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Abstract

Evidence suggests that prolonged water-only fasting is safe and may improve cardiometabolic biomarkers in normal-weight males, but data in normal-weight females are lacking. Given the physiological differences between males and females, research is needed in normal-weight females to assess safety and effectiveness. This article presents preliminary, observational data on adverse events as well as the immediate and sustained effects of water-only fasting followed by whole-plant-food refeeding on body composition and select biomarkers in seven normal-weight females recruited from a residential fasting center. Median fasting, refeeding, and follow-up lengths were 10, 5, and 44 days, respectively, during which there were no severe or serious adverse events. There were also slight changes in some cardiometabolic biomarkers that were sustained after a prolonged follow-up period. Despite substantial limitations, the data support additional research with larger samples in this population.

Keywords: prolonged fasting, water-only fasting, cardiometabolic health, insulin resistance, HOMA-IR, females, lean, normal-weight, fatty liver index, whole-plant-food diet

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Conflict of interest
A.C.G. is the owner of the TrueNorth Health Center and President of the Board of Directors of the TrueNorth Health Foundation. All other authors declare no conflict of interest.

Introduction

Prolonged water-only fasting may help to restore cardiometabolic health in people with dysfunctional metabolism.[1-3] There is also limited evidence in normal-weight males suggesting that prolonged water-only fasting is tolerable, does not appear to be harmful, and may slightly improve some biomarkers of cardiometabolic health risk,[4-6] but similar research in normal-weight females is lacking. Here, we present preliminary, observational data on adverse events as well as changes in body composition and select cardiometabolic biomarkers after medically supervised prolonged water-only fasting and refeeding in seven normal-weight females.

Materials and methods

Ethical statement

This study was approved by the TrueNorth Health Foundation Institutional Review Board in Santa Rosa, California (TNHF-2020-2VAT; April 2, 2020) (1) and registered at clinicaltrials.gov (NCT04514146). Informed consent was obtained from all participants prior to participation.

Participants

We enrolled consecutive volunteer patients who had elected to undergo a medically supervised water-only fast at a residential medically supervised fasting center prior to eligibility screening. Inclusion criteria included adults ≤70 years old with a body mass index (BMI) of 18.5–24 kg/m^2, fasting glucose <5.6 mmol/L or hemoglobin A1C <5.7%, systolic/diastolic blood pressure (SBP/DBP) <120/80 mmHg, and total cholesterol (TC) <5.2 mmol/L, and who were approved by a non-research clinician to water-only fast for at least 5 days. Exclusion criteria included past or present diagnosis of the following: diabetes, malignancy, stroke, hypertension, atherosclerosis, heart failure, cardiac arrhythmia, myocardial infarction, embolism, kidney disease, hyperlipidemia, active inflammatory disorder including classic autoimmune connective tissue disorders, multiple sclerosis, inflammatory bowel disorders, dementia, or other cognitive impairment, substance abuse, or abdominal implant. Participation continued until data collection was completed approximately 6 weeks after participants left the facility.

Study design

Participants attended study visits at baseline (BL), end of fast (EOF), end of refeed (EOR), and 6 weeks after departing the facility (FU) (Figure 1). Study data were collected and managed using REDCap electronic data capture tools.[7,8] Four of the seven participants elected to complete the FU visit remotely. These individuals were provided with the necessary equipment and training to collect the clinical measurements at home and had blood drawn at their local LabCorp. At each visit, 18 ml of blood was collected and clinical measurements, including SBP and DBP, height, body weight (BW), and abdominal circumference (AC), were taken. Blood analysis included fasting blood glucose, insulin, high sensitivity C-reactive protein (hsCRP), TC, low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, and very-low-density-lipoprotein (VLDL) cholesterol, triglycerides (TG), and gamma-glutamyl-transferase (GGT). Demographic information, including age, sex, ethnicity, self-reported diet type, and pre-treatment ICD-10 diagnostic codes, was collected at BL and FU. Dual-energy x-ray absorptiometry (DXA) scans were performed at BL, EOF, and EOR visits. Participants also answered an online dietary screener survey at BL and FU.

