



Theses and Dissertations

2021-03-30

Weekly One-Day Water-Only Fasting Interventional Trial for Low-Density Lipoprotein Cholesterol Reduction (WONDERFUL)

Ciera Lynn Bartholomew
Brigham Young University

Follow this and additional works at: <https://scholarsarchive.byu.edu/etd>



Part of the [Life Sciences Commons](#)

BYU ScholarsArchive Citation

Bartholomew, Ciera Lynn, "Weekly One-Day Water-Only Fasting Interventional Trial for Low-Density Lipoprotein Cholesterol Reduction (WONDERFUL)" (2021). *Theses and Dissertations*. 8880.
<https://scholarsarchive.byu.edu/etd/8880>

This Thesis is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact ellen_amatangelo@byu.edu.

Weekly One-Day Water-Only Fasting Interventional Trial for Low-Density
Lipoprotein Cholesterol Reduction (WONDERFUL)

Ciera Lynn Bartholomew

A thesis prospectus submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Bruce Bailey, Chair
Benjamin Horne
Lance Davidson
Larry Tucker

Department of Exercise Sciences

Brigham Young University

Copyright © 2021 Ciera Lynn Bartholomew

All Rights Reserved

ABSTRACT

Weekly One-Day Water-Only Fasting Interventional Trial for Low-Density Lipoprotein Cholesterol Reduction (WONDERFUL)

Ciera Lynn Bartholomew
Department of Exercise Sciences, BYU
Master of Science

Purpose: Fasting has been promoted as a method of preventing disease and aging for thousands of years. With heart disease being a leading cause of death in the U.S., researchers have explored the effects of fasting on variables that influence cardiovascular disease (CVD), like LDL cholesterol. Therefore, the purpose of this study was to assess the effects of weekly water-only fasting on LDL cholesterol (LDL-C) in men and women with metabolic risk factors for CVD.

Methods: This study was a randomized control trial in adult men and women. Participants were randomized to fasting (treatment) or normal diet (control). The fasting protocol consisted of four weeks of two 24-hour water-only fasts, followed by 22 weeks of once-weekly water-only 24-hour fasts. Measurements such as height, weight, waist circumference and LDL-C were assessed at baseline, 4 weeks, 13 weeks, and 26 weeks.

Results: Intermittent fasting (n = 50) and control (n = 53) participants were 49.3 ± 12.0 and 47.0 ± 9.8 years, respectively, predominantly females (66.0% and 67.9%), overweight (103 ± 24 and 100 ± 21 kg), and with mild LDL-C elevation (124 ± 19 and 128 ± 20 mg/dL). Change in weight was -1.70 ± 4.69 (kg) in the fasting group and 0.20 ± 3.45 (kg) in the control group and not different between conditions ($p = 0.06$). There was no condition-by-period interaction for LDL-C ($p = 0.06$). Similarly, the change in LDL-C from baseline to follow-up was not different between conditions ($t = -0.538$, $p = 0.59$; Cohens D = 0.12)

Conclusions: A once-per-week intermittent fasting regimen did not reduce weight or LDL-C. Further research of such fasting regimens is needed to evaluate their potential impact on cardiometabolic health.

Keywords: intermittent fasting, LDL cholesterol, coronary heart disease, fasting

Table of Contents

Title Page	i
Abstract	ii
Table of Contents	iii
List of Tables	iv
List of Figures	v
Introduction.....	1
Methods.....	3
Design	3
Participants.....	3
Procedures.....	4
Measurements	5
Anthropometric Measurements.....	5
LDL Cholesterol	5
Power Analysis	6
Statistical Analysis.....	6
Results.....	7
Discussion.....	8
References.....	13

List of Tables

Table 1. Demographic and Anthropometric Data by Group at Baseline	16
Table 2. Changes in Participant Parameters by Period and Condition	17
Table 3. Difference in LDL Cholesterol Between Groups	18

List of Figures

Figure 1. CONSORT Flow Diagram	19
--------------------------------------	----

Introduction

For centuries, fasting has been associated with a host of benefits. In ancient Greece, prominent figures such as Pythagoras and Hippocrates promoted the spiritual and intellectual benefits of fasting (Kerndt, Naughton, Driscoll, & Loxterkamp, 1982). In 1915, Otto Folin recommended short periods of starvation as an effective means to reduce weight in obese individuals (Folin & Denis, 1915). In 1946, Anton Carlson took fasting research a step further when he investigated the effects of fasting on longevity in rats. He coined the term ‘intermittent fasting’ and noted that many religious individuals throughout history attributed their long lifespan to periodically abstaining from food (Carlson & Hoelzel, 1946).

Most fasting interventions conducted to date in humans have evaluated the primary endpoint of weight loss (Heilbronn, Smith, Martin, Anton, & Ravussin, 2005; Johnson et al., 2007). However, while weight loss has its benefits, intermittent fasting seems to be beneficial for those who suffer with metabolic diseases such as Diabetes Mellitus (Barnosky, Hoddy, Unterman, & Varady, 2014). Prediabetic men who participated in time-restricted feeding lowered fasting plasma insulin, improved insulin sensitivity, and enhanced beta-cell responsiveness after a short five-week intervention (Sutton et al., 2018). Additionally, intermittent fasting has been linked to improved beta-cell function, decreased evening appetite, and reduced oxidative stress in men with prediabetes (Sutton et al., 2018).

