Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Effects of Intermittent Versus Continuous Energy Intakes on Insulin Sensitivity and Metabolic Risk in Women with Overweight

Amy T. Hutchison[1,2,3], Bo Liu[1,2,3], Rachel E. Wood[4], Andrew D. Vincent[1], Campbell H. Thompson[1], Nathan J. O'Callaghan[3,5], Gary A. Wittert[1,2,3], and Leonie K. Heilbronn[1,2,3]

Objective: This study aimed to compare intermittent fasting (IF) versus continuous energy intakes at 100% or 70% of calculated energy requirements on insulin sensitivity, cardiometabolic risk, body weight, and composition.

Methods: Women with overweight ($n = 88$; 50 ± 1 years, BMI 32.3 ± 0.5 kg/m^2) were randomized to one of four diets (IF70, IF100, dietary restriction [DR70], or control) in a 2:2:2:1 ratio for 8 weeks. IF groups fasted for 24 hours after breakfast on three nonconsecutive days per week. All foods were provided and diets matched for macronutrient composition (35% fat, 15% protein, 50% carbohydrate). Insulin sensitivity by hyperinsulinemic-euglycemic clamp, weight, body composition, and plasma markers were assessed following a "fed" day (12-hour fast) and a 24-hour fast (IF only).

Results: IF70 displayed greater reductions in weight, fat mass, total- and low-density lipoprotein cholesterol, and nonesterified fatty acids compared with DR70 and IF100 (all $P \leq 0.05$). IF100 lost more weight and fat than control. However, fasting insulin was increased. There were no group differences in insulin sensitivity by clamp; however, a 24-hour fast transiently reduced insulin sensitivity.

Conclusions: When prescribed at matched energy restriction, IF reduced weight and fat mass and improved total and low-density lipoprotein cholesterol more than DR. IF prescribed in energy balance did not improve health compared with other groups, despite modest weight loss.

Obesity (2019) **27**, 50-58. doi:10.1002/oby.22345

Introduction

Continuous dietary restriction (DR) remains the cornerstone lifestyle intervention to reduce the risk of developing type 2 diabetes and cardiovascular disease in individuals with overweight (1-5). Because of the inherent difficulty associated with long-term adherence to DR (6,7), alternative approaches are being investigated.

Intermittent fasting (IF) involves alternating periods of eating with fasting periods of up to 24 hours for 1 to 4 days a week. In mice, 24-hour IF results in favorable redistribution of adipose tissue (8), reduced fasting glucose and insulin (9), and improved cardiovascular health (10). In most of these studies, the metabolic health benefits have been observed with minimal weight differences (8) or versus pair fed controls (9), suggesting that fasting may be the stimulus required to improve health.

Studies in humans have shown that IF reduces weight and fat mass, total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, postprandial lipemia, and fasting insulin (11-15), while others have shown no significant improvements in metabolic health despite weight loss (16,17). To our knowledge, five studies have compared an intermittent versus continuous dietary approach for 2 to 12 months (12,18-21).

[1] Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia. Correspondence: Amy T. Hutchison (amy.hutchison@adelaide.edu.au) [2] NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, University of Adelaide, Adelaide, South Australia, Australia [3] Nutrition and Metabolism Theme, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia [4] School of Health Sciences, University of Tasmania, Launceston, Tasmania, Australia [5] Commonwealth Scientific and Industrial Research Organization (CSIRO) Food, Nutrition and Bioproducts Flagship, Adelaide, South Australia, Australia.

See Commentary, pg. 11.

Funding agencies: The research was funded by a National Health and Medical Research Council (NHMRC) 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.

Disclosure: The authors declared no conflicts of interest.

Author contributions: LKH and GAW designed the research. ATH, BL, and REW collected data. GAW and CHT provided clinical support and supervised clamps. NJO'C assisted with analysis of plasma biomarkers. ADV performed the statistical analysis. All authors contributed to data interpretation and preparation of the manuscript. LKH had full access to the data and had primary responsibility for the final publication.

Clinical trial registration: ClinicalTrials.gov. identifier NCT01769976.

Additional Supporting Information may be found in the online version of this article.

Received: 11 April 2018; **Accepted:** 18 September 2018; **Published online 20 December 2018. doi:10.1002/oby.22345**

These studies have shown that IF and DR produce similar reductions in body weight and markers of cardiovascular risk. However, Harvie et al. reported that IF reduced body fat and fasting insulin more than DR (19). Two studies have examined insulin sensitivity by hyperinsulinemic-euglycemic clamp after IF (22,23), but none has compared this with DR. Moreover, changes in metabolic parameters between fed and fasted states have been examined only in lean men (22). This is important because "metabolic switching" between fed and fasting states, rather than weight loss, may underlie the health benefits of IF (24,25).

The aims of this study were to conduct a randomized controlled trial in women with overweight to (1) compare the effects of intermittent versus continuous food intakes at two energy levels on peripheral insulin sensitivity, weight and body composition, and cardiometabolic outcomes; and (2) explore the acute metabolic changes that occur when switching between a fed (i.e., after a 12-hour overnight fast) and fasted (24-hour fast) state.

Methods

Participants

Between March 1, 2013, and September 4, 2015, 119 women were screened following advertisement in local newspapers and media to participate in this single-center, randomized controlled trial in Adelaide, South Australia (Supporting Information Figure S1). A total of 88 women enrolled in the study. Inclusion criteria were aged 35 to 70 years; BMI 25 to 42 kg/m^2; weight stable (±5% of their screening weight) for > 6 months prior to study entry; nondiabetic; nonsmoker; sedentary or lightly active (i.e., < 2 moderate- to high-intensity exercise sessions/week); consumed < 140 g alcohol per week; no history of cardiovascular disease, eating disorders, or psychiatric disorders (including those taking antidepressants); not pregnant or breastfeeding; and not taking medication that may affect study outcomes (e.g., phentermine, orlistat, metformin, excluding antihypertensive/lipid-lowering medication). The Royal Adelaide Hospital Research Ethics Committee approved the study, and all participants provided written informed consent prior to their inclusion. The study was registered with ClinicalTrials.gov (NCT01769976).

