Nutrition 66 (2019) 38–43

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Intermittent fasting increases energy expenditure and promotes adipose tissue browning in mice

Bo Liu Ph.D. [a,b], Amanda J. Page Ph.D. [a,b], Amy T. Hutchison Ph.D. [a,b], Gary A. Wittert M.D. [a,b], Leonie K. Heilbronn Ph.D. [a,b,*]

[a] Centre for Nutrition and Gastrointestinal Disease, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia
[b] Nutrition and Metabolism Theme, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

ARTICLE INFO

Article History:
Received 12 December 2018
Received in revised form 7 March 2019
Accepted 10 March 2019

Keywords:
Adipose tissue browning
Body weight
Energy expenditure
Fat mass
Intermittent fasting
Uncoupling protein 1

ABSTRACT

Objective: Intermittent fasting (IF) may limit metabolic adaptations that reduce energy expenditure, potentially by stimulating white adipose tissue (WAT) browning. The aim of this study was to examine the effects of 8 wk of IF on energy metabolism and markers of WAT browning in lean and diet-induced obese mice and in women who were overweight or obese.
Methods: Male C57 BL/6 J mice were fed chow or a high-fat diet (HFD; 43%) for 8 wk before undergoing IF (3 non-consecutive d/wk) for an additional 8 wk. Food intake, energy expenditure, and inguinal and gonadal fat pads were collected in fed or fasted conditions (22 h, IF mice only). Subcutaneous adipose tissue (SAT) was also collected at baseline, and after 8 wk of IF (in the fed state, and after a 24-h fast), in women with overweight or obesity. Uncoupling protein 1 (UCP1) was assessed by quantitative real-time polymerase chain reaction (mice and humans) and immunohistochemistry (mice).
Results: IF reduced body weight and energy intake in HFD fed mice and reduced gonadal and inguinal fat pad weights in both diet groups. IF increased energy expenditure, meal number, *Ucp1* mRNA levels in inguinal and gonadal fat depots, and UCP1 protein in inguinal fat in both diet groups on fed days. In women, IF reduced body weight and fat mass, but did not alter *UCP1* mRNA levels.
Conclusions: IF increased energy expenditure and promoted WAT browning in mice but did not alter UCP1 mRNA levels in SAT in women.

Introduction

Intermittent fasting (IF) is a dietary intervention that involves periods of minimal or no calorie intake followed by periods of unrestricted eating. IF is effective to extend life span [1–3], reduce fat mass [4–6], improve glucose tolerance and insulin sensitivity [4,7,8], and reduce the risk for cardiovascular diseases [9,10] and cancer [2,11] in rodents fed a chow diet. However, to our knowledge, few studies have investigated the response in obese animals [4,6,8,12,13]. In humans, most studies of IF have been conducted in individuals who are overweight or obese. In these studies, IF promotes weight and fat mass loss [14–17]; reduces total cholesterol, low-density lipoprotein cholesterol, triacylglycerols, and systolic blood pressure [16,18]; and improves markers of insulin sensitivity [14].

Brown adipose tissue (BAT) plays a critical role in energy homeostasis and thermogenesis [19]. This function is achieved via a specialized protein known as uncoupling protein 1 (UCP1) that disassociates mitochondrial respiration from adenosine triphosphate synthesis leading to the dissipation of energy as heat. In addition to the classical brown adipocytes residing in brown fat, brown-like adipocytes located within white adipose tissue (WAT) also have thermogenic properties characterized by expression of UCP1 [19]. A range of external cues, such as cold exposure [20–22], exercise [23–25], and pharmaceutical treatment [26,27], promote the development of beige adipocytes in WAT in mice. This is known as “browning” of WAT. Recently, three animal studies demonstrated that both daily calorie restriction and IF

The human study was funded by a National Health and Medical Research Council Project Grant APP1023401. LKH was supported by an Australian Research Council Future Fellowship FT120100027. BL was supported by an Australian Government Research Training Program Scholarship. BL performed the study, acquired and analyzed the data, and wrote the manuscript. AJP supervised the animal study and assisted the data analysis. ATH performed the human study. GAW provided clinical support to the human study. LKH conceived, designed, and supervised the study. All authors reviewed and approved the final manuscript. The authors have no conflicts of interest to declare.

* Corresponding author: Tel.: +61 88 128 4838.
E-mail address: Leonie.heilbronn@adelaide.edu.au (L.K. Heilbronn).

https://doi.org/10.1016/j.nut.2019.03.015
0899-9007/

promote WAT browning [6,13,28], and suggest this could be linked with the alternative activation of macrophages [6,13]. WAT browning also occurs in response to extreme adrenergic stimulation, such as burn injury and administration of β3-adrenergic receptor agonists, in humans [27,29,30].

The aim of this study was to examine the effects of IF on food intake and energy expenditure and adipose tissue browning in mice fed chow or high-fat diet (HFD) in the fed and fasted state. We also assessed the effects of IF on expression of UCP1 in subcutaneous adipose tissue (SAT) in women who were overweight or obese.