Cardiovascular disease is the leading cause of death for men and women in the United States (Murphy, Xu, Kochanek, & Arias, 2018), and intermittent fasting may be a behavioral method of improving cardiovascular disease risk. According to a study by Horne et al., individuals undergoing coronary angiography had lower risk of coronary artery disease when they participated in routine periodic fasting (Horne et al., 2008). Similarly, rats who were fed

every other day for three months experienced a cardioprotective effect. The fasting rats had fewer ischemic injuries after myocardial infarction and better post-MI cardiac remodeling (Ahmet, Wan, Mattson, Lakatta, & Talan, 2005). Assessing cardiovascular disease risk in humans is often performed using a lipid panel, which consists of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, and triglycerides. LDL-C is of particular interest as it plays an integral part in the development of atherosclerotic plaque by causing endothelial damage (Huff & Jialal, 2019).

Multiple observational trials have assessed the effects of Ramadan fasting on LDL-C (Mirmiran, Bahadoran, Gaeini, Moslehi, & Azizi, 2019) and some results suggest intermittent fasting may influence LDL-C levels. For example, Rahbar et al. found that men participating in Ramadan decreased LDL-C by 5% ($p = 0.04$) (Rahbar et al., 2019), but in contrast, Akaberi et al. found that LDL-C increased ($p = 0.01$) during Ramadan and remained elevated one month after the last blood sample was taken (Akaberi, Golshan, Moojdekanloo, & Hashemian, 2014). Additionally, a few randomized trials have reported the effect of various fasting regimens on LDL-C (Jamshed et al., 2019; Klempel, Kroeger, & Varady, 2013b). However, no randomized trials have assessed LDL-C as the primary outcome. Jamshed et al. found that LDL-C increased in the intervention group by 9 ± 4 mg/dL ($p = 0.02$) (Jamshed et al., 2019). In contrast, Sutton et al. conducted a trial in prediabetic men and found that early time-restricted feeding did not affect LDL-C differently than the control arm ($\Delta = 2 \pm 6$ mg/dL; $p = 0.75$) (Sutton et al., 2018). These are just a few examples of how mixed the results are in the current literature around fasting and LDL-C.

While a number of studies have evaluated the relationship between fasting and LDL-C, the results from these studies are challenging to interpret. This may be the result of a lack of

consistency in the fasting length and what is considered to be fasting in the various studies. Many of the fasts were no longer than 16 hours, or the participants were fed a small meal during the fast. In addition, the frequency of the fasts is also not consistent, and most studies have been relatively short, with some studies as short as two days, or as long as 12 weeks. There is also very little information on lower frequency fasting, which may be more sustainable over long periods of time. Therefore, the primary objective of this study was to evaluate how periodic 24-hour fasting for 26 weeks (twice a week for four weeks and once a week for 22 weeks) influences LDL-C in participants with metabolic risk factors for cardiovascular disease.

Methods

Design

This was a randomized, controlled trial conducted by the Intermountain Heart Institute Cardiovascular Research group (Intermountain). The study was sponsored by Intermountain Research and Medical Foundation and was conducted from 2016–2020. This was a collaborative effort between the Intermountain and researchers in the Exercise Sciences Department at Brigham Young University.

This study evaluated the impact of intermittent fasting in subjects with prediabetes or other metabolic syndrome risk factors and elevated LDL-C who were at increased risk for poor cardiovascular, metabolic, and cognitive outcomes. Subjects were randomized 1:1 to one of two study groups: water-only fasting or ad libitum usual diet. Randomization was done using a permuted blocked design.

Participants

Research personnel recruited 103 participants from patients seen at Intermountain hospitals, and clinics. Local community volunteers were also recruited. Previous patients with

electronic records showing they were eligible for the study were also contacted and invited to be screened for enrollment. Community volunteers were recruited through recruitment materials in the hospital or clinics and through other community or social media outreach. Patients gave written consent prior to initiation of study procedures. Those recruited included men or nonpregnant women aged 21 to 70 years of age. Patients were included in the study if they met the following criteria: not taking statin medication, prediabetic without needing medications, diagnosed type 2 diabetes but not taking medications, or had one or more of the components of metabolic syndrome measured within six months of screening (high blood pressure, elevated fasting glucose, high waist circumference or BMI, low HDL-C, or high triglycerides). Exclusion criteria included pregnant and/or lactating women, individuals who had fasted more than 15 hours per episode more than once per month during the past two years, prior diagnosis of a chronic disease, or participation in another clinical trial.

Procedures

The study lasted 26 weeks (6 months). Following recruitment, patients participated in preliminary screening using lipid panels, pregnancy tests (for child-bearing-age women only), and complete metabolic profiling (CMP). Following screening and recruitment into the study, patients had a baseline visit and follow-up visits at 4, 13, and 26 weeks after randomization. Baseline and follow-up testing consisted of the following measurements: height, weight, waist circumference, and lipid panel. The baseline visit took 2–2.5 hours, and subsequent visits about 1.5 hours.

Following baseline screening and randomization, participants participated in their designated groups. Group 1 (subjects allocated to the fasting arm) underwent a fasting regimen of twice per week: approximately 24-hour fasting (defined as 21–27-hour fast) on

nonconsecutive days during the first four weeks of the study, then once-per-week 24-hour fasting during the rest of the study (22 weeks). Between fasting days, eating was ad libitum. Also, a simple fasting diary was kept by Group 1 subjects to aid in compliance assessment and evaluation of how long each subject fasted on each fasting day. To do so, Group 1 subjects were provided with a logbook and instructions for tracking their fasting and food intake. Logbooks were used to track fasting frequency and fasting duration to ensure adherence. Group 2 (control) ate ad libitum and did not track their food intake.

