysis were observed, the result was greater TG accumulation in the subcutaneous fat pad, with a shift in the ratio of TG between depots. The day-to-day variation in adipose tissue TG metabolism in response to alternating days of feeding and fasting was also examined. We show here that DNL increases with 24 h of feeding and decreases with 24 h of fasting, while TG synthesis and net lipolysis are not affected.

Visceral obesity in humans is closely related to the prevalence of insulin resistance and dyslipidemia [20]. In the present study, we demonstrate that modified ADF (reducing energy intake on the fast day by 75% or 85% of baseline needs) and true ADF (complete energy restriction on the fast day) decrease the proportion of visceral fat and increase the proportion of subcutaneous fat, to a similar extent as CR. While this effect on body fat distribution has been shown previously for CR [9,10,21], there is very limited data testing this effect for ADF [12]. In our previous study [12], we report no effect of modified or true ADF on body fat distribution in male mice. The discrepancy between previous and present findings may be explained by the sex of the mice used in each study. Evidence suggests that white adipose tissue in female mice responds differently to dietary restriction regimens, when compared to that of male mice [22]. Female mice conserve subcutaneous fat during periods of CR, while male CR mice lose adiposity equally in the subcutaneous and visceral depots [22]. Moreover, female mice exhibit a reduced capability to restore visceral fat during periods of CR [22]. Thus, it is possible that these beneficial modulations in body fat distribution may only occur in females as a result of short-term ADF. Extrapolation of body fat distribution from

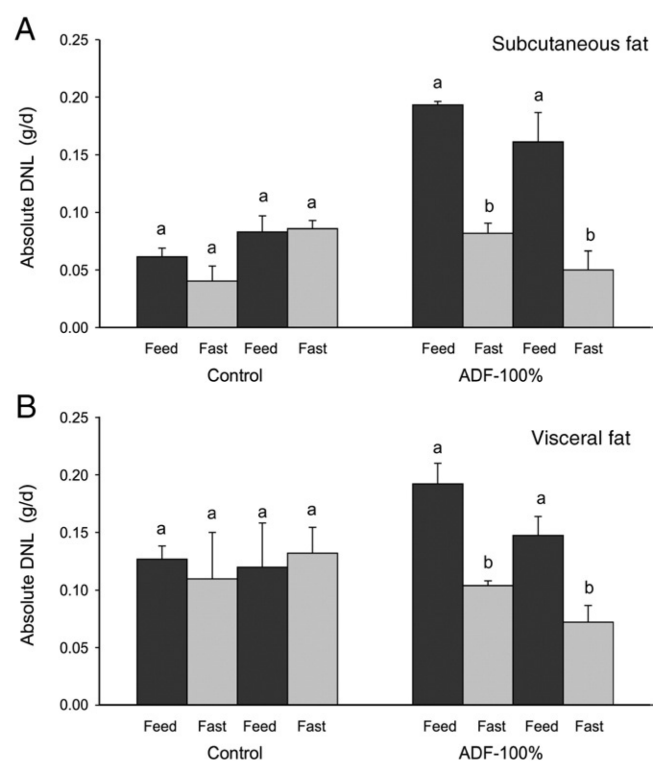

Fig. 8. Response of absolute DNL rates to alternating 24-h periods of feeding or fasting (Study 2). (A) Absolute DNL in subcutaneous fat. (B) Absolute DNL in visceral fat. Values are expressed as mean±S.E.M. Absolute DNL increased (*P*<.001) in response to feeding and decreased (*P*<.001) in response to fasting ADF group in subcutaneous and visceral fat. Absolute DNL in the control group remained stable from day to day. Means not sharing a common superscript letter are significantly different (*P*<.001) (one-way ANOVA with Tukey post hoc test).

Table 2
Response of lipogenic and lipolytic gene expression to alternating 24-h periods of feeding or fasting (Study 2)

	Subcutaneous fat			Visceral fat		
	Control	24-h feed[a]	24-h fast[a]	Control	24-h feed[a]	24-h fast[a]
Lipogenic genes						
FAS	9.0±1.6	8.8±2.2	5.8±0.7	0.7±0.1	3.4±1.2	1.5±0.3
DGAT-1	2.5±0.5	2.2±0.4	5.4±2.2	9.0±5.2 *	3.2±1.1	12.0±8.9
DGAT-2	10.8±3.5	5.3±0.7	3.7±1.3	10.0±5.0	5.4±1.5	3.4±1.8
GPAT	7.7±5.7	2.5±0.6	1.3±0.5	4.7±3.0	2.1±0.4	2.8±1.8
Lipolytic genes						
HSL	1.5±0.1	2.1±0.3	1.8±0.1	2.9±0.9	1.6±0.4	1.6±0.2
CPT-1a	1.0±0.4	0.5±0.1	0.5±0.1	4.0±0.3	1.3±0.4	1.2±0.5
CPT-1b	13.4±2.5	24.7±3.2	43.6±19.3	14.1±6.9	3.9±1.1	1.2±0.4

Values are expressed as mean±S.E.M. 24-h feed, mean values of Groups 1 and 3. 24-h fast, mean values of Groups 2 and 4. CPT-1a, carnitine palmitoyltransferase-1a; CPT-1b, carnitine palmitoyltransferase-1b; DGAT-1, diacylglycerol *O*-acyltransferase-1; DGAT-2, diacylglycerol *O*acyltransferase-2; FAS, fatty acid synthase; GPAT, mitochondrial glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase.

