Thesis

Cardiovascular effects of intermittent fasting

Submitted by

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Graz, June 7th, 2018

Statutory Declaration

I declare on my honour that I have written this thesis independently and without assistance, I have not used other than the specified sources and parts taken from other sources, verbatim or in substance have been identified as such.

Graz, June 7th, 2018 *Barbara Weber eh*

Note of thanks

First I want to express my gratitude for the guidance but also patience during the work on this study and thesis that I received from my supervisors Harald Sourij and Ewald Kolesnik. Norbert Tripolt and Peter Pferschy who conducted the study “Interfast” answered my countless questions patiently and supported me whenever help was needed.

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And thirdly I want to thank Sebastian for coming with me along this journey with all its up and downs.

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1 List of abbreviations

ADF	Alternate day fasting
CR	Caloric restriction
IF	Intermittent fasting
RCT	Randomized controlled trial
OGTT	Oral glucose tolerance test
DXA	Dual-energy-x-ray absorptiometry
DVA	Dynamic vessel analyser
LVMI	Left ventricle mass index
LVM	Left ventricle mass
LAVI	Left atrial volume index
LAV	Left atrial volume
BSA	Body surface area
EF	Ejection Fraction
SV	Stroke Volume
EDV	End-diastolic volume
HOMA IR	Homeostatic model assessment for Insulin Resistance
HOMA Beta	Homeostatic model assessment for Beta-Cells
ISI	Insulin Sensitivity Index
RHI	Reactive Hyperemia Index
LnRHI	Natural log Reactive Hyperemia Index
AI	Augmentation Index
AI Hf75	Augmentation Index normalized to heart rate 75
HDL	High density lipoprotein
VLDL	Very high density lipoprotein
LDL	Low density lipoprotein

2 List of figures

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4 German abstract

Hintergrund: Alternierendes Fasten (ADF) ist eine Unterart von intermittierendem Fasten (IF), das aus einem Fastentag und einem Esstag, an dem beliebige Nahrungsaufnahme möglich ist, besteht. Bisherige Studien haben gezeigt, dass Gewichtsreduktion und Verbesserung von kardiovaskulären Risikofaktoren durch alternierendes Fasten bei übergewichtigen Probanden möglich ist. Daten bezüglich möglicher positiver oder negativer gesundheitlicher Effekte und deren Mechanismen bei gesunden, normalgewichtigen Probanden sind aber immer noch dürftig. Das Ziel dieser Studie ist es, Kurzzeiteffekte von alternierendem Fasten auf kardiovaskuläre Risikofaktoren in gesunden, normalgewichtigen Individuen zu untersuchen.

Material und Methoden: Es handelt sich um eine prospektive, randomisiert kontrollierte Studie, bestehend aus 60 gesunden Probanden. Die Probanden wurden entweder zur ADF Gruppe oder einer Kontrollgruppe randomisiert. Probanden in der ADF Gruppe praktizierten 4 Wochen alternierendes Fasten, nach einem Rhythmus von 36 Stunden fasten und 12 Stunden essen. Die Kontrollgruppe wurde instruiert, die bisherigen Ernährungsgewohnheiten beizubehalten. Durchgeführte Untersuchungen inkludierten die Messung der Body Composition mittels Dual-Röntgen Absorptiometrie, Messung der Endothelfunktion mit dem EndoPAT2000 und der dynamischen Gefäßanalyse über eine Augenhintergrunduntersuchung, dem oralen Glucose Toleranztest (OGTT), 24 Stunden Blutdruckmessung, Echokardiographie und eine Blutabnahme zur Routinelaboruntersuchung.

Ergebnisse: Es zeigte sich eine signifikante Abnahme der Fettmasse von -2.12 ± 1.04 kg in der ADF Gruppe versus -0.15 ± 0.84 kg in der Kontrollgruppe ($p < 0.0001$) und der Magermasse von -1.31 ± 0.99 kg in der ADF Gruppe im Vergleich zur Kontrollgruppe -0.05 ± 0.97 kg ($p < 0.0001$). Ebenfalls eine signifikante Abnahme zeigte der systolische Blutdruck der ADF Gruppe mit -4 ± 8 mmHg (Ausgangsblutdruck 121 ± 11) im Vergleich zur Kontrollgruppe mit 2 ± 14 mmHg (Ausgangsblutdruck 118 ± 8), p-Wert = 0.0475. Keine signifikanten Änderungen zeigten sich beim diastolischen Blutdruck, den Blutfetten, der Endothelfunktion und Parametern der Echokardiographie.

Zusammenfassung: Die Studie hat gezeigt, dass alternierendes Fasten zu einer moderaten Gewichtsabnahme auch bei gesunden, normalgewichtigen Probanden führt. Ebenso scheint alternierendes Fasten zu einer Reduktion des systolischen Blutdrucks zu führen. Eine

Veränderung von anderen kardiovaskulären Markern konnte bei gesunden Probanden, zumindest in diesem kurzen Interventionszeitraum, nicht gezeigt werden.

5 Abstract

Background: alternate day fasting (ADF) is a subtype of intermittent fasting (IF), consisting of fasting days and feast days, where food can be consumed ad-libitum. Previous studies have observed weight loss and improvement of cardiovascular risk factors through ADF in obese subjects. However, data about health effects and their potential mechanisms in healthy, non-obese subjects performing ADF are still rare. It is the aim of this study to investigate short-term effects of ADF on cardiovascular risk factors in healthy, non-obese individuals.

Material and Methods: We performed a prospective, randomized controlled trial consisting of 60 healthy subjects. The participants were randomized either to the ADF group or to the control group. Subjects of the ADF group performed 4 weeks of alternate day fasting with alternating fast days of 36 hours and feast days of 12 hours. The control group was instructed to continue their previous diet. Performed examinations included the measurement of the body composition by using the dual energy x-ray absorptiometry, measurement of the endothelial function with the EndoPAT2000 and dynamic vessel analysis, oral glucose tolerance test (OGTT), 24 hours blood pressure measurement, echocardiography and blood sampling.

Results: The study showed significant reduction in fat mass in the ADF group with -2.12 ± 1.04 kg versus -0.15 ± 0.84 kg in the control group ($p < 0.0001$) and also in the lean mass with -1.31 ± 0.99 kg in the ADF group versus -0.05 ± 0.97 kg in the control group, $p < 0.0001$. Systolic blood pressure significantly decreased in the ADF group with -4 ± 8 mmHg (baseline 121 ± 11) compared to the control group with 2 ± 14 mmHg (baseline 118 ± 8), $p = 0.0475$. No significant changes were found for diastolic blood pressure, blood lipids, endothelial function and parameters of echocardiography.

Conclusion: The study demonstrated that alternate day fasting leads to considerable weight loss within 4 weeks and a decrease in systolic blood pressure even in healthy, non-obese subjects. Improvement of other cardiovascular risk factor could not be observed in healthy individuals, at least not for this short period of time.

6 Introduction

6.1 Obesity

Obesity is defined as an increase of body weight above the norm, commonly measured with the Body Mass Index (BMI). A classification of 4 main groups was made by the World Health Organization (WHO), defining a score of < 18.5 as underweight, 18.5 – 24.99 as normal range, 25.00 – 29.99 as overweight and ≥ 30.00 as obese (World Health Organization, 2006).
A study in 2015 highlighted that 603.7 million adults all over the world were obese, being 12.0% of all adults. (The GBD 2015 Obesity Collaborators, 2017)
A health survey in Austria in 2014 showed that 47% of the population is overweight or obese. The gender difference was big with 55.2 % of men being overweight or obese, but only 38.9 % of the women.

Table 1: Health survey Austria 2014, Statistic Austria, BMI

Gender	Underweight	Normal Range	Overweight	Obese
	< 18.5	18.5 – 24.99	25.00 – 29.99	≥ 30.00
Total (%)	2.8	50.1	32.6	14.4
Male (%)	1.3	43.6	39.5	15.7
Female (%)	4.4	56.7	25.8	13.1

(Statistik Austria, 2015)

Co-morbidities associated with obesity are cardiovascular diseases, such as hypertension, stroke, coronary heart disease and congestive heart failure. Obesity is also related to diseases such as type II diabetes, various types of cancer, gallbladder diseases, pulmonary embolism and diseases within the musculoskeletal system such as chronic back pain or osteoarthritis. Obesity and its comorbidities are associated with a higher risk of disability and death (Djalalinia, et al., 2015). A study by (The GBD 2015 Obesity Collaborators, 2017) found that cardiovascular diseases are the number one cause for death.
Treatment of obesity varies depending on the BMI, waist circumference, health condition and risk factors of the subject. The major aim is to achieve a negative energy balance by a lower calorie intake and/or physical exercise. Additionally to diet and physical activity, drug

treatment can be considered in some cases. Impressive results were also found for surgical treatment in obese patients with type 2 diabetes, including bariatric surgery options such as gastric banding, roux-en-Y gastric bypass, biliopancreatic diversion or sleeve gastrectomy. (Wyatt, 2013)

The study by (Wyatt, 2013) also showed that various kinds of diets such as low fat, moderate fat, low carbohydrate, low glycaemic, high protein and Mediterranean diets both in short and long-term periods had similar weight loss results. Adherence to the diet seems for weight loss results much more important than the type of diet a patient is on. However, the rate of people who have managed to maintain their weight loss 5 years after taking part in a weight-loss program is low (Anderson, et al., 2001), so new strategies need to be found. One approach could be alternate day fasting, which will be described below.