Measurements

Anthropometric Measurements. Waist circumference was measured using a flexible measuring tape in the horizontal plane at the top of the right iliac crest.

Height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg using a height rod affixed to a scale in the cardiology clinic. These measurements were made by the study research coordinator.

LDL Cholesterol. LDL cholesterol was measured on a clinical testing platform in the central clinical lab at Intermountain using the VITROS 950 clinical laboratory system manufactured by Ortho Clinical Diagnostics in Raritan, NJ. The blood testing occurred at the beginning of a study visit and there was one draw per visit. The subject went to the outpatient phlebotomy office with the study research coordinator where they did a sitting blood draw. Blood pressure, height, weight, heart rate, and other physical exam measurements were performed after the blood draw; these tests took about 30 minutes.

The phlebotomist gained access to an antecubital vein with a needle that had a port on the far end. They then attached a blood tube and filled each of the necessary tubes by sticking one tube at a time on the port end of the needle apparatus. For the tests run in the clinical lab,

including the lipid panel, one lavender EDTA plasma tube was drawn and two gold serum separator tubes (SST), each of these being about 5 mL in size. A pneumatic tube system in the medical center was used to send the blood to the clinical lab within moments of being drawn, so that analysis of the samples was immediate. If for any reason the blood could not be analyzed immediately, it was stored in a refrigerator in the lab for a few minutes before being placed on the analyzer. The VITROS had an electronic connection to the laboratory information system which recorded lipid results automatically after completion.

Power Analysis. LDL-C was the primary end point of the study. The trial was powered to detect a 1.5-fold greater reduction in LDL-C than previously recorded in the Fasting II study and was compared to the nonfasting condition (decrease of 20 mg/dL in the intervention arm compared to a 5 mg/dL decrease in the control arm, allowing for regression to the mean in the control group) (Horne, Muhlestein, Butler, Brown, & Anderson, 2014). Alpha was set at 0.05 and beta was set at 0.10. Using a two-sided test, a sample size of 64 in both the fasting and nonfasting groups provided 90% power. Investigators also assumed a drop-out rate of 10% (12 total). On-treatment analyses were also performed to evaluate fasting's effects among subjects who complied with the study intervention compared to those who did not.

Statistical Analysis. Means and standard deviations are to be reported for all variables. Alpha for statistical tests was set to 0.05. A mixed effects model was used to assess how four weeks of two fast days per week followed by five months of weekly fasting affects LDL-C. Condition (fasting, control) and period (baseline, 4 weeks, 13 weeks & 26 weeks) were the fixed effects, and the participant was a random effect. The interaction between condition and period was evaluated. To assess any potential moderating effect of gender on the model, the model was repeated including gender as a fixed effect and the two-way interaction between gender and

condition was evaluated. The LSmeans procedure was used to evaluate significant main and interactive effects. The influence of potential moderating variables, including baseline LDL, waist circumference, change in waist circumference, change in weight, and adherence to the fasting intervention on the relationship between fasting and LDL were assessed through analysis of covariance. Effect size was calculated using Cohens D to describe the impact of fasting on LDL-C from baseline to follow-up.

Results

Of the 164 individuals who were eligible for the trial, 103 were randomized to the fasting (n = 50) and control (n = 53) groups. Average ages were 49.3 ± 12.0 and 47.0 ± 9.8 years, respectively, predominantly females (66.0% and 67.9%), overweight (103 ± 24 and 100 ± 21 kg), and with mild LDL-C elevation (124 ± 19 and 128 ± 20 mg/dL). Of those from the fasting group, 13 were lost to follow-up. The control group had 20 that were lost to follow-up. This resulted in a final sample of n = 71 participants (n = 38 fasting, n = 33 control). While attrition was higher in the control group, there were no differences in age, baseline LDL-C, waist circumference or weight between those who withdrew from the fasting group and those who withdrew from the control group (refer to Table 1 and Figure 1).

Adherence to the fasting regimen was $95\% \pm 12\%$ in the 38 subjects who made it to 26 weeks (based on self-reported data from the study fasting diaries). This included 22 with 100% adherence, six with 97%, three with 93%, three with 90%, two with 80%, one with 70%, and one with 37% adherence. Thus, 95% of subjects in the fasting arm had adherence at or above 80%.

The impact of the intervention on weight and waist circumference are displayed in Table 2 by period. There was no condition-by-period interaction for either weight or waist circumference (p = 0.84 and p = 0.95, respectively) despite a roughly 5 kg decrease in weight in

the fasting group after the first month. Additionally, changes in weight were -1.70 ± 4.69 (kg) in the fasting group and 0.20 ± 3.45 (kg) in the control group ($p = 0.06$). Waist circumference changed -1.13 ± 5.56 (cm) in the fasting group and -0.35 ± 4.41 (cm) in the control group ($p = 0.52$). The change in weight and waist circumference was not significantly different from baseline to follow-up in either condition and was not different between conditions.