Randomization and masking

The trial period was 10 weeks, including a 2-week lead-in and an 8-week intervention. During the lead-in, participants consumed their normal diet and maintained their weight. Following this, participants were randomly assigned in a 2:2:2:1 ratio to one of the following four diets: (1) IF70, an IF diet at 70% of calculated baseline energy requirements per week; (2) IF100, an IF diet at 100% of calculated baseline energy requirements per week (i.e., weight maintenance); (3) DR70, a continuous restriction at 70% of calculated baseline energy requirements daily; or (4) control, 100% of calculated baseline energy requirements daily. Daily energy requirements were calculated by averaging predicted daily energy expenditure from two published equations, both of which use age, gender, height, and weight variables (26,27). Block randomization (four or eight participants) was performed by a research officer, with stratification by BMI (≤ 32.9 or ≥ 33 kg/m^2) and age (≤ 49.9 or ≥ 50 years). Nine participants withdrew from the study. Seven no longer wished to participate, and two were withdrawn by investigators, one for preexisting bronchial issues unrelated to the study and the other because of gastrointestinal surgery that was not disclosed during the screening process (completers: DR70, $n = 24$; IF70, $n = 22$; IF100, $n = 22$; control, $n = 11$).

On fed days, IF70 participants were provided with ~100% and IF100 with ~145% of energy requirements. IF groups consumed breakfast before 8 AM on fasting days (~32% of energy requirements at breakfast on fasting days in IF70 and ~37% in IF100; Supporting Information Table S1), and then commenced a ~24-hour "fast" until 8 AM the following day on three nonconsecutive weekdays per week. During the fast, participants were allowed water, small amounts of energy-free foods (e.g., "diet" drinks, chewing gum/mints), black coffee, and/or tea and were provided with 250 mL of very-low-energy broth (20 kcal/250 mL, 2.0 g protein, 0.1 g fat, 3.0 g carbohydrate) for lunch or dinner. All diets were matched for macronutrient composition (35% fat, 15% protein, 50% carbohydrate). Participants were free-living, and foods were delivered every 2 weeks to their home, excluding fruits and vegetables. Portions of fruits and vegetables were standardized (1 "serving" of fruits = 150 g of fresh fruit or 30 g of dried fruit; 1 "serving" of vegetables = 75 g of raw, steamed, or boiled vegetables), and participants self-selected according to the number of servings specified in their individual menus.

Adherence and perceptions of appetite

Participants completed daily checklists to monitor adherence, and energy intake in weeks 1, 4, and 7 was calculated from 7-day food diaries using FoodWorks (version 8; Xyris Software, Australia; Supporting Information Tables S1 and S2). Participants attended our clinic weekly, where they returned the 7-day checklist from the previous week, were weighed, and received individual counseling to maintain compliance. Perceptions of appetite and symptoms (hunger, fullness, desire to eat, mental alertness, irritability, and perceived difficulty adhering to the diet) were assessed at baseline, week 1, and week 6 using validated visual analogue scales.

Metabolic testing

To minimize the influence of the menstrual cycle, premenopausal women were studied in the follicular phase. Participants consumed a standardized diet (100% of calculated energy requirements, 35% fat, 15% protein, 50% carbohydrate) for 3 days and were instructed to avoid exercise, alcohol, and caffeine for 24 hours prior to the first metabolic testing visit ("baseline"). Participants fasted for 12 hours overnight prior to the baseline and "fed" (week 8) visits. IF groups underwent a third metabolic visit following a 24-hour fast to capture outcomes from fasting days. This visit occurred 2 to 7 days after the fed visit, depending on clinician availability (Supporting Information Figure S2). At all visits, participants arrived at 7:30 AM, were weighed in a gown after voiding, and had waist and hip measurements taken. Blood pressure was measured with the participant seated after 10 minutes of rest. Intravenous cannulas were placed, baseline samples collected, and a primed 120-minute hyperinsulinemic-euglycemic (60 mU/m^2/min) clamp commenced as described (28). Peripheral insulin sensitivity (M) was calculated as mean glucose infusion rate (GIR) during steady state (last 30 minutes), normalized for estimated size of fat-free mass (FFM) as described by others (GIR per kilogram FFM + 17.7) (29,30). Steady-state insulin was significantly lower after 24-hour fasts in IF70 ($P = 0.002$) and IF100 ($P = 0.05$) participants, suggesting increased insulin clearance following a prolonged fast (31,32). We calculated insulin-adjusted GIR by dividing M by I, where I is the steady-state insulin concentration (milliunits per liter) × 100 (29,33). Because

of scheduling conflicts or technical issues, 11 clamps were not conducted. Of these, 5 participants did not have baseline clamps, and therefore subsequent clamps were not scheduled, 2 completed baseline clamps only, and 4 completed baseline and fed visits only. The following numbers of participants were used in the completers analysis of the clamp data: DR70, $n=22$; IF70, $n=17$; IF100, $n=19$; control, $n=10$. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as (fasting serum insulin [milliunits per liter] × fasting plasma glucose [millimoles per liter]) / 22.5. Total body composition was assessed by dual-energy x-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Madison, Wisconsin). All procedures were identical on study days; however, no DXA was performed at the "fast" visit.

Analytical methods

Blood samples were immediately centrifuged and frozen at −80°C. Blood lipids and fasting blood glucose were examined by photometric assays by SA Pathology (Adelaide, South Australia). Serum insulin was measured by radioimmunoassay (HI-14K, Millipore, Burlington, Massachusetts). Serum nonesterified fatty acids (NEFA) were measured by enzymatic colorimetric assay (NEFA-HR (2), Wako Diagnostics, Mountain View, California). Plasma beta-hydroxybutyrate (B-HB) (RANBUT D-3 hydroxybutyrate kit, Randox, Antrim, UK), alanine aminotransferase, aspartate aminotransferase (AST), and high-sensitivity C-reactive protein were measured using commercially available enzymatic kits (Beckman Coulter Inc., Brea, California) on a Beckman AU480 clinical analyzer. Samples from each subject were analyzed within the same run to reduce instrument variation. Serum fibroblast growth factor-21 was measured by enzyme-linked immunosorbent assay (ELISA; R & D Systems, Minneapolis, Minnesota).