Research design and methods

Animals and diets

This study was approved by the animal ethics committees of the South Australian Health and Medical Research Institute (SAHMRI) and the University of Adelaide and was performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Ten-week old male C57 BL/6 J mice (Bioresources, SAHMRI, Australia) were housed four per cage in the Bioresources Facility at SAHMRI under a 12:12 h light/dark cycle, with lights on at 0700 (Zeitgeber time 0, ZT0) and temperature at 21°C ± 3°C. Mice were fed either a lard-based (43% fat, SF04-001, Specialty Feeds, Australia) or a chow diet (18% fat, 2018 SX, Envigo, United States) ad libitum for 8 wk before randomizing mice on each diet into ad libitum feeding (AL, n = 8) or IF (n = 16) for another 8 wk as described previously [31]. IF was initiated at ZT11 for 24-h for 3 non-consecutive d/wk. Food access was controlled by transferring mice daily between cages with or without food. Mice fed ad libitum were also transferred between feeding cages at the same time to standardize handling. All mice had free access to water throughout the study. Body weight and food intake were monitored at ZT11 weekly before IF was introduced and daily after IF was implemented. At 28 wk old, all mice were sacrificed at ZT7-ZT9 with mice in IF groups culled after feeding or 22-h fasting (n = 7–8/group). Inguinal and gonadal adipose tissue were collected.

At 27 wk of age, indirect calorimetry (n = 7–8/group) was assessed. Mice were acclimated to the metabolic cages for 24 h before data collection (Promethium, Sable Systems, Las Vegas, NV, USA). Three days of data were collected after acclimation. This was two consecutive feeding days, followed by a fasting day. Food access for mice in IF groups was controlled using gates connected to the food hoppers. Food spilled by mice and dropped into bedding was carefully removed using forceps before the commencement of data recording and before the fasting day to improve the accuracy of food consumption monitoring. Four mice in each HFD group dragged food from the hopper during monitoring periods. This data was not included in the analysis and an additional four mice were measured to repeat metabolic monitoring. However, this behavior was repeated in some mice and finally four to seven mice per group were included for the analysis of food-related parameters including meal size, meal number, and energy intake. Data analysis of oxygen consumption (VO_2), carbon dioxide expired (VCO_2), respiratory quotient (RQ), energy expenditure (EE), and activity was performed with seven to eight mice per group. VO_2 and VCO_2 were measured at 5 min intervals for 1 min to calculate RQ and EE as described previously [32]. VO_2, VCO_2, and EE were adjusted by a modified body mass, which was determined by subtracting collected inguinal and gonadal fat mass from body mass, lessening the weight bias from this metabolically less active tissue [33]. We also normalized EE by other ways, including body weight, body weight raised to the power of two-thirds or three-fourths, or analysis of covariance, as mentioned previously [33]. For ambulatory activity, consecutive adjacent infrared beam breaks in x-, y-, and z-axes as an activity count were recorded every 5 min as previously described [32]. Data acquisition and food access control were coordinated by MetaScreen v.2.3.4 and raw data were extracted using ExpeData v.1.6.4 (Sable Systems) with built-in macros. All metabolic data were expressed in two ways: hourly and daily. Hourly value was calculated by the real-time output in 1 h. Daily value was determined by the sum of hourly value on each day. Weekly values for energy intake and energy expenditure were calculated using (day 1 + day 2 + day 3)/3 × 7 for AL groups, and (day 1 × 3 + day 2 + day 3*3) for IF groups.

Quantitative real-time polymerase chain reaction (PCR)

The extraction of total RNA, the determination of RNA concentration and purity, and the synthesis of cDNA from RNA were performed as previously reported [34]. Quantitative real-time PCR was performed using the Taqman Ucp1 primer (Mm01244861_m1) and Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The samples were run in duplicate on an ABI 7500 sequence detection system (Applied Biosystems) with corresponding internal negative controls and standard curve (pooled from 10 participants at baseline, and from individual mice, respectively). Relative gene expression was analyzed using the $2^{-\Delta CT}$ method, where $\Delta CT = (CT_{target\ gene} - CT_{reference\ gene})$. *Actb* (Mm00607939_s1) and *B2 m* (Mm00437762_m1) were selected out from six housekeepers (*18s*, *Actb*, *Gapdh*, *Hprt*, *Ppi*, and *B2 m*) using NormFinder as the most stable reference for mice.

Immunohistochemistry

Inguinal adipose tissue from mice (six per group) were fixed in Bouin's solution (HT10132, Sigma-Aldrich, St. Louis, Missouri, USA), dehydrated, paraffin embedded, sectioned at 5 um and mounted on positively charged glass slides. Deparaffinized and rehydrated slides were incubated with ELOXALL solution (SP-600, Vector, Burlingame, California, USA) for 10 min at room temperature to eliminate endogenous peroxidase and alkaline phosphatase. Antigen retrieval was achieved using modified citrate-based buffer (S1700, Dako, Santa Clara, California, USA) and incubation in a 95°C water bath for 20 min. Slides were incubated with a rabbit anti-UCP1 (1:400, Ab10983, Abcam, Cambridge, UK) overnight and then goat anti-rabbit secondary antibody (1:500, Ab6721, Abcam, Cambridge, UK) for 1-h at room temperature. Immunohistochemical detection was performed using 3, 3′-diaminobenzidine (DAB, SK-4105, Vector, Burlingame, California, USA) and slides were counterstained with Mayer's hematoxylin, followed by dehydrating and mounting. All slides were randomly assigned numeric codes by a research officer to blind the investigator (BL) quantifying outcomes. Slides were scanned using the Pannoramic 250 Flash II scanner (3-DHISTECH, Budapest, Hungary). Ucp1 positive areas were analyzed in 10 randomly selected fields at 40 × using Image J built-in macros (National Institutes of Health, Bethesda, MD, USA).