6.2 Intermittent Fasting

Being part of nearly every religion, fasting is practiced all over the world within Islam, Christianity or Hinduism (Kannan, et al., 2016). In recent years various types of intermittent fasting have become more and more popular and bestselling diet books (Mosley, 2012) & (Press, 2013) referring to this topic have been sold millions of times all over the world.

The difference to caloric reduction is that for intermittent fasting one does not need to reduce the caloric intake but has to keep to certain fasting periods.

The table below shows a weekly fasting schedule for the most common types of intermittent fasting as there are the 5:2 diet, alternate day fasting and time restricted feeding. Fasting periods mean a total or partial caloric restriction with 20-25% of normal energy needs. (Antoni R, 2017)

Table 2: Intermittent fasting protocols

	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
5:2 diet	Fast	Fast	Feed	Feed	Feed	Feed	Feed
Time-restricted feeding	≥ 12h fast	≥ 12h fast	≥ 12h fast	≥ 12h fast	≥ 12h fast	≥ 12h fast	≥ 12h fast
Alternate day fasting	Feed	Fast	Feed	Fast	Feed	Fast	Feed

The 5:2 diet involves eating whatever you want for 5 days but eating nothing for 2 consecutive days a week. Time-restricted feeding has fasting periods of 12-20 hours and eating as usual for the rest of the time (Patterson RE, 2016). Alternate day fasting means alternating fast and feast days and will be described detailed below.

6.3 The thrifty gene hypothesis

James V. Neel hypothesized that for gaining optimal metabolic function alternating famine and feast is required, believing that our genome, which was presumably selected 50.000 to 10.000 BC, was influenced by a feast and famine environment, being common during that time. With changing lifestyle famine periods are no longer playing a role for most people in first world countries. According to James V. Neel's thrifty gene hypothesis this could be the reason for the high frequency of diabetes mellitus type 2 nowadays (Neel, 1962), expanding the hypothesis on essential hypertension and obesity in 1998 (Neel, 1999). Since then the thrifty gene hypothesis has been discussed controversially. A study in 2016 questioned the hypothesis that there could be a link between obesity and a selective advantage for surviving periods of famine, as they did not find a positive selection of single nucleotide polymorphism (SNPs) that are associated with human obesity (Wang & Speakman, 2016).

6.4 Alternate day fasting

Alternate day fasting is a new approach for achieving energy intake reduction and has become a popular strategy for weight loss management. ADF is a type of intermittent fasting, where fasting days, in which 0% - 25% of caloric intake is allowed, alternate with fed days, where food can be consumed ad libitum. (Catenacci, et al., 2016) Fasting periods vary from 12 to 36 hours.

6.4.1 ADF effects on body weight and composition

6.4.1.1 Animal studies

(Hatori M, 2012) investigated effects of ADF in mice regarding the prevention of obesity and metabolic diseases. They allocated mice into two groups: ad libitum (high fat diet for 24 hours) or time-restricted (high fat diet for 8 hours a day), with both groups receiving the same amount of calories. Mice with time-restricted feeding were protected against obesity and metabolic diseases, suggesting a promising prevention strategy for obesity and associated metabolic diseases. Another study showed that obese mice on a high-fat ADF diet lost in a 10 weeks diet one-third of their bodyweight (Joslin PMN, 2017).

6.4.1.2 Human trials

A 6 weeks modified ADF regime with very low calorie intake on fast days, resulted in significant decrease in body weight and waist circumference for overweight and obese women (Samira Eshghinia, 2013).

ADF effects on normal-weight and overweight (BMI 20 – 29.9 kg/m²) subjects were investigated by (Varady KA, 2013). 32 participants were allocated to either an ADF group or a control group for 12 weeks. They found significantly decreased body weight and fat mass reduction.

A study from (Heilbronn LK, 2005) with 16 non-obese subjects performing ADF for 3 weeks, assessed body weight and body composition and observed significant body weight reduction and fat loss.

Effects of a high-fat and a low-fat diet on ad-libitum days during an alternate day fasting intervention were examined by a study in 2013 by Klempel et al. The comparison of high-fat and low-fat ADF showed no difference in matters of weight loss (Klempel MC, 2013).

Varady also examined which patient populations benefit the most from alternate day fasting and observed in an 8-weeks ADF intervention with 121 subjects similar weight loss results for men and women. Furthermore the study showed that the degree of weight loss and fat mass reduction was independent from baseline body weight and BMI (Varady KA, 2016).

6.4.2 ADF effects on blood pressure

(Mager DE, 2006) compared effects of alternate day fasting with daily caloric restriction (40%) on heart rate and diastolic blood pressure in rats over a period of 16 weeks. Significant reduction was observed in both ADF and CR, indicating equally beneficial effects for ADF and CR. Reduction in heart rate and blood pressure in rats on an ADF regimen was also shown by (WAN R, 2003).

Neither high-fat nor low-fat ADF regime in obese subjects showed significant changes in systolic and diastolic blood pressure or heart rate in a study by (Klempel MC, 2013).

A study by Heilbronn in 2005 with 16 non obese subjects, performing ADF for 22 days did also show that systolic and diastolic blood pressure were not changed significantly by the intervention (Heilbronn LK, 2005).

(Varady KA, 2009) found that systolic blood pressure and heart rate decreased significantly, whereas diastolic blood pressure showed no changes after 8 weeks of ADF in obese subjects.

6.4.3 ADF on blood lipids

A study in 2006 by Tikoo et al. investigated the effects of intermittent fasting on diabetic nephropathy in diabetic rats and found a significant improvement of HDL cholesterol (Tikoo K & Sharma V, 2007).

A study by (Varady KA, 2011) investigated effects on blood lipids of short-term ADF (8 weeks) in obese (BMI 30 – 39.9 kg/m²) adults. They observed a decrease in LDL cholesterol by 25 % and in triglycerides by 32%, but no change was found in HDL cholesterol. Furthermore LDL particle size was observed: small LDL particles decreased, while large LDL particles increased. An increased number of small LDL particles seems to play a significant role in the development of atherosclerosis (Gardner CD, 1996).

Similar results for the decrease of small LDL particles, again in obese subjects, were also observed by (Cynthia M Kroeger, 2012) in a study with 10 weeks of ADF. The effect of high-fat and low-fat ADF on LDL size was compared and found equally effective (Klempel MC, 2013).

(Varady KA, 2015) conducted another alternate day fasting study in 2015 with 29 obese women (30 – 39.9 kg/m²) and compared 2 types of alternate day fasting. For 8 weeks one group was on a high fat diet on ad-libitum days, the other group on a low fat diet on ad-libitum days. Both groups showed significant decrease in total cholesterol, LDL-cholesterol and triglyceride concentrations.

Different data were found in non-obese and overweight subjects by (Varady KA, 2013), performing ADF for 12 weeks. Triglyceride concentrations decreased and LDL particle size increased both significantly, but no significant changes were found for LDL or HDL cholesterol.

The above mentioned data indicates beneficial effects of ADF for obese subjects on total cholesterol, LDL cholesterol and triglycerides, but no effect on HDL.

For normal weight and overweight subjects ADF seems to be beneficial for triglycerides and LDL particle size, whereas no significant data can be found for LDL or HDL cholesterol in current studies. Data for non-obese subjects performing ADF is rare and will be investigated in this thesis.

6.4.4 ADF on glucose regulation

6.4.4.1 Animal studies

A study by Baumeier et al in 2015 allocated mice on a high-fat diet to either an ad libitum fed group, a caloric restriction group (90% of the food consumed by the ad libitum group) or the intermittent fasting group, who received food ad libitum every other day. Diabetes prevalence in the ad-libitum group was 43% at the age of 14 weeks, whereas no mouse in the caloric restriction group or the intermittent fasting group had diabetes at this age (Baumeier C, 2015).