Table 2 shows the means for LDL-C for each period by condition. As seen in Table 2, there was no condition-by-period interaction ($p = 0.06$). Similarly, there was no change in LDL-C from baseline to follow-up and there was no difference between conditions ($t = -0.538$, $p = 0.59$; Cohens $D = 0.12$) over the course of the study in either condition. Table 3 displays data for the analysis modeling six-month LDL-C and controlled for baseline LDL-C. This model showed that LDL-C was not different between conditions at the end of the study ($p = 0.06$). The model was then adjusted for gender ($p = 0.07$), baseline waist circumference ($p = 0.07$), change in weight circumference ($p = 0.07$), baseline weight ($p = 0.06$) and change in weight ($p = 0.07$) individually (see Table 3). Controlling for these variables had a negligible effect on LDL-C.

Discussion

The objective of this study was to evaluate how periodic 24-hour fasting for 26 weeks (twice a week for four weeks and once a week for 22 weeks) influenced LDL-C in participants with metabolic risk factors for cardiovascular disease. The results of the study show that once-a-week fasting is insufficient to produce a meaningful change in weight or waist circumference. In addition, this frequency of fasting did not produce improvements in LDL-C compared to ad libitum feeding. These results were observed despite participants having elevated LDL-C at the start of the trial and controlling for several potentially confounding variables.

Results from this trial indicate that weekly fasting is insufficient to elicit change in LDL-C, a finding that is consistent with those of several other studies (Gabel et al., 2018; Moro et al., 2016; Sutton et al., 2018; Varady et al., 2013). For example, a pilot study by Gabel et al. found that 16 hours of time-restricted feeding did not change LDL-C after 12 weeks in adults with obesity (Gabel et al., 2018). Similarly, Moro et al found that eight weeks of the same fasting protocol did not alter LDL-C in resistance trained men (Moro et al., 2016). Additionally, Sutton et al. found that 18 hours of early time-restricted feeding also did not change LDL-C in men with prediabetes after five weeks (Sutton et al., 2018). Also, Varady et al. found no changes in LDL-C when obese individuals participate in modified alternate-day fasting (consuming 25% of calories on fast day) for 12 weeks (Varady et al., 2013). Lastly, a 2019 systematic review aimed to assess how intermittent fasting during Ramadan affected lipids and lipoprotein levels in healthy adults found no effect on LDL-C. This analysis included 33 studies, over 1,327 participants, and fast times ranged from 10 to 22 hours (Mirmiran et al., 2019).

However, it is worth noting that not all research supports our findings and several studies have observed a relationship between fasting and LDL-C. For example, a short-term modified alternate-day fast (consuming 25% of calories on fast day) found that LDL-C decreased when compared to follow-up (Varady, Bhutani, Church, & Klempel, 2009). Similarly, Zuo et al. found that a weekly modified 24-hour fast (minimum of 300 liquid kcals) combined with a high-protein calorie-restricted diet was able to reduce LDL-C after 12 weeks of weight loss and 52 weeks of weight maintenance (Zuo et al., 2016). In fact, there have been several modified alternate-day-fasting trials that have resulted in lower LDL-C (Bhutani, Klempel, Kroeger, Trepanowski, & Varady, 2013; Klempel, Kroeger, Bhutani, Trepanowski, & Varady, 2012; Klempel, Kroeger, & Varady, 2013a; Varady et al., 2013).

Finally, there have also been a few studies that have observed increased LDL-C with fasting. A randomized-crossover trial by Horne et al. investigated the effect of one 24-hour water-only fast on cardiovascular biomarkers and found a significant increase in LDL-C (Horne et al., 2013). Similarly, Jamshed et al. found that 18-hour early time-restricted eating resulted in higher LDL-C (Jamshed et al., 2019). In addition, while the results from studies evaluating Ramadan have inconsistent results on LDL-C, there are four that found increased LDL-C (Akaberi et al., 2014; Barkia et al., 2011; Radhakishun et al., 2014; Ziaee et al., 2006).

While many of these results are contradictory to what was found in this trial, there are several differences that may explain this. First, the studies of Ramadan are almost exclusively observational and can show relationships but not establish cause and effect. There are likely other behaviors that go along with this practice that might influence the results. In addition, many of the studies lacked a true control group, which limits the conclusions that can be drawn from these studies. Third, many trials were not water-only fasts. Almost all the alternate-day fasting trials allowed participants to consume a small meal during their fast. This is not representative of a true fast and is more similar to a very low calorie diet. The different results from this trial could also be attributed to the duration of the intervention. Except for a few studies (Trepanowski et al., 2017; Zuo et al., 2016), most of the research assessing the effects of intermittent fasting on LDL-C were 12 weeks in duration or less. Finally, the frequency of fasting in our study was unique and not designed to produce significant weight loss. Reducing the frequency of fasting from two times a week to one after the first four weeks was done by design in order to evaluate a fasting approach that is less intrusive and possibly more sustainable.