Human study

The human study was registered as a clinical trial with Clinicaltrials.gov and was approved by the Research Ethics Committee of the Royal Adelaide Hospital and the University of Adelaide. All participants provided written, informed consent before inclusion. The design of the human study and baseline characteristics of the participants have been reported previously [35]. Briefly, 50 healthy, overweight, or obese women, 35 to 70 y of age, with a body mass index 25 to 42 kg/m^2 were randomly assigned to one of two IF groups for 8 wk. Participants were provided with ~30% of their daily energy requirements for breakfast, and then initiated a 24-h fast on 3 non-consecutive d/wk. On fed days, one group was provided foods at ~100% of energy requirements to achieve an overall 30% energy deficit (IF70). The other group was provided foods at ~145% of their daily energy requirements on fed days, without overall energy restriction (IF100). Periumbilical subcutaneous adipose was obtained by percutaneous biopsy at baseline and after 8 wk of the intervention diet, after a 12-h overnight fast, and 24-h fast as described previously [36]. Twenty-two participants in each group completed the intervention. Owing to the unwillingness of some participants to undergo biopsies and because of scheduling conflicts, biopsy samples were obtained from all three3 visits from only 14 individuals in each group. For the purpose of this study, the two IF groups were combined to assess *UCP1* mRNA levels in SAT. *UCP1* mRNA levels (Hs00222453_m1) in human SAT were normalized by *ACTB* (Hs01060665_g1) and *PPIB* (Hs00168719_m1), which was not different at baseline and following the intervention.

Statistical analysis

Data are shown as mean ± SEM. All statistical analysis was performed using IBM SPSS Statistics 24 (IBM, Armonk, NY, USA). The normality of data distribution was assessed by Shapiro-Wilk test, and data were log10 transformed if not normally distributed. Single comparisons in mouse study were performed using two-way analysis of variance (ANOVA) with diet (chow and HFD) and schedule (AL and IF) as between-group factors. Bonferroni post hoc tests were performed when diet-by-schedule effects were presented. Time effects within group in mouse and human studies were examined by repeated measures ANOVA with Bonferroni post hoc test or paired *t* test (for energy intake and meal numbers in IF mice on two consecutive feeding days only). Significance was accepted as $P < 0.05$.

Results

Body weight and food consumption

As reported previously [31], final body weight was increased in HFD-AL (43 ± 0.8 g) versus HFD-IF (32.9 ± 0.8 g), chow-AL (34.7 ± 1 g) and chow-IF (32.2 ± 0.5 g; all $P < 0.05$), and was not different between chow-AL, chow-IF, and HFD-IF groups. Gonadal and inguinal fat mass was reduced by IF in both diet groups, but was greater in HFD-IF than chow-IF groups (all $P < 0.05$, data not shown).

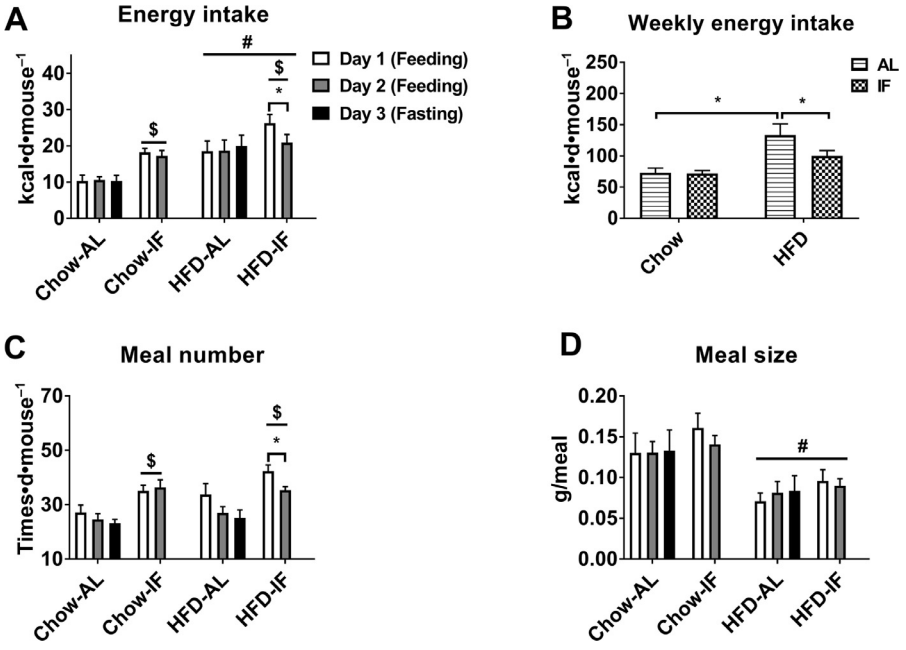

Fig. 1. Energy intake, meal size, and meal number in metabolic cages. (**A** and **B**) Daily and calculated weekly food intake, (**C**) daily meal numbers, and (**D**) daily meal size. Data were presented as mean ± SEM. There were four to seven mice per group. Day 1 and 2 were two consecutive feeding days, followed by a fasting day (day 3). Effects of diet and schedule on each day or in a week (energy intake only) were analyzed by two-way ANOVA with Bonferroni post hoc tests. Within-group effects over days were analyzed using repeated-measures ANOVA with Bonferroni post hoc test (AL groups) or paired *t* test (IF groups). Diet effect: $^{*}P < 0.05$ versus chow on days 1, 2 and 3. Schedule effect: $^{\dagger}P < 0.05$ versus AL on days 1 and 2. Post hoc test: $^{\ddagger}P < 0.05$. AL, ad libitum; ANOVA, analysis of variance; HFD, high-fat diet; IF, intermittent fasting, RQ, respiratory quotient.