Medically supervised water-only fasting and refeeding protocol

The medically supervised water-only fasting and refeeding protocol was implemented at a residential medically supervised fasting center.[9] Briefly, potential participants were screened before arrival and if conditionally approved to water-only fast were instructed to eat a diet consisting of raw or steamed vegetables and fruits for two days prior to arrival. Throughout the fasting period, participants were instructed to remain onsite, consume a minimum of 1.2 liters of distilled water per day, and minimize physical activity. Refeeding length was half of the length of the fast and began with a mixture of fruit and vegetable juices. This was followed by the structured introduction of raw fruits and vegetables, steamed fruits and vegetables, intact grains, and then legumes until participants consumed a diet of exclusively whole-plant foods free of added salt, oil, or sugar (SOS-free diet). Participants were encouraged to continue eating this diet after leaving the center. While fasting and refeeding, participants received 24-hour medical supervision, vital signs and symptoms were monitored twice daily, and required serology was assessed weekly during fasting.

Clinical and laboratory measurements

Clinical and laboratory measurement methods were performed as previously described.[1] Briefly, height (cm) was measured using a Doran Scales Inc. wall-mounted stadiometer (DS5100, Doran Scales Inc., St. Charles, IL, USA). Body weight (kg) was measured using a digital body weight scale (BWB 800A Class III, Tanita Corporation of American Inc., Arlington Heights, IL, USA) while onsite, and with a Conair digital glass scale (WW26 model) for remote FU visits. Body mass index was calculated using the formula weight (kg) ÷ height (m^2).[10] Abdominal circumference (cm) was measured using a tension-sensitive, non-elastic tape (Gullick II, Model 67019, Country Technology Inc., Gay Mills, WI, USA) while onsite, and with a retractable cloth measuring tape for the remote FU visits. Blood pressure was measured on site using a digital BP device (Welch Allyn-Connex ProBP 3400, Hill-Rom Holding Inc. Chicago, IL, USA), and at remote visits using a digital blood pressure device with adjustable cuff size (BP3GX1, Microlife USA Inc, Clearwater, FL, USA). All laboratory measures were conducted using commercially available tests at LabCorp. Homeostatic model assessment of insulin resistance (HOMA-IR) values were calculated as follows: fasting insulin (μU/L) x fasting glucose (nmol/L)/22.5. Fatty liver index (FLI) scores were calculated from BMI, AC, GGT, and TGs using the following formula:

$$FLI = \frac{(e0.953 * logeTG + 0.139 * BMI + 0.718 * logeGGT + 0.053 * AC - 15.745)}{1 + e0.953 * logeTG + 0.139 + BMI + 0.718 * logeGGT + 0.053 * AC - 15.745} * 10$$

Dual-energy x-ray absorptiometry (DXA) scans

Whole-body DXA scans (Horizon Wi (S/N 303354M), Hologic, Marlborough, MA, USA) were performed by certified technicians. Interassay coefficients of variation of the machine are 7.1%, 7.2%, and 2.83% for VAT mass, volume,[11] and area,[12] respectively, and 0.50% and 0.98% for total lean mass and fat mass, respectively.[12]

SOS-free dietary screener

A previously described dietary screener[1] was used to assess adherence to the SOS-free diet over the previous 30 days. The scoring key follows a proposed standardized methodology for measuring dietary adherence[13] with a scale from 0 to 82, where a score of

Author Contributions

Conceptualization: T.R.M & A.C.G.; Methodology, T.R.M & A.C.G.; Formal Analysis, M.N. & N.T.; Investigation, S.G., E.Z., & N.T.; Resources: T.R.M, S.G., & E.Z; Data Curation: N.T. & M.N.; Writing – Original Draft Preparation: N.T. & T.R.M.; Writing – Review & Editing: T.R.M, S.G., M.N. E.Z., N.T., & A.C.G.; Visualization: N.T. & T.R.M; Supervision: T.R.M.; Project Administration: T.R.M., S.G, & E.Z.; Funding Acquisition: A.C.G.