Mice were also on an alternate day fasting diet for a study by (Anson RM, 2003). Overall food intake was not reduced and their body weight was maintained during the period of ADF. Data showed reduced serum glucose and insulin levels, suggesting that ADF could have a positive effect on glucose regulation. (Joslin PMN, 2017) observed similar results with improved glucose tolerance in obese mice on a high-fat alternate day fasting regimen. Correlating results were also found for rats performing ADF by (WAN R, 2003), who found decreased glucose and insulin levels.

Different results were observed by (Mager DE, 2006). The comparison of CR and IF in rats lead to a reduction in plasma glucose in the CR group within 2 weeks, whereas in the IF group no effect on plasma glucose could be observed at any time.

6.4.4.2 Human data

16 non-obese subjects performed alternate day fasting for 22 days in a study by (Heilbronn LK, 2005). Measurements were made at baseline, after 21 days (12 hours of fasting) and 22 days (36 hours of fasting). Fasting plasma glucose concentrations were not significantly different from baseline, neither at day 21 nor at day 22. However, fasting plasma insulin concentrations were similar to baseline at day 21 but significantly lower at day 22 after 36 hours of fasting.

In a study by (Halberg N, 2005) 8 healthy and non-obese men, performed alternate day fasting for two weeks with 20 hour fasting periods every second day. They observed no change before and after the two weeks intervention in fasting plasma glucose and insulin concentrations. They found significantly lower fasting plasma glucose concentrations after 20 hours of fasting on days 4, 6 and 10 compared with a fasting period of 8 hours after an ad-libitum day. Fasting insulin concentrations were not different after 20 hours and 8 hours of fasting.

A study by (Horne B.D, 2013) investigated short-term effects of intermittent fasting after 28 hours. Within their study population of 30 healthy and non-obese subjects they found no change in fasting plasma insulin and glucose concentrations, as well as no change in HOMA IR when comparing 28 hours of fasting to an ad-libitum day (8h fasting).

6.4.5 Alternate day fasting compared to caloric restriction

The question if ADF exceeds the benefits of daily caloric restriction has been examined by (Catenacci, et al., 2016). They compared differences in weight loss, body composition, lipids and insulin sensitivity index (ISI) after 8 weeks of either ADF or daily caloric restriction and additionally to that made an unsupervised follow up after 24 weeks. The outcomes showed similar effects in all those parameters after 8 weeks. There was a numerical benefit without statistical significance for ADF after 24 weeks in changes from fat mass and lean mass compared with baseline. They also examined safety and tolerability of alternate day fasting and found no adverse effects due to ADF.

A study by (Trepanowski, et al., 2017) also compared alternate day fasting with daily caloric restriction. Participants were allocated to either an alternate day fasting group, a daily caloric restriction group or a no-intervention control group. Mean low-density lipoprotein cholesterol levels were significantly higher in the ADF group compared to the caloric restriction group after 12 months. Results also showed that the dropout rate in the ADF group was higher than the one in the caloric restriction group (38% and 29%) compared to the dropout rate in the control group 26%. No significant results were observed for weight loss, blood pressure, heart rate, triglycerides, fasting glucose, fasting insulin and insulin resistance when comparing the ADF group to the daily caloric restriction group.

6.4.6 Interfast

The study “Interfast” is unique in its structure as most of the studies that were investigating effects of ADF included obese, but not healthy subjects. Interfast only included subjects with a BMI between 22.0 – 27.0. The results of ADF in obese subjects were impressive for weight loss but effects on healthy individuals are mostly unexplored.

The primary object of Interfast was to find out to which extend alternate day fasting influences various parameters of healthy individuals. This thesis will focus on metabolic parameters such as HbA1c, OGTT, blood pressure, serum lipids, echocardiography and endothelial function through the EndoPat system and the dynamic vessel analyzation (DVA).

7 Material and Methods

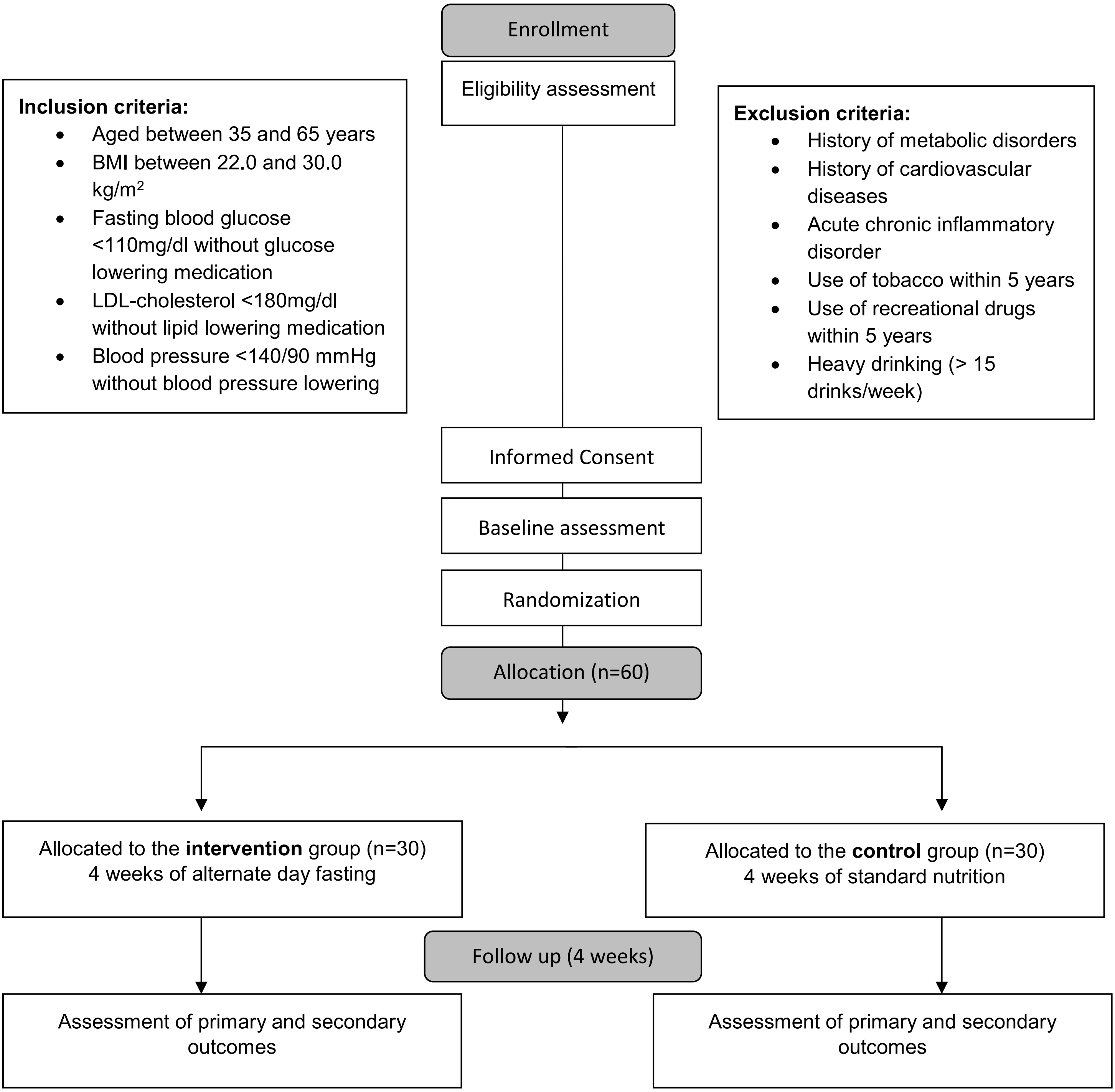

Figure 1: Schematic overview of study design

8 Subjects

First step was recruiting healthy participants for the pilot RCT via Primary Care and adverts. Inclusion and exclusion criteria were defined and eligible subjects were selected.

The inclusion criteria included following parameters: Age between 35 and 65 years, Body mass index in the range of 22.0 – 30.0 kg/m², Fasting blood glucose < 110mg/dl (without medication), LDL-cholesterol < 180 mg/dl (without medication), Blood pressure < 140/90 mmHg (without medication), Stable weight (change ± 10%) for 3 months immediately prior to the study, no history of metabolic disorders or cardiovascular disease, no acute or chronic inflammatory disorder, no current medications to regulate blood sugar and blood pressure or lipids or hormones. No heavy drinking (more than 15 drinks/week), no use of tobacco or recreational drugs within past 5 years and no dietary restrictions (e.g. vegetarianism and veganism).

Patients with known malignancy, women who were pregnant, breast-feeding or trying to become pregnant, patients with history or any chronic disease that could interfere with interpretation of study results, women or men on hormonal supplementation or anti-conceptive hormonal medication for at least two months were also excluded.

