Aus dem Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

# Einfluss des Langzeitfastens auf kardiovaskuläre und metabolische Risikofaktoren bei Gesunden, NAFLD-Patienten und Hypertonikern

zur Erlangung des akademischen Grades
Doctor rerum medicinalium (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Franziska Grundler

aus Überlingen

Datum der Promotion: 04.03.2022

## Vorwort

Die Ergebnisse und Teile der vorliegenden Arbeit wurden veröffentlicht in *PLoS ONE* am 2. Januar 2019 (1), in *Nutrients* am 30. Oktober 2019 (2) und in *Journal of the American Heart Association* am 23. November 2020 (3).

**Inhaltsverzeichnis**


1. Zusammenfassung ..... 1
   1.1 Kurzfassung ..... 1
   1.2 Abstract (Englisch) ..... 2
   1.3 Einführung ..... 3
   1.4 Material und Methodik ..... 5
   1.5 Ergebnisse ..... 9
   1.6 Diskussion ..... 20
   1.7 Literaturverzeichnis ..... 26
2. Eidesstattliche Versicherung und Anteilserklärung ..... 31
3. Übersicht und Druckexemplare der ausgewählten Publikationen ..... 34
4. Lebenslauf ..... 100
5. Komplette Publikationsliste ..... 101
6. Danksagung ..... 102

# 1. Zusammenfassung

## 1.1 Kurzfassung

Fasten zeigte in Zell- und Tiermodellen vielfältige, protektive Wirkungen und eine Verlängerung der gesunden Lebensspanne. Chronische Überernährung und Bewegungsmangel verursachen weltweit einen Anstieg an Adipositas, nicht-alkoholischer Fettlebererkrankung (NAFLD) und Hypertonie. Strategien zur Prävention und Therapie, die kardiovaskuläre Begleit- und Folgeerkrankungen miteinschließen, sind erforderlich. Deshalb untersucht die vorgelegte Arbeit die Effekte des Langzeitfastens (LF) bei Gesunden und Kranken.

Hierzu wurde eine einjährige, prospektive Observationsstudie an einer spezialisierten Klinik durchgeführt. Die Teilnehmenden fasteten mindestens 4 bis zu 41 Tage unter medizinischer Betreuung und nutritiver Energieaufnahme von ca. 250 kcal/Tag. In der ersten Publikation wurde zunächst der Einfluss auf kardiovaskuläre und metabolische Risikofaktoren untersucht. Bei 1422 Probanden reduzierte sich das Gewicht und der Bauchumfang proportional zur Fastenlänge von 5, 10, 15 und 20±2 Tagen, deutlicher bei Männern als bei Frauen. Triglyzeride, Gesamtcholesterin, Low-Density-Lipoprotein-Cholesterin und High-Density-Lipoprotein-Cholesterin sanken signifikant. Blutglukosespiegel wurden auf einen unteren Grenzwert unabhängig von der Fastenlänge reduziert. Ein Anstieg des Acetoacetats wurde im Urin nachgewiesen. In einer zweiten Publikation diente der Fettleberindex (FLI) als Surrogatmarker einer Fettleber (FLI≥60 Punkte). Insgesamt 264 von 697 Probanden hatten zu Beginn nach diesem Index eine Fettleber, die nach dem Fasten bei nur noch 144 Probanden nachweisbar war. Der FLI sank in der gesamten Kohorte um 14,0±11,7 Punkte ($p<0,001$). Bei Patienten mit Typ-2-Diabetes mellitus (T2DM), der bedeutendsten Risikogruppe für NAFLD, senkte sich der FLI um 5,1 Punkte stärker als bei Nicht-T2DM (n=38). Schließlich wurden bei 1610 Probanden Blutdruckveränderungen bei normotensiven (NT; n=920) sowie hypertensiven Subpopulationen (Blutdruck [BD]>140/90 mmHg), mit antihypertensiver (HTM; n=377) und ohne antihypertensiver Medikation (HTNM; n=313), ausführlich analysiert. In der gesamten Kohorte wurde eine Reduktion um 6,5/3,8 mmHg beobachtet. Die HTNM-Gruppe wies den höchsten initialen BD und die deutlichste Reduktion um 16,7/8,8 mmHg auf. Die HTM-Gruppe zeigte eine Reduktion um 7,3/4,7 mmHg, obwohl die Medikamenteneinnahme bei 23,6 % gestoppt und bei 43,5 % reduziert wurde. Eine moderate BD-Senkung wurde in der NT-Gruppe beobachtet. Insgesamt senkte längeres Fasten hohe BD- und FLI-Werte effizienter. Das subjektive Wohlbefinden stieg bei allen Fastenlängen. Unerwünschte Nebenwirkungen waren selten.

Zusammenfassend zeigte das LF in dieser Observationsstudie positiven Einfluss auf kardiovaskuläre und metabolische Risikofaktoren, die mit der Entstehung von NAFLD und Hypertonie assoziiert werden. LF erscheint als eine vielversprechende, nicht-pharmakologische, gut verträgliche, komplementärmedizinische Methode, die in randomisierten kontrollierten Langzeitstudien weiter überprüft werden sollte.

## 1.2 Abstract (Englisch)

Fasting has shown numerous protective effects and a prolonged healthy life span in cell and animal models. Chronic overeating and inactivity lead to an increase in obesity, non-alcoholic fatty liver disease (NAFLD) and hypertension worldwide. Strategies for prevention and therapy for these diseases and secondary cardiovascular diseases are needed. This work investigates the effects of long-term fasting (LF) on healthy and sick individuals.

A one-year prospective observational study was conducted at a specialized clinic. The participants fasted 4 to 41 days under medical supervision with a daily intake of approximately 250 kcal. In a first publication, the influence on cardiovascular and metabolic risk factors were assessed. A reduction in weight and waist circumference was found in 1422 subjects. This was proportional to the fasting duration of 5, 10, 15 and 20±2 days and more pronounced in men than women. Triglycerides, total cholesterol, low-density-lipoprotein-cholesterol, and high-density-lipoprotein-cholesterol decreased significantly. Blood glucose levels dropped to a lower level independently of the fasting duration. Urinary acetoacetate was increased. In a second publication, the fatty liver index (FLI) served as a surrogate marker for a fatty liver (FLI≥60 points). In total, 264 out of 697 subjects had a fatty liver before and only 144 subjects after fasting. The FLI decreased by 14.0±11.7 points ($p<0.001$). In patients with type 2 diabetes mellitus (T2DM), the most important risk group for NAFLD, the FLI decreased by 5.1 points more than in subjects without T2DM (n=38). Thirdly, changes in blood pressure (BP) in 1610 subjects were analysed in-depth in normotensive (NT; n=920) and hypertensive subjects (BP>140/90 mmHg) with (HTM; n=377) or without intake of antihypertensive medication (HTNM; n=313). An average reduction of 6.5/3.8 mmHg was observed. HTNM subjects had the highest initial BP and the strongest reduction by 16.7/8.8 mmHg. HTM subjects experienced a reduction of initial BP by 7.3/4.7 mmHg, even if drug intake was stopped by 23.6 % and reduced by 43.5 %. A moderate BP decrease was observed in NT subjects. Altogether, longer

fasting was more efficient in lowering high BP and FLI. Subjective well-being increased in all fasting duration groups. Adverse effects were rare.

In conclusion, this observational study showed positive effects of LF on cardiovascular and metabolic risk factors associated with the development of NAFLD and hypertension. LF appears to be a promising, non-pharmacological, well-tolerated, complementary approach that should be verified in randomized controlled long-term studies.

## 1.3 Einführung

Das Fasten ist eine evolutionär verankerte Fähigkeit von Mensch und Tier (4). Der zugrundeliegende Fastenstoffwechsel ermöglicht es Phasen eingeschränkter Nahrungsverfügbarkeit zu überdauern (5). In jüngster Zeit besteht großes Interesse am medizinisch eingesetzten Fasten, das als zeitlich begrenzter, freiwilliger Verzicht auf feste Nahrung definiert ist (6, 7). Unterschieden wird je nach Fastenlänge zwischen intermittierendem Fasten, das 12–48 Stunden dauert (8) und dem, in der vorliegenden Arbeit, untersuchten Langzeitfasten (LF), das mindestens drei Tage bis hin zu mehreren Wochen andauern kann (7). Kennzeichnend für das Fasten ist die Umschaltung des Stoffwechsels von der Energiegewinnung aus extern zugeführten Makronährstoffen, hin zur Lipolyse und Ketogenese aus körpereigenen Reserven (8). Freie Fettsäuren dienen im Fasten als Energiesubstrat und werden zu den Ketonkörpern Acetoacetat, β-Hydroxybutyrat und Aceton umgewandelt (8, 9). Diese metabolische Umstellung benötigt ca. 12–24 Stunden (10) und wird mit potentiell gesundheitsfördernden Wirkungen assoziiert, die hauptsächlich in Zell- und Tiermodellen erforscht wurden (4, 10). Dazu zählen eine Erhöhung der zellulären Stressresistenz (11), Verbesserung der kognitiven Leistungsfähigkeit (12), verbesserte Zellregeneration bei anschließender Nahrungswiedereinführung (13) und insgesamt einer Verlängerung der gesunden Lebensspanne (14, 15). Nur wenige und überwiegend ältere Humanstudien mit niedrigen Fallzahlen liegen für das LF vor, die unter anderem folgende Wirkungen beobachteten: Verbesserung kardiovaskulärer Risikofaktoren (16-18), Reduktion von Entzündungen (19, 20), Erhöhung der Autophagie (21) und Stimmungsaufhellung (22).

Der Anstieg der Adipositas geht mit einem Anstieg sekundärer Erkrankungen wie Diabetes mellitus Typ 2, Fettstoffwechselstörungen, nicht-alkoholischer Fettlebererkrankung (NAFLD) und Hypertonie einher (23, 24). Diese erhöhen das Risiko für Schlaganfall und Herzinsuffizienz

(25). Etwa ein Drittel aller weltweiten Todesfälle sind kardiovaskulär bedingt (25). 18 % der Deutschen sind adipös (26). Besonders stammbetonte Adipositas, gekennzeichnet durch viszerale Fettdepots, ist eng mit erhöhtem kardiovaskulären Risiko verbunden (27). Die Hypertrophie und -plasie der Adipozyten verursacht eine Dysfunktion des metabolisch aktiven Fettgewebes, wodurch eine systemische geringgradige Entzündung, Insulinresistenz, und Dyslipidämie entstehen kann (28). Erhöhte Triglyzeride (TG) und Low-Density-Lipoprotein-Cholesterin (LDL-C) sowie erniedrigtes High-Density-Lipoprotein-Cholesterin (HDL-C) tragen zu gesteigertem Arterioskleroserisiko bei (29). Das massive Einströmen freier Fettsäuren sowie ein übermäßiger Fruktosekonsum verursacht die Einlagerung von TG in die Leber (30). Übersteigt der Fettgehalt >5 % des Lebergewichts, unter Ausschluss von erhöhtem Alkoholkonsum, spricht man von einer nicht-alkoholischen Fettleber, die sich zu einer Leberentzündung, Fibrose und anschließenden Zirrhose weiterentwickeln kann (30). Zudem begünstigt die Dysregulation des Stoffwechsels bei Adipösen eine Erhöhung des Blutdrucks (BD) (28). Hypertonie ist definiert als BD-Werte ≥140/90 mmHg (31). Fast ein Drittel der Deutschen leidet unter Hypertonie mit steigender Prävalenz im Alter (32).

Die Therapie kardiovaskulärer Erkrankungen führt zu einer monetären Belastung des Gesundheitssystems (24, 33). Medikamentöse Behandlungsansätze zur BD-Regulation können zu Nebenwirkungen führen (34), die die Compliance beeinträchtigen (35) oder sind im Fall der der NAFLD noch nicht etabliert (36). Ein komplementärmedizinischer Ansatz wie das LF könnte eine nicht-medikamentöse Alternative sein (3). Für die Anwendung dieses traditionell, häufig religiös eingesetzten Verfahrens fehlen jedoch wissenschaftliche Belege (7). Daher wurde eine einjährige, prospektive Observationsstudie mit 1780 Fastenverläufen von Gesunden und Kranken in einer spezialisierten Fastenklinik durchgeführt. Dabei wurden die Effekte eines mehr als viertägigen, modifizierten Fastens nach dem Buchinger Wilhelmi Programm, das die Einnahme von ca. 250 kcal/Tag in Form von Fruchtsaft, Gemüsebrühe und Honig beinhaltete, wissenschaftlich dokumentiert.

Das Ziel der vorliegenden Arbeit ist es zu untersuchen, ob LF kardiovaskuläre und metabolische Risikofaktoren bei Gesunden und Kranken beeinflusst. Folgende primäre Schwerpunkte wurden deshalb in den drei vorgelegten Publikationen in geeigneten Kohorten untersucht:

1. Körpergewicht, Bauchumfang und Parameter des Lipid- und Glukosestoffwechsels (1)
2. Fettleberindex (FLI), ein nicht-invasiver Indikator der Leberverfettung, mit gesonderter Betrachtung von Patienten mit Typ-2-Diabetes mellitus (Typ-2-Diabetiker, T2DM) (2)

3. BD bei normo- und hypertensiven Personen, letztere unterteilt nach Einnahme antihypertensiver Medikation (3)

## 1.4 Material und Methodik

Die vorliegende Arbeit untersuchte die Daten einer einjährigen, prospektiven, unkontrollierten Observationsstudie, die in der Klinik Buchinger Wilhelmi in Überlingen in Kooperation mit dem Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie der Medizinischen Fakultät Charité – Universitätsmedizin Berlin durchgeführt wurde (1-3). Die Durchführung erfolgte nach den Richtlinien der Deklaration von Helsinki. Das Studienprotokoll wurde von den Ethikkommissionen der Charité Universitätsmedizin Berlin (Aktenzeichen EA4/054/15 vom 23.7.2015) und der Landesärztekammer Baden-Württemberg genehmigt (Aktenzeichen B-F-2015-063 vom 8.9.2015) und im Deutschen Register Klinischer Studien registriert (DRKS-ID: 00010111). Die Rekrutierung von 1901 Probanden aus insgesamt 3929 regulären Patienten der Klinik fand zwischen dem 1.1.2016 und dem 31.12.2016 statt (Abbildung 1). Volljährige Patienten, die sich ausreichend auf Deutsch, Englisch oder Französisch verständigen konnten und einen kontinuierlichen Aufenthalt von mehr als zehn Übernachtungen verbrachten, konnten teilnehmen. Das Vorliegen einer Kontraindikation des Fastens, definiert in den Leitlinien zur Fastentherapie (6), führte zum Ausschluss. Zudem wurden 121 Probanden von der Auswertung ausgeschlossen, die sich für eine Diät entschieden. Somit gaben insgesamt 1780 Probanden, nach vorangegangener Aufklärung und Bestätigung an keiner anderen Studie teilzunehmen, ihr schriftliches Einverständnis zur Teilnahme (1-3). Für die einzelnen Publikationen galten darüber hinaus noch folgende Ein- und Ausschlusskriterien (Abbildung 1):

Basis-Studie: Eine Blutabnahme zu Beginn und eine Zweite am Ende des Fastens wurde vorausgesetzt und nur Fastenlängen von 4–21 Tagen berücksichtigt (1).

FLI-Studie: Die Datenauswertung bezog sich auf die ersten 741 eingeschlossenen Probanden. Zwei Blutabnahmen, zu Beginn und am Ende des Fastens, waren obligatorisch, ebenso wie ein Body-Mass-Index (BMI) $\geq$19 kg/m² und eine Fastenlänge von $\geq$6 Tagen. Viral- oder autoimmunausgelöste Lebererkrankungen führten zum Ausschluss (2).

BD-Studie: Die BD-Messung am Morgen des ersten Fastentags musste vorliegen oder eine medikamentöse Behandlung der diagnostizierten Hypertonie bestehen. Eine Fastenlänge von

≥4 Tagen sowie der anschließende Kostaufbau in der Klink wurden vorausgesetzt. Außerdem war eine Blutabnahme zu Beginn erforderlich (3).

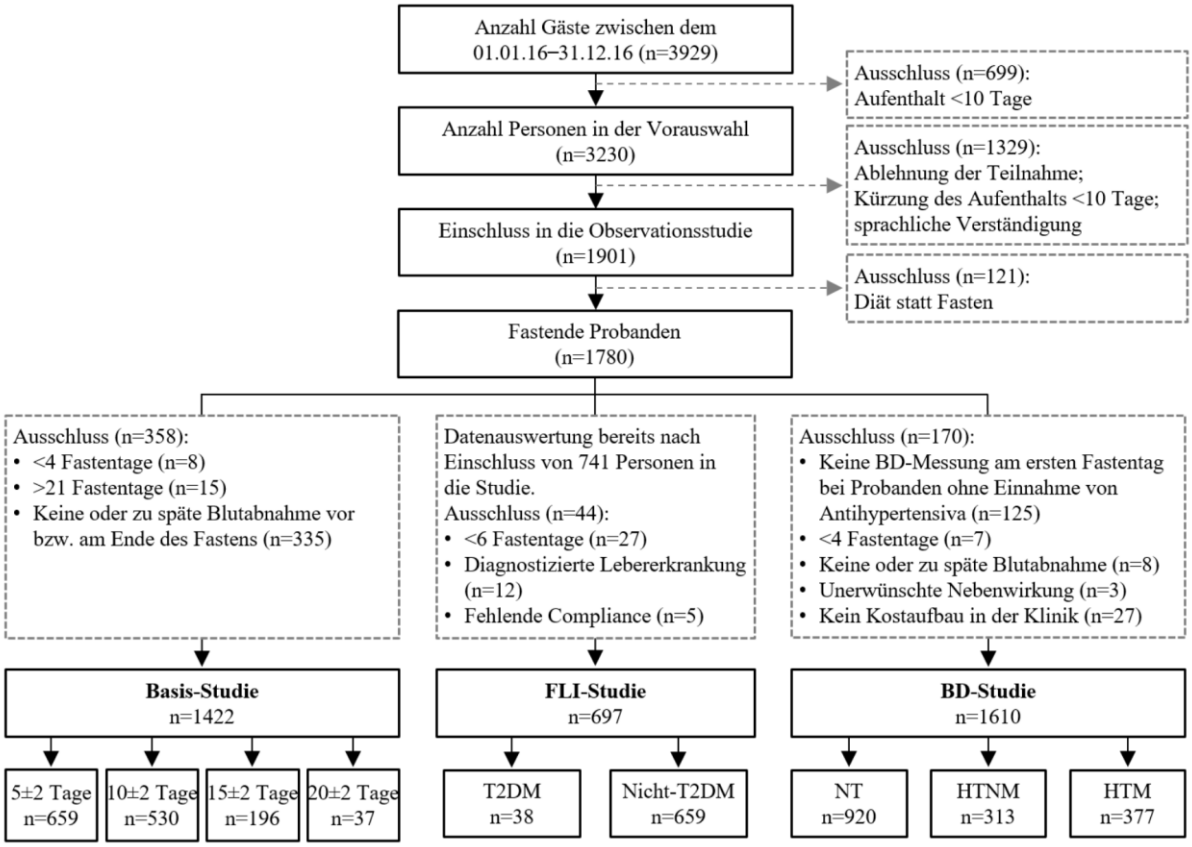



**Abbildung 1. Teilnehmer der einjährigen, prospektiven Observationsstudie und Aufteilung in drei geeignete Kollektive zur Analyse der Subgruppen:** Basis-Studie, unterteilt nach Fastenlänge; FLI-Studie unterteilt nach T2DM und BD-Studie unterteilt nach BD-Kategorie zu Beginn.
FLI, Fettleberindex; BD, Blutdruck; T2DM, Typ-2-Diabetiker; NT, normotensiv; HTNM, nicht-medikamentös behandelte Hypertoniker; HTM, medikamentös behandelte Hypertoniker. Adaptiert von (1-3).

## Fastenprotokoll

Alle Probanden fasteten nach dem Buchinger Wilhelmi Fastenprogramm (1), das auf den Leitlinien zur Fastentherapie basiert (6). Zur Vorbereitung absolvierten die Probanden einen Entlastungstag mit einer Energiereduktion auf 600 kcal, bestehend aus wahlweise Reis- bzw. Gemüsegerichten oder Obst. Das mehrtägige Fasten wurde mit einer Darmentleerung durch Einnahme von 20–40 g Natriumsulfat ($Na_2SO_4$), gelöst in 500 ml Wasser, eingeleitet. Die tägliche Aufnahme von je 250 ml Fruchtsaft und Gemüsebrühe sowie 20 g Honig stellte eine Energiezufuhr von ca. 250 kcal während des Fastens sicher. Zusätzlich wurde eine Flüssigkeitszufuhr von 2–3 l kalorienfreien Getränken empfohlen. Das Fastenbrechen erfolgte durch den Verzehr von Apfelmus zu Mittag und einer Gemüse-/Kartoffelsuppe am Abend des

letzten Fastentags. Die stufenweise Einführung von fester, vegetarischer Nahrung erfolgte über 3–4 Tage von 800–1600 kcal. Körperliche Aktivität, Anwendungen zur Darmreinigung und individuelle Therapieangebote ergänzten das Programm (1-3).

**Datenerhebung**

Die klinischen Daten wurden nach einem standardisierten Protokoll, in Einklang mit den Leitlinien zur guten klinischen Praxis, erhoben. Daten aus den Prüfbögen und die Tagebucheinträge der Probanden wurden in eine elektronische Datenbank (Research Electronic Data Capture (37)) übertragen (1-3). Diese wurden mit studienrelevanten Daten aus den Patientenakten, insbesondere der Medikationen, ergänzt (3). Die Fasteneignung wurde zu Beginn durch eine ärztliche Untersuchung geprüft. Hierbei wurden Diagnosen und die Einnahme von Medikamenten dokumentiert und gegebenenfalls Änderungen im Verlauf des Fastens durch den Arzt erfasst. Zusätzlich wurde die Körpergröße gemessen (Stadiometer: seca 285, Seca, Hamburg, Deutschland) und der Bauchumfang zu Beginn sowie am Ende des Fastens, mittig, horizontal zwischen der letzten Rippe und dem Beckenkamm mit einem Maßband ermittelt (1-3). Jeden Morgen wurde das Körpergewicht (Waage: Seca 704/635, Seca, Hamburg, Deutschland) durch eine Pflegekraft dokumentiert, während die Probanden nüchtern, leicht gekleidet und schuhlos waren. Der BMI wurde durch Körpergewicht (kg) / Körpergröße $(m)^2$ berechnet. BD und Puls wurden nach fünfminütiger Ruhepause einmalig, im Sitzen, am nicht-dominanten Arm gemessen (Boso Carat professional, BOSCH + SOHN GmbH u. Co. KG, Jungingen, Deutschland) (1-3).

Routinemäßig wurde eine Blutabnahme entweder nüchtern am Morgen des Entlastungstags oder dem ersten Fastentag und eine zweite Blutabnahme am Tag des Fastenbrechens oder zuvor durchgeführt. Die Analyse erfolgte in einem zertifizierten Hochdurchsatzlabor (MVZ Labor Ravensburg GbR, Ravensburg, Deutschland) nach Standardverfahren, Details siehe Publikationen (1-3).

Der FLI ist eine Methode zur Bestimmung der Wahrscheinlichkeit einer Leberverfettung (38). Er wurde berechnet mit folgender Formel nach Bedogni et al. (38): $FLI = (e^{0,953 * \log e (TG) + 0,139 * BMI + 0,718 * \log e (\text{Gamma-Glutamyl-Transferase [GGT]}) + 0,053 * \text{Bauchumfang} - 15,745}) / (1 + e^{0,953 * \log e (TG) + 0,139 * BMI + 0,718 * \log e (GGT) + 0,053 * \text{Bauchumfang} - 15,745}) * 100$.

Die semi-quantitative, selbständige Messung des Ketonkörpergehalts (Acetoacetat) im täglichen Morgenurin wurde mit Teststreifen (Ketostix, Bayer AG, Leverkusen, Deutschland) durchgeführt (1). Die Probanden dokumentierten zudem in einem Studientagebuch unter

Aufsicht einer Pflegekraft folgende Daten: Täglich wurde das emotionale und körperliche Wohlbefinden auf numerischen Skalen von 0 (sehr schlecht) bis 10 (hervorragend) bewertet (1). Der WHO-5-Wohlbefindensindex (WHO-5) spiegelte die positive, mentale Grundeinstellung wieder. Fünf Aussagen wurden zwischen 0 (nie) und 5 (zu jeder Zeit) beurteilt, und deren Summe mit vier multipliziert, um einen Wert zwischen 0 % und 100 %, dem bestmöglichen Wohlbefinden, zu erhalten (39). Der WHO-5 bezog sich auf die vergangenen zwei Wochen vor Beginn und die Zeit während des Fastens (3). Das Entspannungsniveau wurde auf einer numerischen Skala von 0 (bedrohliche Spannung) bis 10 (totale Entspannung) vor und am Ende des Fastens dokumentiert (3). Ärzte dokumentierten unerwünschte Nebenwirkungen (1-3).

**Statistische Auswertung**

Aufgrund des Studiendesigns, einer prospektiven Observationsstudie, wurde als Ziel die Rekrutierung von mehr als 1000 Probanden gesetzt, um eine explorative Datenanalyse durchzuführen. Fehlende Werte wurden nicht ersetzt. Die Referenztage vor und nach dem LF variierten zwischen den drei Auswertungen. Sie bezogen sich entweder auf den ersten Fastentag und den Tag der zweiten Laboruntersuchung, falls die Messungen fehlten auf den jeweiligen Vortag (1), oder auf die Messungen am ersten Aufenthaltsmorgen und den Tag der zweiten Laboruntersuchung und wiederum falls die Messungen fehlten auf den Vortag (2) bzw. auf den ersten Fastentag und das Fastenbrechen (3).

Basis-Studie: Gruppenunterschiede zu Beginn wurden mit einer Einweg-Varianzanalyse (ANOVA) mit Tukey's post-hoc Test geprüft. In einem mehrstufigen parsimonischen Modell wurde zunächst mit Hilfe eines linearen gemischten Modells die Wirkung des Fastens berechnet. Dabei wurden Messwiederholungen, Fastenintervention, Dauer, Geschlecht, und die Interaktionen Fasten*Dauer, Fasten*Geschlecht, Geschlecht*Dauer und der Ausgangswert des Outcomes als feste Effekte verwendet. Die Kovarianz-Struktur, entweder „zusammengesetzt symmetrisch", „autoregressiv erster Ordnung" oder „Varianzkomponenten", wurde je nach Bayessches Informationskriterium gewählt. Nicht signifikante Interaktionen wurden anschließend wieder aus dem Modell entfernt, um dem Prinzip der Parsimonität gerecht zu werden. Statistische Signifikanz wurde mit $p<0,01$ definiert und die Daten als Mittelwert±Standardfehler angegeben. Die Datenanalyse wurde mit SAS Version 9.4 (SAS Institute, Cary, North Carolina, USA) durchgeführt und die Graphiken mit GraphPad Prism Version 6 (GraphPad Software, La Jolla, Kalifornien, USA) erstellt (1).

FLI-Studie: Mit Hilfe des D'Agostino-Pearson Normalitätstests wurde die Normalverteilung geprüft. Gruppenunterschiede zu Beginn wurden für stetige Variablen mit dem ungepaarten t-Test bzw. dem Mann-Whitney-U-Test berechnet sowie dem Chi-Quadrat-Anpassungstest und dem exakten Fisher-Test für kategoriale Variablen. Vorher-nachher-Unterschiede wurden mit dem gepaarten t-Test bzw. dem Wilcoxon-Rangsummen-Test untersucht. Korrelationsanalysen wurden mit dem Pearson bzw. Spearman Korrelationskoeffizienten durchgeführt. Statistische Signifikanz wurde mit $p<0,05$ definiert und die Daten als Mittelwert±Standardabweichung angegeben. Die Datenanalyse wurde mit SPSS Version 24.0 (IBM Corp., Armonk, New York, USA) durchgeführt und die Graphiken mit GraphPad Prism Version 6 (GraphPad Software, La Jolla, Kalifornien, USA) erstellt (2).

BD-Studie: Gruppenunterschiede zu Beginn wurden mit einer ANOVA mit anschließendem Tukey's post-hoc Test für stetige sowie dem Chi-Quadrat-Anpassungstest und dem exakten Fisher-Test für kategoriale Variablen durchgeführt. Unterschiede zwischen den verschiedenen Zeitpunkten wurden mit der Methode der kleinsten Quadrate eines gemischten linearen Modells berechnet, wobei der Zeitpunkt als Kovariate und Messwiederholungen als Zufallseffekt verwendet sowie p-Werte für multiple Vergleiche mit Tukey Adjustments bereinigt wurden. Es wurde ein exponentieller Abfall des BD beobachtet, weshalb ein nichtlineares, asymptomatisches Regressionsmodell erstellt wurde. Statistische Signifikanz wurde mit $p<0,05$ definiert und die Daten als Mittelwert±Standardabweichung angegeben. Die Datenanalyse wurde mit R Version 4.0.0 (R Core Team, Wien, Österreich) durchgeführt und die Graphiken mit ggplot2 Version 3.3.0 (40) erstellt (3).

## 1.5 Ergebnisse

Die Ergebnisse der vorliegenden Arbeit wurden in drei Publikationen (1-3) veröffentlicht und werden im Folgenden separat beschrieben.

Basis-Studie:

Unter Berücksichtigung der Ein- und Ausschlusskriterien wurden 1422 Probanden ausgewählt, die zwischen 4–21 Tage fasteten und in vier Gruppen mit Fastenlängen von 5, 10, 15 und 20±2 Tagen eingeteilt (Abbildung 1) (1). Demographische Daten sind in Tabelle 1 gezeigt.

**Tabelle 1. Demographische Daten der Kohorte (n=1422), anthropometrische und kardiovaskuläre Marker, Parameter des Lipid- und Glukosestoffwechsels sowie Acetoacetat im Urin vor und nach dem LF, unterteilt nach Fastendauer.**

| | **Alle** (n=1422) | | **5±2 Tage** (n=659) | | **10±2 Tage** (n=530) | | **15±2 Tage** (n=196) | | **20±2 Tage** (n=37) | | **p-Wert** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | vorher | nachher | vorher | nachher | vorher | nachher | vorher | nachher | vorher | nachher | Fasten | Dauer* | Geschlecht* |
| Alter, Jahre | 55,4±0,4 | – | 54,2±0,5[b,c] | – | 56,3±0,6[a] | – | 56,4±0,9[a] | – | 56,4±2,3 | – | – | – | – |
| Frauen, n (%) | 841 (59,1) | – | 381 (57,8) | – | 316 (59,6) | – | 120 (61,2) | – | 24 (64,9) | – | – | – | – |
| Fastendauer, Tage | 8,2±0,1 | – | 5,4±0,0[b,c,d] | – | 8,6±0,0[a,c,d] | – | 14,1±0,1[a,b,d] | – | 20,1±0,2[a,b,c] | – | – | – | – |
| BMI, kg/m² | 28,2±0,2 | 26,7±0,1 | 27,2±0,2[b,c,d] | 26,1±0,2 | 28,5±0,3[a,c,d] | 27,0±0,2 | 29,7±0,4[a,b,d] | 27,5±0,4 | 33,6±1,1[a,b,c] | 31,0±1,1 | <0,001 | <0,001 | <0,001 |
| Gewicht, kg | 82,0±0,5 | 77,9±0,5 | 79,3±0,8[b,c,d] | 76,1±0,7 | 82,7±0,9[a,c,d] | 78,3±0,8 | 86,6±1,6[a,b,d] | 80,5±1,4 | 96,7±4,0[a,b,c] | 89,6±3,7 | <0,001 | <0,001 | <0,001 |
| Bauchumfang, cm | 94,0±0,4 | 88,0±0,5 | 91,3±0,6[b,c,d] | 86,4±0,7 | 94,8±0,7[a,c,d] | 88,6±0,7 | 98,3±1,2[a,b,d] | 89,4±1,3 | 106,3±2,8[a,b,c] | 96,9±3,1 | <0,001 | <0,001 | <0,001 |
| SBD, mmHg | 130,6±0,6 | 120,6±0,4 | 129,0±0,8 | 122,0±0,6 | 130,3±0,9 | 119,2±0,7 | 136,0±1,5 | 119,9±1,2 | 134,2±3,4 | 118,0±1,8 | <0,001 | <0,001 | – |
| DBD, mmHg | 83,2±0,3 | 77,7±0,3 | 82,5±0,5 | 78,5±0,4 | 83,2±0,5 | 77,0±0,4 | 84,7±0,8 | 77,0±0,7 | 86,3±1,9 | 78,3±1,5 | <0,001 | <0,001 | – |
| Puls, Schläge/min | 69,4±0,3 | 71,3±0,3 | 69,3±0,4 | 72,0±0,4 | 69,2±0,5 | 70,6±0,5 | 70,2±0,7 | 71,0±0,8 | 69,6±1,5 | 68,2±2,0 | 0,04 | 0,007 | – |
| TG, mmol/l | 1,54±0,02 | 1,11±0,01 | 1,47±0,03 | 1,09±0,01 | 1,61±0,04 | 1,10±0,01 | 1,62±0,06 | 1,15±0,02 | 1,45±0,11 | 1,24±0,05 | <0,001 | 0,001 | <0,001 |
| TC, mmol/l | 5,56±0,03 | 4,94±0,03 | 5,52±0,04 | 5,17±0,04 | 5,57±0,05 | 4,88±0,05 | 5,64±0,09 | 4,43±0,08 | 5,67±0,19 | 4,45±0,20 | <0,001 | <0,001 | – |
| LDL-C, mmol/l | 3,49±0,03 | 3,21±0,03 | 3,44±0,04 | 3,42±0,04 | 3,51±0,05 | 3,14±0,05 | 3,57±0,08 | 2,74±0,08 | 3,76±0,16 | 2,85±0,19 | <0,001 | <0,001 | – |
| HDL-C, mmol/l | 1,55±0,01 | 1,32±0,01 | 1,56±0,02 | 1,37±0,02 | 1,55±0,02 | 1,32±0,02 | 1,53±0,03 | 1,23±0,02 | 1,46±0,06 | 1,13±0,05 | <0,001 | <0,001 | <0,001 |
| LDL-C/HDL-C | 2,6±0,1 | 2,6±0,0 | 2,7±0,3 | 2,7±0,0 | 2,5±0,0 | 2,6±0,1 | 2,5±0,1 | 2,4±0,1 | 2,7±0,1 | 2,6±0,2 | 0,66 | – | – |
| Glukose, mmol/l | 5,39±0,03 | 4,68±0,03 | 5,30±0,04 | 4,65±0,05 | 5,42±0,05 | 4,69±0,05 | 5,62±0,09 | 4,68±0,07 | 5,53±0,20 | 4,93±0,16 | <0,001 | 0,03 | <0,001 |
| HbA1c, mmol/mol | 36,0±0,2 | 34,3±0,2 | 35,5±0,3 | 34,2±0,3 | 36,1±0,3 | 34,4±0,3 | 36,7±0,4 | 34,5±0,4 | 37,6±1,3 | 34,9±1,0 | <0,001 | <0,001 | 0,03 |
| Acetoacetat, mg/dl | 2,5±0,3 | 50,2±1,2 | 2,6±0,4 | 50,8±1,7 | 2,6±0,4 | 49,6±2,0 | 2,3±0,7 | 49,5±3,2 | 2,6±1,3 | 51,8±8,6 | <0,001 | – | <0,001 |

Daten sind angegeben als Mittelwert±Standardfehler für die gesamte Kohorte (n=1422) vor und nach dem Fasten, sowie für die Gruppen von 5, 10, 15 und 20±2 Fastentagen. Unterschiede zu Fastenbeginn sind gezeigt als: [a], p<0,05 versus 5±2 Tage; [b], p<0,05 versus 10±2 Tage; [c], p<0,05 versus 15±2 Tage; [d], p<0,05 versus 20±2 Tage. Die Berechnung der p-Werte erfolgte mittels eines linearen gemischten Modells für das Fasten und die Interaktionen Dauer* (Fasten*Dauer) und Geschlecht* (Fasten*Geschlecht).
LF, Langzeitfasten; BMI, Body-Mass-Index; SBD, systolischer Blutdruck; DBD, diastolischer Blutdruck; TG, Triglyzeride; TC, Gesamtcholesterin; LDL-C, Low-Density-Lipoprotein-Cholesterin; HDL-C, High-Density-Lipoprotein-Cholesterin; HbA1c, glykiertes Hämoglobin. Adaptiert von Wilhelmi de Toledo et al. (1).

Das Durchschnittsalter betrug 55,4±0,4 Jahre. Der Frauenanteil lag in allen Gruppen bei ca. 60 %. Der BMI war mit 27,2±0,2 kg/m² in der Gruppe mit 5 Tagen am geringsten und steigerte sich mit zunehmender Fastendauer auf bis zu 33,6±1,1 kg/m² für die am längsten fastende Gruppe ($p<0,05$). Gleiche Beobachtungen galten für das Gewicht, das zwischen 79,3±0,8 kg und 96,7±4,0 kg variierte sowie den Bauchumfang, der zwischen 91,3±0,6 cm und 106,3±2,8 cm lag (Tabelle 1).

LF führte zu einer signifikanten Gewichtsabnahme ($p<0,001$), die proportional zur Fastenlänge anstieg ($p<0,001$), so dass eine mittlere Gewichtsreduktion um 3,2±0,0 kg nach 5 Tagen bis hin zu 8,6±0,3 kg nach 20 Tagen erreicht wurde (Abbildung 2A). Zudem verloren Männer signifikant mehr Gewicht als Frauen ($p<0,001$). Entsprechende Beobachtungen wurden für den Bauchumfang dokumentiert, dessen Reduktion zwischen 4,6±0,1 cm nach 5 Tagen und 8,8±0,8 cm nach 20 Tagen variierte (Abbildung 2B).

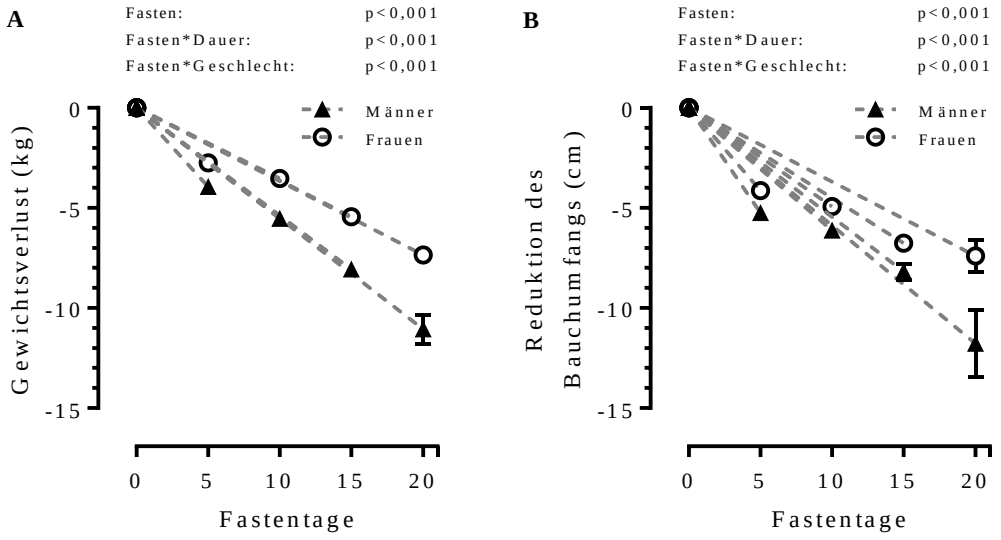



**Abbildung 2. Reduktion des Gewichts (A) und Bauchumfangs (B) je nach Fastenlänge von 5, 10, 15 oder 20±2 Tagen unterschieden nach Männern (Dreiecke) und Frauen (Kreise).** Adaptiert von Wilhelmi de Toledo et al. (1).

Sowohl der systolische BD (SBD) als auch der diastolische BD (DBD) reduzierte sich in allen Gruppen signifikant ($p<0,001$) und erreichte ein ähnliches Niveau von etwa 120/80 mmHg. Dabei wiesen längere Fastendauern höhere Ausgangswerte auf. Der Puls blieb unverändert (Tabelle 1).

Die Blutlipide verminderten sich im Fasten (Tabelle 1). Die TG-Spiegel sanken in der gesamten Kohorte um 0,43±0,02 mmol/l ($p<0,001$) mit deutlicherer Reduktion bei Männern ($p<0,001$), die höhere Ausgangswerte aufwiesen. Das Gesamtcholesterin (TC) sank um 0,62±0,02 mmol/l ($p<0,001$) mit einer stärkeren Reduktion bei längerer Fastendauer, wobei 15 und 20 Fastentage ähnliche Ergebnisse aufwiesen ($p<0,001$). LDL-C erzielte eine durchschnittliche Reduktion von 0,28±0,02 mmol/l ($p<0,001$) und fiel ab einer Fastendauer von 10 Tagen um 0,37±0,03 mmol/l, nach 15 Tagen um 0,83±0,07 mmol/l und nach 20 Tagen um 0,91±0,13 mmol/l ($p<0,001$). Das HDL-C war bei Frauen signifikant höher und wurde durch das Fasten insgesamt um 0,22±0,01 mmol/l gesenkt ($p<0,001$). Hierbei war eine stärkere Senkung bei längeren Fastendauern ($p<0,001$) und bei den Frauen ersichtlich ($p<0,001$). Der LDL-C/HDL-C-Quotient blieb unverändert.

Die Blutglukose sank um 0,72±0,04 mmol/l in allen Gruppen auf ein vergleichbares Niveau ($p<0,001$), jedoch zeigten Männer mit erhöhten Ausgangswerten einen stärkeren Abfall ($p<0,001$; Tabelle 1). Das glykierte Hämoglobin (HbA1c) sank in der gesamten Kohorte um 1,38±0,07 mmol/mol ($p<0,001$) und stärker bei längerer Fastendauer ($p<0,001$). Im Gegensatz zu den fallenden Lipid- und Glukosespiegeln stieg der Acetoacetatgehalt im Urin auf ein konstantes Niveau an. Männer wiesen höhere Konzentrationen als Frauen auf ($p<0,001$; Tabelle 1).

Weitere Blutparameter dienten der Dokumentation der Sicherheit (Tabelle 2). Natrium sank signifikant um durchschnittlich 1,38±0,07 mmol/l ($p<0,001$). Kürzeres Fasten beeinflusste die Natriumspiegel stärker ($p=0,003$). Kalium sank ebenfalls ($p=0,001$), Calcium stieg an ($p<0,001$) und Magnesium blieb unverändert. Die Harnsäure stieg im Fasten um 46,5 % ($p<0,001$), bei Männern stärker als bei Frauen ($p<0,001$). Nach 15 Fastentagen wurden die höchsten Harnsäure-Werte beobachtet ($p=0,01$). Harnstoff hingegen sank signifikant ($p<0,001$) und ausgeprägter bei längerer Fastendauer ($p<0,001$). Kreatinin stieg, unabhängig von der Fastendauer, an ($p<0,001$). Männer wiesen höhere Werte auf ($p<0,001$). Die International Normalized Ratio (INR) und partielle Thromboplastinzeit (PTT) stiegen (je $p<0,001$). Bei längerem Fasten wurde ein stärkerer Anstieg dokumentiert (je $p<0,001$). Im Blutbild zeigten sich signifikante Veränderungen der Zellzahlen innerhalb der Referenzwerte. Leukozyten und Thrombozyten sanken (je $p<0,001$) umso mehr, je länger gefastet wurde (je $p<0,001$), wohingegen Erythrozyten anstiegen ($p<0,001$). Der Hämatokrit blieb unverändert, ebenso wie das mittlere korpuskuläre Hämoglobin (MCH). Der Hämoglobinwert und die mittlere korpuskuläre Hämoglobinkonzentration (MCHC) stiegen an (je $p<0,001$). Das mittlere korpuskuläre Volumen (MCV) sank ($p<0,001$).