0 denotes fully adherent and 82 denotes fully non-adherent. The web-based screening was administered using REDCap at BL and FU visits.

Statistical analysis

Descriptive data analysis was performed in Excel. Variables were initially visually represented using box and whisker plots to summarize the distribution and identify outliers, then summarized using median and interquartile ranges, and mean and standard deviation. After data collection was complete, a power analysis was conducted for a 7-patient sample size at the 0.05 alpha level using effect sizes of independent interest.[14] A repeated measures analysis of variance power analysis was conducted using the wp.rmanova function from the WebPower R package[15] with the arguments set to 1 group, 4 measurements, a within-group effect, and effect sizes defined by Cohen's classifications of effect size magnitude[16]: small (f = 0.10), medium (f = 0.25), and large (f = 0.40). The resulting statistical power was 5.3%, 7.2%, and 10.9% for the small, medium, and large effect size, respectively. A two-sided paired t-test power analysis was conducted using the pwr.t.test function from the pwr R package[17] with effect sizes defined by Cohen's classifications of effect size magnitude[18]: small (d = 0.2), medium (d = 0.5), and large (d = 0.8). The estimated statistical power was 7.4%, 20.1%, and 42.8% for a small, medium, and large effect size, respectively. A study with low statistical power has a higher risk for Type II errors and a lower ability to detect treatment effects.[19] Moreover, when a statistically significant treatment effect is detected in a study with low statistical power, there is a reduced likelihood that the result reflects a true effect and an increased likelihood that the magnitude of the effect size is inflated.[20] Given the small sample size and low statistical power, this study focused on using descriptive statistics and visualizations for in depth data description rather than statistical inference.[21]

Results

We enrolled seven female participants with a baseline median (IQR) age of 44 (36, 55) years, BMI of 21.4 (20.3, 22.3) kg/m^2, fasting glucose of 4.78 (4.64, 4.86) mmol/L, SBP of 110 (101, 112) mmHg, DBP of 73 (67, 75) mmHg, and TC of 4.42 (4.03, 5.08) mmol/L (Table 1). Median (range) fast, refeed, and follow-up lengths were 10 (5, 14), 5 (4, 5), and 44 (37, 48) days, respectively. Retention through FU was 100%, with one participant refusing all DXA scans and one missing AC measurement at FU. There were two unanticipated adverse events reported during fasting. One was a worsening of preexisting Duputren's contracture and the other was an onset of parotitis. The events resolved upon refeeding without medical treatment or change in treatment plan. Median (IQR) SOS-free diet adherence scores were 4.00 (3.08, 8.69) and 5.14 (3.37, 6.34) at BL and FU, respectively. This suggests that participants were highly adherent to an SOS-free diet before and after the intervention.

Median (IQR) BW dropped from 56.3 (52.9, 62.5) kg at BL to 49.7 (47.0, 56.3) kg, 53.4 (49.3, 58.0) kg, and 52.6 (51.0, 58.2) kg at EOF, EOR, and FU, respectively (Table 1). Two participants were temporarily underweight (BMI < 18.5 kg/m^2) at EOF and EOR but normalized at FU. Although there was an increase in median AC between EOF and EOR, median (IQR) AC dropped from 73.7 (66.0, 75.2) cm at BL to 65.8 (64.8, 69.4) cm at FU. The largest drop in median (IQR) SBP and DBP was between BL and EOR, but SBP and DBP returned to near BL values at FU, indicating that long-term BP homeostasis was maintained in this normotensive population.