There were significant diet and schedule effects observed for energy intake measured in the metabolic chamber on two refeeding days, with increased energy intake in HFD- versus chow-fed mice and in IF versus AL mice (all $P < 0.05$, Fig. 1A and Supplementary Fig. 1A). Meal number was increased by IF on refeeding days (schedule effect, both $P < 0.05$, Fig. 1C and Supplementary Fig. 1B). Of note, chow-IF mice maintained similar energy intake and meal numbers on both refeeding days (both $P > 0.05$). However, HFD-IF mice displayed decreased energy intake and meal number on the second versus first refeeding day (both $P < 0.05$). Mathematical extrapolation of this to a weekly value suggested that overall energy intake was not different in chow-IF versus chow-AL mice (−1.4%, $P = 0.94$) but was 25% lower in HFD-IF versus HFD-AL mice ($P < 0.05$, Fig. 1B). This data supports the weighed food intake data [31]. Meal size was not altered by IF, but was smaller in HFD- versus chow-fed mice (all $P < 0.05$, Fig. 1D and Supplementary Fig. 1C).

Energy expenditure, respiratory quotient, and activity

There were significant schedule effects for EE on the two refeeding days, with increased EE in IF versus AL mice (schedule effect, both $P < 0.001$, Fig. 2A and Supplementary Fig. 2A). EE on a fasting day did not differ between IF and AL mice, but was lower in IF mice versus a fed day (schedule effect, $P < 0.05$, Fig. 2A). Mathematical extrapolation of this showed that calculated weekly EE was higher in IF than AL mice (schedule effect, $P < 0.001$, Fig. 2B). This result held when EE was normalized against modified body mass, raw body weight, or body weight raised to the power of two-thirds or three-fourths or by analysis of covariance with body weight as the covariate [33].

There were significant diet-by-schedule effects for averaged RQ on the two refeeding days (all $P < 0.01$, Fig. 2C and Supplementary Fig. 2B). RQ was increased in chow-IF versus chow-AL and HFD-IF mice on both refeeding days (all $P < 0.05$) but was increased in HFD-IF versus HFD-AL mice on the first refeeding day only ($P < 0.05$). As expected, RQ was lower on fasted versus fed days in IF groups (schedule effect, $P < 0.001$), and versus AL mice (both $P < 0.05$). There were significant diet-by-schedule effects for activity, with reduced activity in HFD-AL versus chow-AL and HFD-IF mice (all $P < 0.05$, Fig. 2D and Supplementary Fig. 2C). There was no difference in activity between fed and fasted days in IF mice.

Ucp1 mRNA levels in gonadal fat were higher in HFD- versus chow-fed mice (diet effect, $P < 0.05$, Fig. 3A). After a fed day, *Ucp1* mRNA levels in inguinal and gonadal fat and UCP1 protein in inguinal fat were increased in IF versus AL mice (schedule effect, all $P < 0.05$, Fig. 3A–E). *Ucp1* mRNA levels in both fat pads were lower on a fasted versus fed day in both IF groups (schedule effect, both $P < 0.05$), but this did not differ from AL mice.

In humans, 8 wk of IF reduced body weight and fat mass (−4 ± 0.4 and 3.1 ± 0.3 kg, respectively, both $P < 0.001$). *UCP1* mRNA levels in subcutaneous adipose tissue were detectable in 19 of 28 participants who consented to biopsy with 10 from the IF100 group and 9 from IF70. There was no difference regarding any assessed baseline characteristics in participants who displayed UCP1 mRNA levels that were or were not detectable. There were no changes in the *UCP1* mRNA levels after 8 wk of IF, either when measured after a fed day or after a 24-h fast (Fig. 3F).

Discussion

IF reduces adiposity and improves glucose tolerance and markers of insulin sensitivity in rodent models [4,7,8,14,37,38]. Genetically or pharmaceutically stimulating the development of brown fat in WAT is linked with decreased mass loss, improves glucose tolerance and insulin sensitivity, and ameliorated lipid profiles in animal models [39–41]. Recent evidence suggests that IF induces browning of WAT in mice [6,13]. The present study confirms and extends this, showing that IF promoted visceral adipose tissue (VAT) and SAT browning in chow- and HFD-fed mice, which could be a key mechanism contributing to a healthier phenotype.