**Tabelle 2. Blutparameter vor und nach dem LF, unterteilt nach Fastendauer.**

| | **Alle** (n=1422) | | **5±2 Tage** (n=659) | | **10±2 Tage** (n=530) | | **15±2 Tage** (n=196) | | **20±2 Tage** (n=37) | | **p-Wert** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | vorher | nachher | vorher | nachher | vorher | nachher | vorher | nachher | vorher | nachher | Fasten | Dauer* | Gesch-lecht* |
| Natrium, mmol/l | 140,1±0,1 | 138,7±0,1 | 140,0±0,1 | 138,4±0,1 | 140,1±0,1 | 138,8±0,1 | 139,8±0,3 | 139,2±0,2 | 141,0±0,3 | 139,9±0,4 | <0,001 | 0,003 | – |
| Kalium, mmol/l | 4,4±0,0 | 4,4±0,0 | 4,4±0,0 | 4,4±0,0 | 4,4±0,0 | 4,4±0,0 | 4,3±0,0 | 4,4±0,0 | 4,4±0,1 | 4,4±0,1 | 0,001 | – | 0,008 |
| Calcium, mmol/l | 2,32±0,00 | 2,38±0,00 | 2,33±0,00 | 2,36±0,00 | 2,32±0,00 | 2,39±0,00 | 2,32±0,01 | 2,39±0,01 | 2,33±0,02 | 2,40±0,02 | <0,001 | <0,001 | – |
| Magnesium, mmol/l | 0,86±0,00 | 0,87±0,00 | 0,87±0,00 | 0,89±0,00 | 0,87±0,00 | 0,87±0,00 | 0,85±0,00 | 0,86±0,01 | 0,86±0,01 | 0,85±0,01 | 0,09 | <0,001 | <0,001 |
| Harnsäure, µmol/l | 338,1±2,3 | 495,2±4,4 | 334,0±3,3 | 481,1±6,0 | 339,2±3,8 | 505,5±7,5 | 345,3±6,4 | 513,0±13,5 | 355,9±12,8 | 505,4±30,6 | <0,001 | 0,01 | <0,001 |
| Harnstoff, mmol/l | 4,7±0,0 | 3,1±0,0 | 4,6±0,1 | 3,3±0,1 | 4,7±0,1 | 3,0±0,1 | 4,7±0,1 | 2,7±0,1 | 5,1±0,3 | 2,8±0,3 | <0,001 | <0,001 | <0,001 |
| Kreatinin, µmol/l | 71,9±0,4 | 76,4±0,5 | 72,5±0,6 | 76,5±0,8 | 71,9±0,7 | 76,9±1,0 | 69,9±1,0 | 75,0±1,2 | 72,3±2,5 | 77,2±3,1 | <0,001 | – | <0,001 |
| INR | 0,99±0,00 | 1,08±0,00 | 0,98±0,00 | 1,06±0,00 | 0,99±0,01 | 1,09±0,01 | 0,99±0,02 | 1,11±0,02 | 0,98±0,01 | 1,10±0,02 | <0,001 | <0,001 | – |
| PTT, sek | 31,1±0,1 | 32,7±0,1 | 31,1±0,1 | 32,4±0,1 | 31,0±0,1 | 32,8±0,2 | 31,2±0,2 | 33,7±0,3 | 31,4±0,4 | 32,9±0,5 | <0,001 | <0,001 | – |
| Leukozyten, $10^3$/µl | 5,9±0,0 | 5,4±0,0 | 5,9±0,1 | 5,5±0,1 | 6,0±0,1 | 5,4±0,1 | 6,0±0,1 | 5,0±0,1 | 5,7±0,3 | 4,7±0,2 | <0,001 | <0,001 | – |
| Thrombozyten, $10^3$/µl | 244,1±1,5 | 237,5±1,5 | 242,6±2,2 | 239,2±2,2 | 245,9±2,5 | 238,9±2,6 | 243,9±3,9 | 230,6±4,1 | 245,6±11,4 | 224,4±11,4 | <0,001 | <0,001 | <0,001 |
| Erythrozyten, $10^6$/µl | 4,76±0,01 | 4,82±0,01 | 4,76±0,02 | 4,82±0,02 | 4,76±0,02 | 4,81±0,02 | 4,74±0,03 | 4,81±0,03 | 4,74±0,07 | 4,86±0,07 | <0,001 | – | 0,001 |
| Hämoglobin, mmol/l | 8,9±0,0 | 9,0±0,0 | 8,9±0,0 | 9,0±0,0 | 8,9±0,0 | 9,0±0,0 | 8,9±0,1 | 9,0±0,0 | 8,7±0,1 | 8,9±0,1 | <0,001 | – | <0,001 |
| Hämatokrit, % | 42,2±0,1 | 42,3±0,1 | 42,2±0,1 | 42,3±0,1 | 42,3±0,1 | 42,3±0,1 | 42,2±0,2 | 42,3±0,2 | 41,8±0,5 | 42,2±0,5 | 0,74 | – | 0,02 |
| MCV, fl | 89,0±0,1 | 88,0±0,1 | 88,8±0,2 | 87,9±0,2 | 89,1±0,2 | 88,0±0,2 | 89,3±0,3 | 88,0±0,3 | 88,5±0,9 | 87,0±0,8 | <0,001 | 0,001 | <0,001 |
| MCH, pg | 30,1±0,1 | 30,1±0,1 | 30,1±0,1 | 30,1±0,1 | 30,2±0,1 | 30,2±0,1 | 30,2±0,1 | 30,1±0,1 | 29,7±0,3 | 29,7±0,3 | 0,38 | 0,09 | – |
| MCHC, g/dl | 33,9±0,0 | 34,2±0,0 | 33,9±0,0 | 34,2±0,0 | 33,9±0,0 | 34,3±0,0 | 33,8±0,1 | 34,2±0,1 | 33,6±0,2 | 34,0±0,1 | <0,001 | – | 0,004 |
| GOT, µkat/l | 0,42±0,01 | 0,62±0,01 | 0,41±0,01 | 0,61±0,01 | 0,43±0,01 | 0,62±0,01 | 0,41±0,01 | 0,60±0,02 | 0,44±0,02 | 0,66±0,04 | <0,001 | – | <0,001 |
| GPT, µkat/l | 0,51±0,01 | 0,68±0,01 | 0,49±0,01 | 0,64±0,02 | 0,52±0,02 | 0,70±0,02 | 0,53±0,02 | 0,72±0,03 | 0,57±0,02 | 0,77±0,05 | <0,001 | 0,10 | – |
| GGT, µkat/l | 0,55±0,02 | 0,41±0,01 | 0,52±0,03 | 0,42±0,02 | 0,57±0,05 | 0,41±0,03 | 0,61±0,06 | 0,38±0,03 | 0,51±0,05 | 0,37±0,04 | <0,001 | <0,001 | <0,001 |
| AP, µkat/l | 1,07±0,01 | 1,04±0,01 | 1,02±0,01 | 1,01±0,01 | 1,11±0,02 | 1,06±0,01 | 1,13±0,02 | 1,04±0,02 | 1,21±0,06 | 1,11±0,05 | <0,001 | <0,001 | 0,002 |

Daten sind angegeben als Mittelwert±Standardfehler für die gesamte Kohorte (n=1422) vor und nach dem Fasten, sowie für die Gruppen von 5, 10, 15 und 20±2 Fastentagen. Unterschiede zu Fastenbeginn sind gezeigt als: [a], p<0,05 versus 5±2 Tage; [b], p<0,05 versus 10±2 Tage; [c], p<0,05 versus 15±2 Tage; [d], p<0,05 versus 20±2 Tage. Die Berechnung der p-Werte erfolgte mittels eines linearen gemischten Modells für das Fasten und die Interaktionen Dauer* (Fasten*Dauer) und Geschlecht* (Fasten*Geschlecht).
LF, Langzeitfasten; INR, International Normalized Ratio; PTT, partielle Thromboplastinzeit; MCV, mittleres korpuskuläres Volumen; MCH, mittleres korpuskuläres Hämoglobin; MCHC, mittlere korpuskuläre Hämoglobinkonzentration; GOT, Glutamat-Oxalacetat-Transaminase; GPT, Glutamat-Pyruvat-Transaminase); GGT, Gamma-Glutamyl-Transferase; AP, Alkalische Phosphatase. Adaptiert von Wilhelmi de Toledo et al. (1).

Die Leberenzyme Glutamat-Oxalacetat-Transaminase (GOT) und Glutamat-Pyruvat-Transaminase (GPT) stiegen am Ende des Fastens um 0,20±0,01 µkat/l bzw. 0,17±0,01 µkat/l an (je p<0,001; Tabelle 2). GOT-Werte waren bei Männern höher als bei Frauen (p<0,001). GGT und Alkalische Phosphatase (AP) sanken um 0,14±0,01 µkat/l bzw. 0,04±0,00 µkat/l (je p<0,001), abhängig von der Fastenlänge (je p<0,001) und mit Unterschieden je nach Geschlecht (GGT: p<0,001; AP: p=0,002).

FLI-Studie:

In die Auswertung wurden 697 Probanden gemäß der Ein- und Ausschlusskriterien einbezogen (Abbildung 1) (2). Darunter waren 38 T2DM, von denen 28 Antidiabetika mit folgender Häufigkeit einnahmen: Metformin n=27, Dipeptidylpeptidase-4-Hemmer n=6, Sulfonylharnstoff n=5, Natrium-Glukose-Cotransporter-2-Inhibitor n=2 und Glitazon n=1. Vier Personen erhielten Insulin, drei davon kombinierten dieses mit oralen Antidiabetika. Die 38 T2DM wurden mit den 659 Nicht-T2DM verglichen.

Die mittlere Fastendauer betrug 8,5±4,0 Tage, variierte zwischen 6–38 Tagen und unterschied sich nicht zwischen den Gruppen (Tabelle 3). Die gesamte Kohorte war überwiegend weiblich (63,1 %) und mittleren Alters, wobei die T2DM signifikant älter (p=0,002) und schwerer (p<0,001) waren sowie einen größeren Bauchumfang (p<0,001) aufwiesen. Zu Beginn hatten 273 Probanden keine Fettleber (FLI<30 Punkte), bei 160 Probanden lag der FLI zwischen ≥30 Punkten und <60 Punkten und 264 Patienten hatten eine Fettleber (FLI≥60 Punkte). T2DM hatten mit 78,4±7,0 Punkten einen höheren Ausgangs-FLI als Nicht-T2DM mit 44,9±31,6 Punkten (p<0,001; Tabelle 3).
LF senkte den FLI-Wert signifikant um 14,0±11,7 Punkte (p<0,001) und um 5,4 Punkte stärker bei T2DM als bei Nicht-T2DM (p<0,001; Abbildung 3). Die Anzahl an Probanden mit Fettleber reduzierte sich um 46,3 % bei Nicht-T2DM und um 36,4 % bei T2DM. Dagegen stieg die Anzahl ohne Fettleber um 44,7 % bei Nicht-T2DM und um 75,0 % bei T2DM.
Gewicht, BMI und Bauchumfang reduzierten sich signifikant (je p<0,001) und jeweils stärker bei T2DM (Gewicht: p=0,005; BMI: p=0,021; Bauchumfang: p=0,043). TG, TC, LDL-C und HDL-C wurden reduziert (je p<0,001). Bei T2DM fiel die TG-Absenkung ausgeprägter aus als bei den Nicht-T2DM (p=0,006) und das HDL-C wurde nicht beeinflusst (p<0,001). Glukose und HbA1c sanken (je p<0,001), wiederum deutlicher bei T2DM (p<0,001). GOT und GPT stiegen an (je p<0,001), wohingegen GGT und AP absanken (je p<0,001). Nur GPT zeigte einen signifikant stärkeren Anstieg bei T2DM (p=0,015; Tabelle 3).

**Tabelle 3. Demographische Daten der Kohorte (n=697), anthropometrische Marker, FLI, Parameter des Lipid- und Glukosestoffwechsels und Leberenzyme vor und nach dem LF, unterteilt nach T2DM und Nicht-T2DM.**

| | **Alle (n=697)** | | | **T2DM (n=38)** | | | **Nicht-T2DM (n=659)** | | | **p-Wert** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | vorher | nachher | p-Wert | vorher | nachher | p-Wert | vorher | nachher | p-Wert | Vergleich vorher-Werte | Vergleich T2DM vs. Nicht-T2DM |
| Alter, Jahre | 54,6±13,4 | – | – | 60,9±9,7 | – | – | 54,2±13,5 | – | – | 0,002 | – |
| Frauen, n (%) | 440 (63,1) | – | – | 11 (28,9) | – | – | 429 (65,1) | – | – | – | – |
| Fastendauer, Tage | 8,5±4,0 | – | – | 9,3±4,8 | – | – | 8,4±4,0 | – | – | 0,261 | – |
| BMI, kg/m² | 28,2±5,3 | 26,7±5,0 | <0,001 | 31,8±5,2 | 30,0±4,7 | <0,001 | 28,0±5,3 | 26,5±4,9 | <0,001 | <0,001 | 0,021 |
| Gewicht, kg | 81,3±18,2 | 76,9±18,2 | <0,001 | 95,5±17,8 | 90,2±16,3 | <0,001 | 80,5±17,9 | 76,2±16,7 | <0,001 | <0,001 | 0,005 |
| Bauchumfang, cm | 92,9±15,4 | 87,5±14,5 | <0,001 | 107,1±11,3 | 100,8±11,0 | <0,001 | 92,1±15,2 | 86,7±14,3 | <0,001 | <0,001 | 0,043 |
| FLI, Punkte | 46,8±31,9 | 32,7±28,1 | <0,001 | 78,4±17,0 | 59,2±21,4 | <0,001 | 44,9±31,6 | 31,2±27,6 | <0,001 | <0,001 | 0,002 |
| FLI<30, n | 273 | 399 | – | 1 | 4 | – | 272 | 395 | – | <0,001 | – |
| FLI≥30 – FLI<60, n | 160 | 153 | – | 4 | 13 | – | 156 | 140 | – | | |
| FLI≥60, n | 264 | 145 | – | 33 | 21 | – | 231 | 124 | – | | |
| TG, mmol/l | 1,55±0,80 | 1,10±0,34 | <0,001 | 2,09±0,95 | 1,31±0,33 | <0,001 | 1,52±0,77 | 1,09±0,34 | <0,001 | <0,001 | 0,006 |
| TC, mmol/l | 5,56±1,19 | 4,92±1,21 | <0,001 | 4,83±1,18 | 4,39±0,95 | 0,014 | 5,60±1,18 | 4,95±1,22 | <0,001 | <0,001 | 0,186 |
| LDL-C, mmol/l | 3,46±1,08 | 3,16±1,19 | <0,001 | 3,01±1,02 | 2,80±1,00 | 0,233 | 3,49±1,07 | 3,18±1,19 | <0,001 | 0,008 | 0,614 |
| HDL-C, mmol/l | 1,55±0,48 | 1,33±0,37 | <0,001 | 1,17±0,34 | 1,10±0,28 | 0,091 | 1,57±0,47 | 1,34±0,37 | <0,001 | <0,001 | <0,001 |
| Glukose, mmol/l | 5,35±1,11 | 4,63±1,18 | <0,001 | 8,10±2,40 | 5,41±1,56 | <0,001 | 5,19±0,72 | 4,59±1,14 | <0,001 | <0,001 | <0,001 |
| HbA1c, mmol/mol | 36,4±6,9 | 34,6±6,4 | <0,001 | 55,2±15,0 | 50,8±6,9 | <0,001 | 35,3±3,9 | 33,7±4,3 | <0,001 | <0,001 | <0,001 |
| GGT, µkat/l | 0,48±0,57 | 0,36±0,35 | <0,001 | 0,75±0,95 | 0,57±0,62 | <0,001 | 0,47±0,53 | 0,35±0,32 | <0,001 | <0,001 | 0,069 |
| GOT, µkat/l | 0,41±0,21 | 0,61±0,31 | <0,001 | 0,46±0,20 | 0,75±0,31 | <0,001 | 0,40±0,21 | 0,61±0,31 | <0,001 | 0,055 | 0,061 |
| GPT, µkat/l | 0,50±0,35 | 0,68±0,46 | <0,001 | 0,63±0,38 | 0,97±0,56 | <0,001 | 0,49±0,35 | 0,66±0,44 | <0,001 | <0,001 | 0,015 |
| AP, µkat/l | 1,09±0,30 | 1,05±0,27 | <0,001 | 1,06±0,31 | 1,03±0,26 | 0,330 | 1,09±0,30 | 1,05±0,27 | <0,001 | 0,516 | 0,989 |

Daten sind angegeben als Mittelwert±Standardabweichung für die gesamte Kohorte (n=697) vor und nach dem Fasten, sowie unterteilt nach T2DM (n=38) und nicht-T2DM (n=659).

FLI, Fettleberindex; LF, Langzeitfasten; T2DM, Typ-2-Diabetiker; BMI, Body-Mass-Index; TG, Triglyzeride; TC, Gesamtcholesterin; LDL-C, Low-Density-Lipoprotein-Cholesterin; HDL-C, High-Density-Lipoprotein-Cholesterin; HbA1c, glykiertes Hämoglobin; GOT, Glutamat-Oxalacetat-Transaminase; GPT, Glutamat-Pyruvat-Transaminase; GGT, Gamma-Glutamyl-Transferase; AP, Alkalische Phosphatase. Adaptiert von Drinda et al. (2).

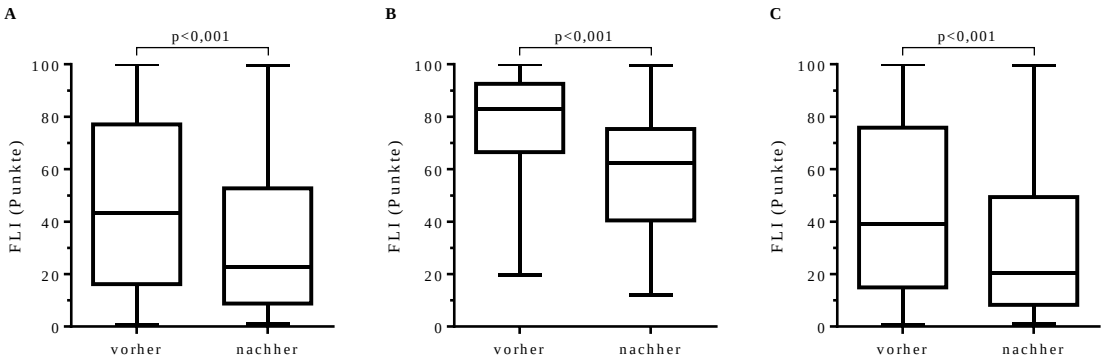



**Abbildung 3. FLI-Werte vor und nach dem LF in der gesamten Kohorte (n=697; A), bei 38 Probanden mit T2DM (B) und 659 Probanden ohne T2DM (C).**
FLI, Fettleberindex; LF, Langzeitfasten; T2DM, Typ-2-Diabetiker. Adaptiert von Drinda et al. (2).

Längeres Fasten reduzierte den FLI stärker (r=-0,20; p<0,001). Eine signifikante Korrelation des FLI wurde zudem mit dem BMI (r=-0,14; p<0,001), Bauchumfang (r=0,29; p<0,001), GGT (r=0,47; p<0,001), GOT (r=-0,10; p=0,012), AP (r=0,18; p<0,001), TC (r=0,29; p<0,001) und TG (r=0,62; p<0,001) dokumentiert. Dagegen korrelierte der FLI nicht mit GPT, Glukose und dem HbA1c-Wert.

<u>BD-Studie:</u>

Eine Kohorte aus 1610 Probanden wurde anhand der BD-Werte zu Beginn nach internationalen Richtlinien (31) in drei Gruppen eingeteilt (Abbildung 1): normotensiv, bei einem BD <140/<90 mmHg und ohne Einnahme von Antihypertonika (NT; n=920), hypertensiv bei einem BD ≥140/≥90 mmHg und ohne Einnahme von Antihypertensiva (HTNM; n=313) sowie medikamentös behandelte Hypertoniker bei Einnahme von Antihypertensiva (HTM; n=377) (3). In dieser Gruppe wurden folgende Medikationen dokumentiert: Angiotensin II Rezeptor Antagonisten n=180, Adrenozeptor-Blocker n=149, Diuretika n=129, Angiotensin-konvertierende Enzyminhibitoren n=104, Calciumkanalblocker n=90 und Renininhibitoren n=4. Am häufigsten wurden Einzelmedikationen (44,8 %) eingenommen, gefolgt von der Einnahme zweier Wirkstoffe (38,7 %) und der Kombination von drei oder vier Wirkstoffen (16,5 %).

Das Durchschnittsalter der Kohorte lag bei 55,4±13,3 Jahren (Tabelle 4). NT-Probanden waren signifikant jünger und überwiegend weiblich, wogegen die HTM-Gruppe am ältesten war und mehr Männer beinhaltete (je p<0,001). Die mittlere Fastendauer lag bei 10,1±4,1 Tagen.

**Tabelle 4. Demographische Daten der Kohorte (n=1610), anthropometrische Marker, BD-Werte, Parameter des Lipid- und Glukosestoffwechsels, Natrium und subjektives Wohlbefinden vor und nach dem LF, unterteilt nach NT-, HTNM- und HTM-Probanden.**

| | **Alle (n=1610)** | | | **NT (n=920)** | | | **HTNM (n=313)** | | | **HTM (n=377)** | | | **p-Wert** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | vorher | nachher | p-Wert | vorher | nachher | p-Wert | vorher | nachher | p-Wert | vorher | nachher | p-Wert | Vergleich vorher-Werte |
| Alter, Jahre | 55,4±13,3 | - | - | 51,3±13,3 | - | - | 59,3±11,3 | - | - | 62,3±10,7 | - | - | <0,001[abc] |
| Frauen, n (%) | 955 (59,3) | - | - | 630 (68,5) | - | - | 167 (53,4) | - | - | 158 (41,9) | - | - | <0,001[abc] |
| Fastendauer, Tage | 10,1±4,1 | - | - | 9,7±3,8 | - | - | 10,6±4,3 | - | - | 10,3±4,5 | - | - | <0,001[bc] |
| BMI, kg/m$^2$ | 27,9±5,6 | 26,5±5,3 | >0,001 | 26,3±4,8 | 25,0±4,5 | >0,001 | 29,9±6,4 | 28,2±6,0 | >0,001 | 30,3±5,4 | 28,8±5,1 | <0,001 | <0,001[bc] |
| Gewicht, kg | 81,1±19,2 | 77,0±18,2 | >0,001 | 76,0±16,7 | 72,3±15,9 | >0,001 | 87,3±21,7 | 82,2±20,7 | >0,001 | 88,9±19,0 | 84,3±17,8 | >0,001 | <0,001[bc] |
| Bauchumfang, cm | 94,1±16,5 | 88,1±15,1 | >0,001 | 88,6±14,4 | 83,0±13,2 | >0,001 | 99,4±16,2 | 92,1±14,6 | >0,001 | 103,2±16,0 | 97,2±14,6 | >0,001 | <0,001[abc] |
| SBD, mmHg | 126,2±18,6 | 119,7±15,9 | >0,001 | 116,6±11,3 | 113,6±12,9 | >0,001 | 145,5±15,3 | 128,8±15,9 | >0,001 | 134,6±19,3 | 127,3±15,7 | >0,001 | <0,001[abc] |
| DBD mmHg | 81,4±11,1 | 77,6±9,8 | >0,001 | 75,9±7,3 | 74,0±8,1 | >0,001 | 92,5±9,4 | 83,7±9,8 | >0,001 | 86,0±11,0 | 81,3±10,0 | >0,001 | <0,001[abc] |
| Puls, Schläge/min | 70,7±10,7 | 71,0±11,6 | 0,18 | 70,2±10,4 | 71,2±12,4 | 0,004 | 72,6±10,7 | 73,0±11,8 | 0,53 | 70,2±11,4 | 68,9±11,3 | 0,002 | 0,002[ab] |
| TG, mmol/l | 1,54±0,81 | 1,11±0,34 | >0,001 | 1,36±0,65 | 1,05±0,32 | >0,001 | 1,70±0,89 | 1,17±0,33 | >0,001 | 1,85±0,98 | 1,21±0,37 | >0,001 | <0,001[abc] |
| TC, mmol/l | 5,54±1,13 | 4,92±1,14 | >0,001 | 5,51±1,10 | 4,91±1,11 | >0,001 | 5,81±1,15 | 5,07±1,17 | >0,001 | 5,39±1,17 | 4,79±1,19 | >0,001 | <0,001[ab] |
| LDL-C, mmol/l | 3,47±1,03 | 3,18±1,12 | >0,001 | 3,42±0,99 | 3,13±1,08 | >0,001 | 3,69±1,07 | 3,37±1,16 | >0,001 | 3,40±1,09 | 3,15±1,18 | >0,001 | 0,01[ab] |
| HDL-C, mmol/l | 1,55±0,46 | 1,32±0,38 | >0,001 | 1,61±0,47 | 1,39±0,39 | >0,001 | 1,55±0,47 | 1,28±0,38 | >0,001 | 1,39±0,40 | 1,19±0,30 | >0,001 | <0,001[ac] |
| Glukose, mmol/l | 5,39±1,09 | 4,71±1,17 | >0,001 | 5,11±0,75 | 4,58±1,15 | >0,001 | 5,60±1,07 | 4,75±1,07 | >0,001 | 5,89±1,51 | 4,97±1,23 | >0,001 | <0,001[abc] |
| Hba1c, mmol/mol | 36,1±0,2 | 34,6±0,1 | >0,001 | 34,6±0,2 | 33,3±0,2 | >0,001 | 36,8±0,4 | 35,2±0,4 | >0,001 | 39,0±0,4 | 34,6±0,4 | >0,001 | <0,001[abc] |
| Natrium, mmol/l | 140,1±2,9 | 139,0±2,6 | >0,001 | 140,0±2,9 | 139,0±2,7 | >0,001 | 140,2±2,2 | 139,0±2,4 | >0,001 | 139,9±3,1 | 139,0±2,7 | >0,001 | 0,47 |
| WHO-5, % | 56,1±16,2 | 73,3±14,8 | >0,001 | 56,2±16,1 | 73,2±14,9 | >0,001 | 56,8±16,8 | 73,1±14,6 | >0,001 | 55,4±15,9 | 73,6±14,9 | >0,001 | 0,69 |
| Entspannungsniveau, 0-10 Punkte | 4,9±2,2 | 7,8±2,0 | >0,001 | 4,8±2,3 | 7,7±2,1 | >0,001 | 4,8±2,1 | 7,8±2,1 | >0,001 | 5,8±2,1 | 7,9±2,0 | >0,001 | 0,06 |

Daten sind angegeben als Mittelwert±Standardabweichung für die gesamte Kohorte (n=1610) sowie NT (n=920), HTNM (n=313) und HTM (n=377) vor und nach dem Fasten. Unterschiede zu Fastenbeginn sind gezeigt als: [a], p<0,05 HTNM versus HTM; [b], p<0,05 HTNM versus NT; [c], p<0,05 HTM versus NT. Die Berechnung der p-Werte erfolgte mittels der Methode der kleinsten Quadrate eines linearen gemischten Modells adjustiert für Zeitpunkt und Messwiederholungen.
BD, Blutdruck; LF, Langzeitfasten; NT, normotensiv; HTNM, nicht-medikamentös behandelte Hypertoniker; HTM, medikamentös behandelte Hypertoniker; BMI, Body-Mass-Index; SBD, systolischer Blutdruck; DBD, diastolischer Blutdruck; TG, Triglyzeride; TC, Gesamtcholesterin; LDL-C, Low-Density-Lipoprotein-Cholesterin; HDL-C, High-Density-Lipoprotein-Cholesterin; HbA1c, glykiertes Hämoglobin; WHO-5, WHO-5-Wohlbefindens-Index. Adaptiert von Grundler et al. (3)

Die NT-Gruppe fastete etwas kürzer, hatte ein niedrigeres Ausgangsgewicht und einen geringeren BMI als die HTNM- und HTM-Gruppe (je p<0,001). Der Bauchumfang war in der NT-Gruppe am geringsten und in der HTM-Gruppe am größten (p<0,001; Tabelle 4). Der BD der NT-Gruppe lag zu Beginn bei 116,6±11,3/75,9±7,3 mmHg, für die HTNM-Gruppe signifikant höher bei 145,5±15,3/92,5±9,4 mmHg und für die HTM-Gruppe bei 134,6±19,3/86,0±11,0 mmHg (p<0,001; Tabelle 4). TG, Glukose, und HbA1c zeigten die niedrigsten Werte für die NT-Gruppe und die höchsten Werte für die HTM-Gruppe (je p<0,001). Dagegen wies die HTM-Gruppe im Vergleich zu den beiden anderen Gruppen das niedrigste HDL-C (p<0,001) auf und hatte zudem signifikant niedrigeres TC (p<0,001) und LDL-C (p=0,01) als die HTNM-Gruppe. Es wurde eine vermehrte Einnahme von Lipidsenkern in der HTM-Gruppe (31,8 %) im Gegensatz zur HTNM- (9,3 %) und NT-Gruppe (6,6 %) dokumentiert. Natriumspiegel waren bei allen vergleichbar und der Puls in der HTNM-Gruppe moderat erhöht (p=0,002; Tabelle 4).

In der Kohorte wurde eine signifikante Reduktion des BD während des Fastens von 126,2±18,6/81,4±11,0 mmHg zu 119,7±15,9/77,6±9,8 mmHg (p<0,001), mit vergleichbaren Ergebnissen für Männer und Frauen, dokumentiert. In der NT-Gruppe wurde eine mittlere BD-Reduktion um 6,5/3,8 mmHg beobachtet, die sich ab dem achten Fastentag signifikant vom Ausgangswert unterschied (SBD: p=0,007; DBD: p=0,006; Abbildung 4A, 4D). Die HTNM-Gruppe erfuhr die stärkste BD-Reduktion um durchschnittlich 16,7/8,8 mmHg, welche bereits ab dem ersten Fastentag signifikant war (SBD: p<0,001; DBD: p<0,001; Abbildung 4B, 4E). Insgesamt 190 der 313 HTNM-Probanden (60,7 %) hatten am Ende des Fastens einen BD innerhalb der Norm. In der HTM-Gruppe wurde eine BD-Reduktion von durchschnittlich 7,3/4,7 mmHg erreicht (Abbildung 4C, 4F). Hierbei unterschieden sich der SBD am fünften (p<0,001) und der DBD am dritten Fastentag signifikant (p<0,001) vom Ausgangswert. Während des Fastens wurde die antihypertensive Medikation nach Bedarf adaptiert, so dass 23,6 % der Probanden die Einnahme stoppten und 43,5 % reduzierten. Bei 19,4 % blieb sie unverändert, bei 1,6 % wurde die Wirksubstanz ausgetauscht und bei 3,2 % aufgrund unzureichender Medikation vor dem Fasten erhöht. Die Dokumentation der BD-Medikation fehlte bei 8,7 % der Probanden.

Sowohl für die HTNM- als auch die HTM-Gruppe führte längeres Fasten zu einer stärkeren Senkung des BD in den Normbereich. Außerdem war für beide Gruppen während des Fastens eine exponentielle Abnahme des BD mit Annäherung an einen unteren Grenzwert sichtbar. Horizontale Asymptoten lagen für die HTNM-Gruppe bei 126,0±1,0/82,6±0,4 mmHg und für die HTM-Gruppe bei 123,2±2,6/80,1±0,6 mmHg (p<0,001).

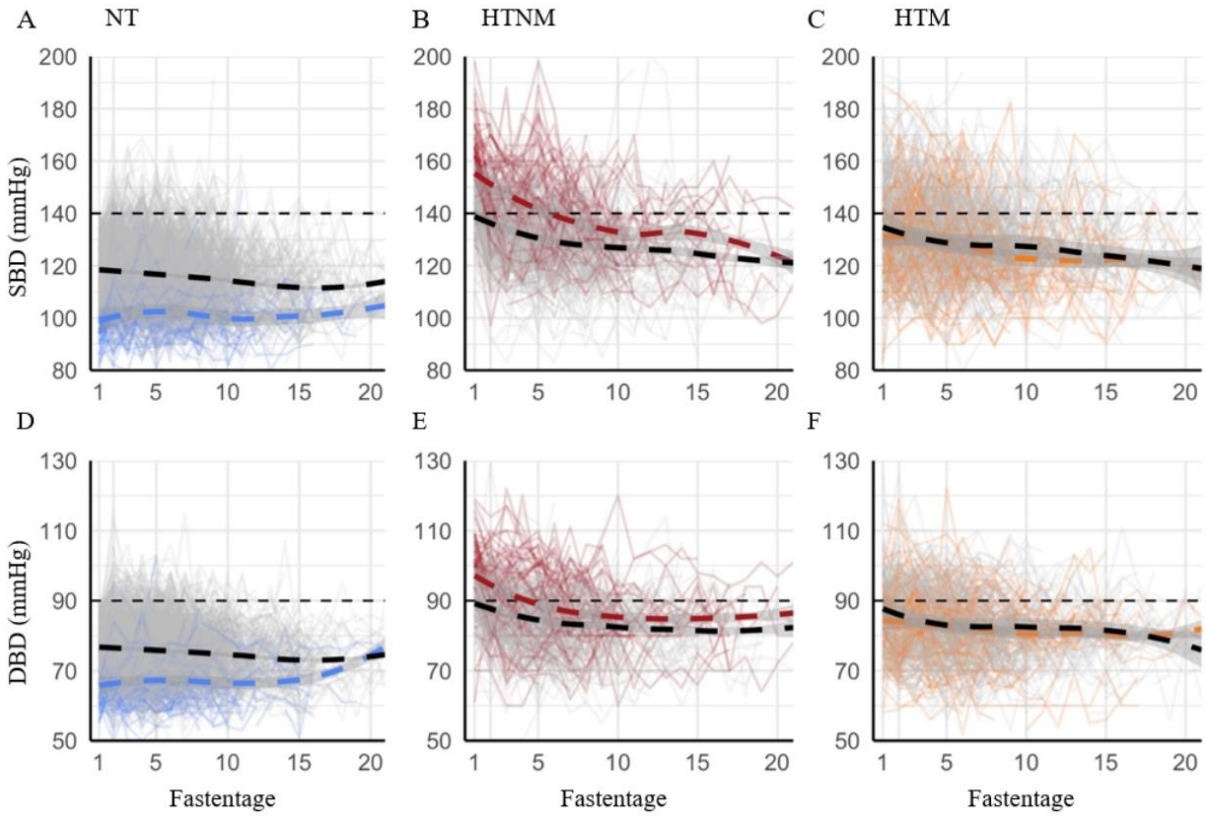



**Abbildung 4. Veränderung des SBD und DBD während dem LF für NT (A, D), HTNM (B, E) sowie HTM (C, F).** Generelle Veränderungen, reflektiert durch gleitende Mittelwerte, sind als gestrichelte Linien dargestellt. Einzelne BD-Verläufe sind als graue Linien gezeigt. Farblich abgesetzt sind Subgruppen: 74 NT-Probanden mit einem initial niedrigem BD (<100/60 mmHg) sind in Blau dargestellt; 76 HTNM-Probanden mit den höchsten Ausgangswerten (>160/90 mmHg) sind in Rot dargestellt; 89 HTM-Probanden, die ihre Medikation stoppten, sind in Orange dargestellt.
NT, normotensiv; HTNM, nicht-medikamentös behandelte Hypertoniker; HTM, medikamentös behandelte Hypertoniker; SBD, systolischer Blutdruck; DBD, diastolischer Blutdruck; LF, Langzeitfasten. Adaptiert von Grundler et al. (3).

In Subgruppen wurde der Einfluss des LF auf die Höhe der BD-Reduktion je nach Ausgangswert untersucht. Bei 76 HTNM-Probanden mit initialen BD-Werten >160/90 mmHg wurde die stärkste BD-Senkung um 23,1/13,0 mmHg ($p<0,001$) beobachtet (Abbildung 4B, 4E). Bei NT-Probanden mit einem BD >100/60 mmHg und <120/80 mmHg war keine Änderung erkennbar, wohingegen der BD bei 74 überwiegend weiblichen NT mit einem initial niedrigem BD <100/60 mmHg während des Fastens um 7,3/3,1 mmHg stieg ($p<0,001$; Abbildung 4A, 4D). HTM-Probanden, die ihre Medikation beibehielten (n=73), reduzierten den BD um 8,7/5,1 mmHg. Diejenigen, die die Medikation stoppten (n=89), reduzierten den BD um 1,3/2,7 mmHg (Abbildung 4C, 4F).

Darüber hinaus senkte Fasten das Gewicht signifikant von 81,1±19,2 kg auf 77,0±18,2 kg ($p<0,001$). Der Bauchumfang verminderte sich um 5,5±3,8 cm ($p<0,001$; Tabelle 4).

Unerwünschte Nebenwirkungen:

Während der einjährigen Observationsstudie traten bei 23 der 1780 Probanden unerwünschte Nebenwirkungen auf. Dazu zählten: Herzrhythmusstörungen n=5, Hyponatriämie n=4, Schwindel n=4, Ekzem n=3, Hospitalisierung n=3, Erbrechen n=2, Hypoglykämie n=2, Hypokaliämie n=2, Ausbruch einer Infektion, Gichtanfall, Hyperventilation, krampfartige Bauchschmerzen, Pleuropneumonie, Sehstörungen, Synkope, Tetanie, Zahnfleischbluten (je n=1). Zwei Probanden mussten das Fasten aufgrund unerwünschter Ereignisse vorzeitig beenden (1-3).

Subjektives Wohlergehen:

Insgesamt wurde eine Verbesserung des subjektiven Wohlergehens der Probanden dokumentiert (1, 3). Das emotionale Wohlbefinden, welches mittels numerischen Skalen von 0 bis 10 Punkten erfasst wurde, erhöhte sich im Fasten von 6,2±0,1 Punkte auf 7,6±0,1 Punkte und das körperliche Wohlbefinden von 5,8±0,1 Punkte auf 7,4±0,1 Punkte (je $p<0,001$) (1). Der WHO-5 stieg um 30,7 % ($p<0,001$) und das Entspannungsniveau, das ebenfalls mittels numerischer Skala von 0 bis 10 Punkten erfasst wurde, stieg um 2,9 Punkte ($p<0,001$; Tabelle 4) (3).

## 1.6 Diskussion

Die vorliegende Arbeit untersuchte die Auswirkungen eines medizinisch begleiteten LF auf kardiovaskuläre und metabolische Risikofaktoren in einer einjährigen, prospektiven Observationsstudie. Die Ergebnisse dieser großen Studienpopulation zeigten eine signifikante Reduktion des Körpergewichts und Bauchumfangs (1). Zudem verbesserten sich TG, TC, LDL-C, Blutglukose und HbA1c-Werte (1). Der FLI, ein Indikator der NAFLD, sank bei Probanden mit und ohne T2DM signifikant (2). Außerdem reduzierte sich der BD bei unbehandelten und medikamentös behandelten Hypertonikern (3).

Viszerale Adipositas, Dyslipidämie, Hyperglykämie und Insulinresistenz sind maßgebliche Risikofaktoren für die Entstehung kardiovaskulärer Krankheiten (28, 41) und einer NAFLD, die als hepatische Manifestation des metabolischen Syndroms bezeichnet wird (23). Die vorliegenden Ergebnisse zeigten eine positive Beeinflussung einzelner Risikofaktoren durch das LF, wodurch eine präventive Wirkung vermutet werden kann (1-3). Übergewicht und die daraus resultierende Dysfunktion des Fettgewebes sind ein zentraler Ausgangspunkt (23, 41). LF senkte Körpergewicht und BMI signifikant mit erwartungsgemäß stärkerer Reduktion bei

längerem Fasten (1). Ein moderater Gewichtsverlust von 5–10 % beeinflusst das Auftreten adipositasbedingter Komorbiditäten positiv (42) und konnte durch LF größtenteils erreicht werden (1). Die Reduktion des Bauchumfangs (1), spiegelte eine Abnahme des metabolisch aktiven, viszeralen Bauchfettes wider und ist mit einer Risikoreduktion für kardiovaskuläre Ereignisse verbunden (27). Einarmige Interventionsstudien, die ebenfalls das hier untersuchte Fastenprogramm verwendeten, bestätigten diese Beobachtungen bei 109 überwiegend Stoffwechselgesunden, die 7–13 Tage fasteten (43) und bei 16 gesunden Männern, die zehn Tage fasteten (44). Im Einklang waren auch die Ergebnisse eines siebentägigen Buchinger Fastens in einer randomisierten kontrollierten Studie bei 32 T2DM (18).

Lipidstoffwechselstörungen führen zu arteriosklerotischen Veränderungen, erhöhen das Risiko von Fetteinlagerungen in der Leber und können zur BD-Erhöhung beitragen (23, 30). Durch die Unterbrechung der Nahrungszufuhr wurde ein prompter Abfall der TG beobachtet (1). LDL-C und TC sanken abhängig von der Fastenlänge (1). Der LDL-C/HDL-C-Quotient blieb konstant (1). Insgesamt deutete das Lipidprofil auf ein reduziertes atherogenes Risiko nach dem Fasten (29). Überdies wurde gezeigt, dass zehntägiges Fasten einen Marker für oxidative Schäden an Lipiden (Thiobarbitursäure-reaktive Substanzen) senkte (43, 45). Vorliegend, wurde außerdem ein positiver Einfluss auf den Glukosestoffwechsel beobachtet (1). Dabei sank die Blutglukose unabhängig von der Fastenlänge auf einen unteren Grenzwert und der HbA1c-Wert wurde reduziert (1). Der günstige Einfluss des LF auf kardiovaskuläre und metabolische Risikofaktoren wurde bei T2DM (18) und Gesunden (43) gezeigt und wird außerdem gestützt durch die Ergebnisse eines zehntägigen, prospektiven, unkontrollierten Wasserfastens von acht Gesunden, deren Metabolitenprofil im Plasma bestimmt wurde (46). Eine einarmige Interventionsstudie bei 19 Probanden mit metabolischem Syndrom, die zwölf Wochen lang das Essen auf zehn Stunden am Tag begrenzten, erfuhren zwar signifikante Abnahmen des Gewichts, Bauchumfangs, TC und LDL-C, konnten aber TG und Glukoseparameter nicht beeinflussen (47).

Unter Berücksichtigung des Nachweises von Acetoacetat im Urin (1), dokumentierten die präsentierten Ergebnisse die Umschaltung in den Fastenstoffwechsel (8). In Übersichtsarbeiten sind die Wirkungen der Ketonkörper, insbesondere des β-Hydroxybutyrats, auf Signalwege beschrieben, die in Zell- und Tierstudien gefunden wurden und zu gesundheitsfördernden Wirkungen führen können (4, 48). Des Weiteren wurde ein Absinken des Insulinspiegels gezeigt (44). Ein kontrollierter Tierversuch mit genetischen T2DM-Mäusen (n=15) zeigte, dass sechs bis acht Zyklen einer viertägigen fasten-nachahmenden Diät (fasting mimicking diet; FMD; erster Tag 50 %-ige Kalorienreduktion [calorie restriction, CR] gefolgt von 90 %-iger

CR, kohlenhydrat- und proteinarm) und anschließender siebentägiger *ad libitum* Fütterung die Insulinsensitivität und β-Zellfunktion verbesserten (49).

LF zeigte in der vorliegenden Arbeit Wirkungen bei Kranken. So wurde die Anzahl an Personen mit manifester Fettleber gesenkt (2). Dies galt auch für T2DM, die zu Beginn in erhöhtem Maße eine NAFLD aufwiesen (2). Auffällig war, dass die FLI-Reduktion bei T2DM, trotz ähnlicher Fastenlänge, signifikant stärker ausfiel. Im Vergleich mit den Nicht-T2DM konnten T2DM Körpergewicht, Bauchumfang, Blutglukose, HbA1c-Werte und TG deutlicher senken (2). Diese Verbesserungen sind besonders für T2DM wünschenswert (50). In einer randomisierten kontrollierten Studie mit NAFLD-Patienten zeigten sich nach 4 und 12 Wochen bei alternierendem, umtägigen Fasten (alternate day fasting, ADF; 75 %-ige CR am Fasttag; n=90) Senkungen der TC- und TG-Spiegel. In der Gruppe, die die tägliche Nahrungsaufnahme auf acht Stunden begrenzte (n=95), reduzierten sich nur die TG-Spiegel. Die Lebersteifigkeit, gemessen mittels Elastographie, wurde weder in den Interventionsgruppen noch in der Kontrollgruppe (20 %-ige CR) beeinflusst (51). Dagegen zeigte eine wöchentliche FMD (70 %-ige CR, kohlenhydrat- und proteinarm) im Wechsel mit *ad libitum* Fütterung über acht Wochen, bei genetischen T2DM-Mäusen (n=8), eine signifikante Reduktion des Fettgehalts in der Leber anhand histologischer Befunde im Gegensatz zur *ad libitum* gefütterten Kontrollgruppe (52). Die fasten-induzierte β-Oxidation und Ketogenese könnte zur Entfettung der Leber beitragen (8, 52).

Auch Hypertoniker konnten durch LF den BD klinisch wirksam, in nicht-linearer Weise abhängig von der Fastenlänge, normalisieren (3). Besonders hervorzuheben ist, dass 164 der 377 HTM-Probanden ihre Medikation reduzierten und 89 stoppten. Trotzdem wurde ein BD <140/90 mmHg bei 70,3 % am Fastenende erreicht (3). Außerdem erfuhren HTNM-Probanden mit den höchsten Ausgangswerten die stärkste Reduktion. Insgesamt 60,7 % der HTNM-Probanden normalisierten am Ende des Fastens ihren BD (3). Im Gegensatz hierzu unterlagen NT-Probanden nur geringfügigen Änderungen und Probanden mit initial niedrigem BD steigerten diesen (3). In einer älteren, unkontrollierten Beobachtungsstudie bei 124 nicht-medikamentös behandelten Hypertonikern von denen 43 adipös und 81 nicht adipös waren und die 14,7 bzw. 12,9 Tage nach der Buchinger Methode fasteten, wurde jeweils eine BD-Reduktion um 34/17 mmHg festgestellt (16). Eine weitere unkontrollierte Studie über elftägiges Wasserfasten bei 174 Hypertonikern, von denen 6,3 % Antihypertensiva einnahmen und stoppten, dokumentierte eine BD-Senkung um 37/13 mmHg. Zudem zeigten Hypertoniker mit einem initialen BD >180/110 mmHg die stärkste Reduktion um 60/17 mmHg (53). In der vorliegenden Observationsstudie profitierten besonders Personen mit hohen FLI- bzw. BD-

Werten vom LF. Längere Fastenperioden waren effektiver (2, 3). Dies lässt vermuten, dass Personen mit Übergewicht und metabolischen Beeinträchtigungen ihren Gesundheitszustand zumindest kurzfristig verbessern können (2, 3).

Lebensstilmodifikationen werden zur Behandlung und Prävention kardiovaskulärer Erkrankungen (54) sowie NAFLD empfohlen (48, 55). LF wird diesen Empfehlungen teils gerecht durch Reduktion des Körpergewichts und Bauchumfangs, den währenddessen eingeschränkten Alkohol- und Salzkonsum, sowie die Entspannungsförderung (1-3). Außerdem entspricht das untersuchte Fastenprogramm den Empfehlungen von 30 Minuten Bewegung am Tag (56). Körperliche Aktivität per se zeigte in einer Metaanalyse von 16 Studien mit 706 Probanden, darunter 639 adipösen NAFLD-Patienten, eine Senkung des Leberfettgehalts um 2,4 % (95 %-Konfidenzintervall [KI]: -3,13 bis -1,66) (57). Eine weitere Metaanalyse von fünf randomisierten kontrollierten Studien, die 229 Probanden mit erhöhtem BD untersuchten, ergab, dass körperliche Aktivität den BD um 8,2/4,1 mmHg (95 %-KI: SBD, -10,9 bis -5,5; DBD, -6,3 bis -1,9) senkte (58).