Table 1: Cardiometabolic markers after fasting, refeeding, and 6-week follow-up

	Median (IQR)			
	BL	EOF	EOR	FU
BW, kg	56.3 (52.9, 62.5)	49.7 (47.0, 56.3)	53.4 (49.3, 58.0)	52.6 (51.0, 58.2)
BMI, kg/m^2 (18.5–24.9 kg/m^2)	21.4 (20.3, 22.3)	19.3 (18.3, 19.9)	20.1 (19.0 20.5)	20.1 (19.3, 20.9)
AC, cm (< 88 cm for females)	73.7 (66.0, 75.2)	66.1 (61.5, 68.6)	73.5 (65.5, 74.3)	65.8 (64.8, 69.4)
SBP, mmHG (<120 mmHg)	110 (101, 112)	103 (103, 109)	99 (94, 104)	106 (99, 110)
DBP, mmHG (<80 mmHg)	73 (67, 75)	73 (72, 77)	65 (64, 72)	70 (65, 73)
TC, mmol/L (2.59– 5.15 mmol/L)	4.42 (4.03, 5.08)	5.74 (5.50, 6.80)	4.63 (3.85, 5.18)	4.29 (3.90, 4.46)
HDL, mmol/L (≥1.01 mmol/L)	1.63 (1.38, 1.75)	1.47 (1.28, 1.62)	1.42 (1.18, 1.45)	1.55 (1.25, 1.80)
LDL, mmol/L (< 2.56 mmol/L)	2.35 (2.19, 3.00)	3.72 (3.58, 4.47)	2.43 (2.13, 3.04)	2.33 (2.16, 2.48)
VLDL, mmol/L (<0.78 mmol/L)	0.34 (0.32, 0.48)	0.57 (0.41, 0.63)	0.59 (0.56, 0.85)	0.34 (0.26, 0.50)
TG, mmol/L (<3.86 mmol/L)	0.80 (0.74, 1.18)	1.37 (1.05, 1.56)	1.46 (1.36, 2.20)	0.80 (0.53, 1.18)
Glucose, mmol/L (3.61- 5.49 mmol/L)	4.78 (4.64, 4.86)	3.78 (3.47, 3.86)	5.00 (4.75, 5.14)	4.61 (4.53, 4.78)
Insulin, pmol/L (15.6- 149.4 pmol/L)	31.2 (23.7, 32.4)	7.2 (5.4, 9)	25.8 (21.9, 30.9)	22.2 (15.9, 26.4)
HOMA -IR (<1.9 insulin sensitive)	1.08 (0.83, 1.13)	0.20 (0.13, 0.23)	0.92 (0.73, 1.25)	0.83 (0.51, 0.92)
GGT, nmol/(s*L) (<1000 nmol/(s*L))	283.4 (191.7, 325.1)	250.1 (175.0, 291.7)	216.7 (166.7, 300.0)	216.7 (158.4, 258.4)
FLI (< 30 optimal)	4.71 (4.0, 7.84)	4.21 (3.88, 4.72)	5.71 (3.79, 11.20)	3.0 (2.25, 6.54)
hsCRP, nmol/L (< 9.5 nmol/L)	4.7 (4.4, 6.3)	8.4 (6.8, 40.3)	4.2 (3.7, 9.3)	6.0 (3.0, 6.7)

Notes: Reference intervals are below the respective variable. *N* = 7 at BL, EOF, EOR, and FU for all variables except AC and FLI at FU, which are missing 1 value each (*N* = 6).; IQR, interquartile range; BL, baseline visit; EOF, end of fast visit; EOR, end of refeed visit; FU, six-week follow-up visit; BW, body weight; AC, abdominal circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; TG, triglycerides; HOMA-IR, homeostatic model assessment for insulin resistance; GGT, gamma-glutamyl transferase; FLI, fatty liver index; hsCRP, high-sensitivity C-reactive protein; kg, kilogram; m, meter; cm, centimeter; mmHg, millimeters of mercury; mmol, millimole; L, liter; pmol, picomole; nmol, nanomole; s, second; mg, milligram.

Dual-energy x-ray absorptiometry scans were used to further assess the effect of water-only fasting and refeeding on body composition (Table 2). From BL to EOR, mean values decreased by -6.9% for total mass (5.75kg vs 5.36 kg), -6.3% for total lean mass (3.85 kg vs 3.61 kg), and -9.0% for total fat mass (1.72 kg vs 1.56 kg) (Figure 1). From EOF to EOR, mean total fat mass decreased an additional -0.52 kg, which accounted for 33% of the total fat mass lost (Figure 1C, D). Conversely, during the same period, mean total lean mass recovered by 2.18 kg and the percentage of lean mass at EOR was the same as at BL (Figure 1A, B). Estimated VAT mass, volume, and area each decreased by 12% from BL to EOR (Figure 1E, F). Substantial changes were not observed in android/gynoid (A/G) ratio or bone mineral content.