Statistical analysis

The number of participants was established from past studies (28,34,35). The primary comparison was insulin sensitivity normalized for FFM and steady-state insulin concentration (M/I; [GIR/kg FFM+17.7]/mU) between DR70 and IF70 groups. With $n=22$ per group (randomized 1:1), a t test would allow detection of a mean difference in M of 15 μmol/kg FFM + 17.7 between groups, based on an SD of 17, with 80% power (two-sided $\alpha=0.05$). This has allowed for a 10% dropout rate, and thus we recruited a total of $n=25$ per group. For completeness, we included an IF100 group and a non-weight-loss group (control) at half sample size.

Statistical methods

All end points were assessed as follows. DR70 and IF70 participants measured on the fed day were compared using linear regressions (one observation per individual), adjusting for baseline levels. Thereafter, we included the IF70 24-hour fast measurements and compared the three levels (DR70 vs. IF70 12 hour vs. IF70 24 hour) using mixed-effects regressions, with a random intercept per individual and compound symmetry correlation structure. Finally, we assessed the diet comparison with and without adjusting for weight loss, which was included as an additional fixed effect. IF100 versus control diets were compared in a similar manner using the same regression models. Finally, IF70 versus IF100 participants were compared using linear mixed-effects regressions using the same random-effect structure as above, with baseline, time (12 hour vs. 24 hour), and diet (IF70 vs. IF100) as fixed effects. Individuals missing outcome data were excluded from each analysis, while those missing baseline data were imputed using cohort means. After examination of residual distributions, all end points measured from plasma (glucose, insulin, B-HB, and liver markers), except for total, high-density lipoprotein, and LDL cholesterol, were log-transformed. Data are shown as mean ± SEM. Diet comparisons are pairwise with significance set at $P<0.05$ (two sided). Statistical analyses were performed using SPSS software (version 21.0; IBM, Armonk, New York) and R (version 3.3.3; The R Foundation, Vienna, Austria).

Results

A total of 88 women (mean age 50 ± 1 years, mean BMI 32.3 ± 0.5) enrolled in the study. Baseline age, weight, BMI, body composition, fasting glucose, insulin, and blood lipids appeared balanced between treatment groups (Table 1).

In addressing aim 1, weight ($P=0.03$; Figure 1B) and fat loss ($P=0.05$; Figure 1C) were greater in IF70 compared with DR70 and compared with IF100 (both $P<0.01$). Weight and fat loss were also greater in IF100 compared with control (both $P<0.001$). The reduction in FFM was not statistically different between IF70 and DR70 ($P=0.07$) or IF70 and IF100 ($P=0.06$), but it was greater in IF100 compared with control ($P=0.04$). The proportion of weight lost as FFM was not significantly different between IF70 and DR70 ($P=0.94$), IF70 and IF100 ($P=0.74$), or IF100 and control ($P=0.11$; Supporting Information Table S3). The reduction in waist circumference was greater in IF70 compared with IF100 ($P=0.04$; Supporting Information Table S3). There were no differences between groups for change in hip circumference (Supporting Information Table S3). Given the unexpected differences in weight loss between DR70 and IF70 as well as IF100 and control, we have reported comparisons unadjusted and adjusted for weight loss.

Self-reported energy intake was not different from that provided in control ($P=0.83$) or DR70 ($P=0.96$) (Supporting Information Table S1). The IF100 group reported consuming 240 ± 336 kcal/d less than provided on fed days and the IF70 group 188 ± 200 kcal/d less than provided on fed days (Supporting Information Table S2). This resulted in an overall average weekly deficit of ~9% and ~2% more than prescribed, respectively. As such, energy restriction was greater in IF70 (−31% ± 2%) compared with DR70 (−30% ± 2%) ($P=0.02$) and greater in IF100 (−9% ± 8%) compared with control (0% ± 5%; $P=0.02$). Perceived difficulty adhering to the diet was higher in IF100 compared with IF70 and control at week 1 (both $P<0.05$) but not at week 6 ($P=0.61$ compared with IF70, $P=0.08$ compared with control). In week 1, self-reported feelings of hunger on a fed day were lower in IF70 compared with DR70 and higher compared with IF100 (Supporting Information Figure S3).

The change in insulin sensitivity by clamp after a fed day was not significantly different between IF70 and DR70 ($P=0.95$), IF70 and IF100 ($P=0.31$), or IF100 and control ($P=0.65$) (Figure 2A). However, there was a trend for insulin sensitivity to be impaired after a fast day in IF70 compared with DR70 ($P=0.08$). Changes in glucose (Figure 2C) and insulin (Figure 2D) were significantly greater after a fast day only in IF70 compared with DR70 (both $P<0.05$) and after a fed day only compared with IF100 (both $P=0.02$). This translated into reduced (improved) HOMA-IR after a fast day in IF70 compared with DR70 ($P=0.01$; Figure 2B) and after a fed day in IF70 compared with

TABLE 1 Baseline characteristics of participants

	Control ($n=12$)	IF100 ($n=25$)	IF70 ($n=25$)	DR70 ($n=26$)
Age at enrollment (y)	49 ± 3	51 ± 2	49 ± 2	51 ± 2
Weight (kg)	83.8 ± 4.8	84.1 ± 2.8	89.4 ± 2.8	88.4 ± 2.8
BMI (kg/m^2)	30.9 ± 1.5	31.2 ± 0.9	32.4 ± 0.8	32.6 ± 1.0
Pre/postmenopausal	6/6	10/15	13/12	12/14
Body fat (%)	44.5 ± 2.6	47.0 ± 1.3	48.3 ± 1.4	48.4 ± 1.4
Waist circumference (cm)	98 ± 6	99 ± 3	101 ± 2	99 ± 2
Hip circumference (cm)	112 ± 4	112 ± 2	115 ± 2	116 ± 2
Fasting glucose (mmol/L)	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Fasting insulin (mU/L)	16.8 ± 2.2	18.6 ± 1.5	19.5 ± 1.5	15.5 ± 1.3
HOMA-IR	3.8 ± 0.6	4.1 ± 0.4	4.3 ± 0.3	3.4 ± 0.3
Total cholesterol (mmol/L)	4.5 ± 0.4	5.0 ± 0.2	4.8 ± 0.1	4.9 ± 0.1
HDL-C (mmol/L)	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.1	1.4 ± 0.1
LDL-C (mmol/L)	2.6 ± 0.3	3.0 ± 0.2	2.9 ± 0.1	3.0 ± 0.1
Triglycerides (mmol/L)	1.4 ± 0.3	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
HS-CRP (mg/dL)	2.1 ± 0.6	2.8 ± 0.5	2.9 ± 0.5	2.7 ± 0.5
ALT (U/L)	16.7 ± 1.9	21.6 ± 2.4	19.5 ± 1.9	19.7 ± 1.7
AST (U/L)	19.7 ± 1.4	21.3 ± 1.3	20.1 ± 1.1	19.5 ± 1.0
FGF-21 (mmol/L)	163.8 ± 32.7	169.1 ± 23.1	142.5 ± 23.1	184.4 ± 22.1