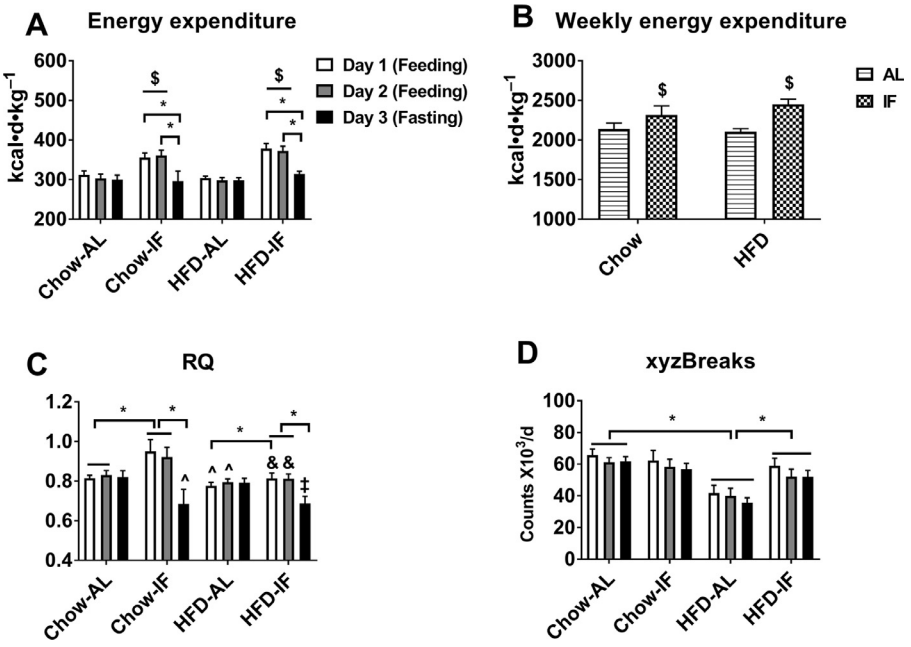

Fig. 2. Energy expenditure, RQ and activity in metabolic cages. (**A** and **B**) Daily and calculated weekly energy expenditure, (**C**) Daily RQ and (**D**) Daily activity. Data presented as mean ± SEM. There were seven to eight mice per group. Days 1 and 2 were two consecutive feeding days, followed by a fasting day (day 3). Effects of diet and schedule on each day, or in a week (energy expenditure only) were analyzed by two-way ANOVA with Bonferroni post hoc tests. Within-group effects over 3 d were analyzed using repeated-measures ANOVA with Bonferroni post hoc test. Schedule effect: $^{*}P < 0.05$ versus AL on days 1 and 2. Post hoc test: $^{\dagger}P < 0.05$; $^{\ddagger}P < 0.05$ versus chow-AL; $^{\S}P < 0.05$ versus chow-IF and $^{\|}P < 0.05$ versus HFD-AL. AL, ad libitum; ANOVA, analysis of variance; HFD, high-fat diet; IF, intermittent fasting RQ, respiratory quotient.

Total daily EE consists of resting energy expenditure and thermoregulation, food-induced thermogenesis, and physical activity [42], and increasing this is one approach to tackle obesity [43]. IF increased EE in both chow- and HFD-fed mice. This was observed without a difference in activity in chow-fed mice. However, activity was lower in mice fed a HFD ad libitum, which likely contributed to some of the difference in EE between HFD groups. We also acknowledge that greater food intakes occurred on refeeding days, and thus greater increases in food-induced thermogenesis may have partially contributed to increased EE. However, EE was not lower on a fast day in IF animals, when no food was consumed. Overall, the present results support recent findings that IF increases EE in mice [6,13]. Similar to previous studies [3,4,7,12,13], the present study also confirmed that chow-IF mice compensate for intermittent food deprivation by overeating on refeeding days and do not lose weight. This is in contrast to IF mice fed an HFD, where food intake was lower and activity and EE was higher, resulting in weight loss. The signal that is preventing overconsumption on an HFD is unclear.

IF increased adipose tissue browning in inguinal and subcutaneous adipose tissue. This is consistent with two recent reports [6,13]. We further demonstrated that the increases in Ucp1 mRNA levels in both inguinal and gonadal adipose tissue were higher after the refeeding day. This data suggests that "intermittent overfeeding," rather than "intermittent fasting" promotes WAT browning. Acute fasting (24–48 h) decreases *Ucp1* expression in both BAT and WAT [44–47], whereas refeeding increased *Ucp1* expression [6,47]. Furthermore, Ding, et al. demonstrated that an acute 24-h fasting suppressed WAT browning via microRNA-149-3p and PR domain containing 16 pathway [45]. Interestingly, recent work by Fabbiano, et al. suggested that daily calorie restriction (−40%) also led to WAT browning in both SAT and VAT. This was mediated by increased eosinophil infiltration, type 2 cytokine signaling, and alternative activation of macrophage in fat [28]. In the present study, IF did not promote alternate activation of macrophages in WAT in mice [31]. Activation of β3-adrenergic receptors via cold exposure or pharmaceutical manipulations has also been linked with WAT browning in mice [21]. This effect has been reported to be associated with the activation of protein kinase A and p38 mitogen-activated protein kinase, which in turn initiate the transcription of UCP1 via phosphorylation of cAMP-dependent transcription factor ATF-2 [48]. IF may also increase sympathetic stimulation [4] and thus adipose tissue browning via this pathway. However, growing evidence also suggests that β3-adrenergic receptor is not essential to increase BAT because mice lacking β3-adrenergic receptors undergo browning in response to cold exposure [49,50]. Indeed, Li, et al. suggested that every-other-day feeding promoted browning but observed decreased mRNA levels of β3- adrenergic receptor in BAT and WAT in mice [6]. The authors demonstrated that the reshaped gut microbiota in every-other -day feeding was linked with this [6], but the mechanisms by which gut microbiota influences browning were not tested in that paper and are thus unknown.