Table 2: Body composition after fasting and refeeding

	Mean (SD)		
	BL	EOF	EOR
Total Mass, g	57580.1 (6459.5)	51963.0 (6112.9)	53616.2 (6070.8)
Total Lean Mass, g	38501.0 (4598.4)	33915.1 (4243.3)	36093.4 (4065.8)
% Lean Mass	66.8 (2.4)	65.3 (3.4)	67.4 (3.4)
A/G Ratio	0.74 (0.14)	0.72 (0.10)	0.67 (0.14)
Total Fat Mass, g	17186.3 (2233.9)	16149.5 (2607.8)	15631.1 (2718.0)
% Total Fat	29.9 (2.1)	31.1 (3.1)	29.1 (3.1)
BMC, g	1892.8 (301.6)	1898.3 (304.1)	1891.8 (309.1)
Est. VAT Mass, g	283 (87)	244 (82)	248 (76)
Est. VAT Volume, cm^3	306 (94)	264 (88)	269 (82)
Est. VAT Area, cm^2	58.7 (18.0)	50.7 (16.9)	51.5 (15.7)
LMI kg/m^2	14.0 (1.2)	12.3 (1.2)	13.1 (1.2)
Bone Density g/cm^2	1.011 (0.107)	1.019 (0.104)	1.028 (0.113)
Append. LMI kg/m^2	5.70 (0.71)	5.02 (0.66)	5.34 (0.80)

Notes: N= 6 at BL, EOF, and EOR; SD, standard deviation; BL, baseline visit; EOF, end of fast visit; EOR, end of refeed visit; g, grams; A/G, android/gynoid; BMC, bone mineral content; Est., estimated; VAT, visceral adipose tissue; cm, centimeter; kg, kilogram; m, meter; LMI, lean mass index; Append, appendicular.

Figure 1: Boxplots of DXA analysis for (A) total lean mass (g), (B) percent lean mass, (C) total fat mass (g), (D) percent fat mass, (E) estimated VAT mass (g), and (F) estimated VAT volume (cm^3) at BL, EOF, and EOR. Boxplots include the minimum value, first (lower) and third (upper) quartiles, the median, and the maximum value. Dots represent individual participants.