Data are shown as mean ± SEM.
There were no significant differences between groups at baseline in any of the outcome measures.
DR70, continuous energy restriction at 70% of baseline energy requirements; IF70, intermittent fasting diet at 70% of baseline energy requirements; IF100, intermittent fasting diet at 100% of baseline energy requirements; control, continuous food intake at 100% of baseline energy requirements.
ALT, alanine transaminase; AST, aspartate transaminase; FGF-21, fibroblast growth factor-21; HDL-C, high-density lipoprotein cholesterol; HS-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol.

IF100 ($P=0.002$). Fasting insulin after a fed day was also increased in IF100 compared with control ($P=0.05$). The change in NEFA was greater after a fed ($P=0.005$) and fast ($P=0.003$) day in IF70 compared with DR70 and after a fed day only compared with IF100 ($P=0.05$; Figure 2E). There were no differences between diets for the change in high-sensitivity C-reactive protein, alanine transaminase (Supporting Information Table S3), AST (Figure 2F), or systolic and diastolic blood pressure (Supporting Information Table S3). The change in fibroblast growth factor-21 was greater in IF70 compared with IF100 ($P=0.008$; Figure 2G). There was a greater increase in B-HB after a fed day in IF70 compared with IF100 ($P=0.001$; Figure 2H) as well as after a fast day in IF70 compared with DR70 ($P<0.0001$) and IF100 compared with control ($P<0.001$). Adjusting for weight loss did not alter the outcomes for HOMA-IR, NEFA, or B-HB; however, differences between IF70 and IF100 for insulin and glucose were lost.

The changes in total and LDL cholesterol were greater in IF70 compared with DR70 (both $P<0.01$) and IF100 (both $P\leq0.05$; Figure 3A-3C). The change in triglycerides was significantly greater in IF70 compared with DR70 ($P=0.05$; Figure 3D). There were no differences between diets for the change in high-density lipoprotein cholesterol (Figure 3B). After adjustment for weight loss, differences between IF70 and DR70, but not between IF70 and IF100, for the changes in total ($P=0.01$) and LDL cholesterol ($P=0.04$) remained.

With regard to aim 2, a 24-hour fast significantly impaired insulin sensitivity by clamp compared with a 12-hour fast ($P=0.002$; Figure 2A; Supporting Information Table S4). Contrary to this, HOMA-IR was improved by a 24-hour fast ($P<0.0001$; Figure 2D). Fasting glucose (Figure 2B) and insulin (Figure 2C) were reduced (both $P=0.01$) and plasma NEFA (Figure 2E) and B-HB (Figure 2H) concentrations were increased (all $P<0.001$). A 24-hour fast also increased AST ($P=0.01$; Figure 2F) and reduced insulin-induced suppression of NEFA ($P=0.002$; Supporting Information Table S4).

Discussion

This randomized controlled trial showed that provision of an energy-restricted IF diet led to greater reductions in weight and fat mass and improvements in total and LDL cholesterol and NEFA versus energy-matched DR. There were no differences in insulin sensitivity by clamp between groups, although the 24-hour fast tended to induce transient insulin resistance. IF prescribed in energy balance resulted in transient increases in risk markers for type 2 diabetes, despite modest weight loss. These data suggest that IF with energy restriction improves metabolic health, while IF in energy balance does not.

One study has shown that IF led to a greater percentage of weight loss over 8 weeks (18). However, the energy intakes of the IF and DR groups were not matched, resulting in a −376 kcal/d greater restriction in the IF group. This deficit, rather than mode of meal delivery, may partially explain this outcome. Harvie et al. prescribed a similar energy deficit between intermittent and continuous energy-restricted groups (19). In that study, weight loss was not statistically different between intermittent and continuous groups after 6 months (−6.4 kg [95% CI: −7.9 to