In humans, WAT browning occurs after burn injury and administration of β3-adrenergic receptor agonists [27,29,30]. However, mild to moderate external stimuli such as cold exposure, exercise, and daily calorie restriction that promote VAT and SAT browning in mice [20–25,28], do not induce SAT browning in humans [5,51–53]. We also did not see changes in *UCP1* mRNA levels in SAT following IF. Weight and fat mass loss by calorie restriction also did not change SAT browning in obese individuals [5]. It should be noted that although brown-like adipocytes are found in both VAT and SAT [54], their distribution patterns are different in mice and humans. As shown in the present study, and previously, mice have higher levels of brown-like adipocytes in SAT, whereas these are higher in visceral fat depots in humans [54]. This indicates that there is likely to be a greater potential for VAT than SAT to brown in response to external stimuli in humans. However, it was not possible to obtain visceral fat samples in this study and thus it is possible that IF could stimulate browning in this

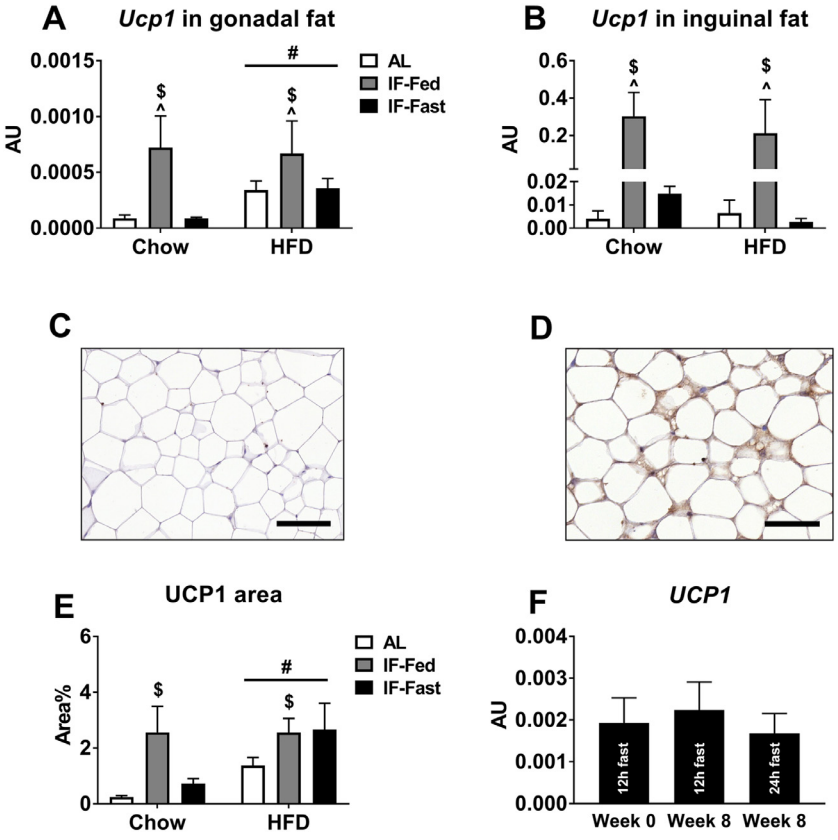

Fig. 3. IF promoted white adipose tissue browning in mice but not in humans. (**A** and **B**): *Ucp1* mRNA levels in gonadal and inguinal fat in mice. (**C** and **D**): Representative images of immunohistochemical staining of UCP1 in chow-AL (**C**) and chow-IF mice (**D**). (**E**): UCP1 positive area in inguinal fat in mice. (**F**): *UCP1* mRNA levels in human subcutaneous adipose tissue. Scale bar: 100 μm. Data presented as mean ± SEM. There were 7 to 8 mice per for Figure A and B, 6 per group for C, and 28 for F. Effects of diet and schedule (A, B, and E) were analyzed using two-way ANOVA with Bonferroni post hoc test. Time effects within group (F) were analyzed using repeated-measures ANOVA with Bonferroni post hoc test. Diet effect: $^{*}P < 0.05$ versus chow; Schedule effect: $^{\dagger}P < 0.05$ versus AL and $^{\ddagger}P < 0.05$ versus IF-Fast. AL, ad libitum; ANOVA, analysis of variance; AU: Arbitrary unit; IF, intermittent fasting; Ucp, uncoupled protein.

tissue. We also only obtained a small abdominal sample of tissue, which may not be representative of the entire depot. We also only studied women in this trial. Sex rather than species differences could also explain the discrepancies in results [55–57], although female mice have been shown to undergo similar browning in response to β3-adrenergic activation [56].

Conclusion

IF increased EE and promoted WAT browning in both chow- and HFD-fed mice, but did not alter *UCP1* mRNA levels in SAT from women.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.03.015.