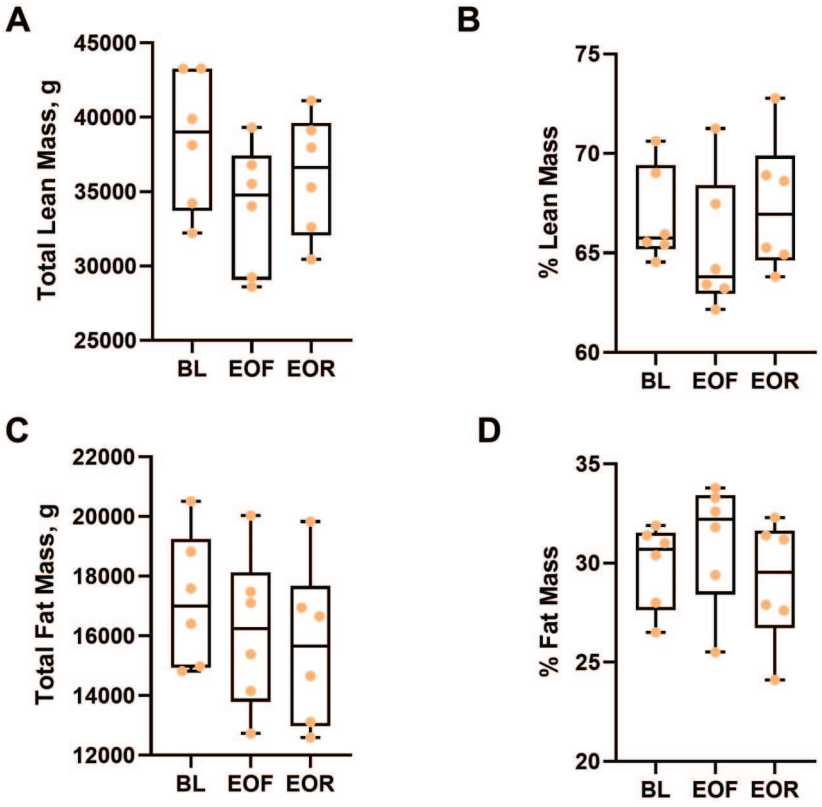
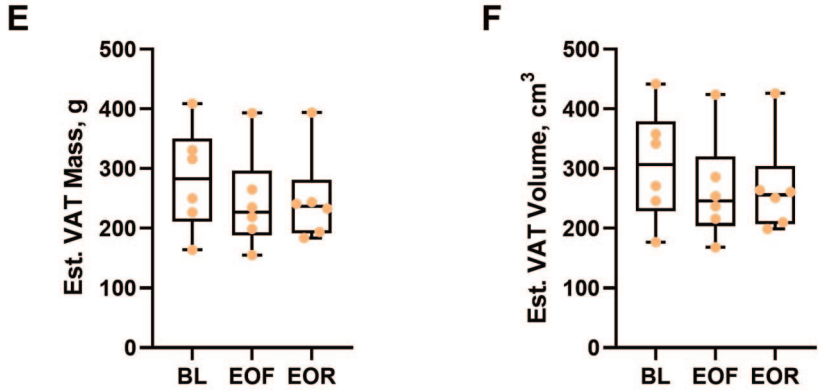


Figure 1 (continued)

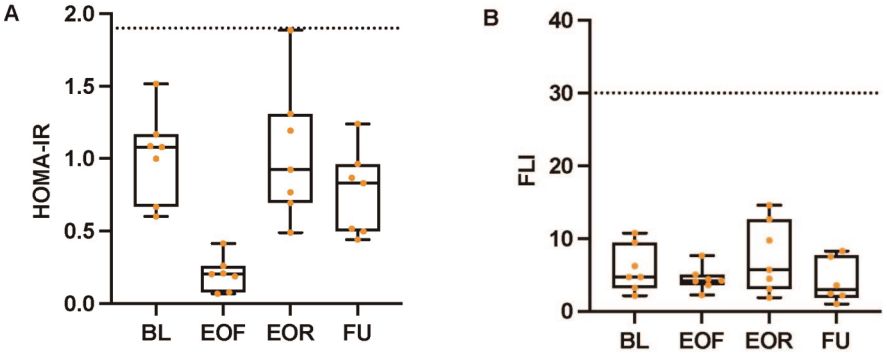


Notes: DXA, dual-energy x-ray absorptiometry; VAT, visceral adipose tissue; BL, baseline visit; EOF, end-of-fast visit; EOR, end-of-refeed visit; g, gram; cm, centimeter.

Median (IQR) TC and LDL increased from 4.42 (4.03, 5.08) mmol/L and 2.35 (2.19, 3.00) mmol/L at BL to 5.74 (5.50, 6.80) mmol/L and 3.72 (3.58, 4.47) mmol/L at EOF, respectively (Table 1). Both median TC and LDL, which increased outside of reference range at EOF, normalized to BL by EOR. There was also a slight increase in median (IQR) VLDL and TG from 0.34 (0.32, 0.48) mmol/L and 0.80 (0.74, 1.18) mmol/L at BL to 0.57 (0.41, 0.63) mmol/L and 1.37 (1.05, 1.56) mmol/l at EOF, respectively. The values remained elevated at EOR but decreased to BL values at FU. Median (IQR) HDL decreased slightly from 1.63 (1.38, 1.75) mmol/L at BL to 1.47 (1.28, 1.62) mmol/L at EOF, 1.42 (1.18, 1.45) mmol/L at EOR, and 1.55 (1.25, 1.80) mmol/L at FU (Table 1). However, the HDL:TC ratio remained unchanged between BL (0.37) and FU (0.36).