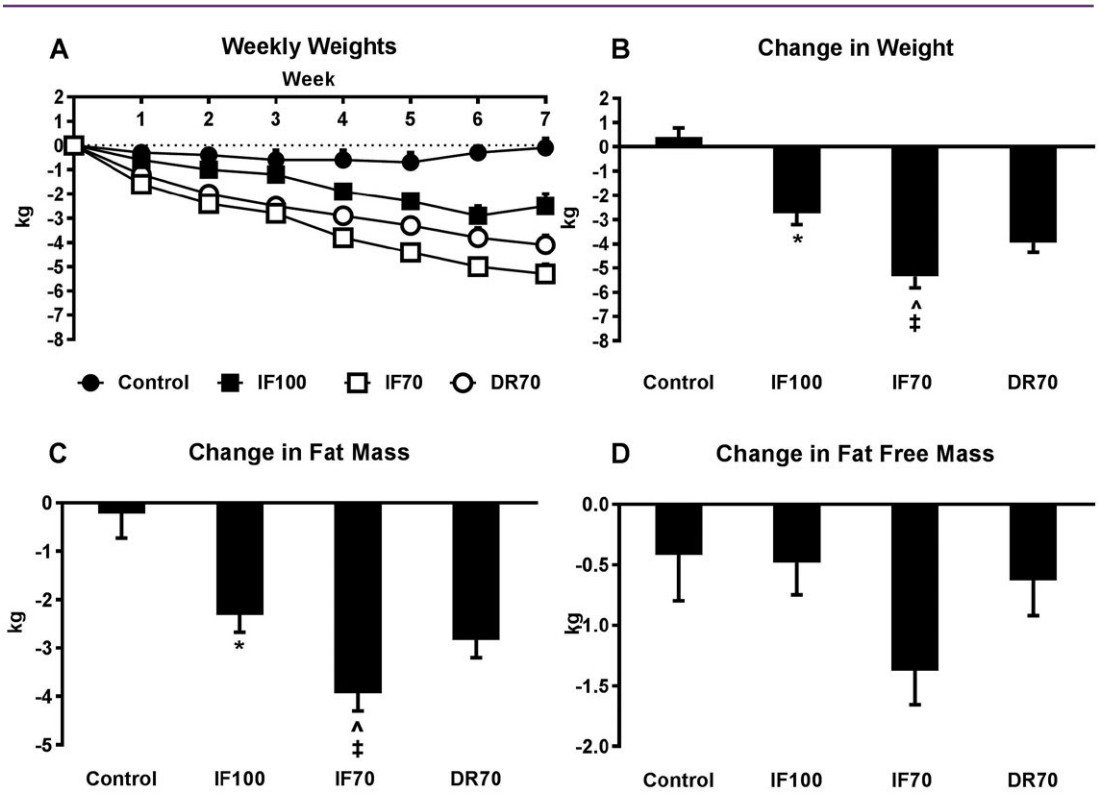

Figure 1 Changes in anthropometric outcomes following 8 weeks of intermittent or continuous intake at 70% and 100% of daily energy requirements. (A) Weekly weights; (B) change in body weight; (C) change in fat mass; (D) change in fat-free mass. Data are shown as mean ± SEM. Pairwise comparisons: *$P<0.05$ vs. control; ^$P<0.05$ vs. IF100; ‡$P<0.05$ vs. DR70. Control: continuous energy intake at 100% of baseline energy requirements; IF100: intermittent fasting diet at 100% of baseline energy requirements; IF70: intermittent fasting diet at 70% of baseline energy requirements; DR70: continuous energy restriction at 70% of baseline energy requirements.

–4.8kg] IF compared with –5.6 kg [–6.9 to –4.4 kg] DR). Although self-reported energy intakes were lower in the intermittent group, they consumed meal replacements on "fasting" days, whereas the continuous group was prescribed a conventional food-based diet daily (19). This study was repeated with conventional food-based diets prescribed to both groups for 3 months, and fat mass losses were greater in the intermittent versus continuous group (20). In contrast, Trepanowski et al. showed that weight and fat loss was not different between intermittent and continuous restriction groups at 6 months or 1 year (21). The authors noted that this study was underpowered to detect weight differences.

In the current study, IF did not preserve FFM, as has been reported previously (13,15), but resulted in significantly more weight and fat mass loss. While participants were instructed to maintain their preenrolment activity levels, we acknowledge that undisclosed changes in activity could have contributed to this outcome. Analysis of self-reported diet records showed excellent adherence in the control and DR70 groups as well as in the IF groups on fasting days, as reported food intake was not significantly different from prescribed. However, IF participants reported consuming less food than prescribed on fed days, resulting in additional energy restriction of 2% in the IF70 group and 9% in the IF100 group. A degree of spontaneous energy restriction on fed days has been reported previously (20,21) and appears to be a benefit of IF.

The mechanistic reason for this remains elusive. However, increased plasma B-HB concentrations may play a role. Physiological ketosis reduces feelings of hunger and increases feelings of fullness in humans (36) and may also mitigate the reduction in postprandial cholecystokinin and increased ghrelin concentrations that occur in response to energy restriction (37). Fasting for 24 hours also reduces ghrelin concentrations (38). However, the impacts of IF on gut peptides are controversial (11,18). In the current study, we observed no differences in perceived hunger between modes of dietary restriction, but this was recorded at a single time point only each day. Previous studies have reported that perceived hunger on a fast day (11) or averaged across eating and fasting days (20) was unchanged, while others have reported reduced hunger at the end of a fasting day (39). The effects of IF on appetite regulation deserves further investigation.

There is controversy in the existing literature over whether IF is superior to DR to improve metabolic health, with four out of five studies reporting greater improvements in markers of diabetes or cardiovascular risk (12,18-20). In this study, we observed greater reductions in total cholesterol, LDL cholesterol, and NEFA in the IF70 compared with DR70 group as well as transiently lower glucose and insulin levels after the fasting day. However, the additional weight loss in the IF70 group may underlie the greater metabolic benefits observed in this study, despite our intentions to match weight loss in these groups. To account for this, we adjusted for the change in body