Ein zugelassenes Medikament gibt es für NAFLD derzeit nicht (36). Ein Geeignetes sollte Wirkmechanismen gegen leber-spezifische Veränderungen, kardiovaskuläre Komplikationen und T2DM vereinen (59). LF beeinflusst einige Ziele antihypertensiver Wirkstoffe (3). Eine erhöhte Natriurese aufgrund renaler Elimination anionischer Ketonkörper findet in den ersten Fastentagen statt, bis die Natriumionen nach Einsetzen der Glukoneogenese in der Niere durch Ammoniumionen ersetzt werden (60). Eine Erhöhung des atrialen natriuretischen Peptids, einhergehend mit gesteigerter Natriurese, wurde bei 25 adipösen Hypertonikern am vierten von zehn Fastentagen im Rahmen einer kontrollierten Interventionsstudie (500 kcal/Tag), beobachtet (61). Die daraus resultierende Reduktion des Plasmavolumens (62) begründet, weshalb Diuretika routinemäßig abgesetzt werden (6). Fasten hat Einfluss auf das vegetative Nervensystem (63). Eine unkontrollierte Interventionsstudie, an der 16 Personen mit metabolischem Syndrom teilnahmen, zeigte, dass bei einem 16-tägigen Fasten (230 kcal/Tag) die Adrenalinspiegel am zweiten Tag vorübergehend erhöht waren und anschließend unter Ausgangsniveau absanken. Die Noradrenalinspiegel sanken unmittelbar mit transientem Anstieg am vierten Tag (64). Ratten, die einem ADF für sechs Monate unterzogen wurden (n=8), zeigten in einem Stresstest niedrigere Adrenalin- und Noradrenalinspiegel als die *ad libitum* gefütterte Kontrollgruppe (65). Ein Anstieg der parasympathischen Aktivität wurde im Tierversuch an Ratten (n=12), von denen die Hälfte eine 40 %-ige CR und die anderen ein ADF über acht Wochen machten, durch Messung der Herzraten- und DBD-Variabilität, gezeigt (66). Ein zugrundeliegender Mechanismus könnte die erhöhte Expression des Brain-derived

neurotrophic factor (BDNF) infolge der Aktivierung durch β-Hydroxybutyrat sein, wie es in kortikalen Nervenzellen nachgewiesen wurde (67). Eine intrazerebroventrikuläre Infusion von BDNF beeinflusste die Herzrate durch Erhöhung der parasympathischen Aktivität im Hirnstamm bei Mäusen (n=24) (68). Der Gewichtsverlust könnte durch Reduktion der Fettmasse Einfluss auf ektopische Fettansammlungen der Nieren sowie Blutgefäße haben, die durch mechanischen Druck und Freisetzung proinflammatorischer Adipokine zur BD-Steigerung beitragen (69). Außerdem könnte die metabolische Aktivität des Fettgewebes moduliert werden und ein Adiponektinanstieg zu einer Vasodilatation führen (70, 71). Eine kontrollierte Studie an Ratten (n=15) wies eine Steigerung des Adiponektins bei ADF über drei Monate nach (72), ebenso bei Mäusen, die entweder ADF (75 %-, 85 %- oder 100 %-ige CR am Fasttag) oder eine 25 %-ige CR über vier Wochen (je n=6) machten (73). Zusätzlich senkte ein achttägiges Buchinger Fasten in einer explorativen, kontrollierten Studie bei 36 Probanden die Leptinspiegel um 58 % (22). Somit könnten BD-steigernde Effekte durch erhöhtes Leptin, wie die Aktivierung des sympathischen Nervensystems und die Ausschüttung von Aldosteron, reduziert werden (74). Des Weiteren wurde in einer Querschnittstudie bei 17 Frauen in der Menopause, die eine 13-wöchige CR auf 600 kcal/Tag mit Gewichtsverlust >5 % durchgeführt hatten, die Senkung einzelner Bestandteile des Renin-Angiotensin-Aldosteron-Systems beobachtet (75). Dies zeigte auch eine einarmige Interventionsstudie, bei der 16 Adipöse eine 40 %-ige CR über fünf Wochen durchführten (76). Ferner könnte die Senkung der Insulinspiegel durch das LF (44) die anti-natriuretischen Effekte einer Hyperinsulinämie aufheben (77).

In der vorliegenden Arbeit traten unerwünschte Nebenwirkungen bei etwa 1 % der großen Studienpopulation auf. Hypoglykämien oder Anzeichen von zu niedrigem BD, wie zum Beispiel Schwindelgefühl, waren selten (1-3). Das moderate Absinken der Natriumspiegel könnte neben der fasteninduzierten Natriurese zu Beginn durch die verminderte Salzzufuhr währenddessen bedingt sein (1, 3). Die Ketosurie wird zudem für den Anstieg der Harnsäure verantwortlich gemacht, da sie deren renale Ausscheidung hemmt (60), wie die intravenöse Verabreichung der Ketonkörper bei Gesunden (n=4) zeigte (78), ohne dabei vermehrt Gichtanfälle beim Fasten auszulösen (1). Von Bedeutung ist die erhöhte Blutungsneigung mit verlängerter Gerinnungszeit (1). Vorliegend wurde eine Abnahme der Zellzahlen von Leukozyten und Thrombozyten beobachtet (1). Es gibt Hinweise auf einen transienten Abfall. Bei zweimal im Monat durchgeführter, viertägiger FMD, gefolgt von *ad libitum* Fütterung ab einem Alter von 16 Monaten, wurde bei Mäusen (n=6–12) beobachtet, dass die altersbedingte Abnahme der Blutbildung unterbrochen wurde und es zu einem Anstieg der Leukozyten,

Thrombozyten, und Erythrozyten kam (79). Die Studie beschrieb außerdem, dass die Lebergröße abnahm und die GPT vorübergehend anstieg, jedoch 24 Stunden nach Nahrungswiedereinführung eine Regeneration dokumentiert wurde (79). Der hier beobachtete moderate Anstieg der Leberenzyme GOT und GPT (1) wurde in älteren, unkontrollierten Beobachtungen bei 88 Adipösen, die ein Wasserfasten für 35 Tage durchführten, festgestellt und auf eine erhöhte Transaminierungsaktivität zurückgeführt (80). LF steigerte in der vorliegenden Observationsstudie das Wohlergehen (1, 3) und die Ergebnisse konnten in zwei weiteren Kohorten repliziert werden (43, 44). Bei 36 Personen mit chronischem Schmerzsyndrom konnte nach achttägigem Buchinger Fasten ebenfalls ein Stimmungsanstieg mittels visueller Skala beobachtet werden (22).

Der größte limitierende Faktor für die Interpretation der vorliegenden Daten ist das beobachtende Studiendesign, trotz der hohen Teilnehmerzahl (1). Eine Trennung der rein fasteninduzierten Effekte von den kurbedingten Effekten, wie Bewegung und Erholung ist dadurch nicht möglich. Randomisierte kontrollierte Studien sind notwendig, um die Beobachtungen wissenschaftlich zu untermauern. Hierbei sollte die Nachhaltigkeit der kurzfristig erreichten Wirkungen in Langzeitstudien untersucht werden (2, 3). Der FLI dient als Surrogatmarker und sollte durch präzise Fettleber-Bestimmungsmethoden geprüft bzw. ersetzt werden (2). Außerdem wurde der BD zwar täglich, jedoch nur einmalig gemessen (1, 3). Bereits bestehende Medikationen wurden ohne Verblindung oberflächlich erfasst, so dass nur semiquantitative Auswertungen möglich waren (1, 3). Die Studie wurde in einer spezialisierten Fastenklinik durchgeführt und die Teilnehmenden fasteten auf eigenen Wunsch (1). Damit besteht bezüglich der Compliance ein Bias (1). Die erzielten Ergebnisse sind somit nicht unmittelbar auf das LF im Allgemeinen übertragbar (1-3).

Zusammenfassend deuten die Ergebnisse der vorliegenden Arbeit auf eine Verbesserung kardiovaskulärer und metabolischer Risikofaktoren, wie Gewicht, Bauchumfang, TG, TC, LDL-C, Blutglukose und HbA1c hin. Dabei scheinen Gesunde und Kranke vom LF zu profitieren, wie die Reduktion des FLI bei T2DM und die BD-Senkung bei Hypertonikern zeigte. Unerwünschte Nebenwirkungen wurden selten beobachtet und das subjektive Wohlergehen gesteigert. LF könnte somit ein vielversprechender, komplementärmedizinischer, nicht-pharmakologischer Ansatz zur Prävention und unterstützenden Therapie übergewichtsbedingter Gesundheitsprobleme wie NAFLD und Hypertonie sein. In weiteren, möglichst randomisierten kontrollierten Langzeitstudien sollten die vielfältigen Wirkmechanismen eruiert werden.

## 1.7 Literaturverzeichnis


1. Wilhelmi de Toledo F, Grundler F, Bergouignan A, Drinda S, Michalsen A. Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects. PLoS One [Internet]. 2019 [cited Jan 2 2019]; 14(1):e0209353. Available from: https://doi.org/10.1371/journal.pone.0209353.
2. Drinda S, Grundler F, Neumann T, Lehmann T, Steckhan N, Michalsen A, Wilhelmi de Toledo F. Effects of Periodic Fasting on Fatty Liver Index—A Prospective Observational Study. Nutrients. 2019;11(11):2601.
3. Grundler F, Mesnage R, Michalsen A, Wilhelmi de Toledo F. Blood pressure changes in 1610 subjects with and without antihypertensive medication during long-term fasting. J Am Heart Assoc [Internet]. 2020 [cited Nov 23 2020]; 9(23):e018649. Available from: https://doi.org/10.1161/JAHA.120.018649.
4. de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. N Engl J Med. 2019;381(26):2541-51.
5. Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metab. 2016;23(6):1048-59.
6. Wilhelmi de Toledo F, Buchinger A, Burggrabe H, Hölz G, Kuhn C, Lischka E, Lischka N, Lützner H, May W, Ritzmann-Widderich M. Fasting therapy-an expert panel update of the 2002 consensus guidelines. Complement Med Res. 2013;20(6):434-43.
7. Wilhelmi de Toledo F, Grundler F, Sirtori CR, Ruscica M. Unravelling the health effects of fasting: a long road from obesity treatment to healthy life span increase and improved cognition. Ann Med. 2020;52(5):147-61.
8. Anton SD, Moehl K, Donahoo WT, Marosi K, Lee SA, Mainous AG, III, Leeuwenburgh C, Mattson MP. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. Obesity (Silver Spring). 2018;26(2):254-68.
9. Cahill GF, Jr. Starvation in man. Clin Endocrinol Metab. 1976;5(2):397-415.
10. Longo VD, Di Tano M, Mattson MP, Guidi N. Intermittent and periodic fasting, longevity and disease. Nat Aging. 2021;1(1):47-59.
11. Raffaghello L, Lee C, Safdie FM, Wei M, Madia F, Bianchi G, Longo VD. Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. Proc Natl Acad Sci USA. 2008;105(24):8215-20.
12. Singh R, Lakhanpal D, Kumar S, Sharma S, Kataria H, Kaur M, Kaur G. Late-onset intermittent fasting dietary restriction as a potential intervention to retard age-associated brain function impairments in male rats. Age. 2012;34(4):917-33.
13. Cheng C-W, Adams GB, Perin L, Wei M, Zhou X, Lam BS, Da Sacco S, Mirisola M, Quinn DI, Dorff TB. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell stem cell. 2014;14(6):810-23.
14. Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider NL. Effects of intermittent feeding upon growth and life span in rats. Gerontology. 1982;28(4):233-41.
15. Uno M, Honjoh S, Matsuda M, Hoshikawa H, Kishimoto S, Yamamoto T, Ebisuya M, Yamamoto T, Matsumoto K, Nishida E. A fasting-responsive signaling pathway that extends life span in C. elegans. Cell Rep. 2013;3(1):79-91.
16. Müller H, Wilhelmi de Toledo F, Schuck P, Resch K. Blutdrucksenkung durch Fasten bei adipösen und nichtadipösen Hypertonikern. Perfusion. 2001;14:108-12.
17. Li C, Ostermann T, Hardt M, Lüdtke R, Broecker-Preuss M, Dobos G, Michalsen A. Metabolic and psychological response to 7-day fasting in obese patients with and without metabolic syndrome. Complement Med Res. 2013;20(6):413-20.
18. Li C, Sadraie B, Steckhan N, Kessler C, Stange R, Jeitler M, Michalsen A. Effects of a one-week fasting therapy in patients with type-2 diabetes mellitus and metabolic

syndrome–A randomized controlled explorative study. Exp Clin Endocrinol Diabetes. 2017;125(09):618-24.
19. Uden A-M, Trang L, Venizelos N, Palmblad J. Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. Ann Rheum Dis. 1983;42(1):45-51.
20. Sköldstam L, Larsson L, Lindström FD. Effects of fasting and lactovegetarian diet on rheumatoid arthritis. Scand J Rheumatol. 1979;8(4):249-55.
21. Pietrocola F, Demont Y, Castoldi F, Enot D, Durand S, Semeraro M, Baracco EE, Pol J, Bravo-San Pedro JM, Bordenave C, Levesque S, Humeau J, Chery A, Métivier D, Madeo F, Maiuri MC, Kroemer G. Metabolic effects of fasting on human and mouse blood in vivo. Autophagy. 2017;13(3):567-78.
22. Michalsen A, Kuhlmann MK, Lüdtke R, Bäcker M, Langhorst J, Dobos GJ. Prolonged fasting in patients with chronic pain syndromes leads to late mood-enhancement not related to weight loss and fasting-induced leptin depletion. Nutr Neurosci. 2006;9(5-6):195-200.
23. Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen H-M. NAFLD and cardiovascular diseases: a clinical review. Clin Res Cardiol [Internet]. 2020 [cited Dec 31 2020]. Available from: https://doi.org/10.1007/s00392-020-01709-7.
24. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020;16(4):1-15.
25. Joseph P, Leong D, McKee M, Anand SS, Schwalm J-D, Teo K, Mente A, Yusuf S. Reducing the Global Burden of Cardiovascular Disease, Part 1. Circ Res. 2017;121(6):677-94.
26. Schienkiewitz A, Mensink GBM, Kuhnert R, Lange C. Übergewicht und Adipositas bei Erwachsenen in Deutschland. Journal of Health Monitoring [Internet]. 2017 [cited Feb 6 2021]; 2(2). Available from: https://doi.org/10.17886/rki-gbe-2017-025.
27. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, Cuevas A, Hu FB, Griffin BA, Zambon A, Barter P, Fruchart J-C, Eckel RH, Matsuzawa Y, Després J-P. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol. 2020;16(3):177-89.
28. Schrover IM, Spiering W, Leiner T, Visseren F. Adipose tissue dysfunction: clinical relevance and diagnostic possibilities. Horm Metab Res. 2016;48(4):213-25.
29. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). Eur Heart J. 2020;41(1):111-88.
30. Hydes TJ, Ravi S, Loomba R, Gray ME. Evidence-based clinical advice for nutrition and dietary weight loss strategies for the management of NAFLD and NASH. Clin Mol Hepatol. 2020;26(4):383-400.
31. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, Ramirez A, Schlaich M, Stergiou GS, Tomaszewski M, Wainford RD, Williams B, Schutte AE. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertension. 2020;75(6):1334-57.
32. Neuhauser H, Kuhnert R, Born S. 12-Monats-Prävalenz von Bluthochdruck in Deutschland. Journal of Health Monitoring [Internet]. 2017 [cited Feb 6 2021]; 2(1). Available from: https://doi.org/10.17886/rki-gbe-2017-007.
33. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE, Mossialos EA, Maggioni AP, Kazakiewicz D, May HT. European Society of Cardiology: cardiovascular disease statistics 2019. Eur Heart J. 2020;41(1):12-85.
34. Laurent S. Antihypertensive drugs. Pharmacol Res. 2017;124:116-25.

35. Nicoll R, Henein M. Caloric restriction and its effect on blood pressure, heart rate variability and arterial stiffness and dilatation: a review of the evidence. Int J Mol Sci [Internet]. 2018 [cited May 24 2019]; 19(3):751. Available from: https://doi.org/10.3390/ijms19030751.
36. Thiagarajan P, Aithal GP. Drug Development for Nonalcoholic Fatty Liver Disease: Landscape and Challenges. J Clin Exp Hepatol. 2019;9(4):515-21.
37. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform [Internet]. 2019 [cited Dec 17 2020]; 95:103208. Available from: https://doi.org/10.1016/j.jbi.2019.103208.
38. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6(1):33.
39. Topp CW, Østergaard SD, Søndergaard S, Bech P. The WHO-5 Well-Being Index: A Systematic Review of the Literature. Psychother Psychosom. 2015;84(3):167-76.
40. Wickham H. ggplot2: elegant graphics for data analysis. 2nd ed. Cham, Switzerland: Springer International Publishing; 2016.
41. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, Beguinot F, Miele C. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci. 2019;20(9):2538.
42. Ryan DH, Yockey SR. Weight Loss and Improvement in Comorbidity: Differences at 5%, 10%, 15%, and Over. Curr Obes Rep. 2017;6(2):187-94.
43. Wilhelmi de Toledo F, Grundler F, Goutzourelas N, Tekos F, Vassi E, Mesnage R, Kouretas D. Influence of Long-Term Fasting on Blood Redox Status in Humans. Antioxidants [Internet]. 2020 [cited June 6 2020]; 9(6):496. Available from: https://doi.org/10.3390/antiox9060496.
44. Mesnage R, Grundler F, Schwiertz A, Le Maho Y, Wilhelmi de Toledo F. Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting. J Nutr Sci [Internet]. 2019 [cited Nov 12 2019]; 8:e36. Available from: https://doi.org/10.1017/jns.2019.33.
45. Grundler F, Mesnage R, Goutzourelas N, Tekos F, Makri S, Brack M, Kouretas D, de Toledo FW. Interplay between oxidative damage, the redox status, and metabolic biomarkers during long-term fasting. Food Chem Toxicol [Internet]. 2020 [cited Aug 25 2020]; 145:111701. Available from: https://doi.org/10.1016/j.fct.2020.111701.
46. Steinhauser ML, Olenchock BA, O'Keefe J, Lun M, Pierce KA, Lee H, Pantano L, Klibanski A, Shulman GI, Clish CB. The circulating metabolome of human starvation. JCI insight [Internet]. 2018 [cited Dec 12 2020]; 3(16):e121434. Available from: https://doi.org/10.1172/jci.insight.121434.
47. Wilkinson MJ, Manoogian EN, Zadourian A, Lo H, Fakhouri S, Shoghi A, Wang X, Fleischer JG, Navlakha S, Panda S. Ten-hour time-restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome. Cell Metab. 2020;31(1):92-104.
48. Carneros D, López-Lluch G, Bustos M. Physiopathology of Lifestyle Interventions in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients. 2020;12(11):3472.
49. Cheng C-W, Villani V, Buono R, Wei M, Kumar S, Yilmaz OH, Cohen P, Sneddon JB, Perin L, Longo VD. Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell. 2017;168(5):775-88.
50. Crupi AN, Haase J, Brandhorst S, Longo VD. Periodic and Intermittent Fasting in Diabetes and Cardiovascular Disease. Curr Diab Rep. 2020;20(12):1-14.

51. Cai H, Qin Y-L, Shi Z-Y, Chen J-H, Zeng M-J, Zhou W, Chen R-Q, Chen Z-Y. Effects of alternate-day fasting on body weight and dyslipidaemia in patients with non-alcoholic fatty liver disease: a randomised controlled trial. BMC Gastroenterol. 2019;19(1):1-8.
52. Wei S, Han R, Zhao J, Wang S, Huang M, Wang Y, Chen Y. Intermittent administration of a fasting-mimicking diet intervenes in diabetes progression, restores β cells and reconstructs gut microbiota in mice. Nutr Metab (Lond). 2018;15(1):80.
53. Goldhamer A, Lisle D, Parpia B, Anderson SV, Campbell TC. Medically supervised water-only fasting in the treatment of hypertension. J Manipulative Physiol Ther. 2001;24(5):335-9.
54. Bakris G, Ali W, Parati G. ACC/AHA Versus ESC/ESH on Hypertension Guidelines: JACC Guideline Comparison. J Am Coll Cardiol. 2019;73(23):3018-26.
55. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. Obesity facts. 2016;9(2):65-90.
56. Mancia G, Fagard R, Narkiewicz K, Redán J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. J Hypertens. 2013;31(10):1925-38.
57. Baker CJ, Martinez-Huenchullan SF, D'Souza M, Xu Y, Li M, Bi Y, Johnson NA, Twigg SM. Effect of exercise on hepatic steatosis: Are benefits seen without dietary intervention? A systematic review and meta-analysis. J Diabetes. 2021;13(1):63-77.
58. de Sousa EC, Abrahin O, Ferreira ALL, Rodrigues RP, Alves EAC, Vieira RP. Resistance training alone reduces systolic and diastolic blood pressure in prehypertensive and hypertensive individuals: meta-analysis. Hypertens Res. 2017;40(11):927-31.
59. Johnston MP, Patel J, Byrne CD. Multi-drug approaches to NASH: what's in the development pipeline? Expert Opin Investig Drugs. 2020;29(2):143-50.
60. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. West J Med. 1982;137(5):379-99.
61. Maoz E, Shamiss A, Peleg E, Salzberg M, Rosenthal T. The role of atrial natriuretic peptide in natriuresis of fasting. J Hypertens. 1992;10(9):1041-4.
62. Davidson MB. Role of Ketogenesis in Urinary Sodium Excretion: Elucidation by Nicotinic Acid Administration during Fasting. J Clin Endocrinol Metab. 1979;49(6):818-23.
63. DeLalio LJ, Sved AF, Stocker SD. Sympathetic nervous system contributions to hypertension: updates and therapeutic relevance. Can J Cardiol. 2020;36(5):712-20.
64. Göhler L, Hahnemann T, Michael N, Oehme P, Steglich H-D, Conradi E, Grune T, Siems W. Reduction of plasma catecholamines in humans during clinically controlled severe underfeeding. Prev Med. 2000;30(2):95-102.
65. Wan R, Camandola S, Mattson MP. Intermittent food deprivation improves cardiovascular and neuroendocrine responses to stress in rats. J Nutr. 2003;133(6):1921-9.
66. Mager DE, Wan R, Brown M, Cheng A, Wareski P, Abernethy DR, Mattson MP. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. The FASEB Journal. 2006;20(6):631-7.
67. Marosi K, Kim SW, Moehl K, Scheibye-Knudsen M, Cheng A, Cutler R, Camandola S, Mattson MP. 3-hydroxybutyrate regulates energy metabolism and induces BDNF expression in cerebral cortical neurons. J Neurochem. 2016;139(5):769-81.
68. Wan R, Weigand LA, Bateman R, Griffioen K, Mendelowitz D, Mattson MP. Evidence that BDNF regulates heart rate by a mechanism involving increased brainstem parasympathetic neuron excitability. J Neurochem. 2014;129(4):573-80.

69. Fantin F, Giani A, Zoico E, Rossi AP, Mazzali G, Zamboni M. Weight loss and hypertension in obese subjects. Nutrients. 2019;11(7):1667.
70. Ohashi K, Ouchi N, Matsuzawa Y. Adiponectin and hypertension. Am J Hypertens. 2011;24(3):263-9.
71. Dorresteijn J, Visseren F, Spiering W. Mechanisms linking obesity to hypertension. Obes Rev. 2012;13(1):17-26.
72. Wan R, Ahmet I, Brown M, Cheng A, Kamimura N, Talan M, Mattson MP. Cardioprotective effect of intermittent fasting is associated with an elevation of adiponectin levels in rats. J Nutr Biochem. 2010;21(5):413-7.
73. Varady KA, Allister CA, Roohk DJ, Hellerstein MK. Improvements in body fat distribution and circulating adiponectin by alternate-day fasting versus calorie restriction. J Nutr Biochem. 2010;21(3):188-95.
74. Beltowski J. Role of leptin in blood pressure regulation and arterial hypertension. J Hypertens. 2006;24(5):789-801.
75. Engeli S, Böhnke J, Gorzelniak K, Janke J, Schling P, Bader M, Luft FC, Sharma AM. Weight Loss and the Renin-Angiotensin-Aldosterone System. Hypertension. 2005;45(3):356-62.
76. Harp JB, Henry SA, DiGirolamo M. Dietary weight loss decreases serum angiotensin-converting enzyme activity in obese adults. Obes Res. 2002;10(10):985-90.
77. Sarzani R, Spannella F, Giulietti F, Balietti P, Cocci G, Bordicchia M. Cardiac natriuretic peptides, hypertension and cardiovascular risk. High Blood Press Cardiovasc Prev. 2017;24(2):115-26.
78. Goldfinger S, Klinenberg JR, Seegmiller JE, Miller J, Bradley K. Renal retention of uric acid induced by infusion of beta-hydroxybutyrate and acetoacetate. N Engl J Med. 1965;272(7):351-5.
79. Brandhorst S, Choi IY, Wei M, Cheng CW, Sedrakyan S, Navarrete G, Dubeau L, Yap LP, Park R, Vinciguerra M. A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. Cell Metab. 2015;22(1):86-99.
80. Ditschuneit H. Der Stoffwechsel bei Fettsucht und bei komplettem Fasten. Medizin und Ernährung. 1971;12:169-77.

## 2. Eidesstattliche Versicherung und Anteilserklärung

„Ich, Franziska Grundler, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema:

*Einfluss des Langzeitfastens auf kardiovaskuläre und metabolische Risikofaktoren bei Gesunden, NAFLD-Patienten und Hypertonikern*

selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

Franziska Grundler hatte folgenden Anteil an den nachfolgenden Publikationen:

Publikation 1:
Françoise Wilhelmi de Toledo*, Franziska Grundler*, Audrey Bergouignan, Stefan Drinda, Andreas Michalsen.
Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects.
*PLoS ONE*, 2019 (* Authors contributed equally)
DOI: 10.1371/journal.pone.0209353; PMID: 30601864; Impact Factor: 2,7

Beitrag im Einzelnen:

- Beteiligung an der Erstellung des Studienprotokolls sowie der Einreichung des Ethikantrags, der Studienregistrierung sowie der Erstellung sämtlicher studienrelevanter Dokumente für die Observationsstudie
- Verantwortlich für die Koordination und Durchführung der einjährigen Observationsstudie und Leitung der Datensammlung inklusive Einrichtung der elektronischen Datenbank
- Mitarbeit an der Datenauswertung (Flow Chart, Nebenwirkungen, gesundheitliche Hauptbeschwerde) nach Rücksprache mit den Co-Autoren
- Beteiligt an der Dateninterpretation
- Literaturrecherche und Beteiligung an der Anfertigung der ersten Version des Manuskripts als Bearbeitungsvorlage
- Erstellung aller Graphiken und Tabellen des Manuskripts nach Rücksprache mit den Co-Autoren
- Formatierung des Manuskripts nach den Richtlinien der Fachzeitschrift und Einreichung des Manuskripts
- Mitarbeit an der Revision des Artikels
- Geteilte Erstautorenschaft

Publikation 2:
Stefan Drinda, Franziska Grundler, Thomas Neumann, Thomas Lehmann, Nico Steckhan, Andreas Michalsen, Françoise Wilhelmi de Toledo.
Effects of Periodic Fasting on Fatty Liver Index—A Prospective Observational Study.
*Nutrients*, 2019
DOI 10.3390/nu11112601; PMID: 31671589; Impact Factor: 4,5

Beitrag im Einzelnen:

- Beteiligung an der Datenauswertung (Vorher-Nachher-Analyse und Korrelationsanalyse) nach Rücksprache mit den Co-Autoren
- Beteiligt an der Dateninterpretation
- Mitarbeit am Schreiben von Teilen des Methoden- und Ergebnisteils des Manuskripts
- Erstellung der Graphiken Figure 1, Figure 2 und Figure 3 sowie der Tabellen 1–3 des Manuskripts nach Rücksprache mit den Co-Autoren
- Mitarbeit an der Revision des Artikels
- Zweitautor

Publikation 3:
Franziska Grundler, Robin Mesnage, Andreas Michalsen, Françoise Wilhelmi de Toledo.
Blood pressure changes in 1610 subjects with and without antihypertensive medication during long-term fasting.
*Journal of the American Heart Association*, 2020
DOI: 10.1161/JAHA.120.018649; PMID: 33222606; Impact Factor: 4,6

Beitrag im Einzelnen:

- Mitarbeit an der Datenauswertung (Flow Chart, Kategorisierung der Diagnosen, Einnahme von Antihypertensiva) nach Rücksprache mit den Co-Autoren
- Beteiligt an der Dateninterpretation
- Anfertigung der ersten Version des Manuskripts
- Erstellung der Graphik Figure 1 sowie der Tabellen 1, 3, 4 des Manuskripts nach Rücksprache mit den Co-Autoren
- Formatierung des Manuskripts nach den Richtlinien der Fachzeitschrift und Einreichung des Manuskripts
- Mitarbeit an der Revision des Artikels
- Erstautorenschaft

_____
Unterschrift, Datum und Stempel des/der erstbetreuenden Hochschullehrers/in

_____
Unterschrift des Doktoranden/der Doktorandin

## 3. Übersicht und Druckexemplare der ausgewählten Publikationen

Folgende Publikationen werden nachfolgend abgedruckt:

Publikation 1:

Françoise Wilhelmi de Toledo*, **Franziska Grundler***, Audrey Bergouignan, Stefan Drinda, Andreas Michalsen. Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects.
*PLoS ONE*, 2019. (* Authors contributed equally)
DOI: 10.1371/journal.pone.0209353; PMID: 30601864; Impact Factor: 2,7

Publikation 2:
Stefan Drinda, **Franziska Grundler**, Thomas Neumann, Thomas Lehmann, Nico Steckhan, Andreas Michalsen, Françoise Wilhelmi de Toledo. Effects of Periodic Fasting on Fatty Liver Index—A Prospective Observational Study.
*Nutrients*, 2019.
DOI: 10.3390/nu11112601; PMID: 31671589; Impact Factor: 4,5

Publikation 3:
**Franziska Grundler**, Robin Mesnage, Andreas Michalsen, Françoise Wilhelmi de Toledo. Blood Pressure Changes in 1610 Subjects With and Without Antihypertensive Medication During Long-Term Fasting.
*Journal of the American Heart Association*, 2020.
DOI: 10.1161/JAHA.120.018649; PMID: 33222606; Impact Factor: 4,6

PLOS ONE

RESEARCH ARTICLE

# Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects

**Françoise Wilhelmi de Toledo**[1☯*], **Franziska Grundler**[1,2☯], **Audrey Bergouignan**[3,4,5,6], **Stefan Drinda**[1], **Andreas Michalsen**[7,8]

**1** Buchinger Wilhelmi Clinic, Überlingen, Germany, **2** Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, **3** Division of Endocrinology, Metabolism, and Diabetes and Anschutz Health and Wellness Center, University of Colorado, School of Medicine, Aurora, Colorado, United States of America, **4** Division of Geriatric Medicine, University of Colorado, School of Medicine, Aurora, Colorado, United States of America, **5** Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, CNRS, Strasbourg, France, **6** UMR 7178 Centre National de la Recherche Scientifique (CNRS), Strasbourg, France, **7** Institute of Social Medicine, Epidemiology and Health Economics, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, **8** Department of Internal and Integrative Medicine, Immanuel Krankenhaus Berlin, Berlin, Germany

☯ These authors contributed equally to this work.
* francoise.wilhelmi@buchinger-wilhelmi.com



OPEN ACCESS

**Citation:** Wilhelmi de Toledo F, Grundler F, Bergouignan A, Drinda S, Michalsen A (2019) Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects. PLoS ONE 14(1): e0209353. https://doi.org/10.1371/journal.pone.0209353

**Editor:** Massimiliano Ruscica, Università degli Studi di Milano, ITALY

**Received:** June 18, 2018

**Accepted:** December 3, 2018

**Published:** January 2, 2019



**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** The study was financed by Amplius GmbH, Überlingen, Germany. This company has the task to develop a research department for the Buchinger Wilhelmi Clinics Überlingen and Marbella who are the funders. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the

## Abstract

Only few studies document longer periods of fasting in large cohorts including non-obese participants. The aim of this study was to document prospectively the safety and any changes in basic health and well-being indicators during Buchinger periodic fasting within a specialised clinic. In a one-year observational study 1422 subjects participated in a fasting program consisting of fasting periods of between 4 and 21 days. Subjects were grouped in fasting period lengths of 5, 10, 15 and 20±2 days. The participants fasted according to the Buchinger guidelines with a daily caloric intake of 200–250 kcal accompanied by a moderate-intensity lifestyle program. Clinical parameters as well as adverse effects and well-being were documented daily. Blood examinations before and at the end of the fasting period complemented the pre-post analysis using mixed-effects linear models. Significant reductions in weight, abdominal circumference and blood pressure were observed in the whole group (each $p<0.001$). A beneficial modulating effect of fasting on blood lipids, glucoregulation and further general health-related blood parameters was shown. In all groups, fasting led to a decrease in blood glucose levels to low norm range and to an increase in ketone bodies levels (each $p<0.001$), documenting the metabolic switch. An increase in physical and emotional well-being (each $p<0.001$) and an absence of hunger feeling in 93.2% of the subjects supported the feasibility of prolonged fasting. Among the 404 subjects with pre-existing health-complaints, 341 (84.4%) reported an improvement. Adverse effects were reported in less than 1% of the participants. The results from 1422 subjects showed for the first time that Buchinger periodic fasting lasting from 4 to 21 days is safe and well tolerated. It led to enhancement of emotional and physical well-being and improvements in relevant cardiovascular and general risk factors, as well as subjective health complaints.

manuscript. No additional external funding received for this study.

**Competing interests:** FWT is member of the Directory Board of the Buchinger Wilhelmi Clinic (BWC), where the study was performed. As managing director of Amplius GmbH, FWT executes the scientific leadership at BWC. Amplius GmbH is a company that conceives, coordinates and develops fasting research on behalf of BWC. FG is currently employed, and SD was formerly employed, at BWC. AM is a consultant at BWC and receives financial compensation for this role from Amplius GmbH. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

## Introduction

There are a growing number of recent publications on intermittent fasting (IF), generally lasting 16 to 48 hours, and calorie restriction (CR). Periodic fasting (PF), recently defined as lasting from 2 to as many as 21 or more days, is less studied in humans, especially for periods longer than 4 days [1, 2]. Results show that lifespan and healthspan are prolonged in several animal models by fasting and CR [2–7] and that parameters of age-related diseases are improved in humans [5, 8, 9]. Fasting leads to pronounced metabolic changes: The shift from carbohydrates and glucose to fatty acids and ketones as the major cellular fuel source for body and brain seems to play a key role. It has recently been referred to as intermittent metabolic switching (IMS) and glucose-to-ketone (G-to-K) switch. The reverse step–ketone-to-glucose (K-to-G) switch–happens upon refeeding [2]. The G-to-K switch includes reduction in blood glucose, insulin and IGF-1 levels, depletion or reduction of glycogen stores, and an increase in lipolysis and ketogenesis [2, 5, 10]. Fasting has been shown to induce differential cellular stress resistance [11] and autophagy [12, 13], as well as triggering the synthesis of detoxification enzymes [9, 14]. Fasting seems to modify the intestinal microbiome [9, 15]. It also leads to changes in the intestinal mucosal walls in rats [16] and to pronounced neuroendocrine adaptation processes [2, 17]. Finally, in the K-to-G switch, fasting has been found to activate stem cells and multiple system regeneration in the refeeding period [4, 18, 19] and to increase the mitochondrial biogenesis in neurons and other body cells [2, 9].

Long periods of fasting, lasting several days to several weeks, are physiologic, e.g. during seasons of low sun exposure, and are still part of the life of most animals [20, 21] as well as of humans living without food conservation technologies [22].

Fasting periods with various patterns are found in most religions [23]. For instance, Ramadan intermittent fasting was linked with improvements in cardiometabolic risk factors [24]. Furthermore, morbid obesity and associated diseases were treated in the 1960s with long periods of fasting that were termed the "zero calorie diet" [25, 26]. In exceptional circumstances these periods could last up to 249 days or more [27, 28]. A medical program of periodic fasting developed by the German physician Otto Buchinger to treat obesity and metabolic and inflammatory pathologies is well-known in central Europe [29–31]. The therapeutic effects of Buchinger periodic fasting are documented in small studies on overweight [32], blood pressure [33], metabolic syndrome [34], fibromyalgia [35], chronic pain syndromes [36] and the enhancement of quality of life [37]. The effects of repeated cycles of Buchinger fasting have also been reported [38, 39].

We are not aware of large studies on PF including normal weight or moderately obese subjects and focused on safety and tolerability. In the present observational study, we documented prospectively the safety, general health-related outcomes and well-being of 1422 subjects. They fasted for periods between 4 to 21 days under medical supervision according to the Buchinger fasting program, as described in peer-reviewed guidelines [31]. The fasting took place in a facility specialized in therapeutic fasting, the Buchinger Wilhelmi clinic (BWC) in Germany. The protocol involved daily clinical monitoring, intake of 2–3 L of water per day and 250 kcal of food, as well as a multi-disciplinary program including health education and physical activity.

The aim of this study was to assess for the first time prospectively the safety, therapeutic efficiency and effects on well-being of Buchinger periodic fasting in a large cohort.

## Materials and methods

### Ethics

This observational and prospective study was approved by the medical council of Baden-Württemberg and the Ethics Committee of the Charité-University Medical Center, Berlin

(application number: EA4/054/15) on 5 May 2015. The study protocol was registered in the German Clinical Trials Register (DRKS-ID: DRKS00010111). The authors confirm that all ongoing and related trials for this intervention are registered. At the time of obtaining the ethical approval by the German authorities it was not mandatory to register an observational study. Only randomized clinical trials were clearly recommended to register. Nevertheless, we decided to register this observational study (on 3 June 2016).

Participants were enrolled after giving their written informed consent between 1 January and 31 December 2016. The study was conducted in the BWC in Überlingen (Germany) in accordance with the principles of the Declaration of Helsinki. The follow-up was completed between 26 January 2016 and 18 December 2017.

## Participants

Our study on Buchinger fasting during periods of 4 to 21 days included 1422 subjects. They were selected out of a total of 3929 subjects who were admitted to the BWC and fulfilled the following criteria: they had a clinic stay of at least 10 nights and signed the informed written consent at the beginning of the inpatient stay after confirming that they would not participate in another study. Subjects were aged between 18–99 years and had no predefined contraindication to Buchinger fasting (e.g. cachexia, anorexia nervosa, advanced kidney, liver or cerebrovascular insufficiency, dementia or other severely debilitating cognitive disease and no existing pregnancy or lactation period) [31]. Furthermore, blood must have been collected on the precise days defined in the protocol (S2 and S3 Text). We excluded subjects who were prescribed other diets than fasting according to predefined criteria as well as those who could not follow the study procedures due to an inability to speak German, English or French. A flow chart reflecting the selection procedure is given in Fig 1.

The subjects came voluntarily to the BWC for preventive or therapeutic reasons. They selected established programs of 5, 10, 15 and 20 fasting days, which are reflected in the 4 groups (F5d, F10d, F15d, F20d). The main personal intentions for the fasting intervention were reduction of cardiovascular risk factors, weight loss in case of obesity and relief of general health problems such as inflammatory diseases, distress and exhaustion. In case of prescribed drug intake, the dosage was adapted during the stay by the 8 physicians of the clinic, who examined all participants 2 to 3 times per week. The subjects had a wide diversity of national and cultural backgrounds. The majority of them came from upper social classes and had high education levels.

## Fasting program

All subjects fasted according to the guidelines of the Buchinger fasting therapy [31] under daily supervision of nurses and specialized physicians. On the day before the beginning of the fast, the participants were given a 600 kcal vegetarian diet divided into 3 meals of either rice and vegetables or fruits, according to individual preference. To initiate the fasting period, the intestinal tract was emptied through the intake of a laxative (20–40 g $NaSO_4$ in 500 ml water). During fasting all subjects were asked to drink 3 L of water or non-caloric herbal teas daily with an optional portion of 20 g honey. Additionally, an organic freshly squeezed fruit or vegetable juice (250 ml) was served at noon and a vegetable soup (250 ml) in the evening, leading to an average total calorie intake of 200–250 kcal and 25–35 g of carbohydrates per day. At the beginning of the fasting period the subjects entered a program of light physical exercise alternating with rest and individual mild non-physical treatments like hydrotherapy or physiotherapy. The exercise program consisted of light to moderate intensity outdoor walks and group gymnastics. The whole program was led by certified trainers. During the fasting period an

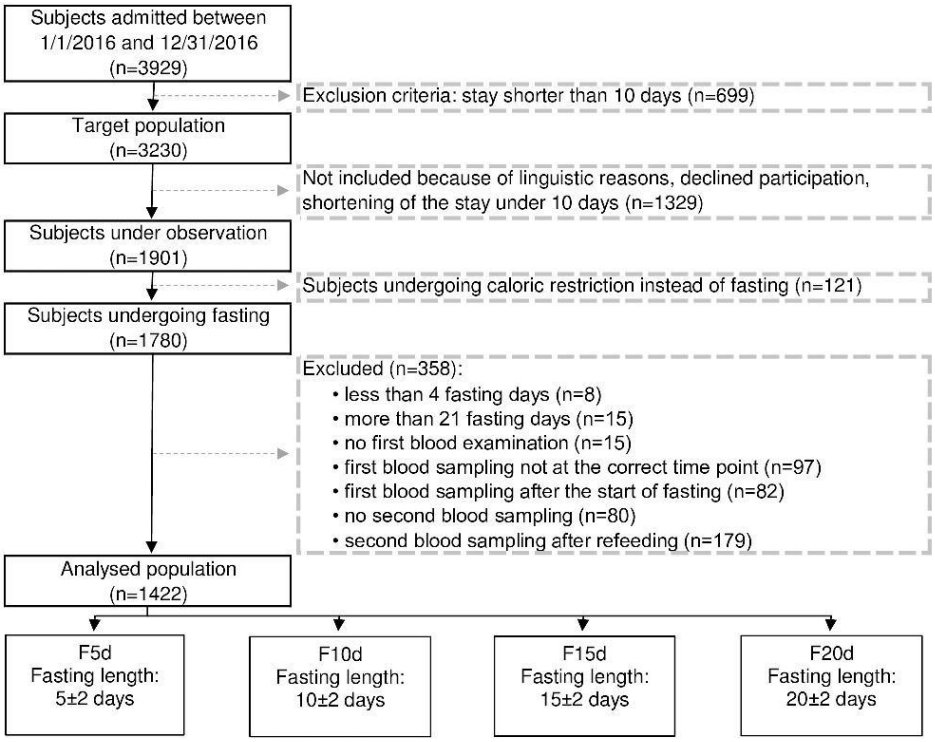



**Fig 1. Flow chart of the selection procedure of study participants.**

https://doi.org/10.1371/journal.pone.0209353.g001

enema or, if preferred by the patient, a mild laxative was applied every second day in order to remove intestinal remnants and desquamated mucosal cells. On the last day of fasting, food was stepwise reintroduced during an average of 4 days, with an ovo-lacto-vegetarian organic diet progressively increasing from 800 to 1600 kcal/day.

## Measurements

To document safety as well as health benefits and well-being during a prolonged periodic fasting program, we performed the predefined following measurements at baseline (pre-) and at the completion of fasting (post-). Before starting the fast all subjects went through a thorough physical examination and their medical history was documented.

Well-being, ketone bodies, mild symptoms and any changes in major health complaints were self-reported under supervision. The results were daily noted in a questionnaire (S4, S5, S6 Text). A total of 1311 subjects out of the 1422 returned the completed questionnaire.

**Weight, abdominal circumference, blood pressure and pulse.** Clinical data were collected by the physicians. Trained nurses documented every morning according to a standardized protocol the body weight of the participants wearing standard clothing (Seca 704, Seca,

Hamburg, Germany). Blood pressure and pulse were measured after a pause, once at the non-dominant arm in sitting position (upper arm blood pressure monitor, boso Carat professional, BOSCH + SOHN GmbH u. Co. KG, Jungingen, Germany). Height was assessed with seca 285 (Seca, Hamburg, Germany) and abdominal circumference was determined with a measuring tape mid-way between the lowest rib and the iliac crest (openmindz GmbH, Heidelberg, Germany).

**Well-being.** To evaluate well-being, the participants self-reported daily their physical (PWB) and emotional well-being (EWB) on numeric rating scales from 0 (very bad) to 10 (excellent), under nurses' supervision. The aim was to document the tolerability of the fasting program.

In a pre-study sample, we evaluated the acceptance of validated questionnaires to assess well-being within the patient population of the BWC, but found that they were regarded as too time-consuming in comparison with the numeric rating scales. To avoid drop-out and missing data, we therefore decided to use numeric rating scales.

**Ketone bodies.** The subjects self-measured the semi-quantitative concentration of ketone bodies in the first morning urine using Ketostix (Bayer AG, Leverkusen, Germany), which reacts according to the concentration of acetoacetic acid.

**Mild symptoms and adverse effects.** The Buchinger periodic fasting program was continuously monitored for safety and supervised by the medical staff: mild symptoms were reported daily by means of a multiple choice questionnaire, completed by the subjects under the supervision of nurses. This questionnaire listed the 19 most frequent mild symptoms that are observed in BWC and mentioned in the guidelines of the Buchinger fasting therapy [31]. We considered a mild symptom as being relevant when it was mentioned at least 3 times. In addition to the listed symptoms, the medical staff reported further mild symptoms that we categorized as "observed symptoms". Furthermore, occasional adverse effects (AE) were documented by the physicians.

**Major health complaint.** A self-evaluation of health status was undertaken at the end of the stay: the subjects were asked to self-rate any changes in their major health complaint (in cases in which they indicated one at the begin of the fast) during the fasting intervention on a visual numeric scale from 0 (much worse) to 7 (much better).

**Blood analysis.** A blood analysis was taken according to international methods (see below): (lipid parameters: total cholesterol [TC], triglycerides [TG], high-density lipoprotein [HDL-C], low-density cholesterol [LDL], LDL-C/HDL-C ratio [LDL/HDL-ratio]; glycaemia: blood glucose and glycated haemoglobin [HbA1c]; blood count: leukocytes, erythrocytes, haemoglobin, haematocrit, mean cell volume [MCV], mean corpuscular haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC], thrombocytes; coagulation: international normalized ratio [INR], Quick, partial thromboplastin time [PTT]; liver function: serum glutamic oxaloacetic transaminase [GOT], serum glutamate pyruvate transaminase [GPT], serum gamma-glutamyl transferase [GGT], alkaline phosphatase [AP]; inflammatory biomarkers: C-reactive protein [CRP], erythrocyte sedimentation rate [ESR] after 1 and 2 hours; renal function: uric acid, urea and creatinine and electrolytes: sodium [Na], potassium [K], calcium [Ca], magnesium [Mg]).

## Laboratory examinations

Blood samples were collected twice, namely before the start of the fasting (thereafter referred as baseline values) and at the end of the fasting period. They were collected by trained medical-technical assistants between 7.30 and 9.30 am and drawn into EDTA (S-Monovette 2.7 ml K3 EDTA), citrate (S-Monovette 3 ml 9NC, Citrate 3.2% [1:10]) and blood sedimentation

tubes (S-Sedivette 3.5 ml 4NC, ESR/Citrate Buffer [1:5]), that were shaken gently after filling. Additionally, serum tubes including serum gel with clotting activator (S-Monovette 9 ml Z-Gel) were used and stored upright for 30 min until coagulation, with subsequent centrifugation at 3920 g (5000 rpm) for 10 min at room temperature. All tubes were manufactured by Sarsteadt AG & Co. (Nürnbrecht, Germany).

The ESR was assessed within a period of 4 hours after blood collection and determined after 1 and 2 hours of blood sedimentation. All further analyses were performed at MVZ Labor Ravensburg, according to the manufacturer's instruction, in a fully- automated laboratory. Blood cell count (leukocytes, erythrocytes, haemoglobin, MCV, MCH, MCHC, thrombocytes) was measured using the blood analyser Sysmex XN-9000 (Sysmex Europe GmbH, Norderstedt, Germany). Coagulation parameters (INR, Quick, PTT) were assessed on ACL Top (Werfen, Kirchheim, Germany). The liver enzymes (GOT, GPT, GGT, AP), kidney parameters (urea, creatinine, uric acid), lipid parameters (TC, TG, HDL-C, LDL-C, LDL/HDL ratio), electrolytes (Na, K, Ca), glucose and CRP were analysed with ADVIA 2400 (Siemens Healthcare GmbH, Erlangen, Germany). The HbA1c was assessed with TOSOH$^{TM}$ (Bio-Rad Laboratories GmbH, München, Germany) and Mg with ICP-MS 7700x series (Agilent, Waldbronn, Germany).