After eligibility for the study had been assessed, a clinician obtained the informed consent and performed a baseline assessment including medical history, medication and dietary habits of the participants.

The Institute of Medical Informatics, Statistics and Documentation of Medical University of Graz provided the “Randomizer tool”, a system used for the randomization in the pilot RCT.

8.1 Study design

The “Interfast” study is as a single centre, prospective, 2 years clinical trial. It is a cohort study with an integrated randomized controlled pilot trial (RCT). This thesis focuses on the randomized controlled pilot trial and specific cardiovascular measurements described below.

After recruiting the 60 participants, 30 of them were allocated to the intervention group and 30 to the control group (Figure 1). The intervention group had to perform 4 weeks of alternate day fasting (ADF), the control group was instructed to continue their standard nutrition for four weeks.

The ADF group followed a dieting regime where fasting days with zero caloric intake for 36 hours alternated with an ad-libitum day for 12 hours.

The visit schedule for participants in the pilot RCT included four visits, with visit two being 9 ± 4 days after visit one, visit three 2 weeks before visit four and visit four 4 weeks ± 1 week after visit 2. Time schedule for the visits is shown in table 3.

Measurements were performed by physicians and nurses specialised in diabetology, cardiology and ophthalmology in the study centre BioPersMed.

All procedures were conducted according to Good Clinical Practice (GCP) and approved by the Ethical Committee of the Medical University of Graz prior to the study.

Visit	1	2	3	4
Enrolment:				
Eligibility screen	X			
Informed consent	X			
Randomization		X		
Allocation	X			
Interventions:				
Alternate Day Fasting (ADF group)		◆——	———	——◆
Ingestion ad libitum (controls)	◆——	———	———	——◆
Measurements				

OGTT		X		X
Laboratory measurements including routine safety lab		X		X
24h ambulatory blood pressure monitoring	X		X	
Endothelial function (EndoPAT)		x		x
Retinal vessel analyser		x		x
Body composition (Dual-energy X-ray absorptiometry)		X		X
Echocardiography		X		X

Table 3 Schedule of enrolment, interventions and measurements

8.2 Measurements

8.2.1 Body composition

Fat and lean mass were measured in a total body scan with dual-energy-x-ray absorptiometry (DXA) by GE Lunar iDEXA (GE Healthcare, Waukesha, WI, US). The participants were asked to lay with their back down to be between the radiation source and the radiation detector.

2 X-rays, which differ on their energy level, are then beamed through the participant's body. The different tissue densities show depending on the energy of the x-ray different attenuation. The attenuation difference of each tissue is used to calculate the fat mass, lean mass and bone mineral content. (MA, 1996)

8.2.2 Blood sampling

Blood was withdrawn from a subcutaneous vein. By using a serum tube, lipid parameters such as total cholesterol, triglycerides, HDL, VLDL and LDL cholesterol were analysed. For the measurement of HbA1c an EDTA tube was used.

8.2.3 Oral Glucose Tolerance Test (OGTT)

For the oral glucose tolerance test (OGTT) participants were asked to undergo overnight fasting (12-h). When coming to the study centre of the Medical University of Graz a standard gauge cannula was laid in a subcutaneous vein, where a pre-meal blood sampling was made. After that, the participants were asked to drink a solution with 75g glucose (Glucoral 75 citron, Germania Pharmazeutika. Vienna) in 2-4 minutes. This was marked as time 0. Blood samples there then made after 15, 30, 60 and 120 minutes. During the process the cannula was flushed with 0,9% NaCl several times to prevent blood clotting. Fluoride oxalate tubes (1mL) were used for measurement of plasma glucose and serum tubes for analysing insulin and c-peptide.

Indices for insulin resistance are HOMA-IR (Homeostasis Model Assessment for Insulin Resistance) (Matthews DR, 1985) and ISI Insulin sensitivity index (Stumvoll M, 2001), for beta-cell function HOMA Beta (Yiqing Song, 2007).

$$HOMA-IR = \frac{FPG\left(^{mmol}/_{l}\right) * FSI(mU/I)}{22.5}$$

$$HOMA\ Beta = \frac{20 \times \mathrm{FSI}(mU/I)}{\left(FPG\ (mmol/l) - 3.5\right)}$$

$$ISI = 0.222 - 0.00333 \times BMI - 0.0000779 \times Ins120 - 0.000422\ \times age$$

FPG = Glc_0_base/Glc_0_end: mmol/L

FSI = Ins_0_base/Ins_0_end: mU/L

8.2.4 Non-invasive 24h blood pressure measurement

Participants were educated on how to put on the ambulatory blood pressure unit (Mobil-O-Graph, I.E.M, Germany) and received the necessary information to undergo the testing on a normal workday for a period of 24 hours. The participants could apply the device on a day appropriate for them. During the 24 hours, the device was programmed to inflate and record the blood pressure every 15 minutes during the daytime and 30 minutes during the night times, providing approximately 50-75 blood pressure recordings during the 24 hour period.

In classification of blood pressure (Whelton PK, 2017) systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg were seen as normal, 120-129 mmHg (systolic) and < 80 mmHg (diastolic) as elevated blood pressure, 130 – 139 mmHg (systolic) or 80-89mmg (diastolic) as hypertension stage 1 and systolic blood pressure above 140mmHg or diastolic blood pressure above 90 mmHg as hypertension stage 2.

8.2.5 Endothelial function measured with EndoPAT System

Endo-Pat 2000 (Itamar Medical Ltd., Casearea, Israel) uses reactive hyperemia induced dilation to measure endothelial function. To obtain a cardiovascular steady state the subject was in supine position for 15 minutes before measurement, being located in a quiet, temperature-controlled room with down-regulated light. Blood pressure was measured on the control arm, important for the occlusion phase, where the cuff inflates 60mmHg above systolic pressure.

The subject's index fingers were then positioned in probes, with a foam anchor ring at the base of the middle finger and a cuff on the non-dominant arm.

The procedure consists of 3 phases, each of which lasting 5 minutes to measure the pulse wave: the baseline recording period, the arterial occlusion recording period in which a cuff on the non-dominant arm is inflated 60 mmHg above systolic pressure to occlude the arterial flow and after rapidly releasing the cuff, the post occlusion period.

Hyperemia induced dilation indices derived from EndoPAT system are RHI and LnRHI. Reactive hyperemia index (RHI) is calculated automatically by the EndoPAT system using post-occlusion to pre-occlusion PAT (peripheral arterial tone) signal. RHI is normalized for baseline signal and indexed to the control arm.

Augmentation Index (AI) measures arterial stiffness, which is an independent risk factor for cardiovascular diseases (CVD) and also calculated by the EndoPat System. PAT pulses, systolic peak and reflected wave's peak are used for calculation of AI. AI HF75 is the augmentation index normalized to a heart rate of 75 (Itamar, 2017).

8.2.6 Dynamic vessel analyser

Dynamic vessel analysis took place at the department of ophthalmology of the Medical University of Graz. The dynamic vessel analyzer (Imedos, Jena, Germany) measures the response of retinal vessels to flicker light. Flicker light induces a NO-mediated dilation of retinal arterioles and venules, a parameter used for measuring the endothelial function (Stefan Palkovits, 2015).

Green illumination light (530-600nm), induced by a charge-couple device camera, and is reflected by the retina and the retinal vessels to a different degree. This difference is used to quantify the diameter of the retinal vessels (Stefan Palkovits, 2015).

Participants were asked to refrain from alcohol and caffeine containing products 12 hours prior to the examination. After arriving at the department of ophthalmology, topical tropicamide (Agepha 0,5%) was applied in the participant's eyes to obtain pupil dilation. Before the measurement, the patients were asked to rest in a light-dimmed room for 20 minutes.

The measurement starts with focusing the camera's illumination to form a ring of light at the anterior of the eye with an unilluminated circle in the centre of the pupil resulting in a fundus image uncontaminated by the reflection or glare from the camera. The brightness of the fundus camera light is adjusted to achieve optimal contrast between vessels and the surrounding tissues. The patient's fixation was improved by using a fixation target, making sure that the site of interest was in the middle of the fundus picture.

A clinician marked, axially with a straight line, a temporal vein and an artery with a distance of one to two disc diameters from the optic disc margin before starting the measurement.

The measurement consists of 3 phases, each of them lasting 1 minute and measuring the diameter of the marked retinal arteriole and venule. The baseline recording is obtained with one minute of steady illumination, followed by one minute of flicker light stimulation. A post-flicker baseline recording for another minute ends the cycle.