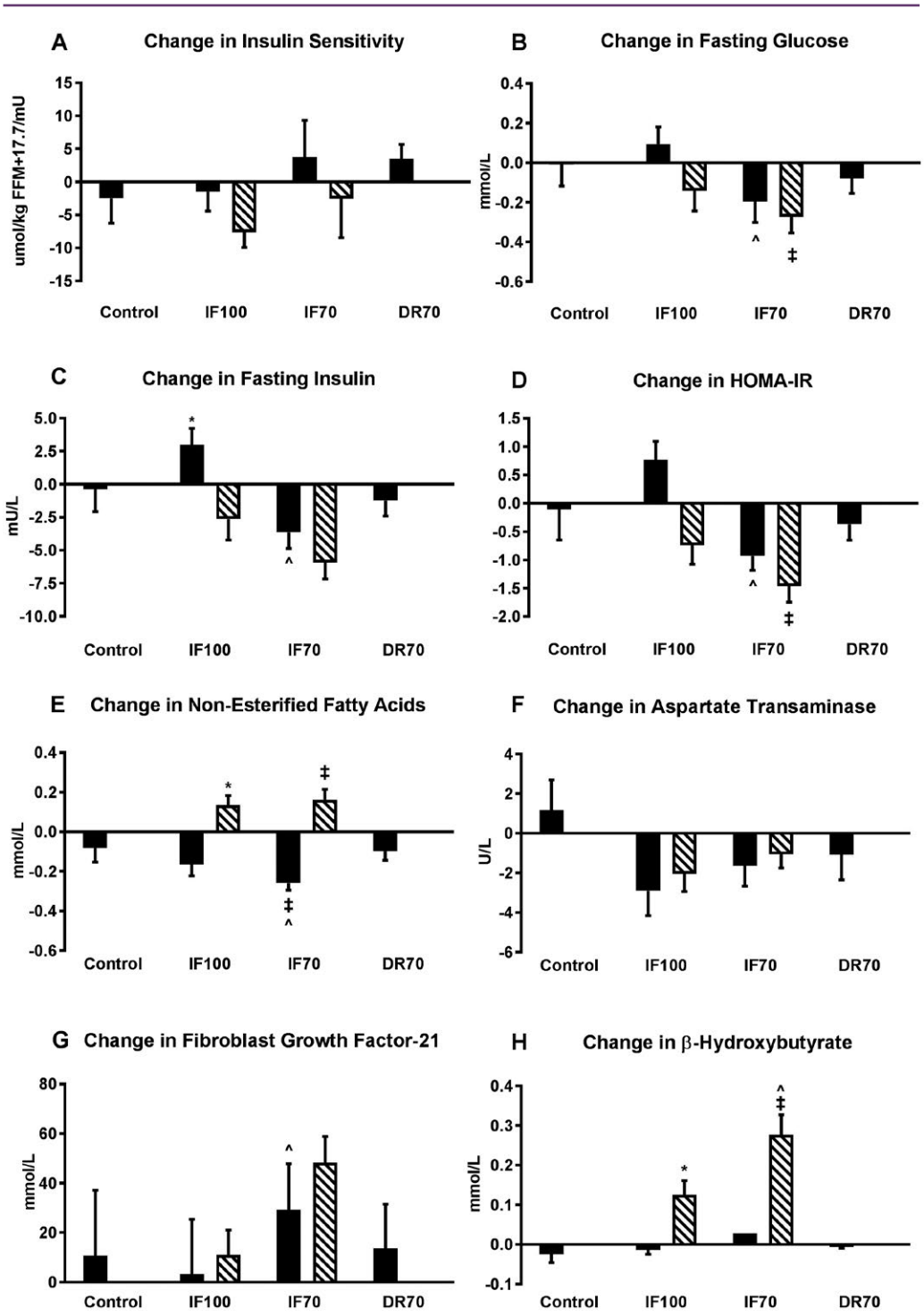

Figure 2 Changes in markers of insulin sensitivity and biochemical markers following 8 weeks of intermittent or continuous intake at 70% and 100% of daily energy requirements. (**A**) Change in insulin sensitivity as assessed by hyperinsulinemic-euglycemic clamp; completers analysis (DR70 $n=22$; IF70 $n=18$; IF100 $n=19$; C $n=10$); (**B**) change in fasting blood glucose; (**C**) change in fasting insulin; (**D**) change in HOMA-IR; (**E**) change in nonesterified fatty acids (NEFA); (**F**) change in aspartate transaminase; (**G**) change in fibroblast growth factor-21; (**H**) change in beta-hydroxybutyrate. Data are shown as mean ± SEM. Filled bars: change from baseline to fed visit; open bars: change from baseline to fasted visit. Pairwise comparisons: $^{*}P<0.05$ vs. C; $^{\wedge}P<0.05$ vs. IF100; $^{\ddagger}P<0.05$ vs. DR70. All end points (excluding insulin sensitivity) were log-transformed before analysis. Control: continuous energy intake at 100% of baseline energy requirements; IF100: intermittent fasting diet at 100% of baseline energy requirements; IF70: intermittent fasting diet at 70% of baseline energy requirements; DR70: continuous energy restriction at 70% of baseline energy requirements.