### Data and statistical analysis

Participants were divided into four groups according to the duration of their fasting period (Fig 1): F5d underwent a fasting period of 5±2 days, with an average of 5.4 (n = 695), F10d underwent a fasting period of 10±2 days, with an average of 8.6 (n = 530), F15d underwent a fasting period of 15±2 days, with an average of 14.1 (n = 196) and F20d underwent a fasting period of 20±2 days, with an average of 20.1 (n = 37). Between-group differences at baseline were tested using a one-way ANOVA test followed by Tukey's post-hoc tests.

We tested the effect of fasting, while taking into account the sex and fasting duration group effects, by using a multistep parsimonious statistical approach. First, for each outcome the effect of fasting was assessed by using mixed linear models taking repeated measurements among subjects into account, with fasting intervention, fasting duration group, sex, fasting duration group-by-fasting-intervention, fasting intervention-by-sex, sex-by-fasting duration group and baseline values of the outcome (pre-fasting) as fixed effects. For each outcome, the covariance structures was selected among three (compound symmetry (CS), autoregressive (AR(1)) and variance components (VC)) using the Bayesian information criteria (BIC). In a last step the interaction effects that were not significant, were removed from the model to obtain a more parsimonious model. To simplify the presentation of the results, sex differences are presented in figures only when the fasting-intervention-by-sex effect was significant. To take into account the multiple tests performed on this dataset, significance was set at a conservative level of $p<0.01$.

Data are shown as mean±standard error of the mean (SEM), if not indicated otherwise. Statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, USA). Graphs were generated using GraphPad Prism version 6 for Windows (GraphPad Software, La Jolla California USA).

## Results

### General parameters

The baseline characteristics of the 1422 adult participants are shown in Table 1. Mean age was 55.4±0.4 with 59.1% women and 40.9% men. In total 63.4% of the subjects were non-obese (BMI<30). Grade I obesity (30≤BMI<35) was present in 19.5% and grade II or higher

**Table 1. Baseline characteristics of the subjects.**

| | All | F5d | F10d | F15d | F20d |
|---|---|---|---|---|---|
| Days (d) | | 5±2 | 10±2 | 15±2 | 20±2 |
| Subjects, n (%) | 1422 (100.0) | 659 (46.3) | 530 (37.3) | 196 (13.8) | 37 (2.6) |
| Men (%) | 581 (40.9) | 278 (42.2) | 214 (40.4) | 76 (38.8) | 13 (35.1) |
| Women (%) | 841 (59.1) | 381 (57.8) | 316 (59.6) | 120 (61.2) | 24 (64.9) |
| Age, years | 55.4±0.4 | 54.2±0.5 [b,c] | 56.3±0.6 [a] | 56.4±0.9 [a] | 56.4±2.3 |
| Fasting length (days) | 8.2±0.1 | 5.4±0.0 [b,c,d] | 8.6±0.0 [a,c,d] | 14.1±0.1 [a,b,d] | 20.1±0.2 [a,b,c] |
| Waist, cm | 94.0±0.4 | 91.3±0.6 [b,c,d] | 94.8±0.7 [a,c,d] | 98.3±1.2 [a,b,d] | 106.3±2.8 [a,b,c] |
| Weight, kg | 82.0±0.5 | 79.3±0.8 [b,c,d] | 82.7±0.9 [a,c,d] | 86.6±1.6 [a,b,d] | 96.7±4.0 [a,b,c] |
| BMI, kg/m$^2$ | 28.2±0.2 | 27.2±0.2 [b,c,d] | 28.5±0.3 [a,c,d] | 29.7±0.4 [a,b,d] | 33.6±1.1 [a,b,c] |
| BMI<25, n (%) | 404 (28.4) | 227 (56.2) | 133 (32.9) | 41 (10.1) | 3 (0.7) |
| 25≤BMI<30, n (%) | 497 (35.0) | 232 (46.7) | 199 (40.0) | 61 (12.3) | 5 (1.0) |
| BMI≥30, n (%) | 425 (29.9) | 155 (36.5) | 160 (37.6) | 84 (19.8) | 26 (6.1) |
| BMI men, kg/m$^2$ | 30.0±0.2 | 29.2±0.3 | 30.3±0.3 | 31.3±0.7 | 34.0±1.5 |
| BMI<25, n (%) | 74 (12.7) | 46 (62.2) | 18 (24.3) | 10 (13.5) | 0 (0.0) |
| 25≤BMI<30, n (%) | 231 (39.8) | 117 (50.6) | 92 (39.8) | 20 (8.7) | 2 (0.9) |
| BMI≥30, n (%) | 230 (39.6) | 94 (40.9) | 87 (37.8) | 40 (17.4) | 9 (3.9) |
| BMI women, kg/m$^2$ | 27.0±0.2 | 25.7±0.2 | 27.3±0.3 | 28.7±0.5 | 33.3±1.4 |
| BMI<25, n (%) | 330 (39.2) | 181 (54.8) | 115 (34.8) | 31 (9.4) | 3 (0.9) |
| 25≤BMI<30, n (%) | 266 (31.6) | 115 (43.2) | 107 (40.2) | 41 (15.4) | 3 (1.1) |
| BMI≥30, n (%) | 195 (23.2) | 61 (31.3) | 73 (37.4) | 44 (22.6) | 17 (8.7) |

Subjects were divided into 4 groups according to the fasting lengths: 5, 10, 15 and 20±2 days. Significant differences between the groups are indicated as

a, $p<0.05$ versus F5d

b, $p<0.05$ versus F10d

c, $p<0.05$ versus F15d

d, $p<0.05$ versus F20d. BMI, body mass index. Data are presented as mean±SEM.

https://doi.org/10.1371/journal.pone.0209353.t001

(BMI≥35) in 10.3%. Subjects who chose to fast on average for 20 days (F20d) had the highest baseline body mass index (BMI), the highest abdominal circumference (waist), and largest weight reduction (-8.6±0.3 kg) (each $p<0.001$). Men had a higher mean BMI at baseline (Table 1).

## Weight, abdominal circumference and blood pressure

As expected, weight and BMI showed a significant decrease (fasting intervention: $p<0.001$) in all 4 groups (S1 Table). The weight loss increased with the fasting period length and varied between 3.2±0.0 kg for F5d and 8.6±0.3 kg for F20d (fasting duration group-by-fasting intervention: $p<0.001$). Abdominal circumference also decreased significantly (fasting intervention: $p<0.001$). The reduction varied between 4.6±0.1 cm for F5d and 8.8±0.8 cm for F20d (fasting duration group-by-fasting intervention: $p<0.001$). Weight and abdominal circumference reduction were significantly higher (fasting-intervention-by-sex: each $p<0.001$) in men in all groups (Fig 2A and 2B), compared with women.

Baseline values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in the groups fasting longer (Fig 3A and 3B). The mean values for the whole cohort decreased significantly from 131.6±0.7 to 120.7±0.4 for SBP (fasting intervention: $p<0.001$) and from 83.7±0.4 to 77.9±0.3 for DBP (fasting intervention: $p<0.001$). The reduction of SBP and DBP was greater in the groups who fasted longer (fasting duration group-by-fasting intervention: each $p<0.001$) without gender difference (Fig 3A and 3B), stabilizing for the whole

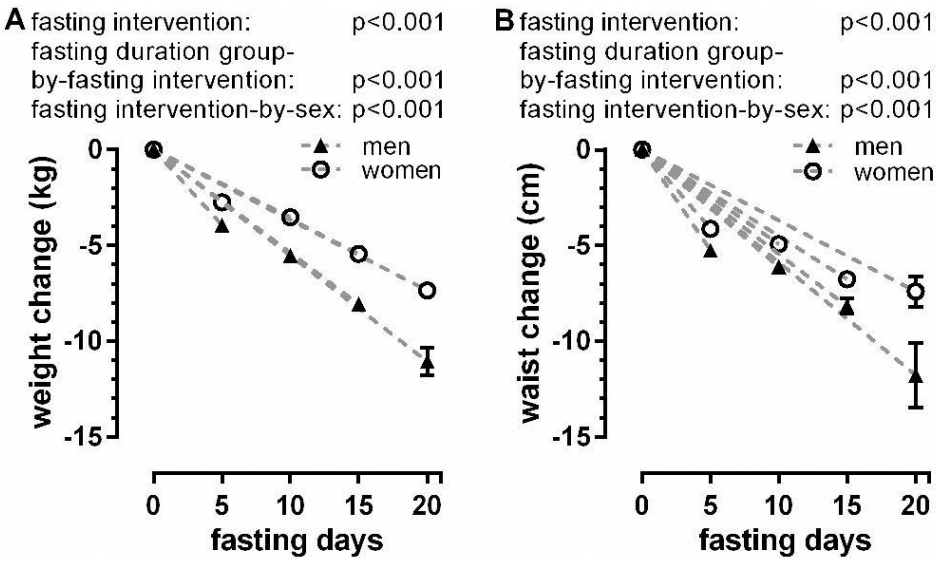



**Fig 2.** Changes in weight (A) and abdominal circumference (B) according to the length of fast and gender.

https://doi.org/10.1371/journal.pone.0209353.g002

cohort around 120/78 mm Hg (S1 Table). We did not observe significant changes in heart rate during fasting in the whole group (S1 Table).

### Well-being

Baseline values of emotional well-being (EWB) and physical well-being (PWB) were lower in the groups that fasted longer. This suggests that subjects choosing longer fasting periods had lower emotional and physical self-ratings at baseline than the ones who selected shorter periods of fast. EWB as well as PWB were both significantly enhanced in the course of the fast (fasting intervention: each $p<0.001$) (S1 Table). There is no difference between genders for those parameters (Fig 4A and 4B). All groups reached similar increased values of well-being at the end of their stay.

### Ketone bodies

Acetoacetic acid, reflecting ketosis, increased significantly from baseline to the end of fast (fasting intervention: $p<0.001$), suggesting a plateau value reached after 5 days. Men had higher scores of acetoacetic acid than women (fasting intervention-by-sex: $p<0.001$) (S1 Table).

### Mild symptoms and adverse effects

The safety of the Buchinger fasting program was assessed by collecting daily all self-reported and observed mild symptoms (Table 2). Of the 1311 participants who returned the filled questionnaire, 0.35% reported muscular cramp, which was the least frequent mild symptom, and 14.94% sleep disturbances, which was the most frequent mild symptom. As shown in S1 Fig.

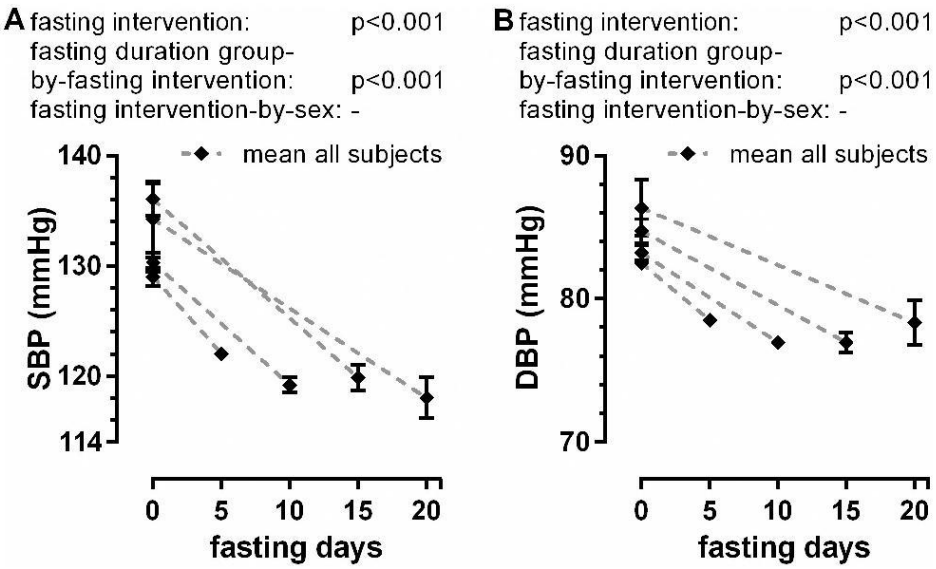



**Fig 3.** Changes in systolic (A) and diastolic blood pressure (B) according to the fasting length.

https://doi.org/10.1371/journal.pone.0209353.g003

the incidence of mild symptoms like muscle pain, sleep disturbances, headaches, and hunger occurred mainly in the first days of the fast.

No fatalities or permanent adverse effects were observed. Two subjects had to be admitted to hospital. A 75-year old man with known coronary artery disease had on the 9th fasting day a non-ST segment elevation myocardial infarction and received uncomplicated percutaneous coronary intervention. After 3 days in the hospital he returned to BWC. The second case was a 67-year old woman who had a one-day hospitalisation because of vomiting with dizziness and diarrhoea on the 4th fasting day. After returning to BWC she received an 800 kcal/d diet. The other AE were transitory and did not lead to an interruption of the fasting therapy. AE (Table 2) such as cardiac arrhythmia were low-grade, transitory and could be treated uncomplicatedly without stopping the fasting. The same applied to transitory hypoglycaemia. We also observed one case of gout attack in a patient treated previous to the fasting with allopurinol for hyperuricemia and frequent gout attacks. He was able to be symptomatically treated and went on fasting.

### Major health complaint

To document the effects of the Buchinger fasting program on their health we asked the participants to self-report if they had a major health complaint before the fasting and how this condition had been influenced by the fast. A group of 404 subjects out of the 1311 who returned the self-report (S2 Fig) mentioned having a major health complaint previous to the fasting. They were asked to evaluate the changes after the fasting. In 84.4% of the 404 subjects the major health complaint had much improved, 8.7% reported that it remained unchanged and 6.9% reported a worsening.

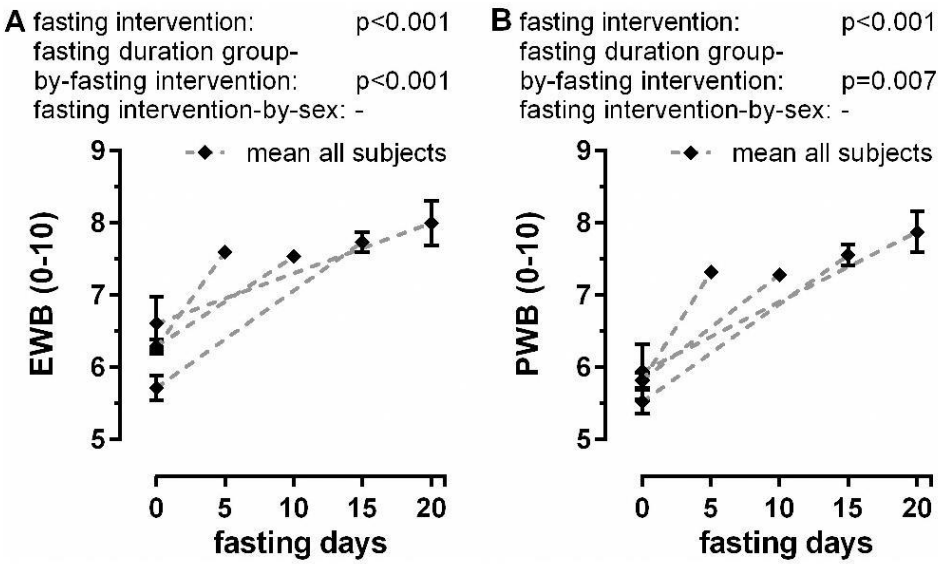



**Fig 4. Changes in emotional (A) and physical well-being (B) during fasting.** Self-recorded data of a 0–10 numeric rating scale for a total of 1074 volunteers are shown.

https://doi.org/10.1371/journal.pone.0209353.g004

### Blood lipids and glycaemia

We assessed the impact of the Buchinger fasting program on metabolism by analysing several blood parameters (S2 Table).

The lipid values are indicated in S2 Table and Fig 5. At baseline, TG values of women were lower than the values of men. Fasting reduced TG levels by 0.44 mmol/L on average (fasting intervention: $p<0.001$) (S2 Table). TG levels at the end of the fasting were similar in all groups, suggesting a floor effect (Fig 5A). The decrease in TC was significant (fasting intervention: $p<0.001$) and higher in the groups who fasted for longer (fasting duration group-by-fasting intervention: $p<0.001$). Fig 5B indicates that F15d and F20d had similar post-values. There was no difference in TC changes during fasting between men and women. Baseline HDL-C values were higher in women (Fig 5C). HDL-C decreased significantly (fasting intervention: $p<0.001$). The reduction was higher in the groups that fasted longer (fasting duration group-by-fasting intervention: $p<0.001$) and in women compared to men (fasting intervention-by-sex: $p<0.001$). LDL-C decreased significantly (fasting intervention: $p<0.001$) (Fig 5D) and again the decrease was higher in the groups that fasted longer (fasting duration group-by-fasting intervention: $p<0.001$). Gender differences for LDL-C were not significant. The LDL/HDL ratio was not influenced by fasting.

The blood glucose parameters are given in S2 Table and Fig 6. Baseline values for glucose were higher in men compared to women (Fig 6). The glucose values decreased significantly (fasting intervention: $p<0.001$) without differences between the fasting period lengths and stabilized at an average of 4.7 mmol/L (S2 Table). Fig 6B shows the significant decrease in HbA1c (fasting intervention: $p<0.001$), which varied between a decrease of 1.2±0.1 for F5d and 2.6 ±0.5 mmol/mol for F20d (fasting duration group-by-fasting intervention: $p<0.001$).

**Table 2. Mild symptoms and adverse effects (AE).**

| Mild symptoms (self-reported) | n | % | Mild symptoms (observed) | n | % |
|---|---|---|---|---|---|
| Sleep Disturbance | 169 | 14.94 | Dizziness | 2 | 0.14 |
| Fatigue | 155 | 13.70 | Eczema | 2 | 0.14 |
| Dry Mouth | 100 | 8.84 | Bleeding gums | 1 | 0.07 |
| Back Pain | 84 | 7.43 | Hyperventilation | 1 | 0.07 |
| Hunger | 77 | 6.81 | Outbreak of infection | 1 | 0.07 |
| Bad Breath | 61 | 5.39 | Pleuropneumonia | 1 | 0.07 |
| Headache | 61 | 5.39 | Tetany | 1 | 0.07 |
| Muscle Pain | 49 | 4.33 | Visual disorder | 1 | 0.07 |
| Abdominal Bloating | 47 | 4.16 | | | |
| Diarrhoea | 38 | 3.36 | | | |
| Sensitivity to Cold | 33 | 2.92 | **AE** | **n** | **%** |
| Cravings | 29 | 2.56 | Cardiac arrhythmia | 3 | 0.21 |
| Vertigo | 28 | 2.48 | Hyponatremia | 3 | 0.21 |
| Blurred Vision | 23 | 2.03 | Hospitalisation | 2 | 0.14 |
| Restless Legs | 23 | 2.03 | Hypoglycaemia | 2 | 0.14 |
| Skin Rash | 19 | 1.68 | Hypokalaemia | 1 | 0.07 |
| Nausea | 13 | 1.15 | Gout | 1 | 0.07 |
| Palpitation | 13 | 1.15 | Vomiting | 1 | 0.07 |
| Dyspepsia | 12 | 1.06 | Spasmodic abdominal pain | 1 | 0.07 |
| Muscular cramp | 4 | 0.35 | | | |

Out of the total of 1422 a group of 1311 subjects completed and returned the daily questionnaire to self-record mild symptoms. In contrast to the observed symptoms and AE, self-reported mild symptoms were recorded if a particular symptom was experienced by the same person more than 3 times during the fasting period. The observed symptoms and AE were documented during the daily nurse visit and/or the medical visit for all subjects. The same subject could mention more than one symptom or AE.

https://doi.org/10.1371/journal.pone.0209353.t002

## Blood count

Table 3 shows the impact of fasting on blood count. Leucocytes decreased significantly in all groups (fasting intervention: $p<0.001$) with stronger reduction in the groups who fasted longer (fasting duration group-by-fasting intervention: $p<0.001$) and without statistical significance between men and women. Erythrocytes showed an increase (fasting intervention: $p<0.001$) of an average of 0.06 $x10^6$/μl in all groups and steadied at around 4.82$x10^6$/μl. Haemoglobin showed also an increase (fasting intervention: $p<0.001$) of about 0.1 mmol/L that was independent of the fasting length. Haematocrit was not influenced by fasting. Thrombocytes showed a significant reduction (fasting intervention: $p<0.001$) during fasting by a mean of 6.6±0.7$x10^3$/μl, with gender difference (fasting intervention-by-sex: $p<0.001$) and influence of the fasting length (fasting duration group-by-fasting intervention: $p<0.001$).

## Coagulation

Table 4 shows changes in blood coagulation parameters, liver function, inflammatory parameters, kidney function and electrolytes. INR and PTT increased (fasting intervention: each $p<0.001$) and Quick value decreased (fasting intervention: $p<0.001$) significantly during fasting. The fasting period length had a significant influence on the coagulation parameters (fasting duration group-by-fasting intervention: each $p<0.001$) and more pronounced changes were observed in groups of longer fasting periods.

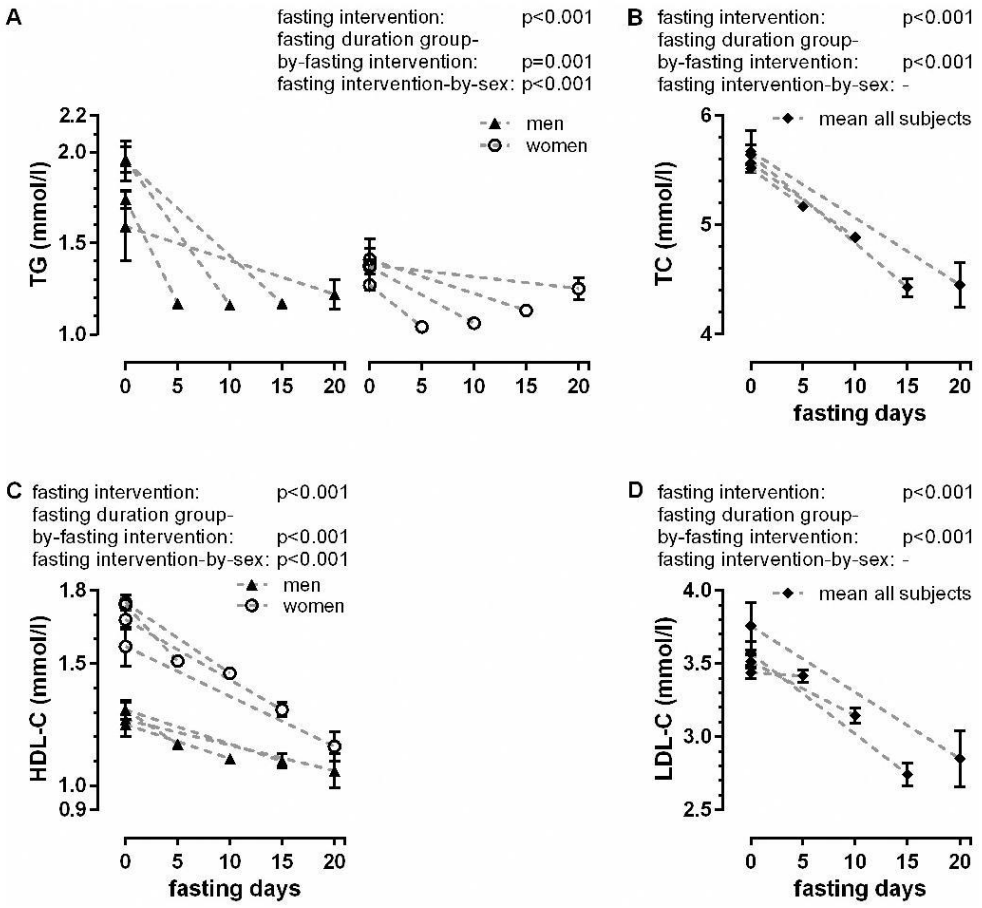



**Fig 5. Fasting-induced changes in lipid metabolism.**

https://doi.org/10.1371/journal.pone.0209353.g005

## Liver function

Regarding liver function, GOT and GPT levels rose significantly during the course of the fast (fasting intervention: each $p<0.001$) without difference between groups. The values at baseline and at the end remained within norm ranges (<0.8 μkat/L) increasing for GOT in average from 0.4 to 0.6 μkat/L and GPT from 0.5 to 0.7 μkat/L. GGT levels decreased significantly from a mean of 0.6 to 0.4 μkat/L (fasting intervention: $p<0.001$). AP showed a significant decrease (fasting intervention: $p<0.001$), more pronounced in the groups that fasted longer (fasting duration group-by-fasting intervention: $p<0.001$), with a slight dependence on gender (fasting intervention-by-sex: $p = 0.002$). The mean values before and after the fast were all in the norm range.

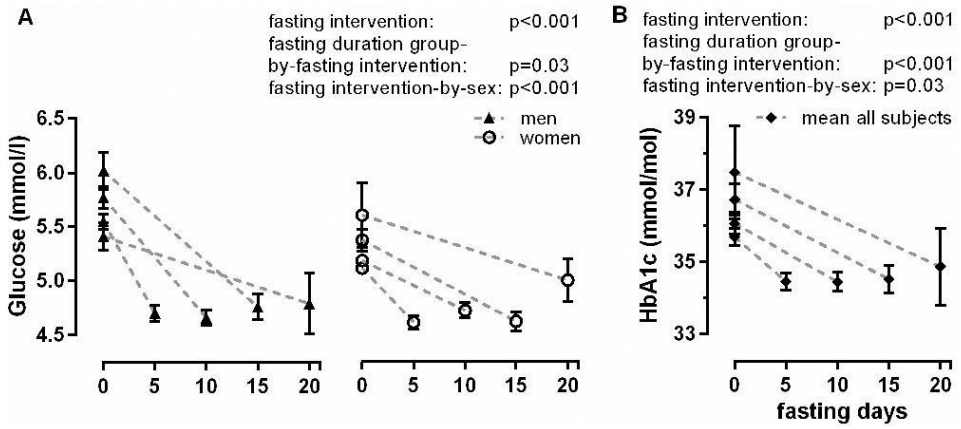



**Fig 6. Changes of blood glucose (A) and glycated haemoglobin (HbA1c) (B).** The panel A was split for gender to increase the readability of the figure.

https://doi.org/10.1371/journal.pone.0209353.g006

## Inflammatory biomarkers

The inflammatory biomarkers CRP and ESR were analysed. The mean values at baseline and at the end were all in norm range (<5.0 mg/L). CRP raised significantly during fasting (fasting

**Table 3. Blood cells pre- and post-fasting.**

| | All (n = 1422) | | F5d 5±2 d | | F10d 10±2 d | | F15d 15±2 d | | F20d 20±2 d | | p-values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pre | post | pre | post | pre | post | Pre | post | pre | post | fasting intervention | fasting duration group | sex | fasting duration group-by-fasting intervention | fasting intervention-by-sex |
| Leukocytes, $10^3$/µl | 5.9 ±0.0 | 5.4 ±0.0 | 5.9 ±0.1 | 5.5 ±0.1 | 6.0 ±0.1 | 5.4 ±0.1 | 6.0 ±0.1 | 5.0 ±0.1 | 5.7 ±0.3 | 4.7 ±0.2 | <0.001 | <0.001 | 0.18 | <0.001 | – |
| Erythrocytes, $10^6$/µl | 4.76 ±0.01 | 4.82 ±0.01 | 4.76 ±0.02 | 4.82 ±0.02 | 4.76 ±0.02 | 4.81 ±0.02 | 4.74 ±0.03 | 4.81 ±0.03 | 4.74 ±0.07 | 4.86 ±0.07 | <0.001 | 0.96 | <0.001 | – | 0.001 |
| Haemoglobin, mmol/L | 8.9 ±0.0 | 9.0 ±0.0 | 8.9 ±0.0 | 9.0 ±0.0 | 8.9 ±0.0 | 9.0 ±0.0 | 8.9 ±0.1 | 9.0 ±0.0 | 8.7 ±0.1 | 8.9 ±0.1 | <0.001 | 0.72 | 0.33 | – | <0.001 |
| Haematocrit, % | 42.2 ±0.1 | 42.3 ±0.1 | 42.2 ±0.1 | 42.3 ±0.1 | 42.3 ±0.1 | 42.3 ±0.1 | 42.2 ±0.2 | 42.3 ±0.2 | 41.8 ±0.5 | 42.2 ±0.5 | 0.74 | 0.83 | 0.06 | – | 0.02 |
| MCV, fl | 89.0 ±0.1 | 88.0 ±0.1 | 88.8 ±0.2 | 87.9 ±0.2 | 89.1 ±0.2 | 88.0 ±0.2 | 89.3 ±0.3 | 88.0 ±0.3 | 88.5 ±0.9 | 87.0 ±0.8 | <0.001 | 0.001 | <0.001 | 0.001 | <0.001 |
| MCH, pg | 30.1 ±0.1 | 30.1 ±0.1 | 30.1 ±0.1 | 30.1 ±0.1 | 30.2 ±0.1 | 30.2 ±0.1 | 30.2 ±0.1 | 30.1 ±0.1 | 29.7 ±0.3 | 29.7 ±0.3 | 0.38 | 0.12 | 0.82 | 0.09 | – |
| MCHC, g/dl | 33.9 ±0.0 | 34.2 ±0.0 | 33.9 ±0.0 | 34.2 ±0.0 | 33.9 ±0.0 | 34.3 ±0.0 | 33.8 ±0.1 | 34.2 ±0.1 | 33.6 ±0.2 | 34.0 ±0.1 | <0.001 | 0.80 | 0.11 | – | 0.004 |
| Thrombocytes, $10^3$/µl | 244.1 ±1.5 | 237.5 ±1.5 | 242.6 ±2.2 | 239.2 ±2.2 | 245.9 ±2.5 | 238.9 ±2.6 | 243.9 ±3.9 | 230.6 ±4.1 | 245.6 ±11.4 | 224.4 ±11.4 | <0.001 | <0.001 | 0.03 | <0.001 | <0.001 |

Values are shown as mean±SEM for all of the groups with different fasting lengths. P-values were calculated for the effects of fasting intervention as well as the effects of the fasting length (fasting duration group) and gender (sex). Interactions between fasting intervention by fasting duration group (fasting duration group-by-fasting intervention) and fasting intervention by gender (fasting intervention-by-sex) are shown.

MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

https://doi.org/10.1371/journal.pone.0209353.t003

**Table 4. Blood parameters pre- and post-fasting.**

| | All (n = 1422) | | F5d 5±2 d | | F10d 10±2 d | | F15d 15±2 d | | F20d 20±2 d | | p-values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pre | post | pre | post | pre | post | Pre | post | pre | post | fasting intervention | fasting duration group | sex | fasting duration group-by-fasting intervention | fasting intervention-by-sex |
| INR | 0.99 ±0.00 | 1.08 ±0.00 | 0.98 ±0.00 | 1.06 ±0.00 | 0.99 ±0.01 | 1.09 ±0.01 | 0.99 ±0.02 | 1.11 ±0.02 | 0.98 ±0.01 | 1.10 ±0.02 | <0.001 | <0.001 | 0.84 | <0.001 | – |
| Quick, % | 104.3 ±0.3 | 91.4 ±0.3 | 104.2 ±0.5 | 92.9 ±0.4 | 104.0 ±0.6 | 90.6 ±0.5 | 105.6 ±1.0 | 89.1 ±0.9 | 104.1 ±1.2 | 88.3 ±1.9 | <0.001 | <0.001 | 0.33 | <0.001 | 0.005 |
| PTT, sec | 31.1 ±0.1 | 32.7 ±0.1 | 31.1 ±0.1 | 32.4 ±0.1 | 31.0 ±0.1 | 32.8 ±0.2 | 31.2 ±0.2 | 33.7 ±0.3 | 31.4 ±0.4 | 32.9 ±0.5 | <0.001 | <0.001 | 0.88 | <0.001 | – |
| GOT, μkat/L | 0.4 ±0.0 | 0.6 ±0.0 | 0.4 ±0.0 | 0.6 ±0.0 | 0.4 ±0.0 | 0.6 ±0.0 | 0.4 ±0.0 | 0.6 ±0.0 | 0.4 ±0.0 | 0.7 ±0.0 | <0.001 | 0.73 | 0.007 | – | <0.001 |
| GPT, μkat/L | 0.5 ±0.0 | 0.7 ±0.0 | 0.5 ±0.0 | 0.6 ±0.0 | 0.5 ±0.0 | 0.7 ±0.0 | 0.5 ±0.0 | 0.7 ±0.0 | 0.6 ±0.0 | 0.8 ±0.1 | <0.001 | 0.07 | 0.65 | 0.10 | – |
| GGT, μkat/L | 0.6 ±0.0 | 0.4 ±0.0 | 0.5 ±0.0 | 0.4 ±0.0 | 0.6 ±0.1 | 0.4 ±0.0 | 0.6 ±0.1 | 0.4 ±0.0 | 0.5 ±0.1 | 0.4 ±0.01 | <0.001 | <0.001 | 0.18 | <0.001 | <0.001 |
| AP, μkat/L | 1.1 ±0.0 | 1.0 ±0.0 | 1.0 ±0.0 | 1.0 ±0.0 | 1.1 ±0.0 | 1.1 ±0.0 | 1.1 ±0.0 | 1.0 ±0.0 | 1.2 ±0.1 | 1.1 ±0.1 | <0.001 | <0.001 | 0.003 | <0.001 | 0.002 |
| CRP, mg/L | 2.85 ±0.14 | 4.30 ±0.20 | 2.49 ±0.19 | 4.11 ±0.27 | 3.02 ±0.28 | 4.35 ±0.36 | 3.37 ±0.32 | 4.74 ±0.63 | 4.08 ±0.67 | 4.67 ±0.82 | 0.001 | 0.97 | 0.99 | 0.81 | 0.53 |
| ESR 1h | 11.6 ±0.2 | 11.4 ±0.2 | 10.9 ±0.3 | 11.7 ±0.3 | 11.8 ±0.4 | 11.5 ±0.4 | 12.8 ±0.7 | 10.6 ±0.6 | 15.1 ±1.6 | 10.2 ±1.4 | <0.001 | <0.001 | 0.002 | <0.001 | <0.001 |
| ESR 2h | 21.7 ±0.4 | 21.3 ±0.4 | 20.4 ±0.5 | 21.8 ±0.5 | 22.1 ±0.6 | 21.4 ±0.6 | 23.9 ±1.1 | 19.7 ±1.0 | 28.2 ±2.8 | 20.1 ±2.4 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Uric acid, μmol/L | 338.1 ±2.3 | 495.2 ±4.4 | 334.0 ±3.3 | 481.1 ±6.0 | 339.2 ±3.8 | 505.5 ±7.5 | 345.3 ±6.4 | 513.0 ±13.5 | 355.9 ±12.8 | 505.4 ±30.6 | <0.001 | 0.01 | <0.001 | 0.01 | <0.001 |
| Urea, mmol/L | 4.7 ±0.0 | 3.1 ±0.0 | 4.6 ±0.1 | 3.3 ±0.1 | 4.7 ±0.1 | 3.0 ±0.1 | 4.7 ±0.1 | 2.7 ±0.1 | 5.1 ±0.3 | 2.8 ±0.3 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Creatinine, μmol/L | 71.92 ±0.40 | 76.43 ±0.54 | 72.53 ±0.58 | 76.45 ±0.76 | 71.88 ±0.68 | 76.88 ±1.00 | 69.86 ±0.98 | 74.98 ±1.21 | 72.27 ±2.53 | 77.17 ±3.13 | <0.001 | 0.34 | <0.001 | – | <0.001 |
| Na,mmol/L | 140.1 ±0.1 | 138.7 ±0.1 | 140.0 ±0.1 | 138.4 ±0.1 | 140.1 ±0.1 | 138.8 ±0.1 | 139.8 ±0.3 | 139.2 ±0.2 | 141.0 ±0.33 | 139.9 ±0.4 | <0.001 | <0.001 | 0.37 | 0.003 | – |
| K, mmol/L | 4.4 ±0.0 | 4.4 ±0.0 | 4.4 ±0.0 | 4.4 ±0.0 | 4.4 ±0.0 | 4.4 ±0.0 | 4.3 ±0.0 | 4.4 ±0.0 | 4.4 ±0.1 | 4.4 ±0.1 | 0.001 | 0.74 | <0.001 | – | 0.008 |
| Ca, mmol/L | 2.32 ±0.00 | 2.38 ±0.00 | 2.33 ±0.00 | 2.36 ±0.00 | 2.32 ±0.00 | 2.39 ±0.00 | 2.32 ±0.01 | 2.39 ±0.01 | 2.33 ±0.02 | 2.40 ±0.02 | <0.001 | <0.001 | 0.14 | <0.001 | – |
| Mg, mmol/L | 0.86 ±0.00 | 0.87 ±0.00 | 0.87 ±0.00 | 0.89 ±0.00 | 0.87 ±0.00 | 0.87 ±0.00 | 0.85 ±0.00 | 0.86 ±0.01 | 0.86 ±0.01 | 0.85 ±0.01 | 0.09 | <0.001 | <0.001 | <0.001 | <0.001 |

Values are shown as mean±SEM for all of the groups with different fasting lengths. P-values were calculated for the effects of fasting (fasting intervention) as well as the effects of the fasting length (fasting duration group) and gender (sex). Interactions between fasting intervention by fasting duration group (fasting duration group-by-fasting intervention) and fasting intervention by gender (fasting intervention-by-sex) are shown.

INR, international normalized ratio; PTT, partial thromboplastin time; GOT, serum glutamic oxaloacetic transaminase; GPT, serum glutamate pyruvate transaminase; GGT, serum gamma-glutamyl transferase; AP, alkaline phosphatase; CRP, C-reactive protein; ESR 1h, erythrocyte sedimentation rate after 1 hour; ESR 2h, erythrocyte sedimentation rate after 2 hours; Na, sodium; K, potassium; Ca, calcium, Mg, magnesium

https://doi.org/10.1371/journal.pone.0209353.t004

intervention: $p<0.001$). There was no difference between groups and gender. ESR after 1 and 2 hours decreased significantly (fasting intervention: each $p<0.001$) and the groups with longer fasting periods displayed stronger reductions (fasting duration group-by-fasting intervention: each $p<0.001$).

### Renal function and uric acid

Uric acid levels, as well as renal function as reflected by urea and creatinine values, are given in Table 4. A significant increase of blood uric acid was observed (338.1±2.3 to 495.2±4.4 μmol/L) (fasting intervention: $p<0.001$). The highest uric acid level was measured in F15d. Urea concentrations decreased significantly in all groups (fasting intervention: $p<0.001$), but the decrease was stronger in the groups with longer fasting periods (fasting duration group-by-fasting intervention: $p<0.001$). Creatinine levels increased significantly (fasting intervention: $p<0.001$) with differences between the sexes (fasting intervention-by-sex: $p<0.001$) but without differences between groups.

### Electrolytes

Sodium concentrations remained in norm range but showed a significant reduction (fasting intervention: $p<0.001$) from a mean of 140.1±0.1 to 138.7±0.1 mmol/L. Calcium levels increased significantly (fasting intervention: $p<0.001$), with an effect of groups (fasting duration group-by-fasting intervention: $p<0.001$) but not of gender. Potassium showed a reduction during fasting (fasting intervention: $p = 0.001$) and magnesium levels remained stable. All values pre- and post-fasting remained in norm range.

## Discussion

The present prospective observational study systematically investigated for the first time the effects of long periods of Buchinger fasting within a specialized clinic in a cohort of 1422 subjects. The results showed that this type of fasting from 4 to 21 days is safe and well tolerated. Furthermore, it led to enhancement of emotional and physical well-being and improvement of relevant cardiovascular risk factors and subjective health complaints.

Fasting resulted as expected in marked weight loss with reduction of abdominal circumference, which was more pronounced in the groups who fasted longer. Thus it can be assumed that preferentially visceral adipose tissue was reduced with weight loss [40]. Of note, no particular rebound in weight gain has been shown after repeated cycles of Buchinger fasting in a previous study [38, 39].

Blood pressure showed a significant decrease, whereby mean values did not fall below the lower norm range, indicating a floor effect. Accordingly, serious hypotensive complications were not reported. Blood pressure reduction might be triggered by factors such as the increase of parasympathetic activity due to the release of brain-derived neurotrophic factor (BDNF) [2, 41, 42], increased renal Na excretion [43] and enhanced receptor sensitivity of natriuretic peptides and insulin [44]. Earlier studies on zero calorie diets and very-low-calorie diets (VLCD) [45–47]–and more recently in smaller studies on Buchinger fasting [33, 34] and water fasting [48]–also described this blood pressure-reducing effect.

We further found decreases in blood lipid levels following the fasting periods: TG levels as well as TC and LDL-C decreased significantly in all groups. Glucose levels and HbA1c decreased also significantly which points out to a positive effect of fasting on glucoregulation. It was also found in two previous smaller studies using Buchinger fasting [49, 50].

Altogether, the positive impact of periodic Buchinger fasting on the above mentioned parameters suggests a general cardioprotective effect that has also been shown in IF [51].

The continuous increase in emotional as well as physical well-being was evident across all groups of different fasting period lengths. This is an important component to increase compliance and has been reported in earlier studies based on daily mood ratings [26, 52, 53]. Weight loss, especially in obese subjects, is linked with mood improvement in many studies [54]. The G-to-K switch has been shown in IF to lead to enhanced performance in cognition, mood,

motor and autonomic nervous system function [2, 55]. In IF and CR this is linked to the release of BDNF [51, 56]. BDNF, associated with neurogenesis and neuron protection, enhances the growth and survival of serotonin neurons [51, 57]. Furthermore, the reduction of insulin and leptin levels appears to act on the hypothalamic-pituitary-adrenal axis, thereby impacting mood positively [58]. Endogenous opioid (β-endorphin) could also contribute to well-being, as documented in a 10-day fasting trial in men [59]. In our study, the reported improvement of a major health complaint, often accompanied by pain relief, could possibly contribute to the increase of well-being. Moreover, it appears likely that a successful completion of a longer fasting period improves the feeling of self-efficacy, thereby enhancing subjective well-being [37]. It has been reported that short fasting periods of two days, as well as alternate-day fasting, are associated with the feeling of hunger [60–62]. This seems to be an obstacle to patient compliance [61, 62]. In contrast, hunger was not reported by 93% of the subjects in our study, which often surprised them positively. This possibly contributed to enhanced well-being.

As expected, we observed a significant increase in urinary ketone bodies excretion up to a maximum level that was similar in all groups. This suggests that in 5 days a plateau was reached. Ketosis and IMS was achieved, although Buchinger fasting provides small quantities of fruit juices and some honey, providing up to 25–35 g carbohydrates/day. Experimental research points to ketosis as the trigger of beneficial effects on brain and neurological diseases [2]. Daily time-restricted feeding causing IMS ameliorates anxiety-like behaviour in mice [63]. IMS enhances also structural and functional synaptic plasticity, cognition and neuronal stress response [64].

In our cohort, no fatal or life-threatening event occurred. Self-reported mild symptoms were observed mainly in the first days and disappeared either spontaneously or with natural remedies. Adverse effects were observed in 0.7% of subjects. Only two subjects had to interrupt the fasting. Adverse effects have also been mentioned in other studies [34, 65].

A recent publication that analysed retrospectively water-only fasting data found relatively more adverse effects, e. g. nausea, presyncope, dyspepsia, vomiting and palpitations [66], which we observed only in single cases. The use of different methodologies to assess and characterise AE limits the comparison with our study, which was prospective. Nevertheless, it could be possible that the supplementation with juices and soups, which slows down initial protein catabolism [67], enhances tolerability by modulating the onset of ketosis.

As already mentioned, we observed a subjective improvement in 85% of cases of a major health complaint. This documents within the limitations of a non-confirmatory study design the therapeutic effectiveness of Buchinger periodic fasting.

To assess further therapeutic effects of fasting, as well as the safety of this procedure, we performed extended laboratory analysis. Within the blood count leukocytes and thrombocytes decreased significantly but not below norm range. In humans after CR as well as in mice fed with cycles of a low calorie fasting mimicking diet, a drop in leukocytes count was followed by an increase in hematopoietic, mesenchymal stem and progenitor cells in the bone marrow. This was associated with the regeneration of all blood cell types and haemoglobin upon refeeding [4, 19]. We cannot extrapolate from our data whether this regeneration applies also to fasting in humans.

The increase in INR values was significant and more marked in the groups who fasted longer. Bleeding time (PTT) was also increased. The increase in INR is well-known [68, 69] and can be relevant for anticoagulated patients. During fasting they should be monitored and their medication adapted.

GOT and GPT levels showed a significant increase within the norm range. GGT instead, dropped significantly and stronger in the groups who fasted longer. A zero-calorie diet in 88 obese people for a duration of up to 35 days showed an increased activity of the GOT and

GPT, with a peak in the 3rd fasting week [25]. This moderate increase was explained by the enhanced transamination processes in the course of fasting. An initial overload of liver detoxifying activity could be postulated and does not seem to have been deleterious effects, since GGT levels decreased and physical well-being increased steadily.

CRP values for all groups showed a significant increase within the norm range. A similar mild CRP increase was explained by the transient increase in circulating levels of catecholamines [34]. In a recent study, the modulation of CRP levels was linked to changes in lipid profiles and associated to cardiovascular outcomes [70]. ESR after 1 h and 2 h decreased significantly. Periodic fasting has been shown to clinically improve symptoms of rheumatoid arthritis, suggesting decreased inflammatory processes [71]. IF boosts levels of antioxidants and reduces levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 [72]. Serum markers of oxidative damage and inflammation are reduced in asthma patients maintained on an alternate day fast [62]. Moreover, the reduction of abdominal circumference which was significant in our study is also associated with a decrease in pro-inflammatory activity [73].

We observed an increase of uric acid in all groups, with a lower value in F20d than in F15d, suggesting that the peak of uric acid concentration was overwhelmed after 15 days. This corresponds to previous observations from a zero-calorie diet [45, 74]. The increase of uric acid concentrations that exceeded the norm range, interestingly caused only one incidence of gout attack in a 72-year-old obese man, treated for hyperuricemia and gout previous to fasting.

The increased concentration of blood uric acid is due to a slight initial increase in protein catabolism, but above all is related to retention caused by competitive tubular secretion with ketone bodies. The latter are preferentially secreted during fasting.

Uric acid has a known antioxidant activity and is a potent scavenger of free radicals in blood plasma [75, 76]. Given that fasting is the product of a long evolutionary survival strategy, the antioxidant power of uric acid should be taken into consideration.

A significant reduction in urea as well as a significant increase in creatinine was observed, both remaining in norm range. This has been previously demonstrated in obese persons undergoing prolonged periods of a zero-calorie diet [45].

We did not observe any renal dysfunction in our cohort such as has been described in cases of extracellular hypovolemia [77]. This is probably explained by the fact that the subjects were asked to drink 3 L of water per day.

Sodium, potassium, calcium and magnesium were in norm range at the beginning and the end of the fast. They remained stable despite a slight elevation in calcium and slight decrease in sodium levels. Enhanced natriuresis has been described in association with ketosuria in the first phase of fasting, diminishing when ammonium, a metabolite of kidney gluconeogenesis, replaces sodium as accompanying cation [43, 47]. We registered six cases of mild hyponatremia in the course of the fast, with the lowest sodium level of 127 mmol/L. They were all non-serious and were normalized by the administration of sodium chloride.