The mechanisms by which intermittent fasting may alter LDL-C were recently explained by Santos and Macedo (Santos & Macedo, 2018). According to these researchers, nuclear

expression of peroxisome proliferator-activated receptor- α and peroxisome proliferator-activated receptor γ coactivator 1 α in the liver lead to increased fatty acid oxidation and ApoA production, while simultaneously decreasing ApoB synthesis. This alteration in ApoB synthesis may be one physiologic factor contributing to changes in LDL-C during intermittent fasting. Additionally, alterations in ApoB synthesis from fasting may also occur by means of altered enzymatic action. For example, fasting decreases the expression of sterol regulatory element-binding protein 2 (SREPB-2) (Horton, Bashmakov, Shimomura, & Shimano, 1998), diminishing the action of several enzymes responsible for cholesterol synthesis (Santos & Macedo, 2018; Tao, Xiong, DePinho, Deng, & Dong, 2013). Some studies have shown intermittent fasting to alter hepatic production of ApoB (Adlouni et al., 1998; Hammouda et al., 2013). Adlouni et al. and Hammouda et al. found a decrease of the ApoB after Ramadan, reflecting the LDL reduction (Adlouni et al., 1998; Hammouda et al., 2013). These mechanisms are also seen in individuals who improve their lipid panel with weight loss (Santos & Macedo, 2018). Given these potential mechanisms for altering LDL-C, it is interesting that our study did not find any change in LDL-C. However, it is possible that this could be because there was also no change in weight or waist circumference. In addition, it is possible that once-a-week fasting for the majority of the study may not have been enough to have a meaningful impact on the mechanisms that alter LDL-C.

Some limitations should be considered when interpreting the results of this study. One limitation of the study was that most of the participants that dropped out of the study were in the control group. This disparity in dropout rate may have been due to lack of a placebo and general interest of the participants to be in the fasting arm of the study. Additionally, this trial did not assess diet at any point in the study. However, this was done by design to prevent participants from changing their diets because they were being observed. Another limitation was that the goal

sample size of the trial was not met, therefore there was lower power than anticipated. However, the effect size we observed was extremely small (0.12). The observed effect size suggests that we would have needed a significantly larger sample size than calculated to reach significance. Finally, this study only addressed changes to LDL-C. Other aspects of metabolic health were not considered and should be the focus of future research.

Despite these limitations, the study makes a considerable addition to the current literature on fasting and LDL-C. This study is the first randomized trial of repeated fasting episodes that uses a standard-diet control arm and evaluates a single outcome other than weight loss. Another strength of this trial was the duration: many fasting studies last 12 weeks or less, and this study was twice as long. Additionally, the sample size was a strength of this study as few fasting trials have sample sizes as large as the one recruited here. Lastly, no study has assessed a fasting protocol like the one in this trial. Many studies have either higher fasting frequency and/or shorter fasts. The lower fasting frequency practiced in the present study resulted in high adherence to the fasting protocol and seems to be sustainable. However, with such high adherence, participants may be able to tolerate a higher fasting frequency in future studies.

In conclusion, the results of this study suggest once-a-week fasting may not be sufficient to elicit changes in LDL-C or weight. This was true even after controlling for weight, waist circumference, and gender. The high adherence of this trial suggests weekly fasting to be sustainable. Future studies should control for diet when evaluating outcomes such as LDL-C to see if fasting alone can improve health outcomes. Additionally, more research is needed to assess fasting frequency, since it is unknown how much fasting is needed to see an improvement in health outcomes.

References

- Adlouni, A., Ghalim, N., Saile, R., Hda, N., Parra, H. J., & Benslimane, A. (1998). Beneficial effect on serum apo AI, apo B and Lp AI levels of Ramadan fasting. *International Journal of Clinical Chemistry*, 271(2), 179-189. doi:10.1016/s0009-8981(97)00245-3
- Ahmet, I., Wan, R., Mattson, M. P., Lakatta, E. G., & Talan, M. (2005). Cardioprotection by intermittent fasting in rats. *Circulation*, 112(20), 3115-3121. doi:10.1161/CIRCULATIONAHA.105.563817
- Akaberi, A., Golshan, A., Moojdekanloo, M., & Hashemian, M. (2014). Does fasting in Ramadan ameliorate lipid profile? A prospective observational study. *Pakistan Journal of Medical Sciences*, 30(4), 708-711. doi:10.12669/pjms.304.4690
- Barkia, A., Mohamed, K., Smaoui, M., Zouari, N., Hammami, M., & Nasri, M. (2011). Change of diet, plasma lipids, lipoproteins, and fatty acids during Ramadan: A controversial association of the considered Ramadan model with atherosclerosis risk. *Journal of Health, Population and Nutrition*, 29(5), 486-493. doi:10.3329/jhpn.v29i5.8902
- Barnosky, A. R., Hoddy, K. K., Unterman, T. G., & Varady, K. A. (2014). Intermittent fasting vs daily calorie restriction for type 2 diabetes prevention: A review of human findings. *Translational Research: The Journal of Laboratory and Clinical Medicine*, 164(4), 302-311. doi:10.1016/j.trsl.2014.05.013
- Bhutani, S., Klempel, M. C., Kroeger, C. M., Trepanowski, J. F., & Varady, K. A. (2013). Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. *Obesity (Silver Spring)*, 21(7), 1370-1379. doi:10.1002/oby.20353
- Carlson, A. J., & Hoelzel, F. (1946). Apparent prolongation of the life span of rats by intermittent fasting. *Journal of Nutrition*, 31, 363-375. doi:10.1093/jn/31.3.363
- Folin, O., & Denis, W. (1915). On Starvation and Obesity with Special Reference to Acidosis. *Journal of Biological Chemistry*, 21(1), 183-192.
- Gabel, K., Hoddy, K. K., Haggerty, N., Song, J., Kroeger, C. M., Trepanowski, J. F., . . . Varady, K. A. (2018). Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study. *Nutrition and Healthy Aging*, 4(4), 345-353. doi:10.3233/NHA-170036
- Hammouda, O., Chtourou, H., Aloui, A., Chahed, H., Kallel, C., Miled, A., . . . Souissi, N. (2013). Concomitant effects of Ramadan fasting and time-of-day on apolipoprotein AI, B, Lp-a and homocysteine responses during aerobic exercise in Tunisian soccer players. *PLoS One*, 8(11), e79873. doi:10.1371/journal.pone.0079873
- Heilbronn, L. K., Smith, S. R., Martin, C. K., Anton, S. D., & Ravussin, E. (2005). Alternate-day fasting in nonobese subjects: Effects on body weight, body composition, and energy metabolism. *American Journal of Clinical Nutrition*, 81(1), 69-73. doi:10.1093/ajcn/81.1.69
- Horne, B. D., May, H. T., Anderson, J. L., Kfoury, A. G., Bailey, B. M., McClure, B. S., . . . Intermountain Heart Collaborative, S. (2008). Usefulness of routine periodic fasting to lower risk of coronary artery disease in patients undergoing coronary angiography. *American Journal of Cardiology*, 102(7), 814-819. doi:10.1016/j.amjcard.2008.05.021
- Horne, B. D., Muhlestein, J. B., Butler, A. R., Brown, H., & Anderson, J. L. (2014). Effects of water-only fasting among prediabetic patients. *Diabetes*, 63(Supp. 1), A590-A590.