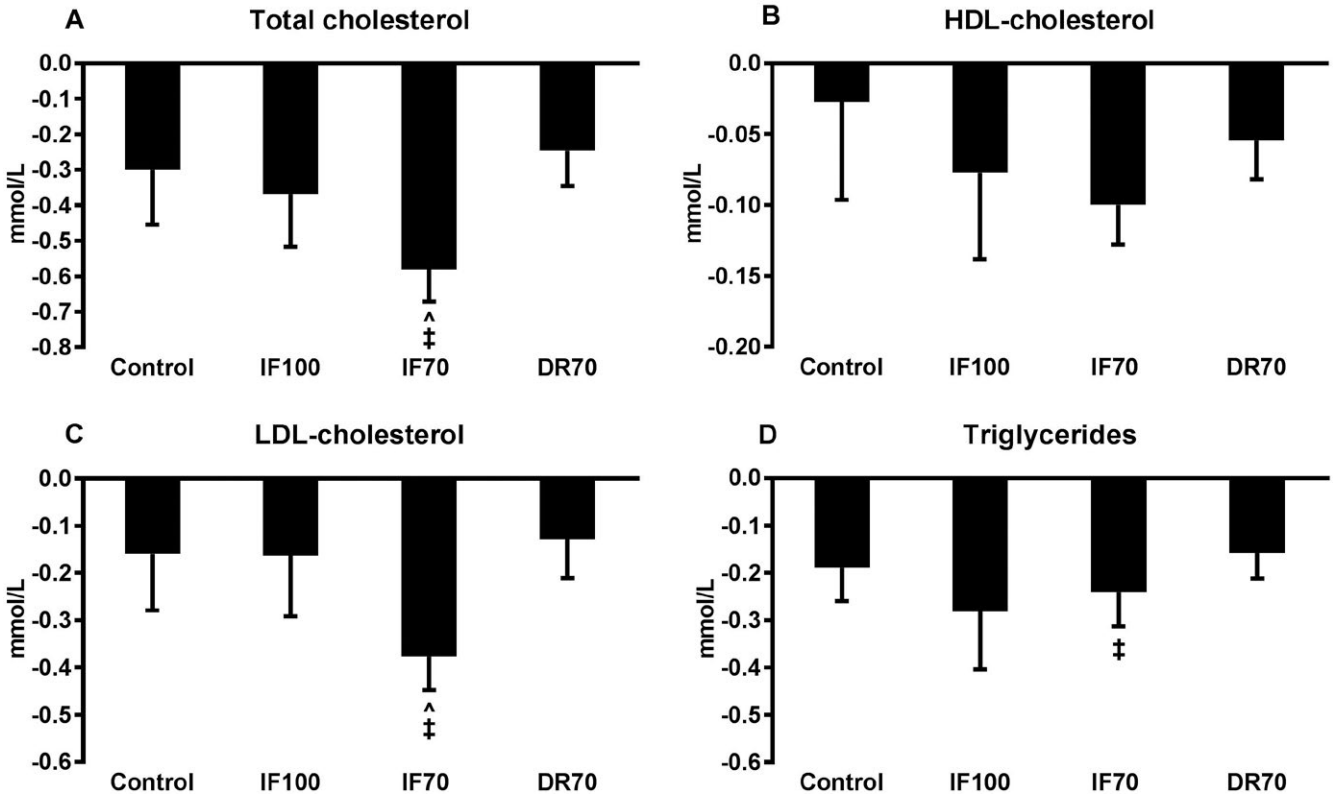

Figure 3 Changes in blood lipid markers following 8 weeks of intermittent or continuous intake at 70% and 100% of daily energy requirements. Changes in (**A**) total cholesterol, (**B**) high-density lipoprotein (HDL) cholesterol, (**C**) low-density lipoprotein (LDL) cholesterol, and (**D**) triglycerides. Data are shown as mean ± SEM. Pairwise comparisons: *significantly different from control ($P < 0.05$); ^significantly different from IF100 ($P < 0.05$); ‡significantly different from DR70 ($P < 0.05$). Control: continuous energy intake at 100% of baseline energy requirements; IF100: intermittent fasting diet at 100% of baseline energy requirements; IF70: intermittent fasting diet at 70% of baseline energy requirements; DR70: continuous energy restriction at 70% of baseline energy requirements.

weight, and observed that greater reductions in NEFA, total cholesterol, and LDL cholesterol levels in the IF70 group occurred independent of weight loss. The reduction in NEFA likely reflects greater improvements in adipose tissue insulin sensitivity and stimulation of fatty acid oxidation after IF.

To establish whether the purported health benefits of IF were attributable to weight loss, or the patterns of regular feeding and fasting as has been established in mice (9), we included the IF100 group. Women in this group were provided food at overall energy balance, which necessitated them eating at ~145% of energy balance for 4 days per week. Aside from modest weight and fat loss, there were no health benefits in the IF100 group versus control or IF70. This is contrary to observations in mice (8-10). Furthermore, transient increases in glucose and insulin were observed after a fed day, as we have noted previously in response to acute overfeeding (40). We speculate this intermittent "overfeeding" underlies the lack of overall benefits observed in this group. The long-term impacts of these transient elevations in risk markers of type 2 diabetes are unclear. However, Trepanowski et al. showed that LDL concentrations were elevated by IF after 12 months (21). While we cannot directly extrapolate our findings to free-living individuals, both studies highlight the necessity of examining the safety of IF long-term, when weight loss typically slows (41).

We also observed marked elevations in blood NEFAs and ketones and decreases in fasting insulin and blood glucose on fasting days, reflecting the switch toward activation of adipose tissue lipolysis and fatty acid oxidation. This is similar to findings by Heilbronn et al., who measured samples following 3 weeks of alternate-day fasting (after a 10-hour overnight fast) and again after a 34-hour fast (42). Few studies have examined the acute changes in metabolic parameters between fed and fasted states. Halberg et al. reported that NEFA and glycerol concentrations were increased, while glucose concentrations were decreased, when measured after a 20-hour fast. They observed no change in B-HB or insulin (22). However, samples were taken immediately before breaking a 20-hour fast (at 5 PM) and compared with samples taken after an overnight fast (8 AM). Therefore, clock differences (i.e., morning vs. evening) could have contributed to this result. Nonetheless, this metabolic switching has been postulated to result in upregulation of mitochondrial fatty acid oxidation and may underpin the benefits to metabolic health by IF (25). In support of this, we noted that the IF70 group displayed greater reductions in NEFA following the fed days compared with DR70, which was independent of the amount of weight lost.

Transient insulin resistance (assessed by clamp) was induced in response to a 24-hour fast in both IF groups. This change was at trend level when comparing a 24-hour fast in IF70 with the fed day in the DR70 group.

This may have been partly because of reduced steady-state insulin concentrations, which are indicative of increased insulin clearance (31) despite adjustment. Nonetheless, this finding contrasts data obtained by HOMA-IR (19,20) and highlights that this method is insufficient to make inferences of "insulin sensitivity" in studies of IF. It also highlights the possibility that tissue-specific changes in insulin sensitivity may occur in response to IF, as HOMA-IR generally reflects hepatic insulin sensitivity, while the clamp mainly reflects muscle insulin sensitivity. To our knowledge, only two studies have previously investigated the impacts of 2 weeks of IF on peripheral insulin sensitivity by clamp in lean men. In the study by Halberg et al., weight was unchanged, insulin sensitivity was improved, and the authors reported an increase in insulin-induced suppression of adipose tissue lipolysis (22). In contrast, Soeters et al. performed a two-step clamp to assess both hepatic and peripheral insulin sensitivity and reported no differences in either measure following 14 days of IF or a standard diet in weight-stable participants (23). In both examples, insulin sensitivity was assessed solely following the fed day. In humans, prolonged fasting (>48 hours) induces insulin resistance; this is likely a protective mechanism to spare glucose for the central nervous system (43). Impaired glucose tolerance was observed after a 36-hour fast and 3 weeks of alternate-day fasting in women (42). Reduced insulin sensitivity has also been detected by intravenous glucose tolerance test after a 24-hour fast in lean individuals (44). This was mitigated by blocking lipolysis with acipimox, suggesting it is mediated by the increase in NEFA (44). In light of this, further understanding of the overall effects of IF on NEFA, lipid metabolism, and ectopic lipid deposition is required.

This was a short-term, highly controlled intervention conducted in women. As such, this data is not translatable beyond 8 weeks or to wider populations, including men or those with established metabolic disturbances, such as type 2 diabetes. This study was powered to detect a 15-unit difference in M with $n=22$/group, and it was therefore underpowered to detect the primary outcome given that we were only able to conduct clamps in $n=17$ from the IF70 group. The randomization pattern of 2:1 for IF100:control also weakens the comparisons between these two groups.