Decreased vasodilation during flicker light simulation is a sign for endothelial dysfunction and was observed to be also reduced in diabetes, hypertension and high blood lipids. (Lim M, 2012)

The DVA measurements assess dilation of the retinal arteriole and venule by comparing baseline recording with flicker-light recording (Stefan Palkovits, 2015).

$$Dilation\ (\%) = \frac{Flicker - Pre_{Flicker}}{Pre_{Flicker}} * 100$$

8.2.7 Echocardiography

Echocardiography was performed transthoracically only (TTE). It took place in either the study centre of the Divison of Endocrinology and Diabetology, Billrothgasse 12, or the outpatient clinic of cardiology, Medical University Graz. The examination took place in a darkened room and was performed with a Vivid 7 or a Vivid 9 (both GE Healthcare, Chalfont St Giles UK), with at least 60 frames per second. All loops and images were recorded by one investigator and then analysed by a second blinded investigator. The participants were asked to undress their upper body and to lie on a patient's bed in the steep left-lateral decubitus position, with their left arm raised. The clinician recorded and analysed images and loops of the heart by using views of the parasternal long and short axis and apical axis (2-, 3-, 4- and 5-chambers), making use of 2-D real-time, M-Mode, PW-, CW-, tissue and colour Doppler. Focus was set on the evaluation of the left ventricular dimensions, the systolic function and the diastolic parameters following the current recommendations of chamber quantification and functional evaluations (Lang RM, 2015). This thesis will set focus on LVMI (Foppa M., 2005), LAVI (Lang RM, 2015), E/e' (Sherif F. Nagueh, 2016) and EF (Lang RM, 2015). Normal value for EF = ≥ 55%, for E/e' = 4 ± 1, for LVMI 43-95 g/m^2 and for LAVI 22 ± 6 mL/m^2 (Lang RM, 2015).

$$\mathrm{LVMI} = \frac{\mathrm{LVM}}{\mathrm{BSA}}$$

$$\mathrm{LAVI} = \frac{\mathrm{LAV}}{\mathrm{BSA}}$$

$$E/e' = E / ((e'_{lateral/}\ e'_{medial}) /2)$$

$$\mathrm{EF} = \left(\frac{\mathrm{SV}}{\mathrm{EDV}}\right) * 100$$

8.3 Statistical analyses

The Kolmogorov-Smirnov test was used for evaluation of data distribution. Normally distributed data are expressed with means ± deviation, non-normally distributed data with median and interquartile range.

To show the differences between baseline and 4 weeks parameters the delta value was calculated. The delta value of RCT ADF und RCT control group was then compared by parametric and non-parametric test as appropriate. A p-value < 0.05 was considered statistically significant. The statistic program used for all calculation was SPSS 19.0 software (SPSS Inc, Chicago, III).

9 Results

9.1 Baseline characteristics

Table 4 Baseline Characteristics

	RCT ADF	RCT control
Number	30	30
Sex (female/male)	12/18	12/18
Age (years)	48.4 ± 7.2	50.1 ± 7.4
Height (cm)	173 ± 8	172 ± 10
Weight (kg)	76.5 ± 9.9	76.4 ± 12.9
Hip circumference (cm)	100.5 ± 6.1	99.2 ± 7.8
Waist circumference (cm)	90.3 ± 8.4	90.1 ± 9.4
Waist to hip ratio	0.9 ± 0.1	0.9 ± 0.1
BMI (kg/m²)	25.5 ± 1.8	25.7 ± 2.4
Blood pressure systolic (mmHg)	121 ± 11	118 ± 8
Blood pressure diastolic (mmHg)	76 ± 10	77 ± 9
HbA1c (mmol/L)	34.7 ± 2.6	34.8 ± 3.4
Fasting blood glucose (mg/dL)	78 ± 8	78 ± 8

60 participants were eligible for the pilot RCT of Interfast. 30 were allocated to the ADF group, 30 to the control group. Each group had 12 (40%) female and 18 (60%) male participants. Summarized, as shown in table 4, no significant difference in baseline characteristics was found between the RCT ADF and RCT control group.

9.2 Body composition

Table 5: Parameters of body composition measured with DXA

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value[1]
Fat mass (kg)	25.10 ± 5.05	22.83 ± 4.97	-2.12 ± 1.04	24.19 ± 6.94	23.54 ± 6.5	-0.15 ± 0.84	< 0.0001
Lean mass (kg)	49.17 ± 9.19	48.13 ± 9.37	-1.31 ± 0.99	50.04 ± 11.53	49.90 ± 11.36	-0.05 ± 0.97	< 0.0001

4 weeks of alternate day fasting resulted in a significant reduction in fat mass and lean mass in the ADF group compared to the control group.

Fat mass in the ADF group was 25.10 ± 5.05 kg at baseline and 22.82 ± 4.97 kg after 4 weeks, whereas the control group had 24.19 ± 6.94 kg at baseline and 23.54 ± 6.50 kg after 4 weeks with a p-value < 0.0001 (for group comparison).

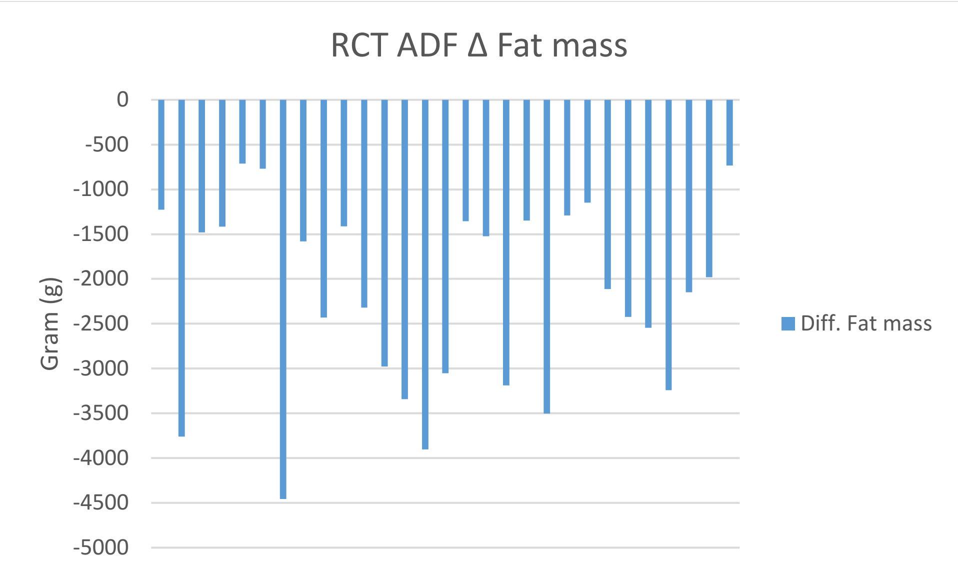

Figure 2: RCT ADF diff. fat mass (single participant data)

[1] For group comparison

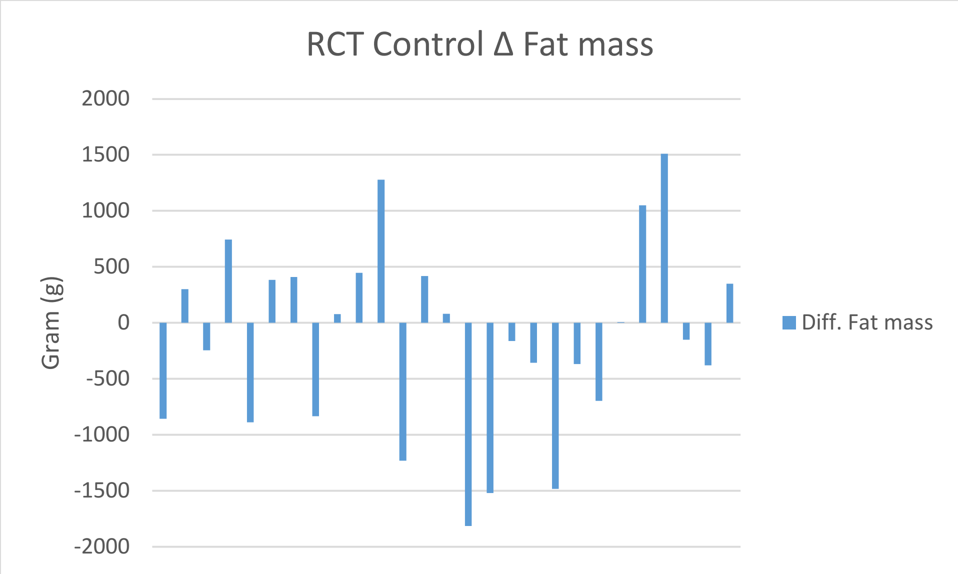

Figure 3: RCT control diff. fat mass (single participant data)

Lean mass in the ADF group changed from 49.17 ± 9.19 kg at baseline to 48.13 ± 9.37 kg after 4 weeks, in the control group 50.04 ± 11.53 kg to 49.88 ± 11.36 kg. P-value < 0.0001 (for group comparison).