- Horne, B. D., Muhlestein, J. B., Lappe, D. L., May, H. T., Carlquist, J. F., Galenko, O., . . . Anderson, J. L. (2013). Randomized cross-over trial of short-term water-only fasting: metabolic and cardiovascular consequences. *Nutrition, Metabolism and Cardiovascular Disease*, 23(11), 1050-1057. doi:10.1016/j.numecd.2012.09.007
- Horton, J. D., Bashmakov, Y., Shimomura, I., & Shimano, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proceedings of the National Academy of Sciences United States of America*, 95(11), 5987-5992. doi:10.1073/pnas.95.11.5987
- Huff, T., Boyd, B. & Jialal, I. (2019). Physiology, Cholesterol. [Updated 2021 Mar 2]. In: StatPearls [Internet]. Treasure Island (FL). StatPearls Publishing. Available from <https://www.ncbi.nlm.nih.gov/books/NBK470561/>
- Jamshed, H., Beyl, R. A., Della Manna, D. L., Yang, E. S., Ravussin, E., & Peterson, C. M. (2019). Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans. *Nutrients*, 11(6). doi:10.3390/nu11061234
- Johnson, J. B., Summer, W., Cutler, R. G., Martin, B., Hyun, D. H., Dixit, V. D., . . . Mattson, M. P. (2007). Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radical Biology Medicine*, 42(5), 665-674. doi:10.1016/j.freeradbiomed.2006.12.005
- Kerndt, P. R., Naughton, J. L., Driscoll, C. E., & Loxterkamp, D. A. (1982). Fasting: The history, pathophysiology and complications. *The Western Journal of Medicine*, 137(5), 379-399. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/6758355>
- Klempel, M. C., Kroeger, C. M., Bhutani, S., Trepanowski, J. F., & Varady, K. A. (2012). Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. *Nutrition Journal*, 11, 98. doi:10.1186/1475-2891-11-98
- Klempel, M. C., Kroeger, C. M., & Varady, K. A. (2013a). Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet. *Metabolism: Clinical and Experimental*, 62(1), 137-143. doi:10.1016/j.metabol.2012.07.002
- Klempel, M. C., Kroeger, C. M., & Varady, K. A. (2013b). Alternate day fasting increases LDL particle size independently of dietary fat content in obese humans. *European Journal of Clinical Nutrition*, 67(7), 783-785. doi:10.1038/ejcn.2013.83
- Mirmiran, P., Bahadoran, Z., Gaeini, Z., Moslehi, N., & Azizi, F. (2019). Effects of Ramadan intermittent fasting on lipid and lipoprotein parameters: An updated meta-analysis. *Nutrition Metabolism and Cardiovascular Disease*, 29(9), 906-915. doi:10.1016/j.numecd.2019.05.056
- Moro, T., Tinsley, G., Bianco, A., Marcolin, G., Pacelli, Q. F., Battaglia, G., . . . Paoli, A. (2016). Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *Journal of Translational Medicine*, 14(1), 290. doi:10.1186/s12967-016-1044-0
- Murphy, S. L., Xu, J., Kochanek, K. D., & Arias, E. (2018). Mortality in the United States, 2017. *NCHS Data Brief*, (328), 1-8.