IF was more effective than DR for reducing body weight and improving metabolic health when prescribed with a similar energy deficit, but it did not differentially impact insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp. When IF was prescribed without energy restriction, there were transient elevations in diabetes risk markers and no overall improvements in metabolic parameters compared with other groups, despite minor weight loss. This study demonstrates that IF approaches using repeated 24-hour fasts improve metabolic health when in energy deficit but not when in energy balance.O

References

1. Neter JE, Stam BE, Kok FJ, Grobbee DE, Geleijnse JM. Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension* 2003;42:878-884.
2. Look AHEAD Research Group; Pi-Sunyer X, Blackburn G, Brancati FL , et al. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the Look AHEAD trial. *Diabetes Care* 2007;30:1374-1383.
3. Wing RR, Lang W, Wadden TA, et al. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care* 2011;34:1481-1486.
4. Diabetes Prevention Program Research Group. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. *Lancet Diabetes Endocrinol* 2015;3:866-875.
5. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 1999;48:839-847.
6. Scheen AJ. The future of obesity: new drugs versus lifestyle interventions. *Expert Opin Investig Drugs* 2008;17:263-267.
7. Moreira EA, Most M, Howard J, Ravussin E. Dietary adherence to long-term controlled feeding in a calorie-restriction study in overweight men and women. *Nutr Clin Pract* 2011;26:309-315.
8. 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;21:188-195.
9. Anson RM, Guo Z, de Cabo R, 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;100:6216-6220.
10. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* 2005;16:129-137.
11. 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.
12. Antoni R, Johnston KL, Collins AL, Robertson MD. Intermittent v. continuous energy restriction: differential effects on postprandial glucose and lipid metabolism following matched weight loss in overweight/obese participants. *Br J Nutr* 2018;119:507-516.
13. 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;90:1138-1143.
14. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Varady KA. Meal timing during alternate day fasting: impact on body weight and cardiovascular disease risk in obese adults. *Obesity (Silver Spring)* 2014;22:2524-2531.
15. Varady KA, Bhutani S, Klempel MC, et al. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr J* 2013;12:146. doi:10.1186/1475-2891-12-146
16. Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Varady KA. Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. *Obesity (Silver Spring)* 2013;21:1370-1379.
17. Williams KV, Mullen ML, Kelley DE, Wing RR. The effect of short periods of caloric restriction on weight loss and glycemic control in type 2 diabetes. *Diabetes Care* 1998;21:2-8.
18. Catenacci VA, Pan Z, Ostendorf D, et al. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity. *Obesity (Silver Spring)* 2016;24:1874-1883.
19. Harvie MN, Pegington M, Mattson MP, 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 (Lond)* 2011;35:714-727.
20. Harvie M, Wright C, Pegington M, et al. The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. *Br J Nutr* 2013;110:1534-1547.
21. Trepanowski JF, Kroeger CM, Barnosky A, 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;177:930-938.
22. Halberg N, Henriksen M, Soderhamn N, et al. Effect of intermittent fasting and refeeding on insulin action in healthy men. *J Appl Physiol* 2005;99:2128-2136.
23. Soeters MR, Lammers NM, Dubbelhuis PF, et al. Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. *Am J Clin Nutr* 2009;90:1244-1251.
24. Antoni R, Johnston KL, Collins AL, Robertson MD. Effects of intermittent fasting on glucose and lipid metabolism. *Proc Nutr Soc* 2017;76:361-368.
25. Anton SD, Moehl K, Donahoo WT, et al. Flipping the metabolic switch: understanding and applying the health benefits of fasting. *Obesity (Silver Spring)* 2018;26:254-268.
26. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).* Washington, DC: National Academies Press; 2005.
27. Redman LM, Heilbronn LK, Martin CK, et al. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. *PLoS One* 2009;4:e4377. doi:10.1371/journal.pone.0004377
28. Heilbronn LK, Rood J, Janderova L, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 2004;89:1844-1848.
29. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214-E223.
30. Tam CS, Xie W, Johnson WD, Cefalu WT, Redman LM, Ravussin E. Defining insulin resistance from hyperinsulinemic-euglycemic clamps. *Diabetes Care* 2012;35:1605-1610.
31. Heijboer AC, Donga E, Voshol PJ, et al. Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. *J Lipid Res* 2005;46:582-588.
32. Soeters MR, Sauerwein HP, Dubbelhuis PF, et al. Muscle adaptation to short-term fasting in healthy lean humans. *J Clin Endocrinol Metab* 2008;93:2900-2903.
33. Rebelos E, Muscelli E, Natali A, et al. Body weight, not insulin sensitivity or secretion, may predict spontaneous weight changes in nondiabetic and prediabetic subjects: the RISC study. *Diabetes* 2011;60:1938-1945.
34. Samocha-Bonet D, Campbell LV, Mori TA, et al. Overfeeding reduces insulin sensitivity and increases oxidative stress, without altering markers of mitochondrial content and function in humans. *PLoS One* 2012;7:e36320. doi:10.1371/journal.pone.0036320
35. Albu JB, Heilbronn LK, Kelley DE, et al. Metabolic changes following a 1-year diet and exercise intervention in patients with type 2 diabetes. *Diabetes* 2010;59:627-633.

36. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med* 2005;142:403-411.
37. Sumithran P, Prendergast LA, Delbridge E, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. *Eur J Clin Nutr* 2013;67:759-764.
38. Koutkia P, Schurgin S, Berry J, et al. Reciprocal changes in endogenous ghrelin and growth hormone during fasting in healthy women. *Am J Physiol Endocrinol Metab* 2005;289:E814-E822.
39. Klempel MC, Bhutani S, Fitzgibbon M, Freels S, Varady KA. Dietary and physical activity adaptations to alternate day modified fasting: implications for optimal weight loss. *Nutr J* 2010;9:35.
40. Samocha-Bonet D, Campbell LV, Viardot A, et al. A family history of type 2 diabetes increases risk factors associated with overfeeding. *Diabetologia* 2010;53:1700-1708.
41. Ravussin E, Redman LM, Rochon J, et al. A 2-year randomized controlled trial of human caloric restriction: feasibility and effects on predictors of health span and longevity. *J Gerontol A Biol Sci Med Sci* 2015;70:1097-1104.
42. Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. *Obes Res* 2005;13:574-581.
43. Hoeks J, van Herpen NA, Mensink M, et al. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes* 2010;59:2117-2125.
44. Salgin B, Marcovecchio ML, Humphreys SM, et al. Effects of prolonged fasting and sustained lipolysis on insulin secretion and insulin sensitivity in normal subjects. *Am J Physiol Endocrinol Metab* 2009;296:E454-E461.