References

[1] Arum O, Bonkowski MS, Rocha JS, Bartke A. The growth hormone receptor gene-disrupted mouse fails to respond to an intermittent fasting diet. Aging Cell 2009;8:756–60.
[2] Xie K, Neff F, Markert A, Rozman J, Aguilar-Pimentel JA, Amarie OV, et al. Every-other-day feeding extends lifespan but fails to delay many symptoms of aging in mice. Nat Commun 2017;8:155.
[3] Goodrick CL, Ingram DK, Reynols MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: Interaction of genotype and age. Mech Ageing Dev 1990;55:69–87.
[4] Gotthardt JD, Verpeut JL, Yeomans BL, Yang JA, Yasrebi A, Roepke TA, et al. Intermittent fasting promotes fat loss with lean mass retention, increased hypothalamic norepinephrine content, and increased neuropeptide Y gene expression in diet-induced obese male mice. Endocrinology 2016;157:679–91.
[5] Barquissau V, Leger B, Beuzelin D, Martins F, Amri EZ, Pisani DF, et al. Caloric restriction and diet-induced weight loss do not induce browning of human subcutaneous white adipose tissue in women and men with obesity. Cell Rep 2018;22:1079–89.
[6] Li G, Xie C, Lu S, Nichols RG, Tian Y, Li L, et al. Intermittent fasting promotes white adipose browning and decreases obesity by shaping the gut microbiota. Cell Metab 2017;26. 672–85.e4.
[7] Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proc Natl Acad Sci U S A 2003;100:6216–20.
[8] Joslin PMN, Bell RK, Swoap SJ. Obese mice on a high-fat alternate-day fasting regimen lose weight and improve glucose tolerance. J Anim Physiol Anim Nutr 2017;101:1036–45.
[9] Krizova E, Simek V. Influence of intermittent fasting and high-fat diet on morphological changes of the digestive system and on changes of lipid metabolism in the laboratory mouse. Physiol Res 1996;45:145–51.
[10] Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. FASEB J 2003;17:1133–4.
[11] Chen Y, Ling L, Su G, Han M, Fan X, Xun P, et al. Effect of intermittent versus chronic calorie restriction on tumor incidence: a systematic review and meta-analysis of animal studies. Sci Rep 2016;6:33739.
[12] Liu H, Javaheri A, Godar RJ, Murphy J, Ma X, Rohatgi N, et al. Intermittent fasting preserves beta-cell mass in obesity-induced diabetes via the autophagy-lysosome pathway. Autophagy 2017;13:1952–68.
[13] Kim KH, Kim YH, Son JE, Lee JH, Kim S, Choe MS, et al. Intermittent fasting promotes adipose thermogenesis and metabolic homeostasis via VEGF-mediated alternative activation of macrophage. Cell Res 2017;27:1309–26.