Changes in lean mass were significant in the RCT ADF group after 4 weeks compared to the RCT control group as shown in figure 4 and 5.

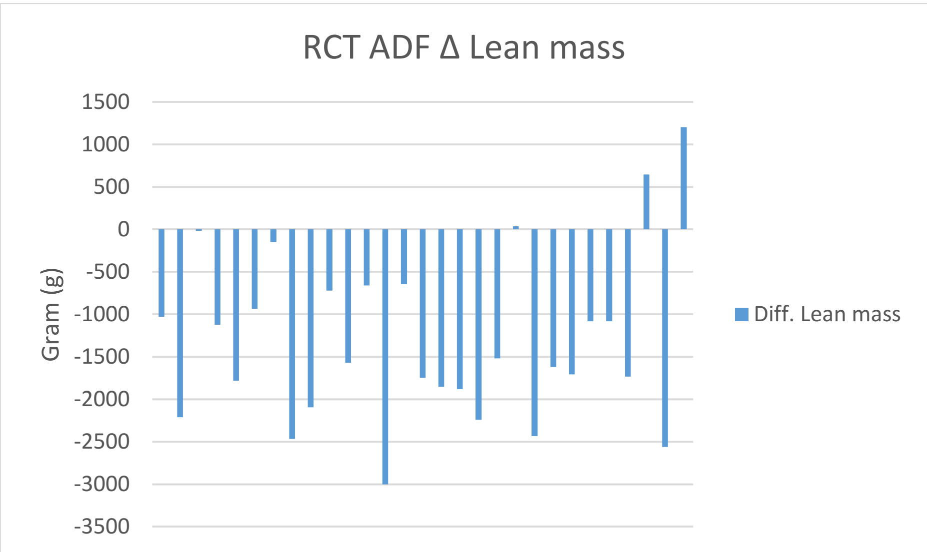

Figure 4: RCT ADF diff. lean mass (single participant data)

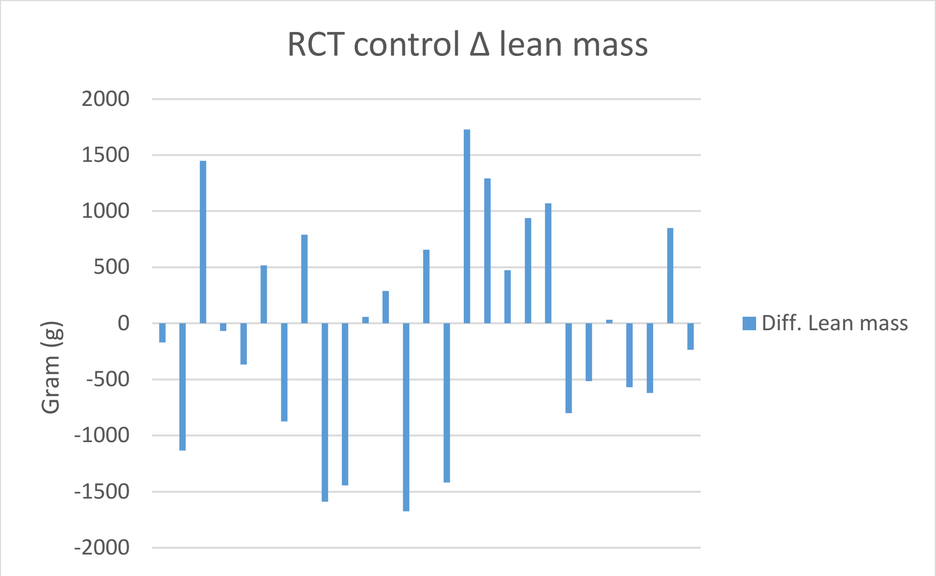

Figure 5: RCT control diff. lean mass (single participant data)

9.3 Non-invasive 24h ambulatory blood pressure monitoring

Table 6: Parameters of non-invasive 24h ambulatory blood pressure monitoring

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value[2]
Systolic blood pressure (mmHg)	121 ± 11	116 ± 10	-5 ± 8	118 ± 8	121 ± 13	2 ± 14	0.0475
Diastolic blood pressure (mmHg)	76 ± 10	74 ± 9	-2 ± 5	77 ± 9	77 ± 8	0 ± 9	0.1968

Alternate day fasting over a period of 4 weeks showed significant improvement of the systolic blood pressure in the ADF group compared to the control group, whereas the diastolic blood pressure did not show any significant difference between the two groups.

Systolic blood pressure in the ADF group was 121 ± 11 mmHg at baseline and 116 ± 10 mmHg after 4 weeks. Diastolic blood pressure in the ADF group was 76 ± 10 mmHg at baseline and 74 ± 9 mmHg after 4 weeks.

In the control group, systolic blood pressure at baseline was 118 ± 8 mmHg and 121 ± 13 mmHg after 4 weeks, diastolic blood pressure was 77 ± 9 mmHg at baseline and 77 ± 8 mmHg after 4 weeks

[2] For group comparison

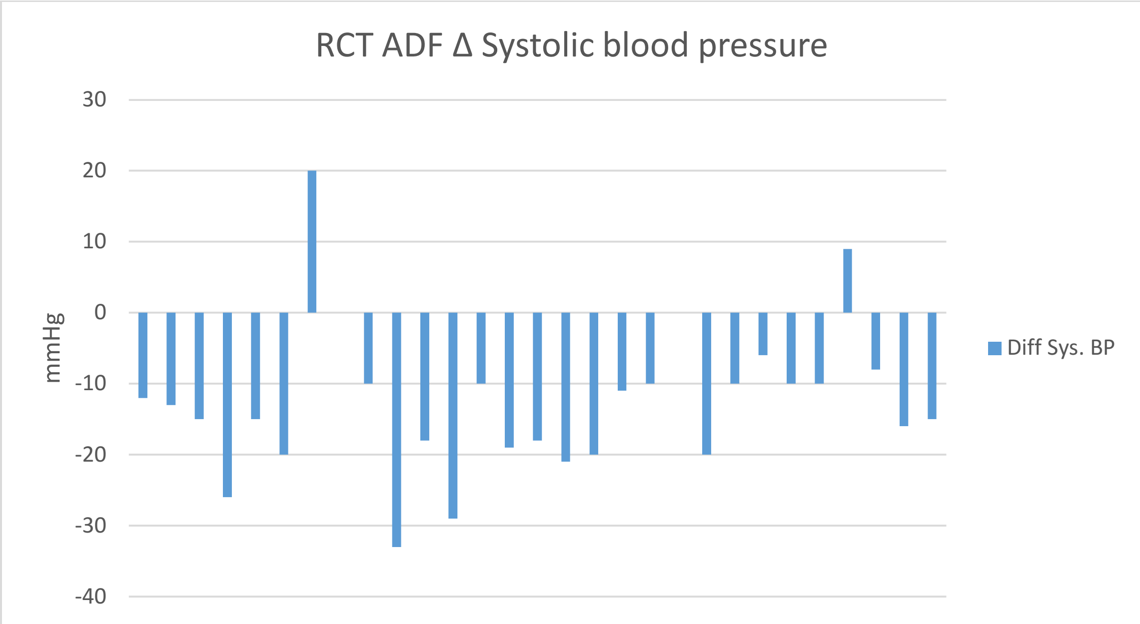

Figure 6: RCT ADF diff. systolic blood pressure (single participant data)