There are some limitations related to our study. First, this was an observational cohort study with its well-known restrictions regarding interpretation of efficacy. Second, our findings are specific for BWC and may not be applicable for other institutions specialised in fasting, or for persons fasting without medical supervision, or outside of facilities specialized in fasting treatments. Third, data assessment and data entry was not blinded and was performed by the staff of the BWC.

## Conclusions

In conclusion, this one-year observational study demonstrates the safety of a periodic Buchinger fast of between 4 and 21 days, as well as its beneficial effects on health and well-being.

Periodic fasting led to marked weight loss and improvements in several cardiovascular risk factors, such as overweight, abdominal circumference and blood pressure. It was accompanied by normalization of numerous blood parameters and led to pronounced improvement of the major health complaint in most participants. Importantly, periodic Buchinger fasting was not linked to relevant perception of hunger. On the contrary, it was subjectively experienced as enjoyable, which is an important factor for compliance.

Further studies should evaluate the long-term specific health-related preventive and therapeutic effects of periodic fasting.

## Supporting information

**S1 Table. Effect of fasting on clinical parameters, well-being and ketosis.**
(PDF)

**S2 Table. Effect of fasting on lipid parameters and glycaemia.**
(PDF)

**S3 Table. Raw data of Fig 2.** Changes in weight and abdominal circumference according to the length of fast and gender.
(XLSX)

**S4 Table. Raw data of Fig 3.** Changes in systolic and diastolic blood pressure according to the fasting length.
(XLSX)

**S5 Table. Raw data of Fig 4.** Changes in emotional and physical well-being during fasting.
(XLSX)

**S6 Table. Raw data of Fig 5.** Fasting-induced changes in lipid metabolism.
(XLSX)

**S7 Table. Raw data of Fig 6.** Changes of blood glucose and glycated haemoglobin.
(XLSX)

**S8 Table. Raw data of Table 1.** Baseline characteristics of the subjects.
(XLSX)

**S9 Table. Raw data of Table 2.** Mild symptoms and adverse effects.
(XLSX)

**S10 Table. Raw data of Table 3.** Blood cells pre- and post-fasting.
(XLSX)

**S11 Table. Raw data of Table 4.** Blood parameters pre- and post-fasting.
(XLSX)

**S12 Table. Raw data of S1 Table.** Effect of fasting on clinical parameters, well-being and ketosis.
(XLSX)

**S13 Table. Raw data of S1 Fig.** Occurrence of self-reported mild symptoms during fasting.
(XLSX)

**S14 Table. Raw data of S2 Fig.** Evolution of a pre-existing health complaint.
(XLSX)

**S15 Table. Raw data of S4 Table.** Effect of fasting on lipid parameters and glycaemia.
(XLSX)

**S1 Fig. Occurrence of self-reported mild symptoms during fasting.**
(PDF)

**S2 Fig. Evolution of a pre-existing health complaint.** A subgroup of 404 subjects indicated to have a major health complaint previous to the fasting.
(PDF)

**S1 Text. STROBE checklist.**
(PDF)

**S2 Text. Study protocol in German.**
(PDF)

**S3 Text. Study protocol translated to English.**
(PDF)

**S4 Text. Questionnaires in German.**
(PDF)

**S5 Text. Questionnaires in English.**
(PDF)

**S6 Text. Questionnaires in French.**
(PDF)

## Acknowledgments

We are grateful to Birgit Maser and the Medical Center, as well as Dr Eva Lischka, Dr Norbert Lischka, Siegfried Bäumler, Martine van Houten, Dr Andrea Siegler, Dr Henning Wittrock and the whole staff of BWC. We thank Dr Diethard Müller and his colleagues at MVZ Labor Ravensburg for the support and laboratory analysis. We are also very grateful to Prof Chantal Simon for her continuous support and the native speaker Claire Robinson for proofreading our manuscript.

## Author Contributions

**Conceptualization:** Françoise Wilhelmi de Toledo, Andreas Michalsen.

**Data curation:** Franziska Grundler, Stefan Drinda.

**Formal analysis:** Franziska Grundler, Audrey Bergouignan.

**Funding acquisition:** Françoise Wilhelmi de Toledo.

**Investigation:** Franziska Grundler.

**Methodology:** Audrey Bergouignan.

**Project administration:** Franziska Grundler.

**Resources:** Françoise Wilhelmi de Toledo.

**Supervision:** Françoise Wilhelmi de Toledo.

**Writing – original draft:** Françoise Wilhelmi de Toledo, Franziska Grundler.

**Writing – review & editing:** Françoise Wilhelmi de Toledo, Franziska Grundler, Audrey Bergouignan, Stefan Drinda, Andreas Michalsen.

## References


1. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. Ageing research reviews. 2017; 39:46–58. https://doi.org/10.1016/j.arr.2016.10.005 PMID: 27810402
2. Mattson MP, Moehl K, Ghena N, Schmaedick M, Cheng A. Intermittent metabolic switching, neuroplasticity and brain health. Nature Reviews Neuroscience. 2018.
3. Yamada Y, Kemnitz JW, Weindruch R, Anderson RM, Schoeller DA, Colman RJ. Caloric Restriction and Healthy Life Span: Frail Phenotype of Nonhuman Primates in the Wisconsin National Primate Research Center Caloric Restriction Study. The Journals of Gerontology: Series A. 2018; 73(3):273–8. https://doi.org/10.1093/gerona/glx059 PMID: 28398464
4. Brandhorst S, Choi IY, Wei M, Cheng CW, Sedrakyan S, Navarrete G, et al. A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. Cell metabolism. 2015; 22(1):86–99. https://doi.org/10.1016/j.cmet.2015.05.012 PMID: 26094889
5. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. Cell metabolism. 2014; 19(2):181–92. https://doi.org/10.1016/j.cmet.2013.12.008 PMID: 24440038
6. Sun S, Hanzawa F, Umeki M, Ikeda S, Mochizuki S, Oda H. Time-restricted feeding suppresses excess sucrose-induced plasma and liver lipid accumulation in rats. PloS one. 2018; 13(8):e0201261. https://doi.org/10.1371/journal.pone.0201261 PMID: 30110343
7. Lettieri-Barbato D, Cannata SM, Casagrande V, Ciriolo MR, Aquilano K. Time-controlled fasting prevents aging-like mitochondrial changes induced by persistent dietary fat overload in skeletal muscle. PloS one. 2018; 13(5):e0195912. https://doi.org/10.1371/journal.pone.0195912 PMID: 29742122
8. Walford RL, Mock D, Verdery R, MacCallum T. Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2002; 57(6):B211–B24.
9. Fontana L, Partridge L. Promoting Health and Longevity through Diet: From Model Organisms to Humans. Cell. 2015; 161(1):106–18. https://doi.org/10.1016/j.cell.2015.02.020. PMID: 25815989
10. Anton SD, Moehl K, Donahoo WT, Marosi K, Lee SA, Mainous AG, et al. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. Obesity. 2017.
11. Raffaghello L, Lee C, Safdie FM, Wei M, Madia F, Bianchi G, et al. Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. Proceedings of the National Academy of Sciences. 2008; 105(24):8215–20. https://doi.org/10.1073/pnas.0708100105 PMID: 18378900
12. López-Otín C, Galluzzi L, Freije JMP, Madeo F, Kroemer G. Metabolic Control of Longevity. Cell. 2016; 166(4):802–21. https://doi.org/10.1016/j.cell.2016.07.031 PMID: 27518560
13. Alirezaei M, Kemball CC, Flynn CT, Wood MR, Whitton JL, Kiosses WB. Short-term fasting induces profound neuronal autophagy. Autophagy. 2010; 6(6):702–10. https://doi.org/10.4161/auto.6.6.12376 PMID: 20534972
14. Lee C, Raffaghello L, Longo VD. Starvation, detoxification, and multidrug resistance in cancer therapy. Drug Resistance Updates. 2012; 15(1):114–22. https://doi.org/10.1016/j.drup.2012.01.004.
15. Remely M, Hippe B, Geretschlaeger I, Stegmayer S, Hoefinger I, Haslberger A. Increased gut microbiota diversity and abundance of Faecalibacterium prausnitzii and Akkermansia after fasting: a pilot study. Wiener klinische Wochenschrift. 2015; 127(9–10):394–8. https://doi.org/10.1007/s00508-015-0755-1 PMID: 25763563
16. Habold C, Reichardt F, Foltzer-Jourdainne C, Lignot J-H. Morphological changes of the rat intestinal lining in relation to body stores depletion during fasting and after refeeding. Pflügers Archiv-European Journal of Physiology. 2007; 455(2):323–32. https://doi.org/10.1007/s00424-007-0289-0 PMID: 17638014
17. Fontana L, Partridge L, Longo VD. Dietary Restriction, Growth Factors and Aging: from yeast to humans. Science (New York, NY). 2010; 328(5976):321–6. https://doi.org/10.1126/science.1172539 PMC3607354. PMID: 20395504
18. Brandhorst S, Harputlugil E, Mitchell JR, Longo VD. Protective effects of short-term dietary restriction in surgical stress and chemotherapy. Ageing Research Reviews. 2017; 39:68–77. https://doi.org/10.1016/j.arr.2017.02.001. PMID: 28216454
19. Cheng C-W, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell stem cell. 2014; 14(6):810–23. https://doi.org/10.1016/j.stem.2014.04.014 PMID: 24905167
20. Bertile F, Fouillen L, Wasselin T, Maes P, Le Maho Y, Van Dorsselaer A, et al. The Safety Limits Of An Extended Fast: Lessons from a Non-Model Organism. Scientific reports. 2016; 6:39008. https://doi.org/10.1038/srep39008 PMID: 27991520

21. McCue MD. Comparative physiology of fasting, starvation, and food limitation: Springer; 2012.
22. Prentice AM, Whitehead RG, Roberts SB, Paul AA. Long-term energy balance in child-bearing Gambian women. The American journal of clinical nutrition. 1981; 34(12):2790–9. https://doi.org/10.1093/ajcn/34.12.2790 PMID: 7315781
23. Persynaki A, Karras S, Pichard C. Unraveling the metabolic health benefits of fasting related to religious beliefs: A narrative review. Nutrition. 2017; 35:14–20. https://doi.org/10.1016/j.nut.2016.10.005 PMID: 28241983
24. Ajabnoor GM, Bahijri S, Shaik NA, Borai A, Alamoudi AA, Al-Aama JY, et al. Ramadan fasting in Saudi Arabia is associated with altered expression of CLOCK, DUSP and IL-1alpha genes, as well as changes in cardiometabolic risk factors. PloS one. 2017; 12(4):e0174342. https://doi.org/10.1371/journal.pone.0174342 PMID: 28384165
25. Ditschuneit H, Faulhaber J, Beil I, Pfeiffer E. Metabolic changes in zero-diet. Der Internist. 1970; 11 (5):176. PMID: 4939022
26. Runcie J, Hilditch T. Energy provision, tissue utilization, and weight loss in prolonged starvation. Br Med J. 1974; 2(5915):352–6. PMID: 4835840
27. Thomson T, Runcie J, Miller V. Treatment of obesity by total fasting for up to 249 days. Lancet. 1966; 2:992–6. PMID: 4162668
28. Stewart W, Fleming LW. Features of a successful therapeutic fast of 382 days´ duration. Postgraduate medical journal. 1973; 49(569):203–9. PMID: 4803438
29. De Toledo FW, Hohler H. Therapeutic Fasting: The Buchinger Amplius Method: The Buchinger Amplius Method: Thieme; 2011.
30. Buchinger O. Das Heilfasten und seine Hilfsmethoden als biologischer Weg: Georg Thieme Verlag; 2005.
31. Wilhelmi de Toledo F, Buchinger A, Burggrabe H, Hölz G, Kuhn C, Lischka E, et al. Fasting therapy-an expert panel update of the 2002 consensus guidelines. Forschende Komplementärmedizin/Research in Complementary Medicine. 2013; 20(6):434–43. https://doi.org/10.1159/000357602 PMID: 24434758
32. Wilhelmi de Toledo F FR, Hebisch D., Kuhn C., Platzer G., Schrag S. The Klinik Buchinger Program For The Treatment of Obesity. Obesity in Europe 1993 / Proceedings of the 5th European congress on obesity, Ulm. 1994:1–9.
33. Müller H, Wilhelmi de Toledo F, Schuck P, Resch K. Blutdrucksenkung durch Fasten bei adipösen und nichtadipösen Hypertonikern. Perfusion. 2001; 14:108–12.
34. Li C, Ostermann T, Hardt M, Lüdtke R, Broecker-Preuss M, Dobos G, et al. Metabolic and psychological response to 7-day fasting in obese patients with and without metabolic syndrome. Complementary Medicine Research. 2013; 20(6):413–20.
35. Michalsen A, Li C, Kaiser K, Lüdtke R, Meier L, Stange R, et al. In-patient treatment of fibromyalgia: a controlled nonrandomized comparison of conventional medicine versus integrative medicine including fasting therapy. Evidence-based complementary and alternative medicine. 2013; 2013.
36. Michalsen A. Prolonged fasting as a method of mood enhancement in chronic pain syndromes: a review of clinical evidence and mechanisms. Current pain and headache reports. 2010; 14(2):80–7. https://doi.org/10.1007/s11916-010-0104-z PMID: 20425196
37. Michalsen A, Grossman P, Lehmann N, Knoblauch NT, Paul A, Moebus S, et al. Psychological and quality-of-life outcomes from a comprehensive stress reduction and lifestyle program in patients with coronary artery disease: results of a randomized trial. Psychotherapy and psychosomatics. 2005; 74 (6):344–52. https://doi.org/10.1159/000087781 PMID: 16244510
38. Brubacher D, Jordan P, Wilhelmi de Toledo F, Brubacher G. Prediction of Weight Development on a 250 kcal/day Diet by a Simple Two-Compartment Model. Aktuelle Ernährungsmedizin. 1998; 23 (6):293–8.
39. Brubacher D, Jordan P, Wilhelmi-de Toledo F, Brubacher G. Relationship between the rate of weight loss in a low caloric diet (250 kcal/day) and age, body mass index, gender, and number of fasting cycles. Aktuelle Ernaehrungsmedizin ( Germany). 1999.
40. Han TS, van Leer EM, Seidell JC, Lean MEJ. Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. BMJ. 1995; 311(7017):1401–5. https://doi.org/10.1136/bmj.311.7017.1401 PMID: 8520275
41. Wan R, Weigand LA, Bateman R, Griffioen K, Mendelowitz D, Mattson MP. Evidence that BDNF regulates heart rate by a mechanism involving increased brainstem parasympathetic neuron excitability. Journal of neurochemistry. 2014; 129(4):573–80. https://doi.org/10.1111/jnc.12656 PMID: 24475741
42. Mager DE, Wan R, Brown M, Cheng A, Wareski P, Abernethy DR, et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. The FASEB Journal. 2006; 20(6):631–7. https://doi.org/10.1096/fj.05-5263com PMID: 16581971

43. Kolanowski J, Bodson A, Desmecht P, Bemelmans S, Stein F, Crabbe J. On the relationship between ketonuria and natriuresis during fasting and upon refeeding in obese patients. European journal of clinical investigation. 1978; 8(5):277–82. PMID: 103718

44. Spark RF, Arky RA, Boulter PR, Saudek CD, O'Brian JT. Renin, aldosterone and glucagon in the natriuresis of fasting. New England Journal of Medicine. 1975; 292(25):1335–40. https://doi.org/10.1056/NEJM197506192922506 PMID: 165411

45. Wechsler JG. Fastentherapie der Adipositas: G. Thieme; 1983.

46. Wadden TA, Foster GD, Letizia KA, Stunkard AJ. A multicenter evaluation of a proprietary weight reduction program for the treatment of marked obesity. Archives of Internal Medicine. 1992; 152 (5):961–6. PMID: 1580722

47. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. Western Journal of Medicine. 1982; 137(5):379–99. PMID: 6758355

48. Goldhamer A, Lisle D, Parpia B, Anderson SV, Campbell TC. Medically supervised water-only fasting in the treatment of hypertension. Journal of manipulative and physiological therapeutics. 2001; 24(5):335–9. https://doi.org/10.1067/mmt.2001.115263 PMID: 11416824

49. Stange R, Pflugbeil C, Michalsen A, Uehleke B. Therapeutic Fasting in Patients with Metabolic Syndrome and Impaired Insulin Resistance. Complementary Medicine Research. 2013; 20(6):421–6.

50. Li C, Sadraie B, Steckhan N, Kessler C, Stange R, Jeitler M, et al. Effects of a one-week fasting therapy in patients with type-2 diabetes mellitus and metabolic syndrome–A randomized controlled explorative study. Experimental and Clinical Endocrinology & Diabetes. 2017; 125(09):618–24.

51. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. The Journal of nutritional biochemistry. 2005; 16(3):129–37. https://doi.org/10.1016/j.jnutbio.2004.12.007 PMID: 15741046

52. Michalsen A, Kuhlmann MK, Lüdtke R, Bäcker M, Langhorst J, Dobos GJ. Prolonged fasting in patients with chronic pain syndromes leads to late mood-enhancement not related to weight loss and fasting-induced leptin depletion. Nutritional neuroscience. 2006:1–6. https://doi.org/10.1080/10284150600627128

53. Michalsen A, Schneider S, Rodenbeck A, Lüdtke R, Huether G, Dobos G. The short-term effects of fasting on the neuroendocrine system in patients with chronic pain syndromes. Nutritional neuroscience. 2003; 6(1):11–8. https://doi.org/10.1080/102841502100004281 1 PMID: 12608732

54. Faulconbridge LF, Wadden TA, Rubin RR, Wing RR, Walkup MP, Fabricatore AN, et al. One-year changes in symptoms of depression and weight in overweight/obese individuals with type 2 diabetes in the look AHEAD study. Obesity. 2012; 20(4):783–93. https://doi.org/10.1038/oby.2011.315 PMID: 22016099

55. Cahill GF Jr, Veech RL. Ketoacids? Good medicine? Transactions of the american clinical and climatological association. 2003; 114:149. PMID: 12813917

56. Marosi K, Kim SW, Moehl K, Scheibye-Knudsen M, Cheng A, Cutler R, et al. 3-hydroxybutyrate regulates energy metabolism and induces BDNF expression in cerebral cortical neurons. Journal of neurochemistry. 2016; 139(5):769–81. https://doi.org/10.1111/jnc.13868 PMID: 27739595

57. Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends in neurosciences. 2004; 27(10):589–94. https://doi.org/10.1016/j.tins.2004.08.001 PMID: 15374669

58. Fond G, Macgregor A, Leboyer M, Michalsen A. Fasting in mood disorders: neurobiology and effectiveness. A review of the literature. Psychiatry research. 2013; 209(3):253–8. https://doi.org/10.1016/j.psychres.2012.12.018 PMID: 23332541

59. Komaki G, Tamai H, Sumioki H, Mori T, Kobayashi N, Mori K, et al. Plasma beta-endorphin during fasting in man. Hormone Research in Paediatrics. 1990; 33(6):239–43.

60. Solianik R, Sujeta A. Two-day fasting evokes stress, but does not affect mood, brain activity, cognitive, psychomotor, and motor performance in overweight women. Behavioural brain research. 2018; 338:166–72. https://doi.org/10.1016/j.bbr.2017.10.028 PMID: 29097329

61. Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. Obesity. 2005; 13(3):574–81.

62. Johnson JB, Summer W, Cutler RG, Martin B, Hyun D-H, Dixit VD, et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radical Biology and Medicine. 2007; 42(5):665–74. https://doi.org/10.1016/j.freeradbiomed.2006.12.005 PMID: 17291990

63. Parikh I, Guo J, Chuang K-H, Zhong Y, Rempe RG, Hoffman JD, et al. Caloric restriction preserves memory and reduces anxiety of aging mice with early enhancement of neurovascular functions. Aging (Albany NY). 2016; 8(11):2814.

64. Stillman CM, Cohen J, Lehman ME, Erickson KI. Mediators of physical activity on neurocognitive function: a review at multiple levels of analysis. Frontiers in human neuroscience. 2016; 10:626. https://doi.org/10.3389/fnhum.2016.00626 PMID: 28018195

65. Mojto V, Gvozdjakova A, Kucharska J, Rausova Z, Vancova O, Valuch J. Effects of complete water fasting and regeneration diet on kidney function, oxidative stress and antioxidants. Bratislavske lekarske listy. 2018; 119(2):107–11. https://doi.org/10.4149/BLL_2018_020 PMID: 29455546

66. Finnell JS, Saul BC, Goldhamer AC, Myers TR. Is fasting safe? A chart review of adverse events during medically supervised, water-only fasting. BMC complementary and alternative medicine. 2018; 18 (1):67. https://doi.org/10.1186/s12906-018-2136-6 PMID: 29458369

67. Cahill GF Jr. Starvation in man. New England Journal of Medicine. 1970; 282(12):668–75. https://doi.org/10.1056/NEJM197003192821209 PMID: 4915800

68. Huber R, Nauck M, Basler N, Haas B, Mattern M, Lüdtke R, et al. Effects of subtotal fasting on plasmatic coagulation, fibrinolytic status and platelet activation: a controlled pilot study in healthy subjects. Nutrition, metabolism and cardiovascular diseases. 2005; 15(3):212–8. https://doi.org/10.1016/j.numecd.2004.12.003 PMID: 15955470

69. Drinda S, Wilhelmi de Toledo F. Heilfasten erhöht die «International Normalized Ratio» bei Gesunden stärker als eine ovo-lacto-vegetarische Diät. Schweizerische Zeitschrift für Ganzheitsmedizin/Swiss Journal of Integrative Medicine. 2012; 24(1):46–9. https://doi.org/10.1159/000335953

70. Ruscica M, Ferri N, Macchi C, Corsini A, Sirtori C. Lipid lowering drugs and inflammatory changes: an impact on cardiovascular outcomes? Annals of medicine. 2018:1–24.

71. Müller H, de Toledo FW, Resch K-L. Fasting followed by vegetarian diet in patients with rheumatoid arthritis: a systematic review. Scandinavian journal of rheumatology. 2001; 30(1):1–10. PMID: 11252685

72. Arumugam T, Phillips T, Cheng A, Morrell C, Mattson M, Wan R. Age and energy intake interact to modify cell stress pathways and stroke outcome. Annals of Neurology. 2010; 67(1):41–52. https://doi.org/10.1002/ana.21798 PMID: 20186857

73. Silva DC, Costa LO, Vasconcelos AA, Cerqueira JC, Fantato D, Torres DC, et al. Waist circumference and menopausal status are independent predictors of endothelial low-grade inflammation. Endocrine Research. 2014; 39(1):22–5. https://doi.org/10.3109/07435800.2013.797431 PMID: 23772608

74. Lennox WG. A study of the retention of uric acid during fasting. Journal of Biological Chemistry. 1925; 66(2):521–72.

75. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant-and radical-caused aging and cancer: a hypothesis. Proceedings of the National Academy of Sciences. 1981; 78(11):6858–62.

76. Becker BF. Towards the physiological function of uric acid. Free Radical Biology and Medicine. 1993; 14(6):615–31. PMID: 8325534

77. Padova J, Bendersky G. Hyperuricemia in diabetic ketoacidosis. New England Journal of Medicine. 1962; 267(11):530–4.

# S1 Table: Effect of fasting on clinical parameters, well-being and ketosis.

| | **All** (n= 1422) | | **F5d 5±2 d** | | **F10d 10±2 d** | | **F15d 15±2 d** | | **F20d 20±2 d** | | **p-values** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pre | post | pre | post | pre | post | pre | post | pre | post | fasting intervention | fasting duration group | sex | fasting duration group-by-fasting intervention | fasting intervention-by-sex |
| Weight, kg | 82.0±0.5 | 77.9±0.5 | 79.3±0.8 | 76.1±0.7 | 82.7±0.9 | 78.3±0.8 | 86.6±1.6 | 80.5±1.4 | 96.7±4.0 | 89.6±3.7 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| BMI, kg/m² | 28.2±0.2 | 26.7±0.1 | 27.2±0.2 | 26.1±0.2 | 28.5±0.3 | 27.0±0.2 | 29.7±0.4 | 27.5±0.4 | 33.6±1.1 | 31.0±1.1 | <0.001 | <0.001 | 0.001 | <0.001 | <0.001 |
| Waist, cm | 94.0±0.4 | 88.0±0.5 | 91.3±0.6 | 86.4±0.7 | 94.8±0.7 | 88.6±0.7 | 98.3±1.2 | 89.4±1.3 | 106.3±2.8 | 96.9±3.1 | <0.001 | <0.001 | 0.024 | <0.001 | <0.001 |
| SBP, mmHg | 130.6±0.6 | 120.6±0.4 | 129.0±0.8 | 122.0±0.6 | 130.3±0.9 | 119.2±0.7 | 136.0±1.5 | 119.9±1.2 | 134.2±3.4 | 118.0±1.8 | <0.001 | <0.001 | 0.007 | <0.001 | – |
| DBP, mmHg | 83.2±0.3 | 77.7±0.3 | 82.5±0.5 | 78.5±0.4 | 83.2±0.5 | 77.0±0.4 | 84.7±0.8 | 77.0±0.7 | 86.3±1.9 | 78.3±1.5 | <0.001 | <0.001 | <0.001 | <0.001 | – |
| Heart rate, beats/min | 69.4±0.3 | 71.3±0.3 | 69.3±0.4 | 72.0±0.4 | 69.2±0.5 | 70.6±0.5 | 70.2±0.7 | 71.0±0.8 | 69.6±1.5 | 68.2±2.0 | 0.04 | 0.003 | 0.003 | 0.007 | – |
| EWB, score 0-10 | 6.2±0.1 | 7.6±0.1 | 6.3±0.1 | 7.6±0.1 | 6.3±0.1 | 7.5±0.1 | 5.7±0.2 | 7.7±0.1 | 6.6±0.4 | 8.0±0.3 | <0.001 | 0.02 | 0.91 | <0.001 | – |
| PWB, score 0-10 | 5.8±0.1 | 7.4±0.1 | 5.8±0.1 | 7.3±0.1 | 5.8±0.1 | 7.3±0.1 | 5.5±0.2 | 7.6±0.1 | 5.9±0.4 | 7.9±0.3 | <0.001 | 0.01 | 0.34 | 0.007 | – |
| Acetoacetic acid, mg/dL | 2.5±0.3 | 50.2±1.2 | 2.6±0.4 | 50.8±1.7 | 2.6±0.4 | 49.6±2.0 | 2.3±0.7 | 49.5±3.2 | 2.6±1.3 | 51.8±8.6 | <0.001 | 0.81 | <0.001 | – | <0.001 |

Values are shown as mean±SEM for all of the groups with different fasting lengths. P-values were calculated for the effects of fasting intervention as well as the effects of the fasting length (fasting duration group) and gender (sex). Interactions between fasting intervention by fasting duration group (fasting duration group-by-fasting intervention) and fasting intervention by gender (fasting intervention-by-sex) are shown.

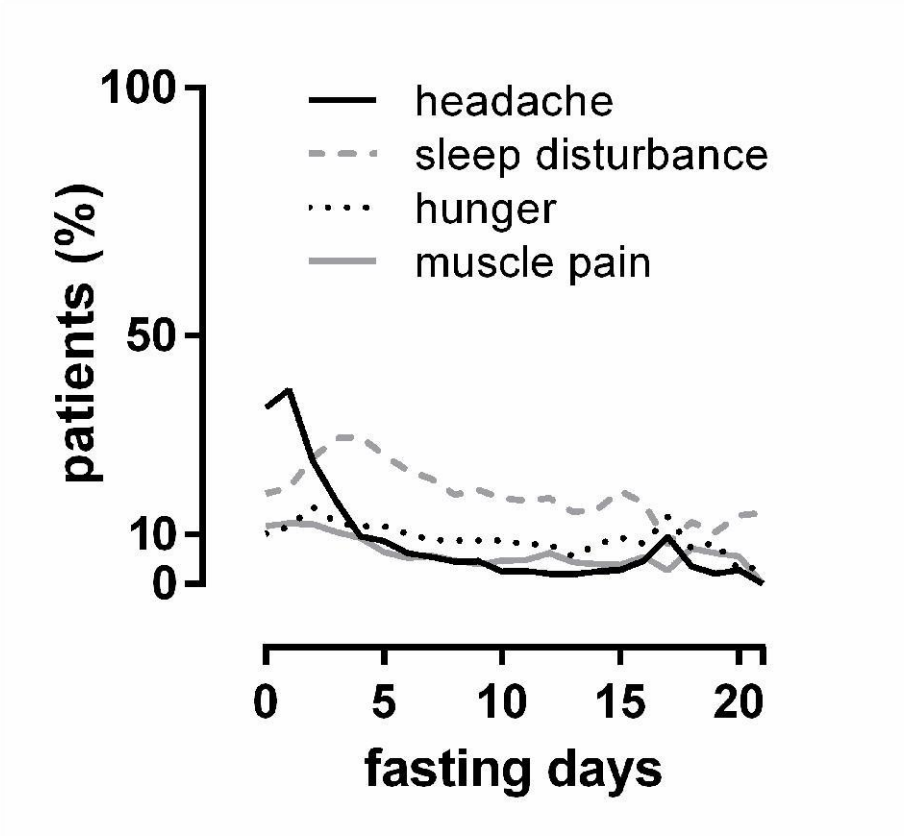
SBP, systolic blood pressure; DBP, diastolic blood pressure; EWB, emotional well-being; PWB, physical well-being.

# S2 Table: Effect of fasting on lipid parameters and glycaemia.

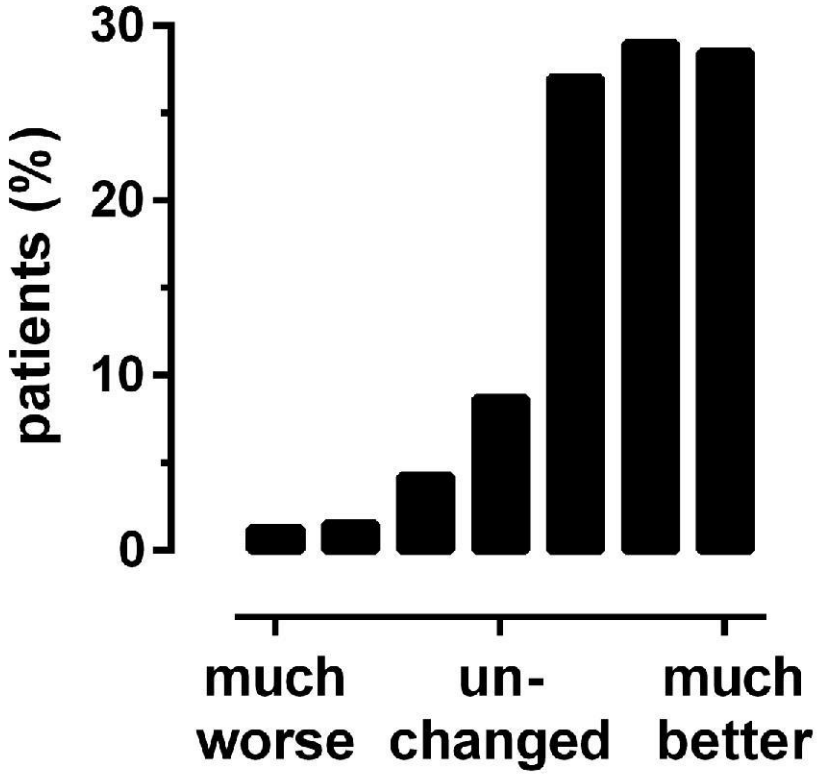
| | **All** (n= 1422) | | **F5d 5±2 d** | | **F10d 10±2 d** | | **F15d 15±2 d** | | **F20d 20±2 d** | | **p-values** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pre | post | pre | post | pre | post | pre | post | pre | post | fasting intervention | fasting duration group | sex | fasting duration group-by-fasting intervention | fasting intervention-by-sex |
| TG, mmol/l | 1.5±0.0 | 1.1±0.0 | 1.5±0.0 | 1.1±0.0 | 1.6±0.0 | 1.1±0.0 | 1.6±0.1 | 1.1±0.0 | 1.5±0.1 | 1.2±0.0 | <0.001 | 0.004 | 0.72 | 0.001 | <0.001 |
| TC, mmol/l | 5.6±0.0 | 4.9±0.0 | 5.5±0.0 | 5.2±0.0 | 5.6±0.0 | 4.9±0.0 | 5.6±0.1 | 4.4±0.1 | 5.7±0.2 | 4.5±0.2 | <0.001 | <0.001 | 0.67 | <0.001 | – |
| HDL-C, mmol/l | 1.5±0.0 | 1.3±0.0 | 1.6±0.0 | 1.4±0.0 | 1.5±0.0 | 1.3±0.0 | 1.5±0.0 | 1.2±0.0 | 1.5±0.1 | 1.1±0.0 | <0.001 | <0.001 | 0.36 | <0.001 | <0.001 |
| LDL-C, mmol/l | 3.5±0.0 | 3.2±0.0 | 3.4±0.0 | 3.4±0.0 | 3.5±0.0 | 3.1±0.0 | 3.6±0.1 | 2.7±0.1 | 3.8±0.2 | 2.8±0.2 | <0.001 | <0.001 | 0.19 | <0.001 | – |
| LDL/HDL ratio | 2.6±0.1 | 2.6±0.0 | 2.7±0.3 | 2.7±0.0 | 2.5±0.0 | 2.6±0.1 | 2.5±0.1 | 2.4±0.1 | 2.7±0.1 | 2.6±0.2 | 0.66 | 0.003 | <0.001 | – | – |
| Glucose, mmol/l | 5.4±0.0 | 4.7±0.0 | 5.3±0.0 | 4.6±0.0 | 5.4±0.1 | 4.7±0.1 | 5.6±0.1 | 4.7±0.1 | 5.5±0.2 | 4.9±0.2 | <0.001 | 0.68 | 0.12 | 0.03 | <0.001 |
| HbA1c, mmol/mol | 36.0±0.2 | 34.3±0.2 | 35.5±0.3 | 34.2±0.3 | 36.1±0.3 | 34.4±0.3 | 36.7±0.4 | 34.5±0.4 | 37.6±1.3 | 34.9±1.0 | <0.001 | <0.001 | 0.67 | <0.001 | 0.03 |

Values are shown as mean±SEM for all of the groups with different fasting lengths. P-values were calculated for the effects of fasting intervention as well as the effects of the fasting length (fasting duration group) and gender (sex). Interactions between fasting intervention by fasting duration group (fasting duration group-by-fasting intervention) and fasting intervention by gender (fasting intervention-by-sex) are shown.

TG, triglyceride; TC, total cholesterol; HDL-C, high-density-lipoprotein; LDL-C, low-density-lipoprotein; HbA1c, glycated haemoglobin.




**S1_Fig. Occurrence of self-reported mild symptoms during fasting.**




**S2_Fig. Evolution of a pre-existing health complaint.** A subgroup of 404 subjects indicated to have a major health complaint previous to the fasting.

nutrients

MDPI

Article

# Effects of Periodic Fasting on Fatty Liver Index—A Prospective Observational Study

**Stefan Drinda [1,2,*], Franziska Grundler [1,3], Thomas Neumann [4], Thomas Lehmann [5], Nico Steckhan [6], Andreas Michalsen [6] and Francoise Wilhelmi de Toledo [1]**

1 Buchinger Wilhelmi Clinic, 88662 Überlingen, Germany; franziska.grundler@buchinger-wilhelmi.com (F.G.); francoise.wilhelmi@buchinger-wilhelmi.com (F.W.d.T.)
2 Clinic St. Katharinental, Department of Rheumatology, 8253 Diessenhofen, Switzerland
3 Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt–Universität zu Berlin and Berlin Institute of Health, 10117 Berlin, Germany
4 Division of Rheumatology, Immunology and Rehabilitation, Kantonsspital St. Gallen, 9007 St. Gallen, Switzerland; thomas.neumann@kssg.ch
5 Institute of Medical Statistics, Computer Sciences and Documentation, Jena University Hospital, 07743 Jena, Germany; thomas.lehmann@med.uni-jena.de
6 Department of Internal and Complementary Medicine, Immanuel Hospital Berlin, 14109 Berlin, Germany; nico.steckhan@charite.de (N.S.); andreas.michalsen@immanuel.de (A.M.)
* Correspondence: stefan.drinda@stgag.ch

Received: 19 September 2019; Accepted: 28 October 2019; Published: 30 October 2019


**Abstract:** This prospective observational trial investigated effects and safety of periodic fasting in subjects with and without type 2 diabetes mellitus (T2DM). The primary end point was set as the change of fatty liver index (FLI) as a surrogate parameter of non-alcoholic fatty liver disease (NAFLD). Six-hundred and ninety-seven subjects (38 with T2DM) were enrolled. A baseline FLI ≥ 60 (the threshold for fatty liver) was found in 264 subjects (37.9%). The mean duration of fasting was 8.5 ± 4.0 days (range 6–38). FLI decreased significantly (−14.02 ± 11.67; $p < 0.0001$), with a larger effect in individuals with T2DM (−19.15 ± 11.0; $p < 0.0001$; $p = 0.002$ compared to non-diabetic subjects). Body mass index (BMI) decreased by −1.51 ± 0.82 kg/m$^2$, and 49.9% of the subjects lost ≥5% body weight. After fasting, nearly half of the 264 subjects with FLI ≥ 60 (highest risk category) shifted to a lower category. The improvement of FLI correlated with the number of fasting days ($r = -0.20$, $p < 0.0001$) and with the magnitude of BMI reduction ($r = 0.14$, $p = 0.0001$). Periodic fasting with concomitant weight reduction leads to significant rapid improvement of FLI in subjects with and without T2DM.

**Keywords:** periodic fasting; obesity; overweight; fatty liver index; diabetes

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered one of the most relevant causes of chronic liver disorders [1], and consists of a disease spectrum including fatty liver, non-alcoholic steatohepatitis, fibrosis, and liver cirrhosis. The least advanced stage of disease, the non-alcoholic fatty liver (NAFL, or simple steatosis), is characterized by an excess of fat in the liver and is mostly asymptomatic [2]. The median prevalence of NAFLD is 20%, ranging from 6% to 33% in industrialized countries [3]. The burden of disease is rapidly increasing, mainly due to the rising prevalence of obesity and sedentary lifestyle, considering that as many as 50% of patients with simple steatosis proceed to develop a non-alcoholic steatohepatitis (NASH) [4].

NAFLD fuels in a closed loop the epidemics of type 2 diabetes mellitus (T2DM) and metabolic syndrome [5], almost doubling the risk of developing these disorders [6]. Notably, NAFLD is regarded as an even stronger predictor for the development of T2DM then waist circumference or obesity [7],

and the degree of histologic liver damage is directly related to the presence of diabetes in morbidly obese patients [8].

NAFLD is a multi-factorial disease resulting from a complex interaction of environmental and genetic factors. The most common cause of NAFLD is an altered whole-body energetic homeostasis resulting from a caloric intake exceeding the caloric expenditure. Energy spill-over is then stored in the form of non-esterified fatty acids (NEFA) from visceral adipose tissue into ectopic fat depots, such as the liver, skeletal muscles, and pancreas [9]. NAFLD has been associated with dietary excess of saturated fatty acids, refined carbohydrates, and fructose [10,11].

The amount of liver fat can be determined by magnetic resonance imaging (MRI) or biopsy, but it can also be calculated by the fatty liver index (FLI), which has been shown to closely correlate with the results of the MRI [12]. The FLI is calculated on the basis of body mass index (BMI), waist circumference, triglycerides (TG), and gamma-glutamyl-transferase (GGT) [13], a calculation that permits an easy detection of NAFLD and also allows a non-instrumental monitoring of treatment effects. The FLI score ranges from 0 to 100, with FLI <30 excluding and ≥60 confirming a diagnosis of fatty liver (including NAFLD).

Several therapeutic strategies can reverse NAFLD [14], but the most important approach is lifestyle modification with diet and exercise [15,16]. A body weight reduction of 7–10% obtained with energy restriction and increased physical activity leads to histological improvement of steatosis, inflammation, and fibrosis [17,18]. Pharmaceutical approaches, in turn, focus directly on hepatic fat accumulation, anti–inflammatory effects (e.g., oxidative stress alleviation and modulation of tumor necrosis factor), insulin sensitization, or anti–obesity drugs [14]. Thus far, however, these approaches have shown rather limited effects on the progression of fatty liver [16,19,20].

While the combination of physical exercise and change of dietary habits is the most effective intervention for weight loss [21], the translation of long-term lifestyle changes into daily routine seems to be difficult to achieve [22]. One of the proposed options to ease this difficulty is fasting therapy, which is the voluntary renouncement of food for a defined period and which results in distinct metabolic changes, i.e., pronounced lipolysis, decreased insulin secretion, increased insulin sensitivity, gluconeogenesis, and production of ketone bodies [23]. Periodic fasting, in contrast to intermittent or Ramadan fasting, is defined as lasting from 2 to 21 days or longer. Periodic fasting has been used since decades as a treatment option of the metabolic syndrome [23]. Additionally, periodic fasting does not only induce beneficial effects on lifestyle modification, with a subsequent better adherence to nutritional recommendations and exercise [24], but can also counteract a prediabetes by restoring beta cell function and overcoming insulin resistance [25].

To the best of our knowledge, there are no clinical data on the impact of fasting therapy on fatty liver, although beneficial effects of periodic and intermittent fasting on fatty liver have already been shown in mice experiments [26]. Currently, it is also unknown if the effects of fasting on fatty liver are similar in non-diabetic and diabetic individuals. The present study was thus performed to examine the short-term effects of fasting therapy on FLI in diabetic and non-diabetic subjects, and to investigate the safety of this approach.

## 2. Materials and Methods

### 2.1. Study Design

This study was conducted according to the Declaration of Helsinki and was registered at the German register of clinical trials (DRKS-ID: 00010111). The study protocol was approved by the ethics committees of the Charité Medical University of Berlin and the Federal Medical Council of Baden-Württemberg, Germany.

The subjects agreed to study participation by signing a written informed consent.

The study was designed as a prospective, uncontrolled, observational cohort study. All subjects were voluntary inpatients at the Buchinger-Wilhelmi Clinic Überlingen (Germany), a medical center

specialized on periodic fasting. The data presented in this study are part of a larger trial aimed at investigating safety, health improvement, and well-being in 1422 individuals undergoing periodic fasting [27].

### 2.2. Subjects

Study subjects were consecutively recruited between January and October 2016. Inclusion criteria were age ≥18 years; BMI ≥19 kg/m$^2$, and a minimum scheduled inpatient stay of 10 days with at least 6 days for periodic fasting. Exclusion criteria were viral or autoimmune liver diseases, cognitive and psychological disorders, pregnancy or lactation, and inadequate language skills to communicate in English, French, or German.

### 2.3. Study Interventions

All participants underwent periodic fasting according to the published guidelines for periodic fasting therapy [28].

The intervention started with a low-calorie transition day (600 kcal/day mono-diet consisting of fruits, rice, oat, or vegetables according to patients' choice). During the fasting period, the subjects received 250 mL fruit juice or vegetable broth at midday, 250 mL vegetable broth in the evening, and optional 20 g honey (maximum total energy intake of 250 kcal/day; maximum total carbohydrate intake 35 g/day). It was recommended to drink at least 2 liters of water/day. The duration of fasting was adapted to the individual therapeutic goal and tolerance, and was then followed by stepwise reintroduction of food. The latter consisted of ovo-lacto-vegetarian food increasing from 800 kcal/day to 1800 kcal/day over at least 3 days. The fasting therapy was accompanied by a physical exercise program with moderate walking and gymnastics as group activities, paralleled by group sessions in mindfulness, and relaxation techniques as autogenic training and meditation.

### 2.4. Outcome Measures

All measurements were done at baseline (day before start of fasting) and at the end of therapy (day after last fasting day). Anthropometrical measurements (height, weight, and waist circumference) and blood samples for laboratory assessments were taken between 7.00 and 10.00 a.m. Glucose was measured using the hexokinase 3 method (Siemens, Erlangen, Germany; coefficients of variation (CV) 11%; normal range 3.9–5.5 mmoL/L); HbA1c by high pressure liquid chromatography (HPLC; Tosoh Bioscience, Griesheim, Germany, CV 10%, normal <42 mmoL/moL); total cholesterol (TC) by enzymatic color reaction (Siemens; CV 7%; normal <5.2 mmoL/L); triglycerides (TG) by glycerol phosphate oxidase (GPO)/Trinder enzymatic color reaction (Siemens; CV 9%; normal <1.7 mmoL/L); high density lipoprotein (HDL) by a two step catalase–elimination reaction (Siemens; CV 9%; range 1.2–3.1 mmoL/L); and low density lipoprotein (LDL) by a two–step catalase–elimination reaction (Siemens; CV 6.2%; normal <4.1 mmoL/L). Glutamate–oxalacetate transferase (GOT), glutamate–pyruvate transferase (GPT), alkaline phosphatase (AP), and gamma glutamyl transferase (GGT) were measured by standard methods.

The FLI was calculated as follows: FLI = (e 0.953 × loge (TG) + 0.139 × BMI + 0.718 × loge (GGT) + 0.053 × WC − 15.745)/(1 + e 0.953 × loge (TG) + 0.139 × BMI + 0.718 × loge (GGT) + 0.053 × WC − 15.745) × 100 [13].

The study data were analyzed as per protocol analysis.

### 2.5. Statistical Analysis

The data were presented as means ± standard deviation (SD), if not otherwise indicated. D'Agostino and Pearson omnibus normality test was used to verify the normal data distribution. Changes between baseline and end of intervention were compared by the paired test for normally distributed data and the Wilcoxon matched-pairs signed rank test for non-parametric data. To determine possible differential effects in diabetic patients, all participants (group all) were clustered in non-diabetic

subjects (fasting glucose <7 mmoL /L and HbA1c <47 mmoL/moL) and patients with T2DM (fasting glucose ≥7 and/or HbA1c ≥47 mmoL/moL). Group differences were calculated with the unpaired test for parametric data or Mann Whitney U test for non-parametric data. Correlations analyses were performed by the Pearson or Spearman correlation coefficients, depending on the normal distribution of the data.

A multivariable linear regression model was fitted for FLI after intervention with gender, age, number of fasting days, GOT, GPT, total cholesterol, diabetes, and baseline FLI as independent variables. Estimated regression coefficients with 95% confidence intervals (CI) were calculated to evaluate the influence of the variables.

Additionally, a binary logistic regression model was applied for a positive outcome (defined as FLI ≤60 after intervention) only for the subjects who had a baseline FLI >60. Independent variables were gender, age, number of fasting days, GOT, GPT, total cholesterol, diabetes, and baseline FLI. Receiver-operating curve (ROC) analysis of predicted probabilities was performed, and the area under curve (AUC) with 95% CI was calculated to measure the accuracy of the model. The discrimination of this model was assessed by comparing the predicted probabilities with the binary outcomes (FLI ≤ 60) of each patient in the ROC analysis.