[14] Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. Am J Clin Nutr 2005;81:69–73.
[15] Klempel MC, Kroeger CM, Varady KA. Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet. Metabolism 2013;62:137–43.
[16] Trepanowski JF, Kroeger CM, Barnosky A, Klempel MC, Bhutani S, Hoddy KK, et al. Effect of alternate-day fasting on weight loss, weight maintenance, and cardioprotection among metabolically healthy obese adults: a randomized clinical trial. JAMA Intern Med 2017;177:930–8.
[17] Byrne NM, Sainsbury A, King NA, Hills AP, Wood RE. Intermittent energy restriction improves weight loss efficiency in obese men: the MATADOR study. Int J Obes 2018;42:129–38.
[18] 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.
[19] Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. J Clin Invest 2015;125:478–86.
[20] Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, et al. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 2010;298:E1244–53.
[21] Lim S, et al. Cold-induced activation of brown adipose tissue and adipose angiogenesis in mice. Nat Protoc 2012;7:606–15.
[22] Fisher FM, Kleiner S, Douris N, Fox ED, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1α and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 2012;26:271–81.
[23] Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463–8.
[24] Xu X, Ying Z, Cai M, Xu Z, Li Y, Jiang SY, et al. Exercise ameliorates high-fat diet-induced metabolic and vascular dysfunction, and increases adipocyte progenitor cell population in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol 2011;300:R1115–25.
[25] Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise effects on white adipose tissue: beiging and metabolic adaptations. Diabetes 2015;64:2361–8.
[26] Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev 2013;27:234–50.
[27] Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, et al. Activation of human brown adipose tissue by a β3-adrenergic receptor agonist. Cell Metab 2015;21:33–8.
[28] Fabbiano S, Suarez-Zamorano N, Rigo D, Veyrat-Durebex C, Stevanovic Dokic A, Colin DJ, et al. Caloric restriction leads to browning of white adipose tissue through type 2 immune signaling. Cell Metab 2016;24:434–46.
[29] Sidossis LS, Porter C, Saraf MK, Borsham E, Radhakrishnan RS, Chao T, et al. Browning of subcutaneous white adipose tissue in humans after severe adrenergic stress. Cell Metabolism 2015;22:219–27.
[30] Patsouris D, Qi P, Abdullahi A, Stanojic M, Chen P, Parousis A, et al. Burn induces browning of the subcutaneous white adipose tissue in mice and humans. Cell Rep 2015;13:1538–44.
[31] Liu B, Page AJ, Hatzinikolas G, Chen M, Wittert GA, Heilbronn LK. Intermittent fasting improves glucose tolerance and promotes adipose tissue remodeling in male mice fed a high fat diet. Endocrinology 2018;160:169–80.
[32] Kaiyala KJ, Morton GJ, Thaler JP, Meek TH, Tylee T, Ogimoto K, et al. Acutely decreased thermoregulatory energy expenditure or decreased activity energy expenditure both acutely reduce food intake in mice. PLoS One 2012;7:e41473.
[33] Tschop MH, Speakman JR, Arch JR, Auwerx J, Bruning JC, Chan L, et al. A guide to analysis of mouse energy metabolism. Nat Methods 2011;9:57–63.
[34] Chen M, Wu L, Zhao J, Wu F, Davies MJ, Wittert GA, et al. Altered glucose metabolism in mouse and humans conceived by IVF. Diabetes 2014;63:3189–98.
[35] Hutchison AT, Liu B, Wood RE, Vincent AD, Thomson CH, O'Callaghan NJ, et al. Effects of intermittent versus continuous energy intakes on insulin sensitivity and metabolic risk in women with overweight. Obesity 2019;27:50–8.
[36] Chen M, Liu B, Thompson CH, Wittert GA, Heilbronn LK. Acute overfeeding does not alter liver or adipose tissue-derived cytokines in healthy humans. Ann Nutr Metab 2016;69:165–70.
[37] Varady KA, Allister CA, Roohk DJ, Hellerstein MK. Improvements in body fat distribution and circulating adiponectin by alternate-day fasting versus calorie restriction. J Nutr Biochem 2010;21:188–95.
[38] Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int J Obes 2011;35:714–27.
[39] Seale P, Conrow HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 2011;121:96–105.
[40] Liu W, Bi P, Shan T, Yang X, Yin H, Wang YX, et al. miR-133 a regulates adipocyte browning in vivo. PLoS Genetics 2013;9:e1003626.
[41] Berbée JFP, Boon MR, Khedoe PP, Bartelt A, Schlein C, Worthmann A, et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat Commun 2015;6:6356.
[42] Speakman JR. Measuring energy metabolism in the mouse—theoretical, practical, and analytical considerations. Front Physiol 2013;4:34.
[43] Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation 2012;126:126–32.
[44] Tang HN, Tang CY, Man XF, Tan SW, Guo Y, Tang J, et al. Plasticity of adipose tissue in response to fasting and refeeding in male mice. Nutr Metab 2017;14:3.
[45] Ding H, Zheng S, Garcia-Ruiz D, Hou D, Wei Z, Liao Z, et al. Fasting induces a subcutaneous-to-visceral fat switch mediated by microRNA-149-3 p and suppression of PRDM16. Nat Commun 2016;7:11533.
[46] Sivitz WI, Fink BD, Donohoue PA. Fasting and leptin modulate adipose and muscle uncoupling protein: divergent effects between messenger ribonucleic acid and protein expression. Endocrinology 1999;140:1511–9.
[47] Champigny O, Ricquier D. Effects of fasting and refeeding on the level of uncoupling protein mRNA in rat brown adipose tissue: evidence for diet-induced and cold-induced responses. J Nutr 1990;120:1730–6.
[48] Cao W, Medvedev AV, Daniel KW, Collins S. Beta-adrenergic activation of p38 MAP kinase in adipocytes: CAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 MAP kinase. J Biol Chem 2001;276:27077–82.
[49] Susulic VS, Frederich RC, Lawitts J, Tozzo E, Kahn BB, Harper ME, et al. Targeted disruption of the beta 3-adrenergic receptor gene. J Biol Chem 1995;270:29483–92.
[50] de Jong JMA, Woulters RTF, Boulet N, Cannon B, Nedergaard J, Petrovic N. The β3-adrenergic receptor is dispensable for browning of adipose tissues. Am J Physiol Endocrinol Metab 2017;312:E508–18.
[51] van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 2013;123:3395–403.
[52] Nakhuda A, Jossee AR, Gburcik V, Crossland H, Raymond F, Metairon S, et al. Biomarkers of browning of white adipose tissue and their regulation during exercise- and diet-induced weight loss. Am J Clin Nutr 2016;104:557–65.
[53] Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, et al. The effects of acute and chronic exercise on PGC-1 alpha, irisin and browning of subcutaneous adipose tissue in humans. FEBS J 2014;281:739–49.
[54] Zuriaga MA, Fuster JJ, Gokce N, Walsh K. Humans and mice display opposing patterns of "browning" gene expression in visceral and subcutaneous white adipose tissue depots. Front Cardiovasc Med 2017;4:27.
[55] Choi DK, Oh TS, Choi JW, Mukherjee R, Wang X, Liu H, et al. Gender difference in proteome of brown adipose tissues between male and female rats exposed to a high fat diet. Cell Physiol Biochem 2011;28:933–48.
[56] Kim SN, Jung YS, Kwon HJ, Seong JK, Granneman JG, Lee YH. Sex differences in sympathetic innervation and browning of white adipose tissue of mice. Biol Sex Differ 2016;7:67.
[57] van den Beukel JC, Grefhorst A, Hoogduijn MJ, Steenbergen J, Mastroberardino PG, Dor FJ, et al. Women have more potential to induce browning of perirenal adipose tissue than men. Obesity 2015;23:1671–9.