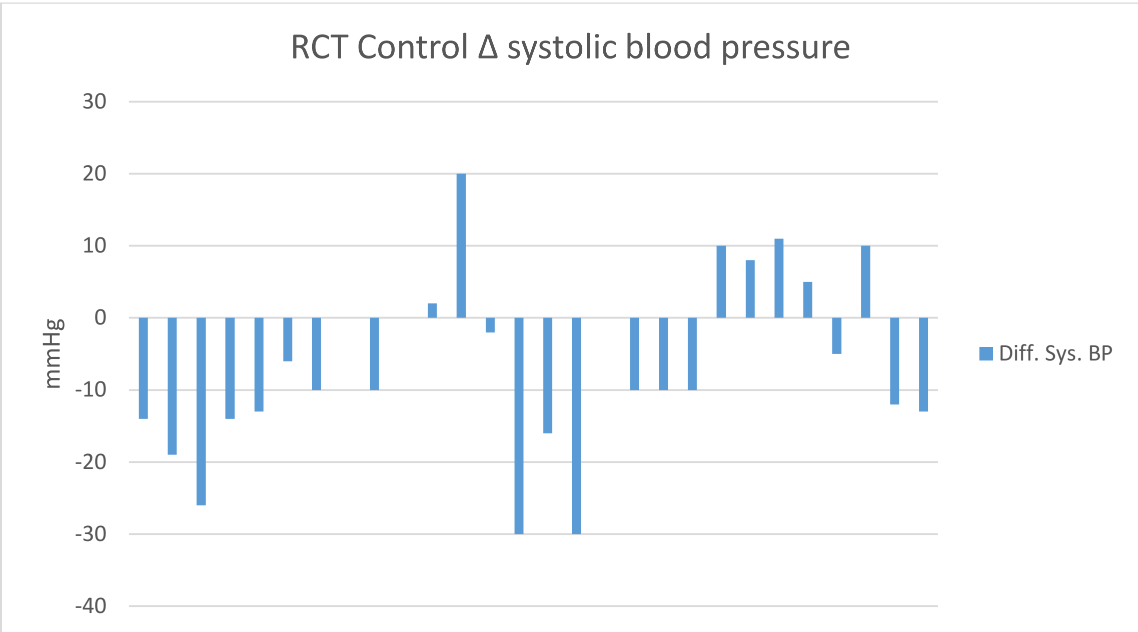

Figure 7: RCT control diff. systolic blood pressure (single participant data)

Systolic blood pressure showed a significant p-value (0.0475) when comparing the RCT ADF and RCT control group as shown in figure 12 and 13. P-Value for diastolic blood pressure was not significant (0.1968).

9.4 Blood lipids

Table 7: Blood lipids measured in serum from blood sampling

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value[3]
Total cholesterol (mg/dL)	209.3 ± 31.6	202.0 ± 34.0	7.1 ± 22.8	234.2 ± 42.5	223.1 ± 34.0	6.4 ± 22.0	0.965
Triglyceride (mg/dL)	85.0 ± 34.9	74.5 ± 31.9	10.8 ± 35.1	88.8 ± 48.7	80.0 ± 40.1	7.5 ± 25.8	0.894
HDL (mg/dL)	59.6 ± 21.8	62.6 ± 18.3	3.0 ±19.9	62.5 ± 20.1	64.4 ± 22.6	2.0 ± 12.0	0.865
VLDL (mg/dL)	19.3 ± 8.3	18.6 ± 8.6	0.6 ± 12.4	20.0 ± 10.1	20.9 ± 9.3	1.0 ± 9.0	0.404
LDL (mg/dL)	127.7 ± 30.0	117.3 ± 24.4	9.8 ± 25.3	148.0 ± 42.1	133.3 ± 34.1	10.5 ± 20.7	0.577

ADF for 4 weeks did not result in significant changes in blood lipids such as total cholesterol, triglycerides, HDL, VLDL or LDL as it is shown in table 7 between the ADF group and the control group.

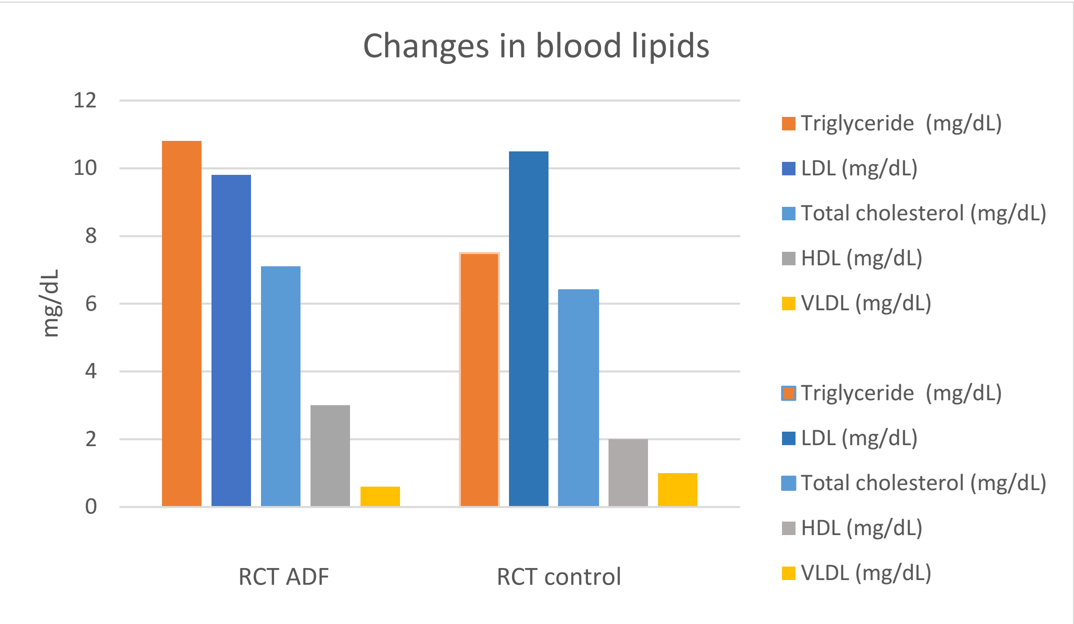

Figure 8: Changes in blood lipids

[3] For group comparison

9.5 HbA1c, fasting glucose, insulin sensitivity and beta-cell function

Table 8: Indices of insulin sensitivity and beta cell function

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value
HbA1c (mmol/L)	34.7 ± 2.6	33.7 ± 2.5	1.0 ± 1.7	34.8 ± 3.4	34.0 ± 3.5	0.7 ± 1.1	0.552
Fasting glucose (mg/dL)	78 ± 8	78 ± 9	1 ± 10	76 ± 8	76 ± 7	1 ± 8	0.316
HOMA IR (Score)	1.43 ± 0.77	1.50 ± 0.80	0.05 ± 0.95	1.34 ± 0.85	1.45 ± 0.87	0.006 ± 0.80	0.8310
HOMA Beta (%)	93.04 ± 73.11	112.22 ± 63.78	17.47 ± 76.22	84.18 ± 53.52	117.17 ± 60.82	33.45 ± 73.62	0.909
ISI	0.10 ± 0.01	0.10 ± 0.01	0.00 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.00 ± 0.02	0.23

HbA1c was measured at baseline and after 4 weeks of alternate day fasting in both the ADF (34.7 ± 2.6 mmol/L to 33.7 ± 2.5 mmol/L) and the control group (34.8 ± 3.4 mmol/L to 34.0 ± 3.5 mmol/L), but no significant difference (p = 0.552) was observed.

In fasting blood glucose the baseline in the ADF group was 78 ± 8 mg/dL and 78 ± 9 mg/dL after 4 weeks, in the control group parameters were 76 ± 8 mg/dL at baseline and 76 ± 7 mg/dL after 4 weeks. (p = 0.316)

The change in HOMA IR was also not different between the two groups (p-value = 0.8310). In the ADF group, baseline to 4 weeks parameters were 1.43 ± 0.77 to 1.50 ± 0.80. In the control group, baseline und 4 weeks HOMA IR were 1.34 ± 0.85 and 1.45 ± 0.87, respectively.

Likewise neither the change in HOMA Beta (p = 0.909) nor in ISI (p = 0.23) was significantly different between the two groups.

9.6 Endothelial function

Table 9: Parameters of endothelial function

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value[4]
EndoPAT							
RHI	1.83 ± 0.65	1.68 ± 0.73	-0.14 ± 0.94	1.99 ± 0.49	2.04 ± 0.42	0.04 ± 0.54	0.0649
LnRHI	0.56 ± 0.40	0.57 ± 0.27	0.02 ± 0.50	0.66 ± 0.24	0.69 ± 0.20	0.03 ± 0.26	0.1183
AI	7.30 ± 16.69	9.22 ± 21.70	2.62 ± 20.94	8.10 ± 21.35	9.28 ± 16.66	2.02 ± 18.11	0.9580
AI Hf75	1.24 ± 15.95	0.27 ± 20.64	0.04 ± 19.09	1.33 ± 17.34	-0.19 ± 16.97	-1.65 ± 13.40	0.7803
DVA							
Dilation Arteriole (%)	3.77 ± 2.40	3.57 ± 2.37	-0.40 ± 1.13	2.70 ± 2.31	3.72 ± 3.03	0.51 ± 1.98	0.276
Dilation Venule (%)	5.07 ± 2.32	5.18 ± 2.39	0.20 ± 1.43	4.42 ± 3.33	3.72 ± 3.03	0.52 ± 1.66	0.656

The EndoPat parameters RHI, LnRHI, AI and AI Hf75 did not show significant changes after 4 weeks of alternate day fasting compared to the control group.

In the ADF group RHI in baseline 1.83 ± 0.65 changed to 1.68 ± 0.73 after 4 weeks. In the control group baseline was 1.99 ± 0.49 and changed to 2.04 ± 042. P-value = 0.0649.