A $p$ value < 0.05 was considered statistically significant. Data analyses were performed by using the statistical software SPSS version 24 (IBM) and GraphPad Prism 6 (GraphPad Inc.).

## 3. Results

Of 1500 screened subjects, 741 were included in the study as per inclusion/exclusion criteria. Of these, 44 dropped out due to: periodic fasting days <6 ($n$ = 27), recently diagnosed liver disease ($n$ = 12), or non-compliance ($n$ = 5). Thus, 697 subjects completed the study and were analyzed per protocol. The mean fasting duration was 8.5 ± 4.0 days (range 6–38), with no significant difference ($p$ = 0.261) between subjects with T2DM (9.3 ± 4.8 days, range 6–31) or without T2DM (8.4 ± 4.0 days, range 6–38).

Of the 697 study subjects, 38 (5.5%) had T2DM. Of those, 28 were treated with anti–diabetic medications as follows: metformin: $n$ = 27, dipeptidyl peptidase-4 (DPP4) inhibitors: $n$ = 6, sulfonylurea: $n$ = 5, sodium-glucose transport 2 (SGLT-2) inhibitors: $n$ = 2, and glitazone: $n$ = 1. Three patients received a combination of oral anti-diabetics and insulin therapy. One subject received insulin treatment only.

Treatment for arterial hypertension was recorded in 122 subjects (17.5%), 99/659 in the non-diabetic subgroup and 23/38 in the T2DM subgroup. Treatment with statins was recorded in 116 subjects (16.6%), 78/659 in the non-diabetic subgroup and 18/38 in the T2DM subgroup.

### 3.1. Baseline Characteristics of Study Participants

Baseline characteristics of all study subjects and categorized for the presence or absence of T2DM are presented in Table 1.

The study population was predominantly female and middle-aged. Patients with T2DM were older than subjects without diabetes (60.92 ± 9.74 years vs. 54.23 ± 13.46 years, $p$ = 0.002), and their baseline BMI and waist circumference were significantly higher than in non-diabetic subjects.

The FLI was normal (<30) in 273 subjects (39.2%), intermediate (between ≥30 and <60) in 160 subjects (23.0%), and indicative of fatty liver (≥60) in 264 subjects (37.9%). The subgroup of patients with T2DM ($n$ = 38) had a significantly higher FLI than subjects without T2DM (78.36 ± 16.97 vs. 44.92 ± 31.57, $p$ < 0.001).

**Table 1.** Baseline characteristics, comparisons of subjects with and without type 2 diabetes mellitus (T2DM).

| | T2DM | No Diabetes | All | *p* Value T2DM vs. No Diabetes |
|---|---|---|---|---|
| *n* | 38 | 659 | 697 | |
| Female, *n* (%) | 11 (28.9) | 429 (65.1) | 440 (63.1) | |
| Age, years | 60.92 ± 9.74 | 54.23 ± 13.46 | 54.60 ± 13.36 | 0.0021 |
| BMI, kg/m$^2$ | 31.79 ± 5.15 | 27.98 ± 5.25 | 28.19 ± 5.31 | <0.0001 |
| BMI categories | | | | |
| <25 kg/m$^2$, *n* (%) | 1 (2.6) | 206 (79.5) | 207 (29.7) | <0.0001 |
| ≥25 kg/m$^2$, *n* (%) | 37 (97.4) | 453 (20.5) | 490 (60.3) | |
| Height, cm | 173.21 ± 0.10 | 169.23 ± 0.09 | 169.45 ± 0.09 | 0.0162 |
| Weight, kg | 95.45 ± 17.78 | 80.46 ± 17.89 | 81.28 ± 18.19 | <0.0001 |
| Waist, cm | 107.13 ± 11.32 | 92.06 ± 15.21 | 92.88 ± 15.40 | <0.0001 |
| Glucose, mmoL/L | 8.10 ± 2.40 | 5.19 ± 0.72 | 5.35 ± 1.11 | <0.0001 |
| HbA1c, mmoL/moL | 55.2 ± 15.0 | 35.28 ± 3.94 | 36.37 ± 6.86 | <0.0001 |
| GGT, µkat/L | 0.75 ± 0.95 | 0.47 ± 0.53 | 0.48 ± 0.57 | 0.0003 |
| GOT, µkat/L | 0.46 ± 0.20 | 0.40 ± 0.21 | 0.41 ± 0.21 | 0.0550 |
| GPT, µkat/L | 0.63 ± 0.38 | 0.49 ± 0.35 | 0.50 ± 0.35 | 0.0006 |
| AP, µkat/L | 1.06 ± 0.31 | 1.09 ± 0.30 | 1.09 ± 0.30 | 0.5164 |
| Cholesterol, mmoL/L | 4.83 ± 1.18 | 5.60 ± 1.18 | 5.56 ± 1.19 | 0.0002 |
| TG, mmoL/L | 2.09 ± 0.95 | 1.52 ± 0.77 | 1.55 ± 0.80 | <0.0001 |
| HDL, mmoL/L | 1.17 ± 0.34 | 1.57 ± 0.47 | 1.55 ± 0.48 | <0.0001 |
| LDL, mmoL/L | 3.01 ± 1.02 | 3.49 ± 1.07 | 3.46 ± 1.08 | 0.0083 |
| FLI, points | 78.36 ± 16.97 | 44.92 ± 31.57 | 46.75 ± 31.86 | <0.0001 |
| FLI category | | | | |
| <30 points | 1 | 272 | 273 | |
| ≥30–<60 points | 4 | 156 | 160 | <0.0001 |
| ≥60 points | 33 | 231 | 264 | |

BMI: body mass index; Waist: abdominal circumference; GGT: gamma glutamyl transferase; GOT: glutamate oxalacetate transferase; GPT: glutamate pyruvate transferase; AP: alkaline phosphatase, Cholesterol: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein. Categorical data were analyzed by Fischer's exact test (BMI categories) and chi-square test (fatty liver index (FLI) categories).

### 3.2. *Changes in FLI*

Overall, periodic fasting reduced the FLI by a mean of −14.02 ± 11.67 points ($p < 0.0001$; Figure 1).

As many as 120 of the 264 subjects with a baseline FLI ≥ 60 (high risk category) shifted to a lower category of FLI risk after therapy. The subgroup of patients with T2DM ($n = 38$) experienced a significantly greater FLI reduction (−19.15 ± 11.0 points, $p < 0.0001$) than non-diabetic subjects (−13.73 ± 11.65 points, $p < 0.0001$; group difference $p = 0.002$; Figure 2).

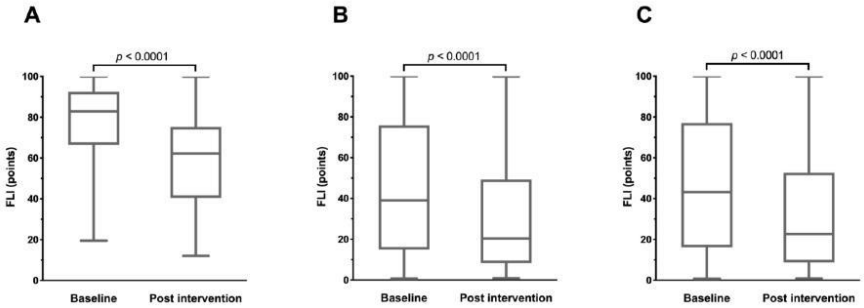



**Figure 1.** Changes in FLI before and after fasting in patients with T2DM (**A**), non-diabetic subjects (**B**), and all subjects (**C**).

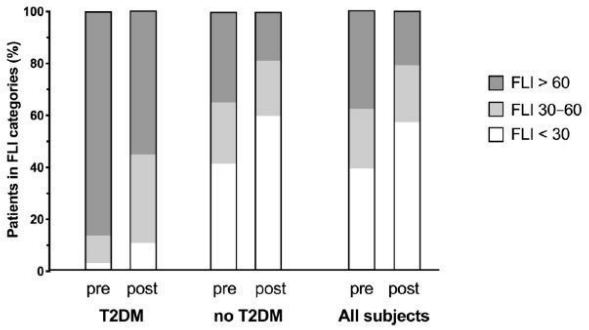



**Figure 2.** Frequency distribution of FLI categories before and after therapeutic fasting in patients with T2DM ($n$ = 38), in non-diabetic subjects (no T2DM; $n$ = 659), and in all subjects ($n$ = 697).

In the non-diabetic subgroup, the number of subjects with fatty liver decreased from 231 to 124 subjects (−46.3%), while the number of subjects with normal FLI (<30 points) increased from 273 to 395 subjects (+44.7%). In the T2DM subgroup, the number of patients with fatty liver decreased from 33 to 21 (−36.4%), while the number of patients with normal FLI increased from 1 to 4. An absolute FLI reduction of >30% was achieved in 60.9% of all subjects who had a weight reduction of ≥5% from baseline.

### 3.3. Changes in Anthropometric and Metabolic Parameters

Changes from baseline are shown in Table 2.

Periodic fasting induced a significant weight loss in the overall population (−4.37 ± 2.42 kg, $p < 0.001$). In the non-diabetic subgroup, the mean changes were −4.31 ± 2.41 kg ($p < 0.001$), in the T2DM subgroup −5.29 ± 2.55 kg ($p < 0.001$). Half of the subjects (348, 49.9%) lost ≥5% of their body weight. Overall, the BMI decreased by −1.51 ± 0.82 kg/m$^2$ ($p < 0.001$). In non-diabetic subjects, the BMI decreased by −1.50 ± 0.81 kg/m$^2$ ($p < 0.001$), and in T2DM patients by −1.75 ± 0.85 kg/m$^2$ ($p < 0.001$).

The waist circumference decreased overall by −5.39 ± 3.28 cm ($p < 0.001$), in non-diabetic subjects by −5.34 ± 3.27 cm ($p < 0.001$), and in T2DM patients by −6.32 ± 3.37 cm ($p < 0.001$).

Fasting plasma glucose and HbA1c levels significantly decreased in all groups. Liver enzymes also decreased after the fasting intervention, except for AP in patients with T2DM. The same was found for blood lipids. All blood lipids markedly dropped, except for HDL cholesterol and LDL cholesterol in patients with T2DM (Table 2).

**Table 2.** Changes from baseline to post fasting, overall and for subjects with and without T2DM.

| | T2DM ($n$ = 38) | | No Diabetes ($n$ = 659) | | All ($n$ = 697) | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SD | $p$ Value | Mean ± SD | $p$ Value | Mean ± SD | $p$ Value | $p$ Value T2DM vs. No Diabetes |
| Weight, kg | −5.29 ± 2.56 | <0.0001 | −4.31 ± 2.41 | <0.0001 | −4.37 ± 2.42 | <0.0001 | 0.0045 |
| BMI, kg/m$^2$ | −1.75 ± 0.85 | <0.0001 | −1.50 ± 0.81 | <0.0001 | −1.51 ± 0.82 | <0.0001 | 0.0213 |
| Waist, cm | −6.32 ± 3.37 | <0.0001 | −5.34 ± 3.27 | <0.0001 | −5.39 ± 3.28 | <0.0001 | 0.0433 |
| FLI, points | −19.15 ± 11.00 | <0.0001 | −13.73 ± 11.65 | <0.0001 | −14.02 ± 11.67 | <0.0001 | 0.0020 |
| Glucose, mmoL/L | −2.69 ± 2.56 | <0.0001 | −0.60 ± 1.24 | <0.0001 | −0.72 ± 1.42 | <0.0001 | <0.0001 |
| HbA1c, mmoL/moL | −4.43 ± 6.65 | <0.0001 | −1.60 ± 2.91 | <0.0001 | −1.76 ± 3.28 | <0.0001 | <0.0001 |
| GGT, µkat/L | −0.18 ± 0.37 | <0.0001 | −0.12 ± 0.27 | <0.0001 | −0.12 ± 0.28 | <0.0001 | 0.0694 |
| GOT, µkat/L | 0.29 ± 0.30 | <0.0001 | 0.20 ± 0.28 | <0.0001 | 0.21 ± 0.29 | <0.0001 | 0.0613 |
| GPT, µkat/L | 0.34 ± 0.45 | <0.0001 | 0.18 ± 0.34 | <0.0001 | 0.18 ± 0.35 | <0.0001 | 0.0147 |
| AP, µkat/L | −0.03 ± 0.19 | 0.3297 | −0.04 ± 0.14 | <0.0001 | −0.04 ± 0.15 | <0.0001 | 0.9886 |
| Cholesterol, mmoL/L | −0.44 ± 1.05 | 0.0136 | −0.66 ± 0.78 | <0.0001 | −0.64 ± 0.79 | <0.0001 | 0.1858 |
| TG, mmoL/L | −0.78 ± 0.96 | <0.0001 | −0.43 ± 0.69 | <0.0001 | −0.44 ± 0.71 | <0.0001 | 0.0057 |
| HDL, mmoL/L | −0.07 ± 0.27 | 0.0905 | −0.24 ± 0.26 | <0.0001 | −0.23 ± 0.27 | <0.0001 | <0.0001 |
| LDL, mmoL/L | −0.20 ± 1.03 | 0.2327 | −0.31 ± 0.79 | <0.0001 | −0.30 ± 0.81 | <0.0001 | 0.6141 |

BMI = body mass index; Waist = abdominal circumference; GGT = gamma glutamyl transferase; GOT = glutamate oxalacetate transferase; GPT = glutamate pyruvate transferase; AP = alkaline phosphatase, Cholesterol = total cholesterol; TG = triglycerides; HDL = high density lipoprotein; LDL = low density lipoprotein. Data were analyzed by a paired $t$–test.

### 3.4. Correlations Analyses

The results of the correlation analyses are shown in Table 3.

**Table 3.** Correlation analyses, overall and for subjects with and without T2DM.

| | T2DM ($n$ = 38) | | No Diabetes ($n$ = 659) | | All ($n$ = 697) | |
|---|---|---|---|---|---|---|
| | r | $p$ Value | r | $p$ Value | r | $p$ Value |
| FLI vs. Fasting days | −0.42 | 0.0091 | −0.18 | <0.0001 | −0.20 | <0.0001 |
| FLI vs. BMI | 0.32 | 0.0474 | 0.27 | <0.0001 | −0.14 | 0.0001 |
| FLI vs. WC | 0.39 | 0.0165 | 0.28 | <0.0001 | 0.29 | <0.0001 |
| FLI vs. GGT | 0.22 | 0.1907 | 0.48 | <0.0001 | 0.47 | <0.0001 |
| FLI vs. GOT | 0.18 | 0.2716 | −0.10 | 0.0102 | −0.10 | 0.0120 |
| FLI vs. GPT | 0.18 | 0.2802 | −0.02 | 0.6154 | −0.02 | 0.5673 |
| FLI vs. AP | 0.35 | 0.0322 | 0.17 | <0.0001 | 0.18 | <0.0001 |
| FLI vs. Cholesterol | 0.33 | 0.0451 | 0.30 | <0.0001 | 0.29 | <0.0001 |
| FLI vs. TG | 0.23 | 0.1576 | 0.63 | <0.0001 | 0.62 | <0.0001 |
| FLI vs. fG | 0.03 | 0.8563 | 0.07 | 0.0942 | −0.02 | 0.6890 |
| FLI vs. HbA1C | 0.14 | 0.4096 | 0.04 | 0.3186 | 0.06 | 0.0891 |

BMI = body mass index; WC = waist circumference; GGT = gamma glutamyl transferase; GOT = glutamate oxalacetate transferase; GPT = glutamate pyruvate transferase; AP = alkaline phosphatase, Cholesterol = total cholesterol; TG = triglycerides; fG = fasting glucose. r = Pearson's correlation coefficient for parametric data or Spearman's correlation coefficient for non-parametric data.

The decreases in FLI induced by fasting correlated with the length of the fasting, in the overall group ($r = -0.20$; $p < 0.0001$) as well as in the subgroups of non-diabetic subjects ($r = -0.18$; $p < 0.0001$) and T2DM patients ($r = -0.36$; $p = 0.0262$; Figure 3).

Likewise, changes in FLI significantly correlated with the decrease of body weight and waist circumference (Table 3, Figure 4).

There were no significant correlations between changes in FLI and fasting plasma glucose or HbA1c levels (Table 3).

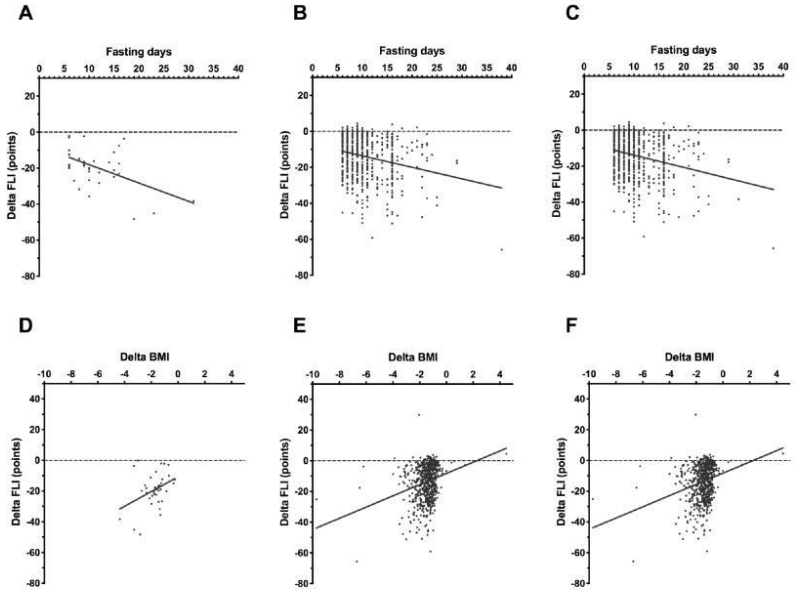



**Figure 3.** Correlation of FLI changes with fasting duration in patients with T2DM (**A**), non-diabetic subjects (**B**), and all subjects (**C**); and with changes of BMI in patients with T2DM (**D**), non-diabetic subjects (E), and all subjects (**F**).

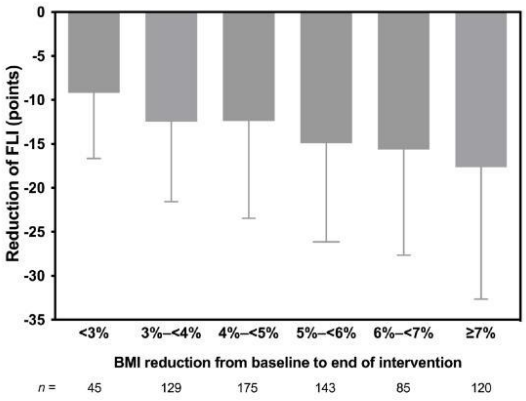



**Figure 4.** Reduction of FLI in relation to percent changes in body mass index (BMI).

Predictors of changes in FLI were analyzed in a multivariate linear regression model with gender, age, number of fasting days, GOT, GPT, total cholesterol, diabetes, and baseline FLI as independent variables. FLI decreased on average by 0.48 points with each additional fasting day (regression coefficient beta: −0.48, 95% CI −0.665 to −0.295, $p < 0.001$). FLI reduction was also nearly 4 points higher for males (−3.94, 95% CI −5.780 to −2.10, $p < 0.001$) than for females. GOT (10.61, 95% CI 4.77 to 16.46, $p < 0.001$), baseline FLI (−0.139, 95% CI −0.169 to −0.110, $p < 0.001$), and total cholesterol (0.027, 95% CI −0.044 to −0.010, $p = 0.002$) were also significant predictors in this model. A ROC curve was calculated to test the performance of this model (input variables: gender, age, number of fasting days, GOT, GPT, total cholesterol, diabetes, and baseline FLI) in discriminating the capability to shift from a FLI > 60 to a FLI ≤ 60 due to periodic fasting (Figure 5). With this model, the ability to discriminate subjects proved relatively high (AUC = 0.947, 95% CI 0.922–0.971, $p < 0.001$).

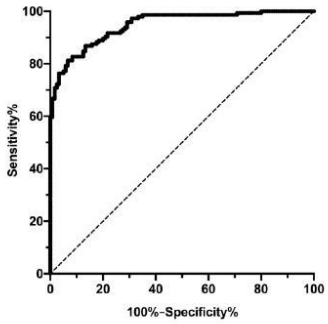



**Figure 5.** Receiving operator characteristic (ROC) curves of gender, age, number of fasting days, and baseline parameters (GOT, GPT, total cholesterol, diabetes status, and FLI) for the prediction of FLI ≤60 after fasting intervention.

### *3.5. Safety*

Adverse events were reported in 10 study subjects (1.4%). None of the events met the criteria of seriousness. The following events occurred in more than one subject: eczema ($n = 3$) and mild hyponatremia ($n = 2$). The following events occurred in one subject each: self-limiting supraventricular tachycardia, intermittent self-limiting paroxysmal atrial fibrillation, mild hypokalemia, bleeding gums, common cold, dizziness, mild hypoglycemia, intermittent visual disorders, and headache. None of the subjects discontinued the fasting because of the events.

A daily self-reporting form for feelings of hunger showed that 363 of the 579 respondents (62.7%; 29 of whom with T2DM) did not experience relevant hunger during the fasting therapy, whereas 217 subjects (37.3%) had at least one episode of hunger. No one indicated to be hungry every day during fasting.

## 4. Discussion

Diet interventions are well-established strategies to reduce body weight and improve glucose metabolism, however no evidence exists about the effects of periodic fasting on NAFLD. This study is the first to show the beneficial effects of periodic fasting on fatty liver. The results of our study supported the hypothesis that FLI, a surrogate parameter of fatty liver, significantly decreases in individuals with and without T2DM after a fasting intervention of at least 6 days. The prospective study design according to a standardized protocol with a well-established fasting technique and a close clinical monitoring was a strength of this study.

Weight reduction and improvement of fatty liver indicators are known to be interrelated [17,18]. Our results indicate that even a modest reduction of BMI improved surrogate markers of fatty liver.

Indeed, in nearly half of the subjects in the highest risk category (FLI > 60), a BMI reduction of less than 5% was sufficient to induce a shift to a lower risk category.

In most patients, NAFLD is associated with features of metabolic syndrome, central obesity, elevated blood pressure, dyslipidemia, hyperglycemia, and insulin resistance [29]. Although these pathologies can be addressed by lifestyle interventions, in daily life the adherence to the necessary lifestyle changes is poor, resulting in suboptimal outcomes. In contrast, in pragmatic programs there is a greater benefit in a more substantial weight loss, particularly at early stages of the intervention period [30]. Hence, periodic fasting can significantly reduce weight, and this effect can be maintained over time [31].

There are several concerns about the adverse effects of fasting. Several non-fatal (e.g., headache) and rarely fatal (e.g., ventricular arrhythmia) events have been reported [32]. In contrast, no severe adverse events were found in a cohort of 1422 subjects treated with a periodic fasting lasting 4–21 days, [27]. Michalsen et al. evaluated the acceptance, safety, effects on health-related outcomes, and lifestyle adherence of fasting therapy in different chronic internal diseases [24]. They found no serious adverse events throughout their study. Our study supports the hypothesis that fasting therapy provided in a controlled clinical setting is a safe intervention.

There is a large body of evidence on the beneficial effects of carbohydrate restriction and hypocaloric diets on NAFLD [33]. It has also been shown that-at equal levels of weight reduction-a carbohydrate-restricted diet leads to a significantly greater intrahepatic triglyceride reduction than low-calorie diet alone [34]. The metabolic advantage of carbohydrate restriction appears to be related to a more pronounced lipid oxidation and enhanced ketogenesis. In recent years, intermittent and periodic fasting has gained popularity as an alternative to continuous caloric restriction. In addition to the weight loss effects, periodic fasting is associated with several metabolic benefits, including the improvement of lipid profiles [27]. This has been also shown for intermittent fasting, e.g., Ramadan fasting [35]. The key mechanism responsible for many of these beneficial effects is the metabolic switch from the utilization of glycogenolysis-derived glucose to fatty acids and fatty acid-derived ketones, i.e., a fundamental switch from lipid synthesis and fat storage to mobilization of fat in the form of free fatty acids and fatty acid-derived ketones. This occurs between 12 and 36 hours after cessation of food consumption [36]. Hyperinsulinemia suppresses ketogenesis and therefore prolongs the time to switch in cases of obesity, insulin resistance, and T2DM [37]. Although there is some evidence of impaired ketogenesis during the progression of liver disease to steatohepatitis, therapies that increase hepatic ketogenesis are expected nonetheless to ameliorate NAFLD [38,39].

Our data provided first evidence that periodic fasting leads to a clearance of liver fat: fasting significantly reduced FLI and increased the proportion of patients without NAFLD (FLI < 30 units; Figure 2). The effects of fasting therapy were stronger in males and in individuals with higher baseline FLI, higher GOT, and higher cholesterol levels. Each additional fasting day further decreased the FLI. The binary logistic regression showed that every day of fasting increase by 40% the chance to improve a manifest fatty liver (FLI > 60) and switch to a lower category of risk. This implies that the duration of fasting must be sufficient to influence fatty liver positively. This should be taken into account when periodic fasting is considered as treatment for NAFLD.

As already mentioned, insulin resistance, T2DM, and the development of NAFLD are closely associated conditions. Taylor et al. have shown that remission of T2DM requires a decrease of liver fat [40]. Patients with T2DM tend to have higher FLI scores, but in this study, we could demonstrate that the fasting intervention was equally effective in T2DM patients and in non-diabetic subjects in terms of FLI, although the improvement of other parameters (e.g., HDL, LDL, and AP) was not as complete. These results are in line with a previous study on periodic fasting in T2DM [31].

Liver enzymes, insulin resistance, and cholesterol levels are related in NAFLD [41,42]. Our results supported a correlation between changes in FLI and changes in liver enzymes (GGT, and GOT) and lipid parameters (TG) after fasting intervention, although this was limited to subjects without T2DM.

The intervention was well tolerated and adverse events were rare. Interestingly, the majority of participants did not feel hungry during fasting, as reported also in other studies [32]. Periodic fasting leads to significant weight loss, reduction of BMI and waist circumference. These observations are interpreted as positive effects, considering that at baseline the participants were pre-obese (BMI > 25 kg/m$^2$; overall and in the non-diabetic subgroup) or obese (BMI > 30 kg/m$^2$; in the T2DM subgroup). Both pre-obesity and obesity are regarded as general health risk factors [43].

Our observational study has some limitations. The analyses were carried out as pre- to post intervention changes, without a control group, and were focused on FLI as surrogate parameter for NAFLD, which have been shown to correlate with MRI assessments of fatty liver [12]. MRI assessments are regarded as the gold standard for the diagnostics of NAFLD, but this type of external control was not feasible in this large observational study. There was also no significant difference in fasting duration between diabetic and non-diabetic patients, but we did not match the groups before intervention because of the very different numbers of individuals in each group. Finally, we could not collect data on long-term effects after the fasting intervention, therefore a prediction of sustainability of the fasting effects on hepatic changes is not possible. Lean et al. reported that at 12 months almost half of T2DM patients achieved remission to a no-diabetic state and off antidiabetic drugs after intervention with 3–5 months of formula diet (825–853 kcal/day). It is reasonable to expect that periodic fasting gains similar effects in shorter time with good tolerance [44].

In conclusion, periodic fasting can be regarded as an easily realizable, well-tolerated, non-pharmaceutical intervention, which effectively reduces the FLI. This effect was seen in individuals with and without T2DM. Further studies with a control group and long-term follow-up are needed to better characterize the positive effects of periodic fasting on fatty liver and the adaptations in glucose and lipid metabolism.

**Author Contributions:** S.D., F.G., A.M., and F.W.d.T. designed the study; F.G. coordinated the study and collected the data, S.D., T.N., T.L. and N.S. interpreted data; S.D. and T.N. and F.G. drafted the manuscript; A.M. and F.W.d.T. reviewed and edited the manuscript; all authors approved the final version of the manuscript.

**Funding:** The study was funded by Amplius GmbH, Überlingen, Germany. This company develops a research department for the Buchinger Wilhelmi Clinics Überlingen and Marbella. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Acknowledgments:** The authors thank Ernesta Palombo–Kinne (Jena) for critical revision of the manuscript.

**Conflicts of Interest:** F.W.d.T. is member of the Directory Board of the Buchinger Wilhelmi Clinic where the study was performed. Amplius GmbH is a company that conceives, coordinates and develops fasting research. A.M. is a consultant at Buchinger Wilhelmi Clinic and receives financial compensation for this role from Amplius GmbH. This does not alter the authors' adherence to *Nutrients* policies on sharing data and materials. S.D., T.N., T.L., F.G. and N.S. have no conflicts to declare.

## References

1. Glen, J.; Floros, L.; Day, C.; Pryke, R.; Grp, G.D. Non–alcoholic fatty liver disease (NAFLD): Summary of NICE guidance. *BMJ Brit. Med. J.* **2016**, *354*. [CrossRef] [PubMed]
2. Younossi, Z.M.; Koenig, A.B.; Abdelatif, D.; Fazel, Y.; Henry, L.; Wymer, M. Global Epidemiology of Nonalcoholic Fatty Liver Disease-Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. *Hepatology* **2016**, *64*, 73–84. [CrossRef] [PubMed]
3. Vernon, G.; Baranova, A.; Younossi, Z.M. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment. Pharm. Ther.* **2011**, *34*, 274–285. [CrossRef] [PubMed]
4. Wong, V.W.; Wong, G.L.; Choi, P.C.; Chan, A.W.; Li, M.K.; Chan, H.Y.; Chim, A.M.; Yu, J.; Sung, J.J.; Chan, H.L. Disease progression of non–alcoholic fatty liver disease: A prospective study with paired liver biopsies at 3 years. *Gut* **2010**, *59*, 969–974. [CrossRef] [PubMed]
5. Calzadilla Bertot, L.; Adams, L.A. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int. J. Mol. Sci.* **2016**, *17*, 774. [CrossRef]

6. Ballestri, S.; Zona, S.; Targher, G.; Romagnoli, D.; Baldelli, E.; Nascimbeni, F.; Roverato, A.; Guaraldi, G.; Lonardo, A. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J. Gastroenterol. Hepatol.* **2016**, *31*, 936–944. [CrossRef]
7. Kantartzis, K.; Machann, J.; Schick, F.; Fritsche, A.; Haring, H.U.; Stefan, N. The impact of liver fat vs. visceral fat in determining categories of prediabetes. *Diabetologia* **2010**, *53*, 882–889. [CrossRef]
8. Souto, K.P.; Meinhardt, N.G.; Ramos, M.J.; Ulbrich–Kulkzynski, J.M.; Stein, A.T.; Damin, D.C. Nonalcoholic fatty liver disease in patients with different baseline glucose status undergoing bariatric surgery: Analysis of intraoperative liver biopsies and literature review. *Surg. Obes. Relat. Dis.* **2018**, *14*, 66–73. [CrossRef]
9. Shulman, G.I. Ectopic Fat in Insulin Resistance, Dyslipidemia, and Cardiometabolic Disease REPLY. *New Engl. J. Med.* **2014**, *371*, 2237–2238. [CrossRef]
10. Barrera, F.; George, J. The Role of Diet and Nutritional Intervention for the Management of Patients with NAFLD. *Clin. Liver Dis.* **2014**, *18*, 91. [CrossRef]
11. Abdelmalek, M.F.; Suzuki, A.; Guy, C.; Unalp–Arida, A.; Colvin, R.; Johnson, R.J.; Diehl, A.M.; Clini, N.S. Increased Fructose Consumption Is Associated with Fibrosis Severity in Patients with Nonalcoholic Fatty Liver Disease. *Hepatology* **2010**, *51*, 1961–1971. [CrossRef] [PubMed]
12. Kahl, S.; Strassburger, K.; Nowotny, B.; Livingstone, R.; Kluppelholz, B.; Kessel, K.; Hwang, J.H.; Giani, G.; Hoffmann, B.; Pacini, G.; et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. *PLoS ONE* **2014**, *9*, e94059. [CrossRef] [PubMed]
13. Bedogni, G.; Bellentani, S.; Miglioli, L.; Masutti, F.; Passalacqua, M.; Castiglione, A.; Tiribelli, C. The Fatty Liver Index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* **2006**, *6*, 33. [CrossRef] [PubMed]
14. Lonardo, A.; Nascimbeni, F.; Targher, G.; Bernardi, M.; Bonino, F.; Bugianesi, E.; Casini, A.; Gastaldelli, A.; Marchesini, G.; Marra, F.; et al. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. *Digest. Liver Dis.* **2017**, *49*, 471–483. [CrossRef] [PubMed]
15. Wong, V.W.; Wong, G.L.; Chan, R.S.; Shu, S.S.; Cheung, B.H.; Li, L.S.; Chim, A.M.; Chan, C.K.; Leung, J.K.; Chu, W.C.; et al. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. *J. Hepatol.* **2018**, *69*, 1349–1356. [CrossRef] [PubMed]
16. Benedict, M.; Zhang, X. Non-alcoholic fatty liver disease: An expanded review. *World J. Hepatol.* **2017**, *9*, 715–732. [CrossRef]
17. Promrat, K.; Kleiner, D.E.; Niemeier, H.M.; Jackvony, E.; Kearns, M.; Wands, J.R.; Fava, J.L.; Wing, R.R. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* **2010**, *51*, 121–129. [CrossRef]
18. Vilar–Gomez, E.; Martinez–Perez, Y.; Calzadilla–Bertot, L.; Torres–Gonzalez, A.; Gra–Oramas, B.; Gonzalez–Fabian, L.; Friedman, S.L.; Diago, M.; Romero–Gomez, M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* **2015**, *149*, 367–378. [CrossRef]
19. Rotman, Y.; Sanyal, A.J. Current and upcoming pharmacotherapy for non-alcoholic fatty liver disease. *Gut* **2017**, *66*, 180–190. [CrossRef]
20. Chalasani, N.; Younossi, Z.; Lavine, J.E.; Diehl, A.M.; Brunt, E.M.; Cusi, K.; Charlton, M.; Sanyal, A.J. The diagnosis and management of non–alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* **2012**, *142*, 1592–1609. [CrossRef]
21. Tuomilehto, J.; Lindstrom, J.; Eriksson, J.G.; Valle, T.T.; Hamalainen, H.; Ilanne–Parikka, P.; Keinanen–Kiukaanniemi, S.; Laakso, M.; Louheranta, A.; Rastas, M.; et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* **2001**, *344*, 1343–1350. [CrossRef] [PubMed]
22. Cardona–Morrell, M.; Rychetnik, L.; Morrell, S.L.; Espinel, P.T.; Bauman, A. Reduction of diabetes risk in routine clinical practice: Are physical activity and nutrition interventions feasible and are the outcomes from reference trials replicable? A systematic review and meta-analysis. *BMC Public Health* **2010**, *10*, 653. [CrossRef] [PubMed]
23. Michalsen, A.; Li, C. Fasting therapy for treating and preventing disease—Current state of evidence. *Forsch Komplementmed* **2013**, *20*, 444–453. [CrossRef] [PubMed]

24. Michalsen, A.; Hoffmann, B.; Moebus, S.; Backer, M.; Langhorst, J.; Dobos, G.J. Incorporation of fasting therapy in an integrative medicine ward: Evaluation of outcome, safety, and effects on lifestyle adherence in a large prospective cohort study. *J. Altern. Complement. Med.* **2005**, *11*, 601–607. [CrossRef]
25. Lim, E.L.; Hollingsworth, K.G.; Aribisala, B.S.; Chen, M.J.; Mathers, J.C.; Taylor, R. Reversal of type 2 diabetes: Normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* **2011**, *54*, 2506–2514. [CrossRef]
26. Baumeier, C.; Kaiser, D.; Heeren, J.; Scheja, L.; John, C.; Weise, C.; Eravci, M.; Lagerpusch, M.; Schulze, G.; Joost, H.G.; et al. Caloric restriction and intermittent fasting alter hepatic lipid droplet proteome and diacylglycerol species and prevent diabetes in NZO mice. *Biochim. Biophys. Acta* **2015**, *1851*, 566–576. [CrossRef]
27. Wilhelmi de Toledo, F.; Grundler, F.; Bergouignan, A.; Drinda, S.; Michalsen, A. Safety, health improvement and well–being during a 4 to 21–day fasting period in an observational study including 1422 subjects. *PLoS ONE* **2019**, *14*, e0209353. [CrossRef]
28. Wilhelmi de Toledo, F.; Buchinger, A.; Burggrabe, H.; Holz, G.; Kuhn, C.; Lischka, E.; Lischka, N.; Lutzner, H.; May, W.; Ritzmann–Widderich, M.; et al. Fasting therapy—An expert panel update of the 2002 consensus guidelines. *Forsch Komplementmed* **2013**, *20*, 434–443. [CrossRef]
29. Al-Dayyat, H.M.; Rayyan, Y.M.; Tayyem, R.F. Non-alcoholic fatty liver disease and associated dietary and lifestyle risk factors. *Diabetes Metab. Syndr.* **2018**, *12*, 569–575. [CrossRef]
30. Dunkley, A.J.; Bodicoat, D.H.; Greaves, C.J.; Russell, C.; Yates, T.; Davies, M.J.; Khunti, K. Diabetes prevention in the real world: Effectiveness of pragmatic lifestyle interventions for the prevention of type 2 diabetes and of the impact of adherence to guideline recommendations: A systematic review and meta-analysis. *Diabetes Care* **2014**, *37*, 922–933. [CrossRef]
31. Li, C.; Sadraie, B.; Steckhan, N.; Kessler, C.; Stange, R.; Jeitler, M.; Michalsen, A. Effects of A One-Week Fasting Therapy in Patients with Type-2 Diabetes Mellitus and Metabolic Syndrome—A Randomized Controlled Explorative Study. *Exp. Clin. Endocrinol. Diabetes* **2017**, *125*, 618–624. [CrossRef] [PubMed]
32. Kerndt, P.R.; Naughton, J.L.; Driscoll, C.E.; Loxterkamp, D.A. Fasting: The history, pathophysiology and complications. *West. J. Med.* **1982**, *137*, 379–399. [PubMed]
33. Peng, L.; Wang, J.; Li, F. Weight reduction for non–alcoholic fatty liver disease. *Cochrane Database Syst. Rev.* **2011**. [CrossRef] [PubMed]
34. Browning, J.D.; Baker, J.A.; Rogers, T.; Davis, J.; Satapati, S.; Burgess, S.C. Short–term weight loss and hepatic triglyceride reduction: Evidence of a metabolic advantage with dietary carbohydrate restriction. *Am. J. Clin. Nutr.* **2011**, *93*, 1048–1052. [CrossRef]
35. Dabbous, Z.; Bashir, M.; Elzouki, A.N.; Ahmed, M.S.; Farouk, S.; Hassanien, M.; Malik, R.A.; Abou Samra, A.B.; Elhadd, T.; Group, P.S. Differential effects of gender and patient background diversity on the changes in metabolic and biophysical profiles in people with type-2 diabetes from different ethnicities who fast during Ramadan (H1439): A prospective study from Qatar. *Diabetes Res. Clin. Pract.* **2019**, *152*, 171–176. [CrossRef]
36. Cahill, G.F., Jr. Fuel metabolism in starvation. *Annu Rev. Nutr* **2006**, *26*, 1–22. [CrossRef]
37. Puchalska, P.; Crawford, P.A. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metab.* **2017**, *25*, 262–284. [CrossRef]
38. Mannisto, V.T.; Simonen, M.; Hyysalo, J.; Soininen, P.; Kangas, A.J.; Kaminska, D.; Matte, A.K.; Venesmaa, S.; Kakela, P.; Karja, V.; et al. Ketone body production is differentially altered in steatosis and non-alcoholic steatohepatitis in obese humans. *Liver Int.* **2015**, *35*, 1853–1861. [CrossRef]
39. Degirolamo, C.; Sabba, C.; Moschetta, A. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat. Rev. Drug Discov.* **2016**, *15*, 51–69. [CrossRef]
40. Taylor, R.; Al–Mrabeh, A.; Zhyzhneuskaya, S.; Peters, C.; Barnes, A.C.; Aribisala, B.S.; Hollingsworth, K.G.; Mathers, J.C.; Sattar, N.; Lean, M.E.J. Remission of Human Type 2 Diabetes Requires Decrease in Liver and Pancreas Fat Content but Is Dependent upon Capacity for β Cell Recovery. *Cell Metab.* **2018**, *28*, 547–556. [CrossRef]
41. Cruz, M.A.; Cruz, J.F.; Macena, L.B.; de Santana, D.S.; Oliveira, C.C.; Lima, S.O.; Franca, A.V. Association of the Nonalcoholic Hepatic Steatosis and Its Degrees with the Values of Liver Enzymes and Homeostasis Model Assessment—Insulin Resistance Index. *Gastroenterol. Res.* **2015**, *8*, 260–264. [CrossRef] [PubMed]

42. Van den Berg, E.H.; Amini, M.; Schreuder, T.C.; Dullaart, R.P.; Faber, K.N.; Alizadeh, B.Z.; Blokzijl, H. Prevalence and determinants of non-alcoholic fatty liver disease in lifelines: A large Dutch population cohort. *PLoS ONE* **2017**, *12*, e0171502. [CrossRef] [PubMed]
43. Mameli, C.; Krakauer, N.Y.; Krakauer, J.C.; Bosetti, A.; Ferrari, C.M.; Moiana, N.; Schneider, L.; Borsani, B.; Genoni, T.; Zuccotti, G. The association between a body shape index and cardiovascular risk in overweight and obese children and adolescents. *PLoS ONE* **2018**, *13*, e0190426. [CrossRef] [PubMed]
44. Lean, M.E.; Leslie, W.S.; Barnes, A.C.; Brosnahan, N.; Thom, G.; McCombie, L.; Peters, C.; Zhyzhneuskaya, S.; Al-Mrabeh, A.; Hollingsworth, K.G.; et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): An open-label, cluster-randomised trial. *Lancet* **2018**, *391*, 541–551. [CrossRef]

Journal of the American Heart Association

ORIGINAL RESEARCH

# Blood Pressure Changes in 1610 Subjects With and Without Antihypertensive Medication During Long-Term Fasting

Franziska Grundler, MSc; Robin Mesnage, PhD; Andreas Michalsen, MD, PhD; Françoise Wilhelmi de Toledo, MD

**BACKGROUND:** We investigated daily blood pressure (BP) changes during fasting periods ranging from 4 to 41 (10.0±3.8) days in a cohort of 1610 subjects, including 920 normotensive, 313 hypertensive nonmedicated, and 377 hypertensive medicated individuals.

**METHODS AND RESULTS:** Subjects underwent a multidisciplinary fasting program with a daily intake of ≈250 kcal. Weight and stress scores decreased during fasting, and the well-being index increased, documenting a good tolerability. BP mean values decreased from 126.2±18.6/81.4±11.0 to 119.7±15.9/77.6±9.8 mm Hg (mean change, −6.5/3.8 mm Hg). BP changes were larger for hypertensive nonmedicated subjects (>140/90 mm Hg) and reduced by 16.7/8.8 mm Hg. This reduction reached 24.7/13.1 mm Hg for hypertensive nonmedicated subjects (n=76) with the highest BP (>160/100 mm Hg). In the normotensive group, BP decreased moderately by 3.0/1.9 mm Hg. Interestingly, we documented an increase of 6.3/2.2 mm Hg in a subgroup of 69 female subjects with BP <100/60 mm Hg. In the hypertensive medicated group, although BP decreased from 134.6/86.0 to 127.3/81.3 mm Hg, medication was stopped in 23.6% of the subjects, whereas dosage was reduced in 43.5% and remained unchanged in 19.4%. The decrease in BP was larger in subjects fasting longer. Baseline metabolic parameters, such as body mass index and glucose levels, as well as age, can be used to predict the amplitude of the BP decrease during fasting with a machine learning model.

**CONCLUSIONS:** Long-term fasting tends to decrease BP in subjects with elevated BP values. This effect persisted during the 4 days of stepwise food reintroduction, even when subjects stopped their antihypertensive medication.

**REGISTRATION:** URL: https://www.drks.de/drks_web/; Unique identifier: DRKS00010111.

**Key Words:** Buchinger fasting ■ hypertension ■ weight loss

The management of high blood pressure (BP) is an important public health issue because it is one of the leading risk factors for cardiovascular diseases. Values ≥140/90 mm Hg are defined as hypertension by the International Society of Hypertension guidelines.[1] Genetic, environmental, and lifestyle factors contribute to the development of high BP, including stress, obesity, sedentary lifestyle, excess of alcohol consumption, smoking, and high salt intake.[2] Aging and insulin resistance are also associated with an increase in BP. Uncontrolled hypertension increases the risk of stroke, myocardial infarction, cardiac failure, dementia, renal failure, and blindness.[3]

Lifestyle interventions are recommended as a first step to treat elevated BP. This can include 30 minutes of moderate physical exercise 5 to 7 days per week, moderation of alcohol consumption and salt intake (<6 g/d), cessation of smoking, and stress reduction.[4,5]

Correspondence to: Françoise Wilhelmi de Toledo, MD, Wilhelm-Beck-Straße 27, 88662 Überlingen, Germany. E-mail: francoise.wilhelmi@buchinger-wilhelmi.com

Supplementary Materials for this article are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.018649

For Sources of Funding and Disclosures, see page 12.



*JAHA* is available at: www.ahajournals.org/journal/jaha

## CLINICAL PERSPECTIVE

### What Is New?

- This study investigated the effects of long-term fasting on blood pressure (BP) variations in a large cohort of 1610 subjects.
- We grouped the subjects into normotensive, hypertensive nonmedicated, and hypertensive medicated subjects, documenting beneficial effects, especially on medicated hypertensive subjects who could diminish or stop their medication during the procedure.
- Longer fasting periods modulated the BP more strongly than shorter periods.

### What Are the Clinical Implications?

- Long-term fasting, as a specific nutritional intervention, could be complementary to medication in achieving normal and controlled BP, especially in cases where antihypertensive drugs fail to control BP.
- Hypertensive nonmedicated as well as medicated subjects normalized their BP during long-term fasting; remarkably, two thirds of the medicated subjects could reduce their antihypertensive medication.
- Normotensive subjects stayed in the normal range, whereas female subjects with low baseline BP increased their BP within the normal range during long-term fasting.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **BWC** | Buchinger Wilhelmi Clinic |
| **DBP** | diastolic blood pressure |
| **HTM** | hypertensive medicated |
| **HTNM** | hypertensive nonmedicated |
| **LF** | long-term fasting |
| **NP** | natriuretic peptide |
| **SBP** | systolic blood pressure |
| **WC** | waist circumference |

Individuals are also advised to maintain a normal body mass index (BMI) and waist circumference (WC) (<102 cm in men and 88 cm in women).