Median insulin remained below BL at each time point, while median glucose, which decreased to below BL at EOF, increased above BL at EOR and returned to BL at FU (Table 1). Median (IQR) HOMA-IR decreased from 1.01 (0.80, 1.09) at BL to 0.20 (0.13, 0.23), 0.92 (0.73, 1.25), 0.83 (0.51, 0.92) at EOF, EOR, and FU, respectively (Table 1 and Figure 2A). Median GGT decreased from BL at each time point. Median (IQR) FLI was essentially unchanged from 4.71 (4.0, 7.84) at BL to 4.21 (3.88, 4.72) at EOF but increased to 5.71 (3.79, 11.20) at EOR and then reduced to 3.0 (2.25, 6.54) at FU (Table 1 and Figure 2B). Median (IQR) hsCRP increased from 4.7 (4.4, 6.3) nmol/L at BL to 8.4 (6.8, 40.3) nmol/L at EOF (Table 1). It then decreased to 4.2 (3.7, 9.3) nmol/L at EOR, and increased to 6.0 (3.0, 6.7) nmol/L at FU.

Figure 2: Boxplots of HOMA-IR (A) and FLI (B) at BL, EOF, EOR, and FU. HOMA-IR values < 1.9 represent insulin sensitivity[36] and FLI values < 30 rule out hepatic steatosis.[33] Boxplots include the minimum value, first (lower) and third (upper) quartiles, the median, and the maximum value. Dots represent individual participants.



Notes: HOMA-IR, homeostatic model assessment for insulin resistance; FLI, fatty liver index; BL, baseline; EOF, end-of-fast visit; EOR, end-of-refeed visit; FU, six-week follow-up visit.

Discussion

Periodic (e.g., annual) prolonged water-only fasting may prevent the onset of cardiometabolic dysfunction in healthy people. Here, we present preliminary, observational data in seven normal-weight females as a step toward conducting the large-scale, longitudinal studies necessary to test this assertion. The participants in this study completed their initially proposed fast lengths without interruption, and retention through the FU visit was 100%. There were no severe or serious adverse events during the fasting or refeeding periods. Although two participants (28%) experienced adverse events while fasting, the events resolved upon refeeding and did not require medical treatment. Electrolyte analysis was not completed during refeeding, but there were also no clinical symptoms associated with refeeding syndrome.[22] Overall, this suggests that prolonged water-only fasting is feasible and well tolerated in this population.

To our knowledge, this is the first study utilizing DXA to observe changes in body composition after fasting and refeeding in normal-weight females. Notably, during refeeding, total lean mass increased while total fat mass continued to decrease so that by the end of refeeding, participants had a slightly higher percentage of total lean mass compared with baseline. This is meaningful because lean mass loss during fasting is a speculative concern. Dual-energy x-ray absorptiometry lean mass estimates are affected by hydration status,[23] which fasting can affect through at least two mechanisms involving sodium diuresis in early fasting[24] and glycogen loss from skeletal muscle.[25] Therefore, lean mass measurements after refeeding may provide a better estimate of actual lean mass loss since glycogen and hydration repletion should have occurred by then. Furthermore, it is reported that VAT mass above 700-800 g indicates a high risk of developing cardiometabolic disease,[26] but the lower healthy limit of VAT mass has not been established. Participants in this study began fasting with normal insulin sensitivity and a median VAT mass of 283 g, which reduced by 12% at the EOR visit. The clinical meaningfulness of this loss is unclear.