LnRHI in the ADF group was 0.56 ± 0.40 at baseline and 0.57 ± 0.27 after 4 weeks. The control group had 0.66 ± 0.24 at baseline and 0.69 ± 0.20 after 4 weeks. (p = 0.1183)

AI baseline to 4 weeks in the ADF group was 7.30 ± 16.69 to 9.22 ± 21.70. Baseline to 4 weeks in the control group was 8.10 ± 21.35 to 9.28 ± 16.66, overall p-value was not significant (0.9580).

[4] For group comparison

AI Hf75 baseline in the ADF group was 1.24 ± 15.95 and 0.27 ± 20.64 after 4 weeks. Baseline in the control group was 1.33 ± 17.34, after 4 weeks -0.19 ± 16.97, also not being significant (P-value = 0.7803).

Parameters of the DVA (dilation of arteriole and venule in %) both did not show significant changes. Dilation of the arteriole in the RCT ADF group was 3.77 ± 2.40 % at baseline and 3.57 ± 2.37 % after 4 weeks. In the RCT control group dilation of the arteriole was 2.70 ± 2.31 % at baseline and 3.72 ± 3.03 % after 4 weeks. Dilation of the venule was in the RCT ADF group at baseline 5.07 ± 2.32 % and 5.18 ± 2.39 % after 4 weeks. Baseline to 4 weeks in the RCT control group was 4.42 ± 3.33 % and 3.72 ± 3.03 %. P-value for the comparison between the RCT ADF group and the RCT control group after 4 weeks for dilation of the arteriole was 0.276 and for dilation of the venule 0.656.

9.7 Echocardiography

Table 10: Parameters of echocardiography

	RCT ADF Baseline	RCT ADF 4 weeks	Δ	RCT control Baseline	RCT control 4 weeks	Δ	p-Value
LVMI (g/m²)	88.25 ± 21.41	89.23 ± 19.33	-1.08 ± 19.67	92.01 ± 20.71	89.00 ± 19.39	-2.41 ± 15.25	0.9589
LAVI (ml/m²)	27.26 ± 7.56	29.83 ± 6.73	0.98 ± 7.29	28.25 ± 7.39	29.23 ± 7.40	1.04 ± 6.33	0.7142
EF (%)	63.3 ± 4.8	60.9 ± 6.1	-2.6 ± 6.7	61.3 ± 6.5	61.8 ± 8.9	1.4 ± 8.6	0.128
E/e’	7.09 ± 1.80	7.28 ± 2.07	-0.04 ± 1.61	7.55 ± 2.17	7.37 ± 2.04	-0.32 ± 1.28	0.7536

Within echocardiography, also no significant results could be observed.

LVMI in the ADF group changed from 88.25 ± 21.41 g/m² in the baseline to 89.23 ± 19.33 g/m² after 4 weeks. The control group had the following parameters at baseline and after 4 weeks: 92.01 ± 20.71 g/m² and 89.00 ± 19.38 g/m². P-value was 0.9589.

LAVI was in the ADF group at 27.26 ± 7.56 mL/m² at baseline and at 29.83 ± 6.73 mL/m² after 4 weeks. The control group had 28.25 ± 7.39 mL/m² at baseline and 29.23 ± 7.40 mL/m² after 4 weeks. The p-value for LAVI was 0.7142.

EF parameters also were not significant (p-value = 0.128). Baseline to week-4 parameters in the ADF group were 63.3 ± 4.8 % and 60.9 ±6.1 %. In the control group, parameters were 61.3 ± 6.5 % (baseline) and 61.8 ± 8.9 % (4 weeks).

E/e’ was 7.09 ± 1.80 at baseline and 7.28 ± 2.07 after 4 weeks in the ADF group. In the control group, parameters at baseline were 7.55 ± 2.17 and after 4 weeks 7.37 ± 2.04. P-value was 0.7536.

10 Discussion

Our study shows, that alternate day fasting for a period of 4 weeks is an effective strategy for moderate weight loss in healthy individuals, in particular reduction of fat mass. Also, a reduction on lean body mass was observed.

Beneficial effects of 4 weeks of ADF have also been observed for systolic blood pressure, whereas diastolic blood pressure was not affected. No significant changes in blood lipids, glucose metabolism, endothelial function and heart parameters which were analysed through echocardiography could be observed. Adverse effects were not reported during our study.

The primary goal of this study was to investigate short term effects of alternate day fasting on weight loss and cardiovascular parameters in healthy subjects, as most of the previous ADF studies included overweight or obese subjects.

Conflicting results for weight loss in healthy subjects were found in two short-term ADF studies. 3 weeks of ADF in 16 subjects lead to a significant weight loss and reduction of fat mass in a study by (Heilbronn LK, 2005). 2 weeks of ADF in 8 subjects did not lead to change in body weight as reported by (Halberg N, 2005). This may be due to a small number of subjects and the short trial duration. We show, that 4 weeks of ADF in 30 healthy subjects lead to a significant decrease in fat mass and lean body mass compared to a control group who did not practice ADF.

Between the ADF and the RCT group we observed a significant decrease in the systolic blood pressure. The diastolic blood pressure was not changed significantly. A study by (Varady KA, 2009), where 16 obese subjects performed alternate day fasting for 8 weeks, showed similar results. In this study, systolic blood pressure was decreased significantly, whereas the diastolic blood pressure was not affected. No changes in systolic or diastolic blood pressure were reported by (Heilbronn LK, 2005), where healthy subjects were on an ADF diet for 3 weeks. No change in systolic or diastolic blood pressure was also observed by (Trepanowski, et al., 2017) in a study with obese subjects who were performing ADF for 6 months. The results are conflicting regarding systolic blood pressure but it seems to be evident that diastolic blood pressure cannot be influenced by ADF neither in healthy nor obese and neither short-term nor long-term.

Blood lipids such as total cholesterol, HDL, LDL, VLDL and triacylglycerols were investigated in our study but did not show significant changes after 4 weeks of ADF. (Varady KA, 2013) found significantly decreased triacylglycerols in 16 healthy subjects after performing ADF for a period of 12 weeks compared to a control group but also no significant changes for total cholesterol, LDL and HDL. Other effects were found in obese subjects by (Varady KA, 2015) indicating that obese subjects on an ADF diet benefit more regarding blood lipids than healthy individuals. For this study 29 obese women performed ADF for a period of 8 weeks and total cholesterol, LDL cholesterol and triacylglycerol decreased significantly.

We also investigated effects on glucose metabolism by measuring HbA1c, fasting glucose, HOMA IR, HOMA beta and ISI but found no significant changes compared to our control group. No change in fasting blood glucose was also observed by (Heilbronn LK, 2005) after 3 weeks of ADF in healthy individuals. Interestingly, also in overweight and obese subjects no changes in fasting blood glucose could be observed by (Samira Eshghinia, 2013) after 4 weeks of ADF. In a study with obese subjects, fasting plasma glucose did also not change significantly after 6 months of ADF compared to a control group (Trepanowski, et al., 2017).

Our study was the first to investigate endothelial function via EndoPAT and DVA for alternate day fasting. Measured parameters were the RHI and LnRHI via EndoPat and Dilation in % of arterioles and venules via the dynamic vessel analysis (DVA). No significant changes after 4 weeks of ADF could be found in relation to the control group. We were also the first to measure arterial stiffness, again with the EndoPat system, via the AI (Augmentation Index) but did not observe significant changes in relation to the control group.

Echocardiography parameters such as the left ventricular mass index, the left atrial volume index, ejection fraction and E/e’ for diastolic function were investigated in our study but showed no significant results.

Dietary adherence was very high during our 4 week trial with only three recorded dropouts. There was one dropout of a female participant in the ADF group due to strong feelings of hunger and therefore difficulty in adhering to the fasting protocol. One dropout was in the control group because of not meeting the inclusion criteria (too high blood lipids) and another dropout was a female subject from the control group that wanted to be in the ADF group. A dropout rate of 3.33% in the ADF group is comparable to another study by (Varady KA, 2013) with one dropout in 16 healthy subjects performing ADF for 12 weeks. This may indicate that adherence to the ADF protocol is manageable for healthy, non-obese subjects.

In conclusion, alternate day fasting is an effective fasting protocol for achieving moderate weight loss in healthy individuals for a period of 4 weeks. Systolic blood pressure does improve, but other cardiovascular risk factors did not change significantly. Subjects performing ADF had no problem adhering to our fasting protocol.

Further studies, investigating long-term effects of ADF in healthy subjects and also in different patient populations, such as type 2 diabetes, need to be performed to further understand effects and mechanism behind alternate day fasting.

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