When lifestyle modification fails, the use of antihypertensive medication is recommended. Patients can receive combinations of diuretics, adrenergic receptor blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and renin inhibitors.[6] Antihypertensive drug treatments are effective and have a high medical importance. However, adverse drug effects are common.[7] They can reduce quality of life and impair compliance[2] and thus reduce the control of BP.[8–10] This is an unresolved medical problem. Further nonpharmacological strategies are thus needed to complement drug therapies. We aimed to evaluate the impact of fasting regimens in hypertensive medicated and nonmedicated subjects.

Fasting regimens have become increasingly popular in the past decade. Fasting is an important adaptive mechanism developed by animals to cope with cyclical seasonal variations in food availability. The mobilization of energy stored in fat tissues ensured survival of animals and humans when food was not readily available. Fasting has also been shown to be associated with important health benefits, including a decrease in BP.[11,12]

In the medical context, fasting is defined as a voluntary abstinence from food, or a reduction of total food intake, for short periods of 12 to 48 hours (intermittent fasting and time-restricted eating) or longer periods of 48 hours to 21 days or more (long-term fasting [LF] or periodic fasting[11,12]). We investigated a LF program documented previously in a peer-reviewed guideline.[13]

Early studies documented that very long periods of fasting (up to 382 days) were used to treat massive obesity and comorbidities, including high BP.[14] A decrease in BP has been widely documented in obese subjects in other weight reduction programs.[15] LF as water-only fasting,[16] or Buchinger type fasting,[17,18] was shown to diminish BP. A similar reduction in BP was documented for intermittent fasting[19] and calorie restriction.[2] We previously reported health outcomes in 1422 subjects undergoing LF therapy at the Buchinger Wilhelmi Clinic (BWC).[20] Herein, we report a comprehensive analysis of daily BP variation on an augmented sample size, including 1610 subjects who fasted in a specialized in-patient medical center during a mean of 10.0±4.1 days followed by 3.0±0.9 days of food reintroduction. We focused on 313 hypertensive nonmedicated (HTNM), 377 hypertensive medicated (HTM), and 920 normotensive subjects to provide more insights into the effectiveness of fasting as a complementary therapy to mitigate high BP.

## METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

### Participants

In total, 1610 subjects were included in this observational study. Inclusion criteria were a stay of ≥10 nights at the BWC, during which the subjects had to comply with its multidisciplinary fasting program.

All participants gave their informed written consent and declared that during this period they were not participating in any other study. The age range was 18 to 99 years. Predefined contraindications were a BMI <16 kg/m$^2$ and/or cachexia; anorexia nervosa or eating disorder; advanced kidney, liver, or cerebrovascular insufficiency; dementia or other debilitating cognitive disease; and pregnancy or lactation period.[13] Moreover, inclusion criteria were a minimal fasting length of 4 days and the obligation to stay at BWC during the period of food reintroduction. At the beginning of the stay, a blood examination was done. All participants had their baseline BP measured in the morning of the first fasting day.

Fasting periods ranged from 4 to 41 (10.0±3.8) days (Table S1). Subjects were divided according to their baseline BP values into 3 groups: normotensive nonmedicated (BP <140/<90 mm Hg), HTNM (BP ≥140/≥90 mm Hg), and HTM, either normotensive or still hypertensive. This corresponded to most major guidelines to classify an individual as hypertensive according to the last International Society of Hypertension practice guidelines.[1]

## Ethical Approval

The medical council of Baden-Württemberg and the Ethics Committee of the Charité–University Medical Center, Berlin (application No. EA4/054/15), approved the prospective observational study on May 5, 2015. The study protocol was registered on June 3, 2016, in the German Clinical Trials Register (DRKS-ID DRKS00010111). Written informed consent was obtained from all participants who were enrolled between January 1, 2016, and December 31, 2016. This study was conducted in accordance with the principles of the Declaration of Helsinki in the BWC in Überlingen (Germany). The follow-up phase, consisting of a questionnaire administered 2 weeks, 3 months, and 11 months after the participants left the BWC, took place between January 26, 2016, and December 18, 2017. The follow-up questionnaires were administered online using Typeform, Barcelona. The subjects received a link and an individual code in a standardized mail. Subjects had to enter their pseudonymized code and answer the follow-up questions. To motivate the subjects to respond to the follow-up questionnaires, they were given explanations by the nurse at departure. They could win a treatment voucher if they filled in the 3 follow-up questionnaires. Despite this effort to motivate the subjects, the response to the follow-up was limited after 2 weeks (n=448), 3 months (n=256), and 11 months (n=188). Because we could not exclude a response bias, we did not include this data set in the analysis. All the raw data are provided in supplementary material for the sake of clarity (Data S1). Correlations between the persistence of BP changes during the follow-up period and the persistence of the weight loss are provided (Figure S1).

## Fasting Program

The course of the fasting included daily supervision by trained nurses and physicians. One day before the start of the fasting, a 600-kcal vegetarian diet of either rice and vegetables or fruits was served in 3 meals. All subjects underwent a physical examination before initiating the fasting period. On the first fasting day, the intestinal tract was emptied through the intake of a laxative (20–40 g $NaSO_4$ in 500 mL water). During fasting, subjects were advised to drink 2 to 3 L/d of water or herbal teas. In addition, 20 g of honey was provided daily, as well as 250 mL organic fresh fruit juice at noon and 250 mL vegetable soup in the evening. On average, the total calorie intake was 200 to 250 kcal/d. The fasting was accompanied by physical activity, alternating with rest, in an environment that promotes calmness and mindfulness. During fasting every second day, an enema or a mild laxative was applied. The period of fasting was followed by a phase of refeeding, during which food was progressively reintroduced from 800 to 1600 kcal/d. This lasted up to 4 days.

## Measurements

Clinical examinations were conducted according to the BWC standards. Data, including baseline demographic and clinical information, adverse effects, and laboratory results, were abstracted from medical records and captured in a secure, web-based software platform called Research Electronic Data Capture.[21] Plausibility of the recorded data was verified continuously by automated checks (eg, for data range) during data entry.

## BP, Medication, and Clinical Parameters

BP and pulse were measured by trained nurses every morning between 7:00 AM and 9:00 AM just after getting up from the night sleep and having only some steps to take place in the calm waiting space of the nurse ward. The measurement was conducted once on the nondominant arm in sitting position after resting for 5 minutes with an upper arm BP monitor (boso Carat professional; BOSCH+SOHN GmbH u. Co. KG, Jungingen, Germany). During follow-up, subjects self-reported their BP values 2 weeks, 3 months, and 11 months after fasting via online questionnaires. For the self-measurement of BP, the subjects received an instruction to measure their BP as done in the clinic. Subjects without an upper arm BP monitor at home

were advised to go to a pharmacy or their family physician. BP medication was regularly documented and monitored for adaptations of the medication. The drug intake was grouped according to the number of antihypertensive classes. Because fasting is known to reduce BP, medication had to be reduced in most of the cases to avoid hypotensive episodes. A fasting treatment guideline recommends to diminish and if possible to stop diuretics when fasting is initiated.[13] This was carefully monitored.

Body weight was measured by a nurse while subjects were lightly dressed (Seca 704; Seca, Hamburg, Germany) during the fasting program and the follow-up stage. WC was measured before and at the end of fasting using a tape measure placed halfway between the lowest rib and the iliac crest (openmindz GmbH, Heidelberg, Germany). Height was determined with Seca 285 (Seca).

## Well-Being

Subjects self-reported daily their well-being. This happened under nurses' supervision and later by means of an online follow-up questionnaire.

The Well-Being Index (World Health Organization 5 [WHO-5]) is a questionnaire reflecting the positive mental attitude. Five statements had to be answered with responses between 0 (at no time) and 5 (all of the time). By adding up the factors for each response and multiplying by 4, we obtained ranges between 0 and 100, whereby 100 reflected the best possible level of well-being.[22] The Well-Being Index normally refers to the past 14 days, but in our study the time span was variable. In this study, we collected the data before and after fasting, as well as in the follow-up period.

## Lifestyle

Smoking habits were documented before and after the fasting period. Subjective stress level was rated on a scale from 0 (threatening tension) to 10 (total relaxation) before fasting and daily during fasting, as well as in the follow-up questionnaires, which were administered to the subjects 2 weeks, 3 months, and 11 months afterwards.

## Safety

Nurses and physicians monitored the subjects constantly over 24 hours. Adverse events were recorded in an adverse effects report form.

## Clinical Blood Parameters

Baseline blood samples were collected at the beginning of the stay at the BWC by trained medical technical assistants in the morning. Blood glucose, glycated hemoglobin, cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides, as well as sodium, were determined, as described in detail in a previously published report.[20]

## Categorization of Diagnoses

Subjects were categorized into groups of diagnoses that are usually associated with high BP, such as diabetes mellitus, when glycated hemoglobin levels were ≥6.5% or an assured diagnosis was noted in the medical report by the physician. Hyperlipidemia was documented when low-density lipoprotein cholesterol levels were ≥4.14 mmol/L, and hypertriglyceridemia was documented when triglyceride levels were ≥1.7 mmol/L.

Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel III conventions. Of the following 5 criteria, 3 have to be met for metabolic syndrome diagnosis[23]: WC >102 cm for men and >88 cm for women, BP ≥130/85 mm Hg, fasting glucose ≥5.6 mmol/L or known diabetes mellitus, triglycerides ≥1.7 mmol/L, and high-density lipoprotein cholesterol levels <1.0 mmol/L for men and <1.3 mmol/L for women.

## Statistical Analysis

The statistical analysis was performed using R (version 4.0.0) on a MacBook Pro with MacOS Catalina version 10.15.5. Data were formatted with dplyr (version 0.8.5). Differences at baseline (Table 1) were evaluated using an ANOVA test (continuous variables) or a $\chi^2$ goodness-of-fit test (categorical variables), with post hoc comparisons made using Tukey honestly significant difference tests (stats::TukeyHSD, version 4.0.0) or pairwise Fisher exact tests (rcompanion::pairwiseNominalIndependence version 2.3.25). We used a Self-Starting Nls Asymptotic Regression Model (stats::Ssasymp) to fit an exponential decay to the variations in systolic BP (SBP) and diastolic BP (DBP) observed over the course of the fasting intervention using a nonlinear model (stats::nls). Differences in BP between the different time points or the different categories were evaluated using least squares means (emmeans:: emmeans, version 1.4.7) of a linear mixed model, considering the time point as a covariate and the repeated measure (patient grouping) as a random effect. *P* values were adjusted for multiple comparisons using Tukey adjustments. Figures were created using ggplot2 (version 3.3.0).

We used a variety of metabolic parameters (presented in Table 1) to predict the amplitude of the BP changes with the caret package (Classification And Regression Training) in R (version 6.0.86) on a training set constituting 75% of the patients randomly

**Table 1. Summary Statistics Showing the Stratification of the Subjects in Clinically Relevant BP Categories**

| Variable | Overall (n=1610) | Normotensive (n=920) | HTNM (n=313) | HTM (n=377) | *P* Value |
|---|---|---|---|---|---|
| Sex distribution, n (%) | | | | | <0.001*,†,‡ |
| Women | 955 (59) | 630 (68) | 167 (53) | 158 (42) | |
| Men | 655 (41) | 290 (32) | 146 (47) | 219 (58) | |
| Age, y | 55.4±13.3 | 51.3±13.3 | 59.3±11.3 | 62.3±10.7 | <0.001*,†,‡ |
| Fasting length, d | 10.1±4.1 | 9.7±3.8 | 10.6±4.3 | 10.3±4.5 | <0.001†,‡ |
| Body mass index, kg/m$^2$ | 27.9±5.6 | 26.3±4.8 | 29.9±6.4 | 30.3±5.4 | <0.001†,‡ |
| Weight, kg | 81.1±19.2 | 76.0±16.7 | 87.3±21.7 | 88.9±19.0 | <0.001†,‡ |
| WC, cm | 94.1±16.5 | 88.6±14.4 | 99.4±16.2 | 103.2±16.0 | <0.001*,†,‡ |
| DBP, mm Hg | 81.4±11.1 | 75.9±7.3 | 92.5±9.4 | 86.0±11.0 | <0.001*,†,‡ |
| SBP, mm Hg | 126.2±18.6 | 116.6±11.3 | 145.5±15.3 | 134.6±19.3 | <0.001*,†,‡ |
| Heart rate, bpm | 70.7±10.7 | 70.2±10.4 | 72.6±10.7 | 70.2±11.4 | 0.002*,† |
| Glucose, mmol/L | 5.4±1.1 | 5.1±0.7 | 5.6±1.1 | 5.9±1.5 | <0.001*,†,‡ |
| Triglycerides, mmol/L | 1.5±0.8 | 1.4±0.6 | 1.7±0.9 | 1.8±1.0 | <0.001*,†,‡ |
| Cholesterol, mmol/L | 5.5±1.1 | 5.5±1.1 | 5.8±1.2 | 5.4±1.2 | <0.001*,† |
| HDL-C, mmol/L | 1.6±0.5 | 1.6±0.5 | 1.5±0.5 | 1.4±0.4 | <0.001*,‡ |
| LDL-C, mmol/L | 3.5±1.0 | 0.6±1.3 | 0.8±1.5 | 0.6±1.3 | 0.01*,† |
| HbA1c, % | 5.5±0.6 | 5.3±0.4 | 5.5±0.6 | 5.7±0.8 | <0.001*,†,‡ |
| Sodium, mmol/L | 140.1±2.9 | 140.0±2.9 | 140.2±2.2 | 139.9±3.1 | 0.47 |
| Well-Being Index, % | 56.1±16.2 | 56.2±16.1 | 56.8±16.8 | 55.4±15.9 | 0.69 |
| Nicotine abuse, (%) | | | | | 0.20 |
| No | 941 (58) | 532 (58) | 187 (60) | 222 (59) | |
| Yes | 176 (11) | 111 (12) | 26 (8) | 39 (10) | |
| Hyperlipidemia, n (%) | | | | | 0.003† |
| No | 1193 (74) | 711 (77) | 211 (67) | 271 (72) | |
| Yes | 386 (24) | 198 (22) | 96 (31) | 92 (24) | |
| Hypertriglyceridemia, n (%) | | | | | <0.001†,‡ |
| No | 1099 (68) | 708 (77) | 190 (61) | 201 (53) | |
| Yes | 494 (31) | 206 (22) | 120 (38) | 168 (45) | |
| Diabetes mellitus, n (%) | | | | | <0.001†,‡ |
| No | 1503 (93) | 894 (97) | 287 (92) | 322 (85) | |
| Yes | 107 (27) | 26 (3) | 26 (8) | 55 (15) | |
| Metabolic syndrome, n (%) | | | | | <0.001*,†,‡ |
| No | 358 (22) | 128 (14) | 147 (47) | 83 (22) | |
| Yes | 399 (25) | 81 (9) | 155 (50) | 163 (43) | |

The 1610 subjects were divided into different clinically relevant BP categories (normotensive, HTNM, and HTM) based on the BP value determined in the morning of the first fasting day or the intake of antihypertensive medication. The *P* value is indicated for the multigroup comparisons. Values represent the mean±SD unless otherwise indicated. BP indicates blood pressure; bpm, beats per minute; DBP, diastolic BP; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HTM, hypertensive medicated; HTNM, hypertensive nonmedicated; LDL-C, low-density lipoprotein cholesterol; SBP, systolic BP; and WC, waist circumference.

*Adjusted *P*<0.05 for hypertensive vs medicated.

†Adjusted *P*<0.05 for hypertensive vs normal.

‡Adjusted *P*<0.05 for medicated vs normal.

selected from our data set (seed number 123). The parameters "weight" and "waist circumference" had large variance inflation factors (calculated with car::vif[] function, version 3.0.7), and were thus dropped. They were highly collinear with BMI, which was left as an indicator of weight status. Missing values were imputed as column medians with randomForest:: na.roughfix(), version 4.6.14. Variables in the training sets were scaled and centered before a linear model was used to evaluate which are the most important predictors of the amplitude of the BP decrease with caret::train().

## RESULTS

During the observation period, 3929 subjects were admitted at the BWC. Of the 1901 eligible subjects, 121 did not undergo a fasting treatment and 170 were

excluded because of a too-short fasting length (n=7), not conducting the food reintroduction at BWC (n=27), incorrect or missing blood sampling (n=8), occurrence of an adverse effect (n=3), or missing baseline BP measurement in the nonmedicated subjects (n=125) (Figure 1).

We evaluated changes in BP caused by LF in 1610 subjects stratified into normotensive (n=920), HTNM (n=313), and HTM (n=377) categories (Table 1). Subjects with normotensive BP were predominantly women. The proportion of men was higher in HTNM and HTM categories. Normotensive subjects were younger than HTNM and HTM subjects (Table 1). In general, HTNM and HTM subjects had a poorer metabolic health. This is reflected by the increased proportion of individuals with metabolic syndrome in the HTNM and HTM categories. Overall, body weight, BMI, and WC were significantly lower in normotensive subjects than in HTNM and HTM subjects (Table 1). In addition, glucose, glycated hemoglobin, and triglyceride levels were highest in HTM subjects, whereas total cholesterol level was lower. The latter effect could be caused by the fact that 31.8% of the subjects in the HTM group took lipid-lowering drugs, by contrast to 9.3% in the HTNM group and 6.6% in the normotensive subjects. Sodium levels, Well-Being Index, and smoking status did not differ between the 3 groups.

Adverse effects were reported in 19 of 1610 subjects (1.2%). The following effects were documented: cardiac arrhythmia, n=3; eczema, n=3; dizziness, n=2; hypoglycemia, n=2; hypokalemia, n=2; hyponatremia, n=2; and single cases of bleeding gums, spasmodic abdominal pain, visual disorder, gout, hyperventilation, outbreak of infection, pleuropneumonia, syncope, tetany, and vomiting. Two subjects were hospitalized because of myocardial infarction and vomiting. Except for the 2 latter participants, all subjects continued fasting.

## Effects of LF on BP

We first examined the daily changes in BP. The baseline BP levels varied between 116.6±11.3/75.9±7.3 mm Hg for normotensive and 145.5±15.3/92.5±9.4 mm Hg for HTNM subjects. Of the 1610 subjects, 377 took

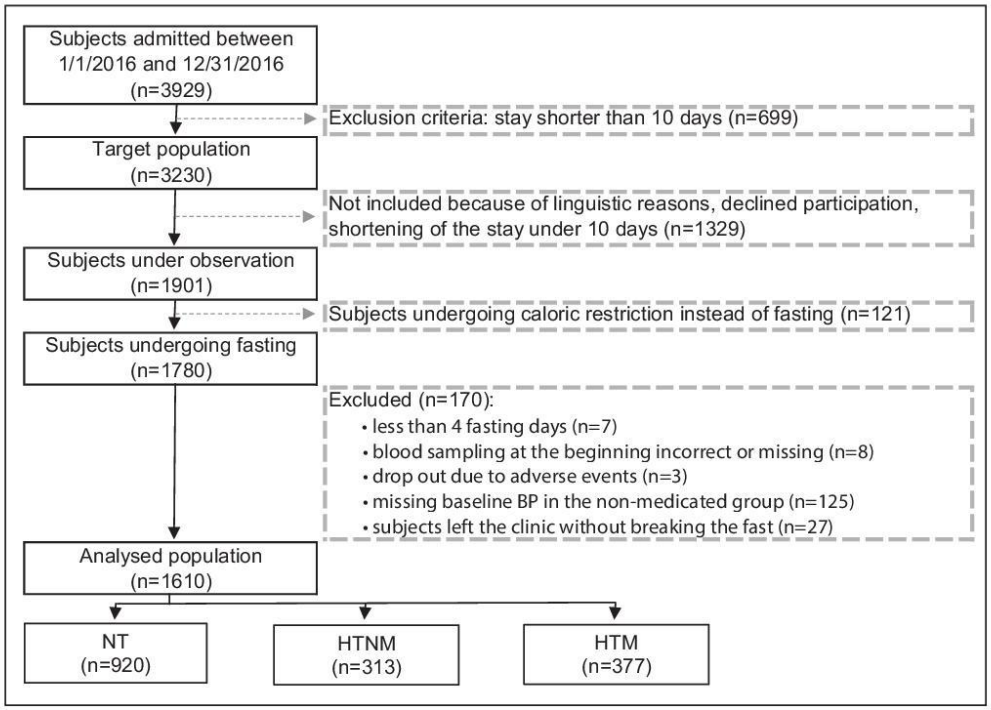



**Figure 1. Flowchart of the study population.**
BP indicates blood pressure; HTM, hypertensive medicated; HTNM, hypertensive nonmedicated; and NT, normotensive.

antihypertensive drugs, with a mean baseline BP of 134.6±19.3/86.0±11.0 mm Hg. Linear-mixed models of the day-to-day changes in BP, adjusted for BMI and sex, showed that a significant decrease in SBP (Table S2) and DBP (Table S3) was documented for the HTNM group from the first day of the fast (Figure 2A and 2C). For normotensive subjects, SBP and DBP significantly differed from baseline from the 8+ day of fast (Figure 2B and 2D). For HTM subjects, BP significantly starting to differ from baseline on the third day for DBP (Figure 2F), and from the fifth day for SBP (Figure 2E). The effect of LF on BP (Tables S4 through S7) was comparable in men (Figure S2) and women (Figure S3). The group of normotensive subjects included 74 individuals (69 women and 5 men) with a BP <100/60 mm Hg. Their BP increased during the course of the intervention (Figure 2A and 2C). The least square mean increase from baseline to the end of the fast was 7.3±1.5/3.1±1.2 mm Hg ($P$<0.001) (Figure 2G and 2H). Considering women only, we documented an increase of 6.3/2.2 mm Hg.

In HTNM and HTM subjects (Figure 2), the decrease in SBP and DBP over the fasting course was exponential and showed an asymptotic floor effect. Horizontal asymptotes from the nonlinear model were reached for the HTNM at 126.0±1.0/82.6±0.4 mm Hg and the HTM at 123.2±2.6/80.1±0.6 mm Hg ($P$<0.001). The reduction in BP was the highest for the HTNM subjects with the most elevated starting BP values (Figure 2G and 2H). Among 76 HTNM subjects with BP levels >160/100 mm Hg, SBP and DBP were reduced by 23.1±2.0/13.0±1.3 mm Hg ($P$<0.001). In contrast, BP was unchanged by fasting in the group of subjects with a healthy BP (>100/60 and <120/80 mm Hg), as evidenced by the mean BP changing from 110.3±5.0/71.8±4.5 to 108.9±10.7/71.4±6.8.

Of the 377 HTM subjects, 89 stopped their BP treatment according to the physicians' prescription.

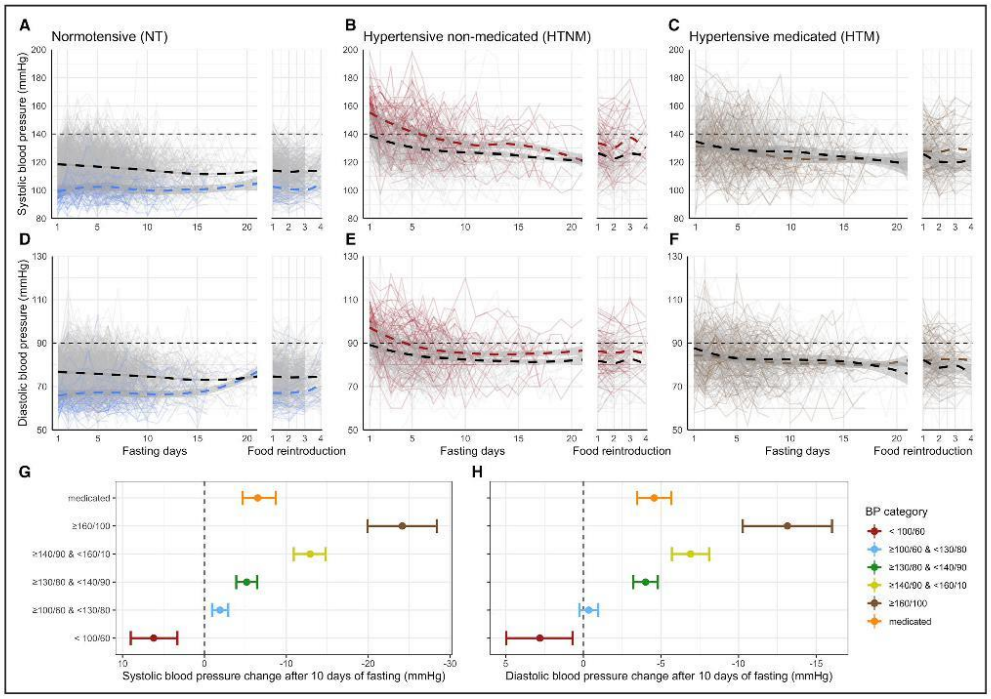



**Figure 2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels are normalized during long-term fasting.** The blood pressure (BP) in normal (**A**, SBP; **D**, DBP), hypertensive (**B**, SBP; **E**, DBP), and medicated subjects (**C**, SBP; **F**, DBP) is presented during the first 20 days of fast and the consecutive refeeding. Individual changes in BP are displayed as gray lines. Smoothed conditional means are plotted as dashed lines to illustrate global trends. A color code is used to highlight subgroups consisting of 74 subjects with low BP (<100/60 mm Hg; in blue in **A** and **D**), 76 subjects with high BP (>160/100 mm Hg; in red in **B** and **E**), as well as 89 subjects who stopped their antihypertensive treatment (in brown in **C** and **F**). We also provided a summary of the changes in BP amplitude for SBP after 10 days of fasting (**G**) and DBP (**H**). Sex-specific changes in BP are presented in supplementary material Figure S2 (men) and Figure S3 (women).

Although the changes in BP from baseline were not statistically significant for these 89 subjects (reduction of 1.2±1.7/2.5±1.3 mm Hg; $P$=0.44), it is noteworthy that the BP was substantially decreased in some individuals who stopped their BP treatment (Figure 2E and 2F).

## Persistence of Metabolic Changes

In the HTNM group, 190 of the 313 subjects shifted at the end of the fasting to the normal BP category (Table 2). As many as 265 of the 377 medicated subjects normalized their BP at the end of fasting, despite reductions in medication.

There were no differences in SBP or DBP levels between the measure taken during the examination on the last day of the fast and the measure at the fourth day of the food reintroduction phase, showing that the reduction of BP caused by fasting persisted during the food reintroduction phase.

LF induced a significant weight reduction in the whole cohort from 81.1±19.2 to 77.0±18.4 kg ($P$<0.001) (Figure 3). In total, 636 subjects (39.5%) reduced their body weight ≥5% during fasting. WC decreased for all from 94.1±16.5 to 88.1±15.1 cm ($P$<0.001) during fasting. The Well-Being Index was significantly enhanced for the whole cohort by 17.2% during the fasting program ($P$<0.001). Moreover, self-reported relaxation level improved during the fasting from 4.9±2.2 to 7.8±2.1 on a visual scale from 0 to 10 points ($P$<0.001).

## Effect of LF on BP Medication

Subjects under drug treatment took on average 1.74 drugs at baseline (Table 3). The following antihypertensive medication was documented: angiotensin II receptor antagonist, n=180; adrenergic receptor blocker use, n=149; diuretic use, n=129; angiotensin-converting enzyme inhibitor use, n=104; calcium channel blocker use, n=90; and renin inhibitor use, n=4. A single medication was prescribed in 44.8%, a combination of 2 drugs in 38.7%, and a combination of 3 or 4 drugs in 16.5% of the subjects.

The antihypertensive medication was adapted according to the BP changes during fasting (Table 4). As many as 89 of the 377 medicated subjects (23.6%) stopped the intake of BP drugs. Their BP values went concomitantly from 129.1/84.3 to 127.8/81.6 mm Hg after an average of 10 days of fasting. Furthermore, 43.5% were able to reduce the medication. In 19.4%, the medication was unchanged and BP values went from 136.8/86.2 to 128.1/81.1 mm Hg. The medication had to be changed to other active substances in 6 subjects; and in 12 subjects, the dosage was increased, because of insufficient dosage of the medication at baseline. It was not possible to evaluate the BP drug change in 8.7% because of missing data.

## Influence of Other Metabolic Parameters on the BP Reduction

It appeared that BP response to fasting was highly variable and individualized. To understand why BP normalized in some subjects while it decreased in some others, we then evaluated whether clinical parameters measured before the fasting intervention or demographic factors could inform on the amplitude of the BP decrease. Subjects were then split in 2 groups, one constituting 75% of the subjects, which was used to train a machine learning algorithm, whereas the remaining 25% of subjects were used to test this algorithm on an independent sample. The correlation between the predicted values and actual values in the test set was statistically significant ($P$<2.2e-16 for SBP and DBP). This was because of the large influence of the baseline BP, which

**Table 2. Number of Individuals in the Different BP Categories After Fasting and Subsequent Food Reintroduction, Reflecting the BP Normalization Divided According to the Fasting Lengths of 5±2, 10±2, and 15±2 and More Days**

| | Hypertensive | | | | | |
|---|---|---|---|---|---|---|
| | Nonmedicated (n=313) | | | Medicated (n=377) | | |
| | 5 | 10 | ≥15 | 5 | 10 | ≥15 |
| Variable | (n=75) | (n=159) | (n=79) | (n=116) | (n=178) | (n=83) |
| BP after fasting, n (%) | | | | | | |
| Normalized | 42 (56) | 98 (62) | 50 (63) | 78 (67) | 126 (71) | 61 (73) |
| Hypertensive | 33 (44) | 61 (38) | 29 (37) | 37 (32) | 52 (29) | 22 (27) |
| BP after food reintroduction, n (%) | | | | | | |
| Normalized | 43 (57) | 93 (58) | 56 (71) | 80 (69) | 120 (67) | 59 (71) |
| Hypertensive | 24 (32) | 49 (31) | 17 (22) | 33 (28) | 51 (29) | 23 (28) |
| Missing | 8 (10.7) | 17 (10.7) | 6 (7.6) | 3 (2.6) | 7 (3.9) | 1 (1.2) |

BP indicates blood pressure.

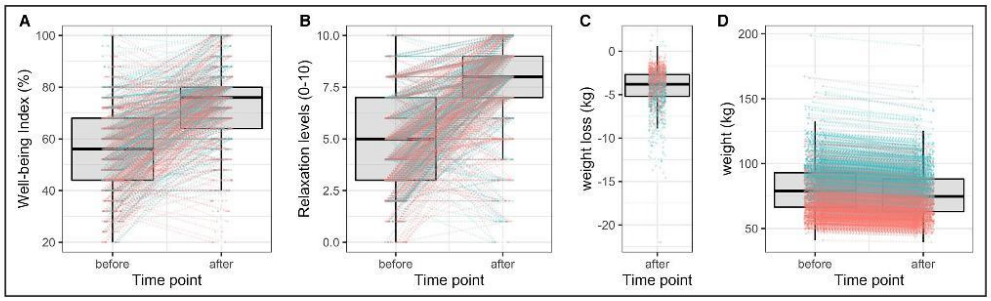



**Figure 3. Changes in blood pressure are concomitant to an increased well-being and a weight loss. Well-being (A), relaxation levels (B), weight loss (C), and weight change (D) were evaluated before and after long-term fasting (men, blue; women, red).**

is strongly linked to the changes in BP observed during LF (Figure 4), as described above. Even when the BP baseline parameters were dropped, the prediction of the changes in SBP caused by fasting was still statistically significant (Pearson coefficient, 0.15; 95% CI, 0.06–0.25; $P$=0.002).

Aside from the roles of the baseline BP and the fasting length, which are extensively described above, the metabolic parameters that were the most important to predict BP changes were age, BMI, and lipid and glucose parameters indicative of metabolic syndrome (Figure 4). The predictive ability of the body weight and the WC was the same as BMI, but these factors were not included in the final model to avoid multicollinearity. As a matter of fact, a linear mixed model using daily weights as predictors showed that the decrease in body weight has an influence on the decrease in BP ($P$<2e-16). Collectively, this analysis shows that individuals with poor metabolic health are more likely to display substantial reductions in BP. However, most of the parameters investigated were collinear because BMI generally increases with age, associated with metabolic impairments: All of these are intimately linked to the BP.

**Table 3. Antihypertensive Drug Intake at Baseline**

| Variable | All | | Men | | Women | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Total BP drug intake | 377 | 100.0 | 219 | 100.0 | 158 | 100.0 |
| Intake of 1 BP drug | 169 | 44.8 | 98 | 44.7 | 71 | 44.9 |
| Intake of 2 BP drugs | 146 | 38.7 | 83 | 37.9 | 63 | 39.9 |
| Intake of 3 BP drugs | 53 | 14.1 | 31 | 14.2 | 22 | 13.9 |
| Intake of 4 BP drugs | 9 | 2.4 | 7 | 3.2 | 2 | 1.3 |
| Mean intake of antihypertensive substances | 1.74 | | 1.78 | | 1.72 | |
| Diuretic use | 129 | 19.7 | 67 | 17.4 | 62 | 22.9 |
| Adrenergic receptor blocker use | 149 | 22.7 | 85 | 22.1 | 64 | 23.6 |
| Calcium channel blocker use | 90 | 13.7 | 56 | 14.5 | 34 | 12.5 |
| ACE inhibitor use | 104 | 15.9 | 70 | 18.2 | 34 | 12.5 |
| Angiotensin II receptor antagonist | 180 | 27.4 | 107 | 27.8 | 73 | 26.9 |
| Renin inhibitor use | 4 | 0.6 | 0 | 0.0 | 4 | 1.5 |

ACE indicates angiotensin-converting enzyme; and BP, blood pressure.

## DISCUSSION

The present study reports BP variations during LF in a large cohort of 1610 subjects, documenting concomitant changes in antihypertensive medication. We found that LF affects BP with a nonlinear time response, leading to a normalization and a floor effect. A common issue occurring when drugs that lower BP are stopped is a rebound effect.[24] In our study, the BP-lowering effect of LF led to the reduction of the intake of antihypertensive drugs in 43.5% of the medicated subjects. In addition, 23.6% of subjects were able to stop the medication. In both cases, reduction and stopping of medication, the decrease in BP was maintained during the whole fasting period as well as during the period of food reintroduction. The food was reintroduced stepwise during 4 days, increasing from 800 to 1600 kcal, thus remaining hypocaloric. Further studies are needed to close the gap of knowledge on the long-term maintenance of the effects observed in this study. Although BP decreased in hypertensive subjects during fasting, in accordance with the results of earlier studies,[17,18,25] we reported that BP increased in a subgroup of female subjects with a BP <100/60 mm Hg. It is not clear if this subgroup had hypotensive symptoms, and therefore we cannot evaluate the clinical relevance of the observed increase in BP. Remarkably, BP was unchanged in subjects with a healthy BP of ≈110/70 mm Hg.

**Table 4. Change in the Intake of Antihypertensive Drug Intake During LF. Subjects were grouped according to the fasting lengths of 5±2, 10±2, and 15±2 and more days.**

| Variable | 5 (n=116) | 10 (n=178) | ≥15 (n=83) | Overall (n=377) |
|---|---|---|---|---|
| Changed | 4 (3.4) | 1 (0.6) | 1 (1.2) | 6 (1.6) |
| Increased | 3 (2.6) | 6 (3.4) | 3 (3.6) | 12 (3.2) |
| Reduced | 51 (44.0) | 73 (41.0) | 40 (48.2) | 164 (43.5) |
| Stopped | 28 (24.1) | 42 (23.6) | 19 (22.9) | 89 (23.6) |
| Unchanged | 20 (17.2) | 36 (20.2) | 17 (20.5) | 73 (19.4) |
| Unknown | 10 (8.6) | 18 (11.2) | 3 (3.6) | 31 (8.7) |

Data are given as number (percentage). LF indicates long-term fasting.

Previous scientific studies were mostly performed on relatively small groups of individuals. A period of 10.5 days of water-only fasting in 174 hypertensive subjects led to an average reduction of 37/13 mm Hg.[16] In another study, 10 to 21 days of fasting, according to the BWC program in 124 subjects with nontreated high BP, led to a mean reduction of 34/17 mm Hg.[18]

An explanation for the observed results could be that the lifestyle recommendations to treat elevated BP[5] were all addressed by the fasting program of the BWC. A significant mean weight loss and a diminution of WC were documented. Natriuresis contributes to the rapid weight loss at the beginning of the fast until sodium and protein sparing mechanisms occur after a few days in humans and animals.[11,26,27] Furthermore, daily physical exercise is part of the multidisciplinary fasting program, as well as low sodium intake and abstinence from alcohol. Stress reduction in the course of fasting was documented on visual scales as well as by an increase in the Well-Being Index. Absence of daily life stress during the trial, and thus the potentially improved sleep, or the increased relaxation, could have contributed to the BP-lowering effect. However, we cannot quantify the role of these factors with the present study design.

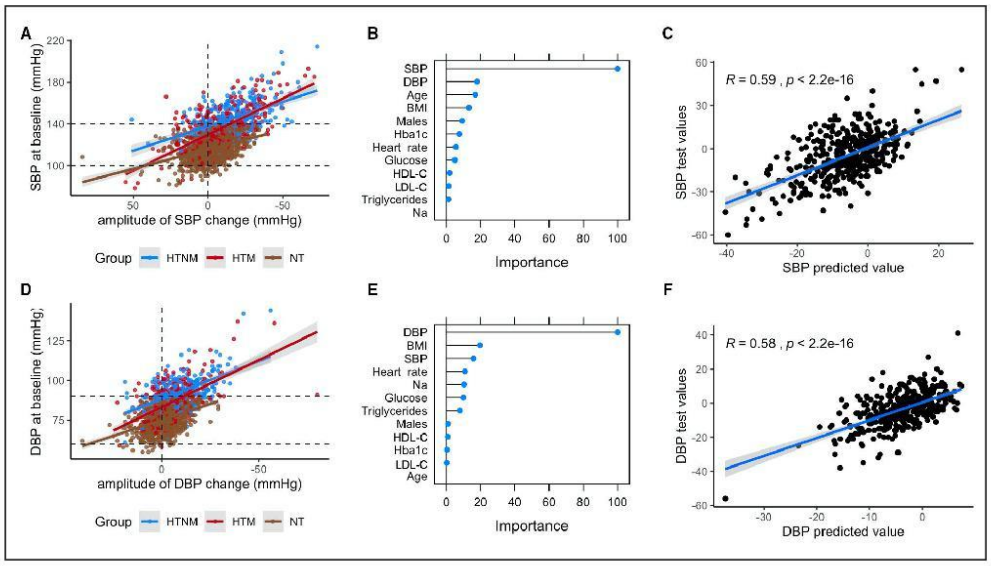



**Figure 4. Baseline parameters can predict the changes in blood pressure (BP) during long-term fasting (LF).**
Baseline BP is strongly associated to the amplitude of systolic BP (SBP) (**A**) and diastolic BP (DBP) (**D**) changes during LF. A machine learning model including baseline metabolic parameters was trained on 75% of the data. This model was used to predict BP changes on a test data set constituted of the remaining 25% of the data. The most important predictors are presented (**B**, SBP; **E**, DBP), along with the reliability of the predictions on the test data set (**C**, SBP; **F**, DBP). BMI indicates body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HTM, hypertensive medicated; HTNM, hypertensive nonmedicated; LDL-C, low-density lipoprotein cholesterol; Na, sodium; and NT, normotensive.

Randomized trials should be performed to further evaluate the BP reducing effect of LF and the role of unspecific factors.

Further explanations could be the similarities between the effects of fasting and antihypertensive drugs on key mechanisms of BP regulation at the level of natriuresis, the autonomous nervous system, the renin-angiotensin-aldosterone system, and the endothelial function. The main BP lowering occurred during the first 5 days, pointing to the physiological changes that happen at the onset of fasting. Sodium is eliminated along with ketone bodies until ammonium replaces sodium after a few days, when gluconeogenesis takes place mainly in the kidneys rather than in the liver.[28] Furthermore, an elevation of cardiac natriuretic peptides (NPs) has been observed.[29,30] NPs enhance sodium and chloride excretion in the kidney, inhibit adipocyte proliferation, activate lipolysis, and increase the availability of free fatty acids for substrate oxidation.[31] In obese hypertensive subjects, the expression of the clearance receptor for NPs is enhanced. Fasting inhibits its expression, thus leading to normalized levels of NPs.[32,33] Natriuresis could partly explain the effects of LF on BP by modulating blood volume,[34] and it is therefore generally recommended to discontinue diuretic medication during fasting, to avoid hyponatremia or hypotensive episodes.[13,35]

Profound hormonal changes during fasting contribute to the BP normalization. Catecholamine levels, reflecting sympathetic nervous system activity, decrease after an initial activation.[36] A 16-day fast (230 kcal/d) in obese subjects documented the largest decrease in norepinephrine and BP on the second fasting day.[37] In addition, an increase in parasympathetic activity was documented in rodents on either alternate-day fasting or 40% calorie restriction, triggered by brain-derived neurotrophic factor via cholinergic neurons and diminished resting heart rate.[38,39] Because a large number of BP drugs act at the level of the sympathetic nervous system, it could be hypothesized that the sustained attenuation of sympathetic nervous system, concomitant to an increase in parasympathetic activity, during fasting could explain the absence of a rebound effect.[40]

Furthermore, insulin resistance is also known to contribute to the pathogenesis of hypertension by inhibiting the physiological vasodilatory effect of insulin.[41,42] Hyperinsulinemia has antinatriuretic effects. It stimulates directly renal tubular reabsorption and decreases circulating levels of NPs.[31] The insulin lowering effect of fasting is well documented.[43] We reported that 10 days of LF led to a significant reduction of the insulin levels in 15 healthy men.[44] In addition, 4 to 21 fasting days in 1422 subjects improved blood glucose and glycated hemoglobin levels.[20]

In our study, the significant weight loss (≈5%) is likely to have a profound impact on BP reduction through lowering the renin-angiotensin-aldosterone system. Thirteen weeks of calorie restriction reduced levels of angiotensinogen (–27%), renin (–43%), aldosterone (–31%), angiotensin-converting enzyme activity (–12%), and angiotensinogen expression in adipose tissue (–20%) in postmenopausal women.[45] This was concomitant to a 5% weight loss and an SBP reduction of 7 mm Hg. In another study, a 5-week dietary intervention with 40% calorie restriction showed similarly reduced plasma renin activity, aldosterone levels, and angiotensin-converting enzyme activity.[46]

Fasting can impact the peripheral vascular system through adiponectin-mediated endothelial nitrogen monoxide production, leading to vasodilatation.[47,48] Intermittent fasting increased the circulating adiponectin levels in rats,[49] as well as alternate-day fasting in mice.[50] In obese adults, an 8-week alternate-day fasting did not change adiponectin levels.[51] Moreover, chronic hyperleptinemia is also related to obesity-associated hypertension. Leptin stimulates the secretion of aldosterone, leading to endothelial dysfunction, and further increases sympathetic nerve activity by binding to leptin receptors in the central nervous system.[52–54] LF reduces circulating leptin levels.[55] Although we did not measure adipokines, the positive effects seen in other fasting studies could also contribute to the observed BP normalization in our cohort.

Because the response to our follow-up questionnaires was low (27.8% after 2 weeks, 15.9% after 3 months, and 11.7% after 11 months), because of difficulties in questioning the participants several months after they left the clinic, we did not include these data in our analysis because it was not possible to control for a possible response bias. The persistence of long-term effects has to be tested in a more accurate follow-up group in future studies.

Altogether, 253 of the 377 medicated subjects reduced or stopped their antihypertensive medication. In 12 subjects, the BP treatment had to be increased because of the fact that the baseline prescription of the antihypertensive drugs was insufficient. Hypertension is often characterized by long-term drug treatment. Long-term intake could lead to a resistance to the medical treatment and to an increase in the needed dosage.[56] Furthermore, dose-related adverse effects (eg, dizziness, fatigue, headaches, renal insufficiency, tachycardia, hyponatremia, hyperkalemia, hyperglycemia, depression, and impotence) are well known under antihypertensive intake.[7,9,10] The acceptance is poor, leading to patients skipping doses or quitting the drug treatment.[57] In contrast, compliance is high when subjects undergo a voluntary fasting therapy.[20] Reasons therefore could be the above-demonstrated increase in well-being, the absence of hunger feelings, and the improvement of individual major health complaints.[20] It might be

comprehensible that subjects under antihypertensive treatment feel relieved when they can stop their drug intake and improve their BP profile during fasting. Furthermore, it can be hypothesized that the fasting-dependent interruption in antihypertensive drug intake, as well as improvements in weight and thus fat mass, insulin and leptin sensitivity, or NP clearance receptors in adipose tissue, could contribute to a resensitization of the BP regulation. This could at least temporarily reduce the prescribed drug dosage, leading to reduced adverse effects.

Some limitations apply to our study. The BP measurements, repeated daily, were conducted only once every day, and details on preexisting antihypertensive treatments were not documented. Despite the large number of participants, it was not possible to match the groups because of the size of the groups of hypertensive and medicated subjects and the observational study design. In addition, the follow-up response was low, introducing selection bias and limiting interpretation. Long-term effects remain to be investigated. This present study nonetheless lays the foundation for further studies focusing on the long-term effects of LF on BP. A key point for further studies would be to understand if BP changes caused by LF can differ between individuals treated with different types of drugs.

## CONCLUSIONS

In conclusion, the LF protocol used in our study is a safe and well-tolerated approach to normalize elevated BP in subjects with and without antihypertensive drug intake. LF affects BP through several mechanisms, such as weight reduction and lifestyle changes. The mechanisms by which LF decreases BP are similar to those of antihypertensive drugs that act on natriuresis, sympathetic nervous system, renin-angiotensin-aldosterone system, and endothelial function. We describe how LF can be a strategy to reduce hypertension, and to transiently reduce or even stop antihypertensive treatments. Fasting appears to be a promising nonpharmacological complementary approach in the treatment of hypertension.

## ARTICLE INFORMATION

Received July 24, 2020; accepted October 16, 2020.

### Affiliations

From the Buchinger Wilhelmi Clinic, Überlingen, Germany (F.G., F.W.d.); Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt–Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany (F.G.); Gene Expression and Therapy Group, Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, Guy's Hospital, London, United Kingdom (R.M.); Institute of Social Medicine, Epidemiology and Health Economics, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt–Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany (A.M.); and Department of Internal and Integrative Medicine, Immanuel Krankenhaus Berlin, Berlin, Germany (A.M.)

### Acknowledgments

We thank the study patients for their participation and the physicians and nursing staff of the Buchinger Wilhelmi Clinic for their cooperation.

Author contributions: Dr Wilhelmi de Toledo, F. Grundler, and Dr Michalsen conceived and conceptualized the study. F. Grundler was project manager and coordinated study conduction and data collection. Dr Mesnage performed the bioinformatics and statistical analysis. F. Grundler, Dr Mesnage, and Dr Wilhelmi de Toledo drafted the manuscript. All authors contributed to data interpretation and the revision and editing of the final manuscript.

### Sources of Funding

The study was financed by Amplius GmbH, Überlingen, Germany. This company has the task to develop a research department for the Buchinger Wilhelmi Clinics Überlingen and Marbella, who are the funders. Amplius GmbH had no role in the design, analysis, or writing of this article. No additional external funding was received for this study.