- Radhakishun, N., Blokhuis, C., van Vliet, M., von Rosenstiel, I., Weijer, O., Heymans, M., Diamant, M. (2014). Intermittent fasting during Ramadan causes a transient increase in total, LDL, and HDL cholesterol and hs-CRP in ethnic obese adolescents. *European Journal of Pediatrics*, 173(8), 1103-1106. doi:10.1007/s00431-014-2276-8
- Rahbar, A. R., Safavi, E., Rooholamini, M., Jaafari, F., Darvishi, S., & Rahbar, A. (2019). Effects of intermittent fasting during Ramadan on insulin-like growth factor-1, interleukin 2, and lipid profile in healthy muslims. *International Journal of Preventative Medicine*, 10, 7. doi:10.4103/ijpvm.IJPVM_252_17
- Santos, H. O., & Macedo, R. C. O. (2018). Impact of intermittent fasting on the lipid profile: Assessment associated with diet and weight loss. *Clinical Nutrition ESPEN*, 24, 14-21. doi:10.1016/j.clnesp.2018.01.002
- Sutton, E. F., Beyl, R., Early, K. S., Cefalu, W. T., Ravussin, E., & Peterson, C. M. (2018). Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metabolism*, 27(6), 1212-1221.e1213. doi:10.1016/j.cmet.2018.04.010
- Tao, R., Xiong, X., DePinho, R. A., Deng, C. X., & Dong, X. C. (2013). Hepatic SREBP-2 and cholesterol biosynthesis are regulated by FoxO3 and Sirt6. *Journal of Lipid Research*, 54(10), 2745-2753. doi:10.1194/jlr.M039339
- Trepanowski, J. F., Kroeger, C. M., Barnosky, A., Klempel, M. C., Bhutani, S., Hoddy, K. K., . . . Varady, K. A. (2017). Effect of alternate-day fasting on weight loss, weight maintenance, and cardioprotection among metabolically healthy obese adults: A randomized clinical trial. *JAMA Internal Medicine*, 177(7), 930-938. doi:10.1001/jamainternmed.2017.0936
- Varady, K. A., Bhutani, S., Church, E. C., & Klempel, M. C. (2009). Short-term modified alternate-day fasting: A novel dietary strategy for weight loss and cardioprotection in obese adults. *American Journal of Clinical Nutrition*, 90(5), 1138-1143. doi:10.3945/ajcn.2009.28380
- Varady, K. A., Bhutani, S., Klempel, M. C., Kroeger, C. M., Trepanowski, J. F., Haus, J. M., . . . Calvo, Y. (2013). Alternate day fasting for weight loss in normal weight and overweight subjects: A randomized controlled trial. *Nutrition Journal*, 12(1), 146-146. doi:10.1186/1475-2891-12-146
- Ziaee, V., Razaee, M., Ahmadinejad, Z., Shaikh, H., Yousefi, R., Yarmohammadi, L., . . . Behjati, M. J. (2006). The changes of metabolic profile and weight during Ramadan fasting. *Singapore Medical Journal*, 47(5), 409-414. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/16645692>
- Zuo, L., He, F., Tinsley, G. M., Pannell, B. K., Ward, E., & Arciero, P. J. (2016). Comparison of high-protein, intermittent fasting low-calorie diet and heart healthy diet for vascular health of the obese. *Frontiers in Physiology*, 7, 350-350. doi:10.3389/fphys.2016.00350

Table 1. Demographic and Anthropometric Data by Group at Baseline

	Fasting, n = 50	Control, n = 53	p-value
Demographics			
Age, years	49.3 ± 12.0	47.0 ± 9.8	0.30
Sex, male	17 (34.0%)	17 (32.1%)	0.84
Ethnicity			
Hispanic/Latino	3 (6.0%)	5 (9.4%)	
Non-Hispanic/Latino	47 (94.0%)	47 (88.7%)	
Unknown	0 (0%)	1 (1.9%)	
Lipids			
Total cholesterol (mg/dL)	196.1 ± 25.4	201.8 ± 27.8	0.28
LDL-C (mg/dL)	123.9 ± 19.2	130.0 ± 17.0	0.35
HDL-C (mg/dL)	46.1 ± 10.2	47.0 ± 14.2	0.73
Triglycerides (mg/dL)	131.7 ± 76.6	137.8 ± 63.3	0.66
VLDL-C (mg/dL)	24.9 ± 13.1	27.6 ± 12.7	0.30
ApoB (mg/dL)	81.6 ± 50.5	75.9 ± 45.0	0.56
Vitals			
SBP (mmHg)	127.5 ± 12.2	127.7 ± 13.8	0.96
DBP (mmHg)	82.1 ± 8.0	81.2 ± 10.4	0.65
BMI (kg/m ²)	35.1 ± 8.2	34.0 ± 7.3	0.49
Weight (kg)	103.0 ± 24.2	99.7 ± 20.7	0.45
Height (cm)	171.5 ± 10.2	171.5 ± 9.9	0.97
Waist circumference (cm)	107.4 ± 20.2	106.2 ± 17.1	0.73
Other Laboratory Measurements			
HOMA-IR	2.3 ± 1.2	2.8 ± 1.9	0.16
Fasting insulin (mIU/L)	11.5 ± 11.5	11.9 ± 7.0	0.85
Fasting glucose (mg/dL)	91.7 ± 14.4	90.2 ± 9.5	0.53

P-values refer to the comparison of groups at baseline. Mean ± SD.

Table 2. Changes in Participant Parameters by Period and Condition

Variable	Fasting (n = 38)				Control (n = 33)				F-statistic	P-value
	Baseline	W4	W13	W26	Baseline	W4	W13	W26		
LDL (mg/dL)	124 ± 21	123 ± 23	121 ± 22	124 ± 24	130 ± 17	137 ± 32	129 ± 21	133 ± 25	3.5	0.06
Waist Circumference (cm)	107 ± 22	108 ± 16	109 ± 15	109 ± 15	106 ± 19	108 ± 15	108 ± 14	109 ± 15	0.0	0.95
Weight (kg)	105 ± 25	100 ± 27	103 ± 25	104 ± 24	102 ± 20	102 ± 22	102 ± 20	102 ± 20	0.0	0.84
BMI (kg/m ²)	33 ± 8	32 ± 8	33 ± 8	33 ± 8	34 ± 7	34 ± 7	34 ± 7	34 ± 7	0.4	0.52

F and P values refer to the comparison between groups by period. The analysis was longitudinal with analyses occurring across multiple periods. Mean ± SD.