Homeostatic model assessment of insulin resistance is a proxy for estimating insulin resistance[27] It is well documented that HOMA-IR increases immediately after fasting in multiple animal and human studies,[28-30] which occurs at least in part due to delayed insulin release and reduced whole-body insulin sensitivity.[31] Because abnormal glucose tolerance is transient and observed across multiple species, it may be a physiological adaptation to fasting and refeeding that confers a survival advantage[32] rather than a deleterious effect of fasting. In this population, there was an uncharacteristic decrease in median HOMA-IR at all time-points. However, 4 of the 7 participants had increased HOMA-IR values after refeeding, which corrected to below baseline values at the FU visit, similar to overweight/obese males and females.[1] Baseline factors of these 4 participants included higher mean age (53 vs 34 years), BMI (21.9 vs 20.3 kg/m^2), AC (72.9 vs 68.5 cm), VAT mass (320.8 vs 207.0 g), and FLI score (7.4 vs 3.9), as well as lower mean HOMA-IR (0.9 vs 1.1) and lower reported levels of dietary adherence (7.93 vs 4.96). It is likely that all seven participants experienced an increase in HOMA-IR shortly after refeeding, but the three participants who had a lower HOMA-IR at the EOR visit experienced a reversal within the median refeed length of 5 days, which potentially indicates superior metabolic flexibility in shifting from fasting to feeding physiology. This could be determined by calculating HOMA-IR daily during refeeding in future studies. Nonetheless, a 29% decrease in median HOMA-IR at the FU visit was observed, suggesting an overall sustained improvement in insulin sensitivity in this normal-weight population.

Fatty liver index is a proxy for estimating fatty liver disease, including metabolic-induced fatty liver disease.[33] The slight increase in median FLI at the EOR visit was due to increased median TG. Nevertheless, there was a 36% reduction in median FLI at the FU visit. The clinical meaningfulness of these changes are unknown because the cutoff to rule out hepatic steatosis is < 30, but they may indicate a sustained loss of liver fat and/or improved metabolic health in these participants. It is interesting to note that dietary adherence scores were similar between the BL and FU visits, indicating the observed changes may be attributable to fasting alone rather than dietary compliance to an exclusively whole-plant-food diet.

As observed in normal-weight males,[5,34,35] median TC, LDL, VLDL, and TG levels increased after fasting by 30%, 58%, 67%, and 72%, respectively, in normal-weight females. Of these, only median TC and LDL increased above reference range during fasting, and all lipids returned to baseline levels at the FU visit, suggesting that these changes are transient physiological adaptations to fasting due to increased lipolysis. Similar to previous reports,[1,2] we also observed a transient increase in median hsCRP during fasting that decreased during refeeding. There was also a slight increase at the FU visit, which may be due to an uncharacteristically high reading in one participant who had an invasive dental procedure the day prior to testing. There is no evidence that these transient changes are detrimental in the short term or would contribute to adverse health overtime**,** and they may actually be beneficial hormetic effects.

Although these data are encouraging, this study has several limitations including that the observational, single-arm design is unable to establish causality. Other major limitations include that the study enrolled a small number of health-conscious, normal-weight females and did not include any control groups. These limitations potentially bias the results such that they are not representative of the normal-weight female population or generalizable to females with other body types or health conditions. The small sample size also precludes statistical analysis with sufficient statistical power to produce inferences that are reliable and reproducible. Furthermore, changes in biomarkers, especially VAT mass and FLI, that are already within normal range before fasting may not be clinically meaningful or represent decreased disease risk. Given this limitation, the best way to assess any long-term effects of prolonged fasting on overall health is with longitudinal analysis over multiple years. Another limitation is that participants' diets before and after the intervention were assessed using a dietary screener in which food intake was self-reported and may be unreliable. Additionally, the study did not measure electrolytes during refeeding, which would provide a better overall idea of the acute effects of fasting. Future studies should enroll a larger number of participants from the general population, include dietary, body type, and other study-specific control groups, include additional refeeding analysis, and assess the effects of periodic, prolonged water-only fasting over several years.

Conclusion

Overall, water-only fasting did not cause any severe or serious adverse effects and resulted in sustained reductions in BW, AC, VAT mass, HOMA-IR, and FLI. Although additional research is necessary, these data indicate that the intervention is not harmful and, given the potential benefits, support further investigation into safety and effectiveness in this population.

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