[a] Alternating 24-h periods of feeding and fasting had no effect on lipogenic or lipolytic gene expression in either fat pad.

rodents to humans is uncertain, however; hence, these findings will need to be confirmed in man.

This redistribution in body fat may explain why plasma adiponectin levels increased in the CR and ADF groups. Adiponectin was augmented by an average of 62% in the modified ADF groups, by 86% in the true ADF group and by 69% in the daily CR group, relative to controls. Additionally, we demonstrate a modest but significant inverse relationship ($r=-.37$, $P=.04$) between adiponectin concentrations and the proportion of visceral fat. Evidence suggests that plasma adiponectin is inversely related to visceral fat accumulation [6,20]. Thus, the redistribution in body fat by ADF and CR may be linked to the increases in plasma adiponectin observed. Similar CR-induced increases in adiponectin (60% relative to controls) have been demonstrated in young, nonobese rats [23]. In our earlier study [12], we observed no effect of true ADF on circulating adiponectin levels in male mice. Recent studies examining sex-specific differences in adiponectin release suggest that adiponectin levels in females may be more responsive to dietary interventions than that of males [19,24]. This sexual dimorphism may once again explain the conflicting data noted between past and present findings. No effect of either CR or ADF was noted for circulating leptin levels. Plasma leptin is positively correlated to body mass index and fat mass [20]. Since body weights of the ADF animals did not differ from that of controls, this may explain why leptin was unaltered by this dietary intervention.

Adipose tissue TG kinetics were measured by the incorporation of ^{2}H into the glycerol moiety of acylglycerides [15]. This technique allows for the measurement of adipose tissue TG synthesis and net lipolysis (TG breakdown) [15]. Results indicate that after 4 weeks of ADF or CR, TG synthesis was significantly augmented in subcutaneous fat but was not affected in visceral fat. New FA synthesis (DNL) was also up-regulated in the subcutaneous fat depot of ADF and CR animals but was not changed in the visceral fat depot. Net lipolysis was not affected in either fat pad. For all kinetic parameters measured, similar effects were seen for ADF and CR regimens. Taken together, these kinetic data suggest that the redistribution in fat from visceral to subcutaneous depots by ADF or CR occurred primarily via an increase in lipid accumulation in subcutaneous fat, with no concomitant increase in visceral fat.

In a separate study, the extent to which there is day-to-day variations in adipose tissue TG and FA metabolism in response to ADF was also evaluated. Our findings suggest that TG synthesis and lipolysis are not significantly altered in a day-to-day manner by the preceding 24 h of feeding or fasting. The de novo synthesis of FA, on the other hand, was very responsive to the preceding 24-h period of ad libitum feeding or complete fasting. Specifically, there was a twofold increase in DNL rates on the day of ad libitum feeding, when compared to the fasting day. This response in DNL occurred in both the subcutaneous and visceral fat pads, suggesting that this effect occurs generally in fat tissue. These findings suggest that DNL in adipose tissue is acutely responsive to short-term signals of energy sufficiency, as has been observed for hepatic DNL [25]. In contrast, the effects on total TG synthesis are evidently not as responsive to short-term dietary signals but persist across the fasting and feeding days of an ADF regimen. It may be speculated that insulin concentrations or nutrient availability modulate adipose tissue DNL over the short term but TG synthesis is regulated by longer-term factors.

Comparisons between adipose tissue lipid metabolic rates and the expression levels of related genes were also carried out. The lipolytic enzyme HSL is found on the lipid droplet of adipocytes and functions to hydrolyze TG. CPT-1a and CPT-1b are enzymes that reside on the outer mitochondrial membrane and participate in the transport of fatty acyl-CoA into the mitochondria for oxidation. No changes in the expression of these lipolytic genes were noted in response to 24 h of feeding or fasting. These gene expression data are consistent with our kinetic findings, which indicate that lipolysis is also not affected by acute feeding and fasting. Lipogenic gene expression was also evaluated. The lipogenic genes DGAT-1, DGAT-2 and GPAT are involved in the synthesis of glycerol phosphate and TG, while FAS is involved in the synthesis of FA from acetyl-CoA and malonyl-CoA. Although DNL was responsive to 24-h feeding and fasting periods, no simultaneous increases in lipogenic gene expression were observed. These findings are consistent with previous observations of dissonance between gene expression and actual lipid biosynthetic fluxes in adipose tissue [26].

In summary, these findings indicate that modified and true ADF regimens produce similar beneficial modulations in body fat distribution and adiponectin as daily CR. The mechanism underlying this redistribution was shown to involve an increase in lipid accumulation within the subcutaneous depot, with no concomitant increase in the visceral depot. Since some individuals find it difficult to adhere to daily CR, these data suggest that ADF may be implemented in place of CR to improve body fat distribution and circulating adiponectin, which may, in turn, confer protection against the development of obesity-related disorders. Whether these effects can be reproduced in males and in other species still requires confirmation, however.

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