Table 3. Difference in LDL Cholesterol Between Groups

	Fasting Group		Control Group		F-Value	P-Value
	Mean	95% CI	Mean	95% CI		
Main Model						
Fasting LDL mg/dL	123	117, 130	132	125, 139	3.5	0.06
Adjusted Model						
Gender	123	117, 130	132	125, 139	3.3	0.07
Baseline Waist Circumference	123	117, 130	132	125, 139	3.7	0.06
Change in Waist Circumference	123	117, 130	132	125, 139	3.4	0.07
Baseline Weight	123	117, 129	132	125, 139	3.7	0.06
Change in Weight	123	117, 130	132	125, 139	3.4	0.07

The main model represents the nonadjusted model of six-month LDL after controlling for baseline LDL. Variables under the adjusted model are controlled variables. Each variable was adjusted for individually. F and P values indicate a period-by-condition interaction. There was no impact on the model after adjusting for the variables.

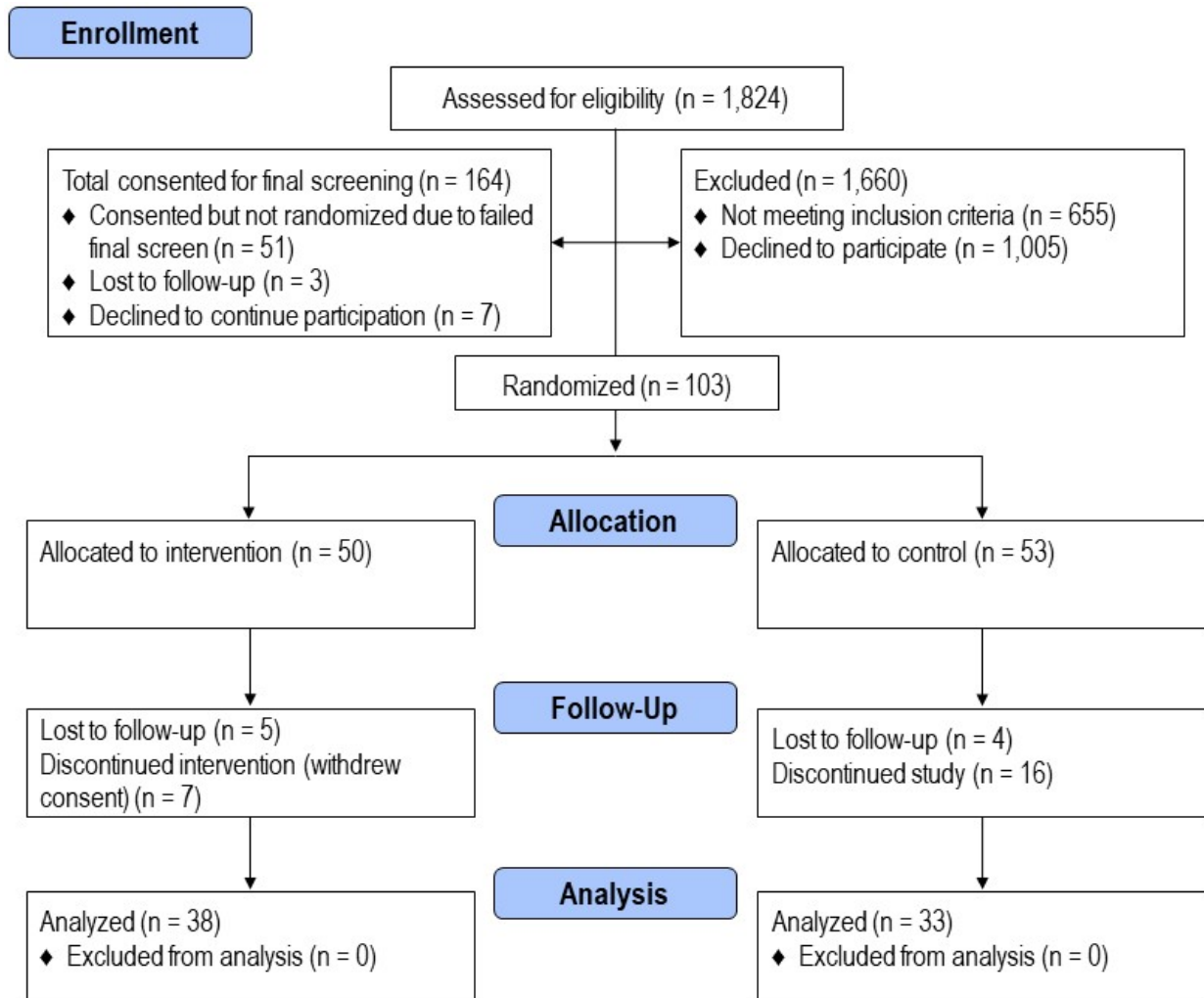


Figure 1. CONSORT Flow Diagram