### Disclosures

Dr Wilhelmi de Toledo is managing director of Amplius GmbH. Dr Mesnage and Dr Michalsen are consultants of Amplius GmbH and receive financial compensation for this role. Dr Wilhelmi de Toledo and F. Grundler are employees of Buchinger Wilhelmi Clinic. The remaining authors have no disclosures to report.

### Supplementary Material

**Tables S1–S7**
**Figures S1–S3**
**Data S1**

## REFERENCES

1. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, Ramirez A, Schlaich M, Stergiou GS, Tomaszewski M, et al. 2020 International Society of Hypertension global hypertension practice guidelines. *Hypertension*. 2020;75:1334–1357.
2. Nicoll R, Henein M. Caloric restriction and its effect on blood pressure, heart rate variability and arterial stiffness and dilatation: a review of the evidence. *Int J Mol Sci*. 2018;19:751.
3. Dorans KS, Mills KT, Liu Y, He J. Trends in prevalence and control of hypertension according to the 2017 American College of Cardiology/American Heart Association (ACC/AHA) Guideline. *J Am Heart Assoc*. 2018;7:e008888. DOI: 10.1161/JAHA.118.008888.
4. Mancia G, Fagard R, Narkiewicz K, Redán J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. *J Hypertens*. 2013;31:1925–1938.
5. Bakris G, Ali W, Parati G. ACC/AHA versus ESC/ESH on hypertension guidelines: JACC guideline comparison. *J Am Coll Cardiol*. 2019;73:3018–3026.
6. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Himmelfarb CD, DePalma SM, Gidding S, Jamerson KA, Jones DW. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2018;138:e484–e594.
7. Laurent S. Antihypertensive drugs. *Pharmacol Res*. 2017;124:116–125.
8. Suthar J, Pathak A, Shelat B. Comparison of safety and efficacy of different class of anti-hypertensive drugs prescribed in patients with hypertension. *Indian J Pharm Pract*. 2019;12:257.
9. Cao Y, Xiang X, Song J, Tian Y, Wang M, Wang X, Li M, Huang Z, Wu Y, Wu T. Distinct effects of antihypertensives on depression in the real-world setting: a retrospective cohort study. *J Affect Disord*. 2019;259:386–391.
10. Tarukallo N, Rasyid H. Anti-hypertensive drugs and sexual dysfunction in men. *J Health Sci*. 2020;13:1–11.
11. Wilhelmi de Toledo F, Grundler F, Sirtori CR, Ruscica M. Unravelling the health effects of fasting: a long road from obesity treatment to healthy life span increase and improved cognition. *Ann Med*. 2020;52:147–161.

12. de Cabo R, Mattson MP. Effects of intermittent fasting on health, aging, and disease. *N Engl J Med*. 2019;381:2541–2551.
13. Wilhelmi de Toledo F, Buchinger A, Burggrabe H, Hölz G, Kuhn C, Lischka E, Lischka N, Lützner H, May W, Ritzmann-Widderich M. Fasting therapy-an expert panel update of the 2002 consensus guidelines. *Forsch Komplementmed*. 2013;20:434–443.
14. Stewart W, Fleming LW. Features of a successful therapeutic fast of 382 days' duration. *Postgrad Med J*. 1973;49:203–209.
15. Bricarello LP, Poltronieri F, Fernandes R, Retondario A, de Moraes Trindade EBS, de Vasconcelos FdAG. Effects of the dietary approach to stop hypertension (DASH) diet on blood pressure, overweight and obesity in adolescents: a systematic review. *Clin Nutr ESPEN*. 2018;28:1–11.
16. Goldhamer A, Lisle D, Parpia B, Anderson SV, Campbell TC. Medically supervised water-only fasting in the treatment of hypertension. *J Manipulative Physiol Ther*. 2001;24:335–339.
17. Li C, Ostermann T, Hardt M, Lüdtke R, Broecker-Preuss M, Dobos G, Michalsen A. Metabolic and psychological response to 7-day fasting in obese patients with and without metabolic syndrome. *Complement Med Res*. 2013;20:413–420.
18. Müller H, Wilhelmi de Toledo F, Schuck P, Resch K. Blutdrucksenkung durch Fasten bei adipösen und nichtadipösen Hypertonikern. *Perfusion*. 2001;14:108–112.
19. Stekovic S, Hofer SJ, Tripolt N, Aon MA, Royer P, Pein L, Stadler JT, Pendl T, Prietl B, Url J. Alternate day fasting improves physiological and molecular markers of aging in healthy, non-obese humans. *Cell Metab*. 2019;30:462–476.e465.
20. Wilhelmi de Toledo F, Grundler F, Bergouignan A, Drinda S, Michalsen A. Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects. *PLoS One*. 2019;14:e0209353.
21. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208.
22. Bech P. Measuring the dimension of psychological general well-being by the WHO-5. *Quality of Life Newsletter*. 2004;32:15–16.
23. Moebus S, Hanisch JU, Neuhäuser M, Aidelsburger P, Wasem J, Jöckel K-H. Assessing the prevalence of the metabolic syndrome according to NCEP ATP III in Germany: feasibility and quality aspects of a two step approach in 1550 randomly selected primary health care practices. *Ger Med Sci*. 2006;4:Doc07.
24. Reidenberg MM. Drug discontinuation effects are part of the pharmacology of a drug. *J Pharmacol Exp Ther*. 2011;339:324–328.
25. Goldhamer AC, Lisle DJ, Sultana P, Anderson SV, Parpia B, Hughes B, Campbell TC. Medically supervised water-only fasting in the treatment of borderline hypertension. *J Altern Complement Med*. 2002;8:643–650.
26. Lignot J-H, LeMaho Y. A history of modern research into fasting, starvation, and inanition. In: McCue MD, ed. *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Berlin, Heidelberg: Springer; 2012:7–23.
27. Viallon M, Leporq B, Drinda S, Wilhelmi de Toledo F, Galusca B, Ratiney H, Croisille P. Chemical-shift-encoded magnetic resonance imaging and spectroscopy to reveal immediate and long-term multi-organs composition changes of a 14-days periodic fasting intervention: a technological and case report. *Front Nutr*. 2019;6:5.
28. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. *West J Med*. 1982;137:379–399.
29. Maoz E, Shamiss A, Peleg E, Salzberg M, Rosenthal T. The role of atrial natriuretic peptide in natriuresis of fasting. *J Hypertens*. 1992;10:1041–1044.
30. Spark RF, Arky RA, Boulter PR, Saudek CD, O'Brian JT. Renin, aldosterone and glucagon in the natriuresis of fasting. *N Engl J Med*. 1975;292:1335–1340.
31. Sarzani R, Spannella F, Giulietti F, Balietti P, Cocci G, Bordicchia M. Cardiac natriuretic peptides, hypertension and cardiovascular risk. *High Blood Press Cardiovasc Prev*. 2017;24:115–126.
32. Cannone V, Cabassi A, Volpi R, Burnett JC. Atrial natriuretic peptide: a molecular target of novel therapeutic approaches to cardio-metabolic disease. *Int J Mol Sci*. 2019;20:3265.
33. Dessì-Fulgheri P, Sarzani R, Serenelli M, Tamburrini P, Spagnolo D, Giantomassi L, Espinosa E, Rappelli A. Low calorie diet enhances renal, hemodynamic, and humoral effects of exogenous atrial natriuretic peptide in obese hypertensives. *Hypertension*. 1999;33:658–662.
34. Davidson MB. Role of ketogenesis in urinary sodium excretion: elucidation by nicotinic acid administration during fasting*. *J Clin Endocrinol Metab*. 1979;49:818–823.
35. Lischka E. Kombinierte Fastentherapie zur Behandlung der primären arteriellen Hypertonie. *Erfahrungsheilkunde*. 2014;63:36–41.
36. Wan R, Camandola S, Mattson MP. Intermittent food deprivation improves cardiovascular and neuroendocrine responses to stress in rats. *J Nutr*. 2003;133:1921–1929.
37. Göhler L, Hahnemann T, Michael N, Oehme P, Steglich H-D, Conradi E, Grune T, Siems W. Reduction of plasma catecholamines in humans during clinically controlled severe underfeeding. *Prev Med*. 2000;30:95–102.
38. Mager DE, Wan R, Brown M, Cheng A, Wareski P, Abernethy DR, Mattson MP. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. *FASEB J*. 2006;20:631–637.
39. Wan R, Weigand LA, Bateman R, Griffioen K, Mendelowitz D, Mattson MP. Evidence that BDNF regulates heart rate by a mechanism involving increased brainstem parasympathetic neuron excitability. *J Neurochem*. 2014;129:573–580.
40. Young JB, Landsberg L. Suppression of sympathetic nervous system during fasting. *Obes Res*. 1997;5:646–649.
41. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Järvinen H; for the European Group for the Study of Insulin Resistance (EGIR). Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. *Hypertension*. 1997;30:1144–1149.
42. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest*. 1994;94:1172–1179.
43. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. *Ageing Res Rev*. 2017;39:46–58.
44. Mesnage R, Grundler F, Schwiertz A, Le Maho Y, Wilhelmi de Toledo F. Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting. *J Nutr Sci*. 2019;8:e36.
45. Engeli S, Böhnke J, Gorzelniak K, Janke J, Schling P, Bader M, Luft FC, Sharma AM. Weight loss and the renin-angiotensin-aldosterone system. *Hypertension*. 2005;45:356–362.
46. Harp JB, Henry SA, DiGirolamo M. Dietary weight loss decreases serum angiotensin-converting enzyme activity in obese adults. *Obes Res*. 2002;10:985–990.
47. Ohashi K, Ouchi N, Matsuzawa Y. Adiponectin and hypertension. *Am J Hypertens*. 2011;24:263–269.
48. Dorresteijn J, Visseren F, Spiering W. Mechanisms linking obesity to hypertension. *Obes Rev*. 2012;13:17–26.
49. Wan R, Ahmet I, Brown M, Cheng A, Kamimura N, Talan M, Mattson MP. Cardioprotective effect of intermittent fasting is associated with an elevation of adiponectin levels in rats. *J Nutr Biochem*. 2010;21:413–417.
50. Varady KA, Allister CA, Roohk DJ, Hellerstein MK. Improvements in body fat distribution and circulating adiponectin by alternate-day fasting versus calorie restriction. *J Nutr Biochem*. 2010;21:188–195.
51. Hoddy KK, Bhutani S, Phillips SA, Varady KA. Effects of different degrees of insulin resistance on endothelial function in obese adults undergoing alternate day fasting. *Nutr Healthy Aging*. 2016;4:63–71.
52. Beltowski J. Role of leptin in blood pressure regulation and arterial hypertension. *J Hypertens*. 2006;24:789–801.
53. da Silva AA, do Carmo JM, Li X, Wang Z, Mouton AJ, Hall JE. Role of hyperinsulinemia and insulin resistance in hypertension: metabolic syndrome revisited. *Can J Cardiol*. 2020;36:671–682.
54. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity, kidney dysfunction and hypertension: mechanistic links. *Nat Rev Nephrol*. 2019;15:367–385.
55. Michalsen A, Kuhlmann MK, Lüdtke R, Bäcker M, Langhorst J, Dobos GJ. Prolonged fasting in patients with chronic pain syndromes leads to late mood-enhancement not related to weight loss and fasting-induced leptin depletion. *Nutr Neurosci*. 2006;9:195–200.
56. Faselis C, Doumas M, Papademetriou V. Common secondary causes of resistant hypertension and rational for treatment. *Int J Hypertens*. 2011;2011:236239.
57. Cohen JS. Adverse drug effects, compliance, and initial doses of antihypertensive drugs recommended by the Joint National Committee vs the Physicians' Desk Reference. *Arch Intern Med*. 2001;161:880–885.

# SUPPLEMENTAL MATERIAL

**Table S1. Number of individuals with available measurements.** The first three columns indicate the number of normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM) subjects at a given day of the study. Numbers are decreasing since fasting days were different for different subjects. We also presented the number of SBP (N_sbp) and DBP (N_dbp) values available as some of the measurements were missing.

| | **N (individuals in study)** | | | **N_sbp (N with SBP available)** | | | **N_dbp (N with DBP available)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | HTNM | HTM | NT | HTNM | HTM | NT | HTNM | HTM | NT |
| Day 1 | 313 | 377 | 920 | 306 | 358 | 920 | 306 | 358 | 920 |
| Day 2 | 313 | 377 | 920 | 292 | 359 | 879 | 292 | 359 | 879 |
| Day 3 | 313 | 377 | 920 | 294 | 361 | 886 | 294 | 361 | 886 |
| Day 4 | 313 | 377 | 920 | 304 | 366 | 874 | 304 | 366 | 872 |
| Day 5 | 313 | 375 | 919 | 298 | 365 | 884 | 298 | 365 | 884 |
| Day 6 | 309 | 371 | 911 | 287 | 359 | 868 | 287 | 359 | 865 |
| Day 7 | 281 | 303 | 760 | 263 | 290 | 723 | 263 | 290 | 723 |
| Day 8 | 238 | 261 | 609 | 222 | 253 | 576 | 222 | 253 | 575 |
| Day 9 | 194 | 229 | 502 | 180 | 217 | 477 | 180 | 217 | 477 |
| Day 10 | 154 | 169 | 386 | 142 | 161 | 369 | 142 | 162 | 369 |
| Day 11 | 109 | 124 | 261 | 98 | 121 | 251 | 98 | 121 | 253 |
| Day 12 | 91 | 99 | 190 | 84 | 96 | 176 | 85 | 96 | 176 |
| Day 13 | 79 | 83 | 161 | 76 | 79 | 144 | 76 | 79 | 144 |
| Day 14 | 71 | 77 | 141 | 68 | 74 | 131 | 68 | 74 | 131 |
| Day 15 | 60 | 73 | 125 | 55 | 71 | 120 | 55 | 70 | 120 |
| Day 16 | 47 | 61 | 92 | 46 | 60 | 86 | 46 | 60 | 86 |
| Day 17 | 27 | 33 | 46 | 27 | 33 | 45 | 27 | 33 | 45 |
| Day 18 | 18 | 25 | 31 | 18 | 24 | 31 | 18 | 24 | 31 |
| Day 19 | 14 | 20 | 23 | 13 | 20 | 23 | 13 | 20 | 24 |
| Day 20 | 12 | 19 | 18 | 12 | 18 | 17 | 12 | 18 | 17 |
| REF1 | 312 | 377 | 917 | 269 | 350 | 809 | 269 | 349 | 809 |
| REF2 | 290 | 360 | 872 | 234 | 305 | 713 | 234 | 305 | 714 |
| REF3 | 210 | 287 | 654 | 152 | 206 | 456 | 152 | 206 | 456 |
| REF4 | 102 | 120 | 268 | 63 | 74 | 162 | 63 | 74 | 162 |
| FUP 1 | 313 | 377 | 920 | 88 | 123 | 237 | 87 | 125 | 237 |
| FUP 2 | 313 | 377 | 920 | 49 | 71 | 136 | 49 | 71 | 135 |
| FUP 3 | 313 | 377 | 920 | 29 | 55 | 105 | 28 | 55 | 105 |

SBP indicates systolic blood pressure; N_sbp, number of available SBP values; DBP, diastolic blood pressure; N_dbp, number of available DBP values; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S2. Statistical significance of the day-to-day difference to baselines for SBP.**
Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for sex and BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| SBP | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| **contrast** | **estimate** | **SE** | **p-value** | **estimate** | **SE** | **p-value** | **estimate** | **SE** | **p-value** |
| Day 1 - Day 2 | 7.5 | 0.8 | 3.6E-13 | 0.9 | 0.7 | 1.0E+00 | -0.9 | 0.5 | 1E+00 |
| Day 1 - Day 3 | 9.4 | 0.8 | 0.0E+00 | 1.7 | 0.7 | 8.8E-01 | 0.7 | 0.5 | 1E+00 |
| Day 1 - Day 4 | 10.3 | 0.8 | 0.0E+00 | 2.9 | 0.7 | 2.7E-02 | 0.6 | 0.5 | 1E+00 |
| Day 1 - Day 5 | 11.9 | 0.8 | 0.0E+00 | 4.0 | 0.7 | 2.2E-05 | 0.9 | 0.5 | 1E+00 |
| Day 1 - Day 6 | 12.8 | 0.8 | 0.0E+00 | 5.0 | 0.7 | 8.9E-09 | 1.5 | 0.5 | 3E-01 |
| Day 1 - Day 7 | 13.1 | 0.8 | 0.0E+00 | 6.1 | 0.8 | 5.6E-12 | 1.8 | 0.5 | 8E-02 |
| Day 1 - Day 8 | 15.6 | 0.9 | 0.0E+00 | 7.1 | 0.8 | 2.6E-13 | 2.3 | 0.5 | 7E-03 |
| Day 1 - Day 9 | 15.9 | 1.0 | 0.0E+00 | 7.7 | 0.9 | 2.1E-13 | 2.1 | 0.6 | 4E-02 |
| Day 1 - Day 10 | 15.8 | 1.0 | 0.0E+00 | 8.5 | 1.0 | 2.2E-13 | 3.5 | 0.6 | 5E-06 |
| Day 1 - Day 11 | 16.9 | 1.2 | 0.0E+00 | 9.0 | 1.1 | 3.8E-13 | 4.1 | 0.7 | 6E-06 |
| Day 1 - Day 12 | 17.1 | 1.3 | 0.0E+00 | 8.0 | 1.2 | 4.7E-09 | 3.6 | 0.8 | 6E-03 |
| Day 1 - Day 13 | 18.2 | 1.3 | 0.0E+00 | 9.4 | 1.3 | 7.8E-11 | 5.0 | 0.9 | 7E-06 |
| Day 1 - Day 14 | 17.4 | 1.4 | 0.0E+00 | 9.7 | 1.3 | 1.1E-10 | 5.8 | 0.9 | 2E-07 |
| Day 1 - Day 15 | 19.2 | 1.5 | 0.0E+00 | 10.6 | 1.3 | 1.3E-12 | 6.3 | 1.0 | 4E-08 |
| Day 1 - Day 16 | 18.2 | 1.6 | 5.0E-14 | 11.3 | 1.4 | 2.2E-12 | 6.2 | 1.1 | 1E-05 |
| Day 1 - Day 17 | 20.6 | 2.1 | 2.2E-13 | 11.7 | 1.9 | 6.5E-07 | 4.6 | 1.5 | 3E-01 |
| Day 1 - Day 18 | 20.0 | 2.5 | 5.0E-13 | 10.9 | 2.3 | 9.6E-04 | 8.0 | 1.8 | 3E-03 |
| Day 1 - Day 19 | 22.3 | 2.9 | 5.1E-12 | 14.1 | 2.6 | 2.2E-05 | 6.0 | 2.0 | 4E-01 |
| Day 1 - Day 20 | 23.4 | 3.0 | 3.9E-12 | 10.9 | 2.6 | 8.6E-03 | 6.4 | 2.4 | 6E-01 |
| Day 1 - Day 21 | 19.4 | 3.3 | 2.0E-06 | 13.8 | 2.9 | 6.4E-04 | 3.6 | 2.5 | 1E+00 |
| Day 1 - REF1 | 16.7 | 0.8 | 0.0E+00 | 6.9 | 0.8 | 3.1E-13 | 3.2 | 0.5 | 4E-09 |
| Day 1 - REF2 | 20.2 | 0.9 | 0.0E+00 | 9.3 | 0.8 | 0.0E+00 | 4.2 | 0.5 | 3E-13 |
| Day 1 - REF3 | 16.6 | 1.0 | 0.0E+00 | 7.8 | 0.9 | 2.2E-13 | 3.6 | 0.6 | 1E-07 |
| Day 1 - REF4 | 18.2 | 1.4 | 0.0E+00 | 7.2 | 1.3 | 1.7E-05 | 3.2 | 0.9 | 5E-02 |
| Day 1 - FUP 1 | 16.1 | 1.2 | 0.0E+00 | 6.4 | 1.1 | 9.7E-07 | -0.7 | 0.7 | 1E+00 |
| Day 1 - FUP 2 | 17.6 | 1.6 | 1.1E-13 | 6.5 | 1.4 | 6.2E-04 | -1.7 | 0.9 | 1E+00 |
| Day 1 - FUP 3 | 15.2 | 2.1 | 6.6E-11 | 3.2 | 1.5 | 9.5E-01 | -1.5 | 1.1 | 1E+00 |

SBP indicates systolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S3. Statistical significance of the day-to-day difference to baselines for DBP.**

Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for sex and BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| DBP | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| contrast | estimate | SE | p-value | estimate | SE | p-value | estimate | SE | p-value |
| Day 1 - Day 2 | 3.9 | 0.5 | 2E-11 | 1.3 | 0.5 | 5.0E-01 | -0.1 | 0.3 | 1.0E+00 |
| Day 1 - Day 3 | 5.4 | 0.5 | 3E-13 | 1.8 | 0.5 | 2.9E-02 | 0.7 | 0.3 | 7.9E-01 |
| Day 1 - Day 4 | 6.4 | 0.5 | 0 | 3.0 | 0.5 | 2.3E-08 | 0.8 | 0.3 | 6.7E-01 |
| Day 1 - Day 5 | 7.2 | 0.5 | 0 | 3.2 | 0.5 | 2.0E-09 | 0.7 | 0.3 | 8.9E-01 |
| Day 1 - Day 6 | 7.5 | 0.5 | 0 | 3.9 | 0.5 | 3.3E-13 | 0.7 | 0.3 | 9.1E-01 |
| Day 1 - Day 7 | 8.2 | 0.5 | 0 | 4.1 | 0.5 | 4.7E-13 | 0.9 | 0.3 | 4.1E-01 |
| Day 1 - Day 8 | 8.3 | 0.6 | 0 | 4.6 | 0.5 | 2.1E-13 | 1.4 | 0.3 | 5.7E-03 |
| Day 1 - Day 9 | 8.8 | 0.6 | 0 | 4.6 | 0.5 | 2.8E-13 | 1.3 | 0.4 | 4.6E-02 |
| Day 1 - Day 10 | 9.2 | 0.7 | 0 | 5.3 | 0.6 | 2.5E-13 | 1.8 | 0.4 | 7.8E-04 |
| Day 1 - Day 11 | 9.6 | 0.8 | 0 | 6.2 | 0.7 | 3.8E-13 | 2.8 | 0.5 | 2.8E-07 |
| Day 1 - Day 12 | 9.6 | 0.8 | 0 | 6.4 | 0.7 | 2.6E-13 | 2.0 | 0.5 | 2.7E-02 |
| Day 1 - Day 13 | 10.2 | 0.8 | 0 | 5.6 | 0.8 | 7.6E-10 | 2.4 | 0.6 | 7.6E-03 |
| Day 1 - Day 14 | 10.3 | 0.9 | 0 | 5.8 | 0.8 | 1.8E-09 | 2.8 | 0.6 | 1.1E-03 |
| Day 1 - Day 15 | 10.1 | 0.9 | 3E-13 | 6.6 | 0.8 | 3.6E-12 | 4.3 | 0.6 | 1.3E-09 |
| Day 1 - Day 16 | 9.7 | 1.0 | 2E-13 | 6.5 | 0.9 | 2.7E-10 | 4.4 | 0.7 | 2.0E-07 |
| Day 1 - Day 17 | 12.0 | 1.3 | 3E-13 | 6.7 | 1.2 | 1.7E-05 | 2.8 | 0.9 | 4.0E-01 |
| Day 1 - Day 18 | 13.1 | 1.5 | 3E-13 | 6.0 | 1.5 | 1.1E-02 | 3.6 | 1.1 | 2.4E-01 |
| Day 1 - Day 19 | 12.2 | 1.8 | 1E-08 | 10.3 | 1.6 | 1.1E-07 | 2.6 | 1.3 | 9.6E-01 |
| Day 1 - Day 20 | 10.2 | 1.9 | 3E-05 | 4.8 | 1.6 | 3.9E-01 | 3.6 | 1.5 | 8.4E-01 |
| Day 1 - Day 21 | 12.3 | 2.1 | 2E-06 | 8.4 | 1.8 | 1.2E-03 | 2.4 | 1.6 | 1.0E+00 |
| Day 1 - REF1 | 9.2 | 0.5 | 0 | 4.6 | 0.5 | 2.8E-13 | 1.7 | 0.3 | 5.3E-06 |
| Day 1 - REF2 | 10.0 | 0.5 | 0 | 5.1 | 0.5 | 2.8E-13 | 2.2 | 0.3 | 3.1E-10 |
| Day 1 - REF3 | 8.8 | 0.6 | 0 | 5.0 | 0.6 | 1.9E-13 | 2.0 | 0.4 | 6.8E-06 |
| Day 1 - REF4 | 10.1 | 0.9 | 0 | 5.9 | 0.8 | 3.5E-10 | 1.4 | 0.5 | 7.1E-01 |
| Day 1 - FUP 1 | 10.3 | 0.8 | 0 | 5.2 | 0.7 | 4.3E-12 | 2.3 | 0.5 | 2.0E-04 |
| Day 1 - FUP 2 | 10.1 | 1.0 | 3E-13 | 6.9 | 0.9 | 8.3E-13 | 1.6 | 0.6 | 6.0E-01 |
| Day 1 - FUP 3 | 9.4 | 1.3 | 2E-10 | 4.0 | 0.9 | 6.8E-03 | 1.2 | 0.7 | 9.9E-01 |

DBP indicates diastolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S4. Statistical significance of the day-to-day difference to baselines for SBP in males.** Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| SBP MALES | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| contrast | estimate | SE | p-value | estimate | SE | p-value | estimate | SE | p-value |
| Day 1 - Day 2 | 6.3 | 1.2 | 1E-04 | 0.9 | 1.0 | 1E+00 | -2.0 | 0.8 | 8E-01 |
| Day 1 - Day 3 | 8.7 | 1.2 | 4E-10 | 1.0 | 1.0 | 1E+00 | 0.4 | 0.8 | 1E+00 |
| Day 1 - Day 4 | 9.7 | 1.2 | 2E-12 | 3.1 | 1.0 | 2E-01 | -0.3 | 0.8 | 1E+00 |
| Day 1 - Day 5 | 11.0 | 1.2 | 6E-12 | 3.5 | 1.0 | 9E-02 | 0.3 | 0.8 | 1E+00 |
| Day 1 - Day 6 | 11.4 | 1.2 | 4E-12 | 5.2 | 1.0 | 6E-05 | 1.0 | 0.8 | 1E+00 |
| Day 1 - Day 7 | 12.2 | 1.3 | 5E-13 | 7.2 | 1.0 | 2E-09 | 1.3 | 0.9 | 1E+00 |
| Day 1 - Day 8 | 16.2 | 1.3 | 7E-12 | 7.5 | 1.1 | 3E-09 | 0.9 | 1.0 | 1E+00 |
| Day 1 - Day 9 | 15.9 | 1.4 | 5E-12 | 8.0 | 1.2 | 2E-09 | 2.3 | 1.0 | 9E-01 |
| Day 1 - Day 10 | 15.0 | 1.6 | 3E-12 | 11.4 | 1.3 | 4E-12 | 3.8 | 1.1 | 1E-01 |
| Day 1 - Day 11 | 17.9 | 1.8 | 1E-11 | 11.1 | 1.4 | 5E-12 | 4.9 | 1.4 | 8E-02 |
| Day 1 - Day 12 | 15.4 | 1.9 | 5E-12 | 10.6 | 1.6 | 8E-09 | 4.5 | 1.6 | 4E-01 |
| Day 1 - Day 13 | 18.5 | 2.0 | 3E-13 | 10.8 | 1.7 | 8E-08 | 5.4 | 1.7 | 3E-01 |
| Day 1 - Day 14 | 17.3 | 2.0 | 4E-12 | 11.2 | 1.7 | 4E-08 | 7.9 | 1.9 | 1E-02 |
| Day 1 - Day 15 | 19.2 | 2.3 | 2E-12 | 12.4 | 1.8 | 1E-09 | 7.4 | 1.8 | 2E-02 |
| Day 1 - Day 16 | 18.4 | 2.5 | 3E-11 | 12.6 | 1.9 | 2E-08 | 7.0 | 2.2 | 2E-01 |
| Day 1 - Day 17 | 22.4 | 3.0 | 5E-11 | 13.6 | 2.5 | 3E-05 | 7.0 | 3.0 | 8E-01 |
| Day 1 - Day 18 | 24.1 | 3.4 | 1E-09 | 17.3 | 3.1 | 8E-06 | 11.1 | 3.6 | 3E-01 |
| Day 1 - Day 19 | 23.8 | 4.2 | 5E-06 | 15.6 | 3.4 | 2E-03 | 11.8 | 5.8 | 1E+00 |
| Day 1 - Day 20 | 24.7 | 4.6 | 2E-05 | 12.0 | 3.4 | 9E-02 | 7.4 | 5.8 | 1E+00 |
| Day 1 - Day 21 | 25.7 | 5.1 | 1E-04 | 17.9 | 4.1 | 5E-03 | 15.0 | 7.1 | 9E-01 |
| Day 1 - REF1 | 18.4 | 1.3 | 4E-14 | 8.4 | 1.0 | 0E+00 | 2.2 | 0.9 | 7E-01 |
| Day 1 - REF2 | 20.8 | 1.3 | 5E-12 | 10.5 | 1.1 | 4E-12 | 4.6 | 0.9 | 2E-04 |
| Day 1 - REF3 | 17.1 | 1.5 | 2E-12 | 9.1 | 1.2 | 3E-11 | 3.7 | 1.1 | 1E-01 |
| Day 1 - REF4 | 21.4 | 2.2 | 4E-12 | 7.0 | 1.7 | 1E-02 | 5.6 | 1.8 | 3E-01 |
| Day 1 - FUP 1 | 13.1 | 1.9 | 2E-09 | 7.6 | 1.5 | 9E-05 | -2.8 | 1.3 | 9E-01 |
| Day 1 - FUP 2 | 14.4 | 2.4 | 4E-07 | 6.2 | 2.0 | 2E-01 | -1.6 | 1.9 | 1E+00 |
| Day 1 - FUP 3 | 8.5 | 3.3 | 7E-01 | 5.4 | 2.4 | 9E-01 | -3.0 | 1.8 | 1E+00 |

SBP indicates systolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S5. Statistical significance of the day-to-day difference to baselines for DBP in males.** Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| DBP MALES | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| contrast | estimate | SE | p-value | estimate | SE | p-value | estimate | SE | p-value |
| Day 1 - Day 2 | 3.8 | 0.8 | 2E-04 | 1.7 | 0.6 | 6E-01 | -0.8 | 0.5 | 1E+00 |
| Day 1 - Day 3 | 5.2 | 0.8 | 2E-09 | 2.0 | 0.6 | 2E-01 | 0.5 | 0.5 | 1E+00 |
| Day 1 - Day 4 | 7.1 | 0.7 | 0E+00 | 4.1 | 0.6 | 9E-09 | 0.9 | 0.5 | 1E+00 |
| Day 1 - Day 5 | 7.3 | 0.8 | 7E-13 | 3.9 | 0.6 | 8E-08 | 0.2 | 0.5 | 1E+00 |
| Day 1 - Day 6 | 7.8 | 0.8 | 3E-12 | 5.0 | 0.6 | 4E-12 | 1.2 | 0.5 | 9E-01 |
| Day 1 - Day 7 | 7.5 | 0.8 | 2E-12 | 4.9 | 0.7 | 4E-11 | 1.3 | 0.6 | 9E-01 |
| Day 1 - Day 8 | 8.5 | 0.8 | 1E-12 | 4.8 | 0.7 | 6E-10 | 1.6 | 0.6 | 6E-01 |
| Day 1 - Day 9 | 8.9 | 0.9 | 4E-12 | 5.1 | 0.7 | 5E-10 | 2.1 | 0.6 | 2E-01 |
| Day 1 - Day 10 | 9.9 | 1.0 | 4E-12 | 7.1 | 0.8 | 4E-12 | 1.5 | 0.7 | 9E-01 |
| Day 1 - Day 11 | 9.9 | 1.1 | 2E-12 | 7.6 | 0.9 | 6E-12 | 3.8 | 0.9 | 2E-03 |
| Day 1 - Day 12 | 9.3 | 1.2 | 4E-13 | 7.2 | 1.0 | 9E-11 | 2.9 | 1.0 | 4E-01 |
| Day 1 - Day 13 | 10.0 | 1.2 | 1E-13 | 5.8 | 1.1 | 2E-05 | 2.4 | 1.1 | 9E-01 |
| Day 1 - Day 14 | 11.0 | 1.3 | 2E-12 | 5.8 | 1.1 | 3E-05 | 5.1 | 1.2 | 5E-03 |
| Day 1 - Day 15 | 10.2 | 1.4 | 2E-10 | 7.2 | 1.1 | 3E-08 | 6.1 | 1.2 | 5E-05 |
| Day 1 - Day 16 | 11.5 | 1.5 | 3E-11 | 6.6 | 1.2 | 1E-05 | 5.1 | 1.4 | 4E-02 |
| Day 1 - Day 17 | 13.7 | 1.9 | 2E-10 | 7.2 | 1.6 | 2E-03 | 3.8 | 1.8 | 1E+00 |
| Day 1 - Day 18 | 15.0 | 2.2 | 1E-09 | 8.8 | 1.9 | 2E-03 | 3.8 | 2.2 | 1E+00 |
| Day 1 - Day 19 | 15.6 | 2.6 | 9E-07 | 13.3 | 2.1 | 2E-07 | 5.7 | 3.6 | 1E+00 |
| Day 1 - Day 20 | 11.9 | 2.8 | 8E-03 | 3.9 | 2.1 | 1E+00 | 6.0 | 3.6 | 1E+00 |
| Day 1 - Day 21 | 14.5 | 3.2 | 1E-03 | 6.7 | 2.6 | 7E-01 | 11.6 | 4.4 | 7E-01 |
| Day 1 - REF1 | 10.0 | 0.8 | 3E-12 | 6.3 | 0.6 | 5E-12 | 1.9 | 0.5 | 9E-02 |
| Day 1 - REF2 | 10.9 | 0.8 | 2E-12 | 5.9 | 0.7 | 2E-13 | 3.0 | 0.6 | 8E-05 |
| Day 1 - REF3 | 9.7 | 1.0 | 4E-12 | 6.1 | 0.8 | 2E-12 | 2.6 | 0.7 | 3E-02 |
| Day 1 - REF4 | 12.4 | 1.4 | 4E-12 | 5.9 | 1.0 | 6E-06 | 1.5 | 1.1 | 1E+00 |
| Day 1 - FUP 1 | 8.7 | 1.2 | 7E-11 | 6.5 | 0.9 | 7E-10 | -1.1 | 0.8 | 1E+00 |
| Day 1 - FUP 2 | 7.4 | 1.5 | 2E-04 | 7.7 | 1.2 | 1E-07 | -1.8 | 1.2 | 1E+00 |
| Day 1 - FUP 3 | 6.1 | 2.1 | 4E-01 | 5.3 | 1.5 | 8E-02 | -2.9 | 1.1 | 6E-01 |

DBP indicates diastolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S6. Statistical significance of the day-to-day difference to baselines for SBP in females.** Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| SBP FEMALES | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| contrast | estimate | SE | p-value | estimate | SE | p-value | estimate | SE | p-value |
| Day 1 - Day 2 | 8.5 | 1.1 | 0E+00 | 0.8 | 1.1 | 1E+00 | -0.4 | 0.6 | 1E+00 |
| Day 1 - Day 3 | 10.1 | 1.1 | 0E+00 | 2.7 | 1.1 | 8E-01 | 0.9 | 0.6 | 1E+00 |
| Day 1 - Day 4 | 10.9 | 1.1 | 0E+00 | 2.5 | 1.1 | 9E-01 | 1.0 | 0.6 | 1E+00 |
| Day 1 - Day 5 | 12.7 | 1.1 | 0E+00 | 4.7 | 1.1 | 1E-02 | 1.2 | 0.6 | 9E-01 |
| Day 1 - Day 6 | 14.0 | 1.1 | 0E+00 | 4.7 | 1.1 | 1E-02 | 1.7 | 0.6 | 4E-01 |
| Day 1 - Day 7 | 13.9 | 1.1 | 0E+00 | 4.5 | 1.2 | 5E-02 | 2.0 | 0.6 | 1E-01 |
| Day 1 - Day 8 | 15.1 | 1.2 | 0E+00 | 6.5 | 1.3 | 1E-04 | 2.9 | 0.6 | 2E-03 |
| Day 1 - Day 9 | 16.0 | 1.3 | 0E+00 | 7.3 | 1.3 | 1E-05 | 2.0 | 0.7 | 3E-01 |
| Day 1 - Day 10 | 16.5 | 1.4 | 0E+00 | 4.8 | 1.5 | 2E-01 | 3.4 | 0.7 | 1E-03 |
| Day 1 - Day 11 | 16.0 | 1.6 | 0E+00 | 5.9 | 1.7 | 1E-01 | 3.8 | 0.8 | 2E-03 |
| Day 1 - Day 12 | 18.8 | 1.7 | 0E+00 | 4.5 | 1.8 | 8E-01 | 3.2 | 1.0 | 2E-01 |
| Day 1 - Day 13 | 17.9 | 1.8 | 0E+00 | 7.4 | 2.0 | 4E-02 | 4.9 | 1.0 | 8E-04 |
| Day 1 - Day 14 | 17.4 | 1.9 | 0E+00 | 7.4 | 2.1 | 8E-02 | 5.2 | 1.1 | 5E-04 |
| Day 1 - Day 15 | 19.3 | 2.0 | 0E+00 | 8.1 | 2.1 | 3E-02 | 5.9 | 1.1 | 9E-05 |
| Day 1 - Day 16 | 18.0 | 2.1 | 0E+00 | 9.5 | 2.2 | 7E-03 | 6.0 | 1.3 | 2E-03 |
| Day 1 - Day 17 | 18.9 | 2.8 | 7E-09 | 8.8 | 3.0 | 4E-01 | 3.8 | 1.7 | 9E-01 |
| Day 1 - Day 18 | 15.3 | 3.5 | 4E-03 | 1.9 | 3.5 | 1E+00 | 7.0 | 2.1 | 1E-01 |
| Day 1 - Day 19 | 21.0 | 4.0 | 8E-05 | 11.8 | 4.1 | 4E-01 | 5.1 | 2.2 | 8E-01 |
| Day 1 - Day 20 | 22.4 | 4.0 | 1E-05 | 9.2 | 4.1 | 9E-01 | 6.2 | 2.6 | 8E-01 |
| Day 1 - Day 21 | 14.4 | 4.4 | 2E-01 | 9.2 | 4.1 | 9E-01 | 1.9 | 2.6 | 1E+00 |
| Day 1 - REF1 | 15.3 | 1.1 | 0E+00 | 4.8 | 1.1 | 7E-03 | 3.7 | 0.6 | 2E-08 |
| Day 1 - REF2 | 19.7 | 1.2 | 0E+00 | 7.7 | 1.2 | 3E-08 | 4.1 | 0.6 | 9E-10 |
| Day 1 - REF3 | 16.3 | 1.3 | 0E+00 | 6.2 | 1.3 | 8E-04 | 3.6 | 0.7 | 4E-05 |
| Day 1 - REF4 | 15.7 | 1.9 | 0E+00 | 8.2 | 2.2 | 5E-02 | 2.5 | 1.0 | 7E-01 |
| Day 1 - FUP 1 | 18.5 | 1.6 | 0E+00 | 4.8 | 1.6 | 3E-01 | 0.4 | 0.9 | 1E+00 |
| Day 1 - FUP 2 | 20.5 | 2.1 | 0E+00 | 6.6 | 1.9 | 1E-01 | -1.7 | 1.1 | 1E+00 |
| Day 1 - FUP 3 | 19.8 | 2.6 | 0E+00 | 1.3 | 2.0 | 1E+00 | -0.5 | 1.3 | 1E+00 |

SBP indicates systolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

**Table S7. Statistical significance of the day-to-day difference to baselines for DBP in females.** Estimates and their SE of least square means, as well as the Tukey adjusted p-value, from a linear-mixed model adjusting for BMI are provided for normotensive (NT), hypertensive non-medicated (HTNM), and medicated (HTM).

| DBP FEMALES | HTNM | | | HTM | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|
| contrast | estimate | SE | p-value | estimate | SE | p-value | estimate | SE | p-value |
| Day 1 - Day 2 | 3.9 | 0.7 | 7E-06 | 0.8 | 0.7 | 1E+00 | 0.3 | 0.4 | 1E+00 |
| Day 1 - Day 3 | 5.5 | 0.7 | 0E+00 | 1.6 | 0.7 | 9E-01 | 0.8 | 0.3 | 8E-01 |
| Day 1 - Day 4 | 5.9 | 0.7 | 0E+00 | 1.6 | 0.7 | 9E-01 | 0.7 | 0.4 | 1E+00 |
| Day 1 - Day 5 | 7.1 | 0.7 | 0E+00 | 2.2 | 0.7 | 3E-01 | 0.9 | 0.3 | 7E-01 |
| Day 1 - Day 6 | 7.2 | 0.7 | 0E+00 | 2.4 | 0.7 | 1E-01 | 0.4 | 0.4 | 1E+00 |
| Day 1 - Day 7 | 8.8 | 0.7 | 0E+00 | 2.9 | 0.8 | 4E-02 | 0.7 | 0.4 | 1E+00 |
| Day 1 - Day 8 | 8.1 | 0.8 | 0E+00 | 4.3 | 0.8 | 3E-05 | 1.3 | 0.4 | 2E-01 |
| Day 1 - Day 9 | 8.6 | 0.8 | 0E+00 | 3.9 | 0.8 | 9E-04 | 1.0 | 0.4 | 9E-01 |
| Day 1 - Day 10 | 8.6 | 0.9 | 0E+00 | 2.8 | 0.9 | 3E-01 | 2.0 | 0.5 | 5E-03 |
| Day 1 - Day 11 | 9.2 | 1.0 | 0E+00 | 4.2 | 1.1 | 3E-02 | 2.4 | 0.5 | 2E-03 |
| Day 1 - Day 12 | 9.9 | 1.1 | 0E+00 | 5.2 | 1.1 | 2E-03 | 1.7 | 0.6 | 6E-01 |
| Day 1 - Day 13 | 10.4 | 1.1 | 0E+00 | 5.5 | 1.2 | 3E-03 | 2.4 | 0.7 | 7E-02 |
| Day 1 - Day 14 | 9.7 | 1.2 | 0E+00 | 5.8 | 1.3 | 3E-03 | 2.0 | 0.7 | 4E-01 |
| Day 1 - Day 15 | 10.0 | 1.2 | 0E+00 | 5.7 | 1.3 | 5E-03 | 3.6 | 0.7 | 2E-04 |
| Day 1 - Day 16 | 8.2 | 1.3 | 4E-07 | 6.5 | 1.4 | 1E-03 | 4.1 | 0.8 | 2E-04 |
| Day 1 - Day 17 | 10.4 | 1.8 | 1E-06 | 5.9 | 1.9 | 3E-01 | 2.4 | 1.1 | 9E-01 |
| Day 1 - Day 18 | 11.1 | 2.2 | 2E-04 | 2.3 | 2.2 | 1E+00 | 3.5 | 1.3 | 6E-01 |
| Day 1 - Day 19 | 8.9 | 2.5 | 1E-01 | 5.9 | 2.6 | 9E-01 | 2.1 | 1.4 | 1E+00 |
| Day 1 - Day 20 | 8.8 | 2.5 | 1E-01 | 6.4 | 2.6 | 8E-01 | 3.0 | 1.7 | 1E+00 |
| Day 1 - Day 21 | 10.4 | 2.8 | 5E-02 | 9.9 | 2.6 | 3E-02 | 1.0 | 1.7 | 1E+00 |
| Day 1 - REF1 | 8.6 | 0.7 | 0E+00 | 2.4 | 0.7 | 2E-01 | 1.6 | 0.4 | 2E-03 |
| Day 1 - REF2 | 9.3 | 0.7 | 0E+00 | 4.1 | 0.7 | 1E-05 | 1.9 | 0.4 | 6E-05 |
| Day 1 - REF3 | 8.0 | 0.8 | 0E+00 | 3.6 | 0.8 | 4E-03 | 1.8 | 0.4 | 7E-03 |
| Day 1 - REF4 | 8.3 | 1.2 | 1E-10 | 6.4 | 1.4 | 1E-03 | 1.3 | 0.6 | 9E-01 |
| Day 1 - FUP 1 | 11.4 | 1.0 | 0E+00 | 3.5 | 1.0 | 7E-02 | 4.1 | 0.6 | 0E+00 |
| Day 1 - FUP 2 | 12.5 | 1.4 | 0E+00 | 5.7 | 1.2 | 6E-04 | 2.8 | 0.7 | 1E-02 |
| Day 1 - FUP 3 | 11.6 | 1.7 | 1E-09 | 2.7 | 1.2 | 9E-01 | 3.7 | 0.8 | 2E-03 |

DBP indicates diastolic blood pressure; SE, standard error; BMI, body mass index; HTNM, hypertensive non-medicated; HTM, hypertensive medicated; NT, normotensive; REF, food reintroduction; FUP, follow-up.

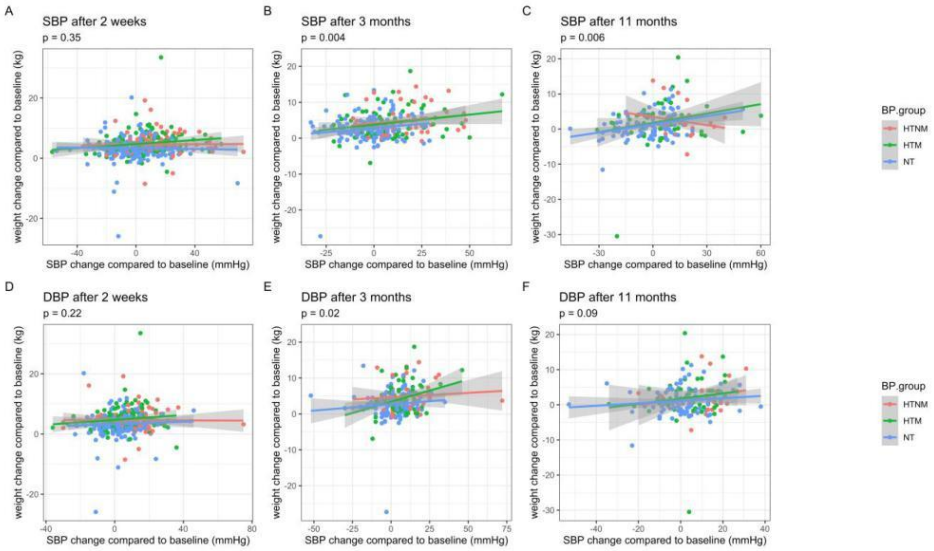



**Figure S1. The persistence of BP changes during the follow-up period correlates with the persistence of the weight loss.** Although the response to the follow-up were limited after two weeks (n=448), three months (n=256) and eleven months (n=188), the correlation of the persistence of weight loss and BP changes suggests that long-term effects on BP could be caused by a persistence of the weight loss.

BP indicates blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

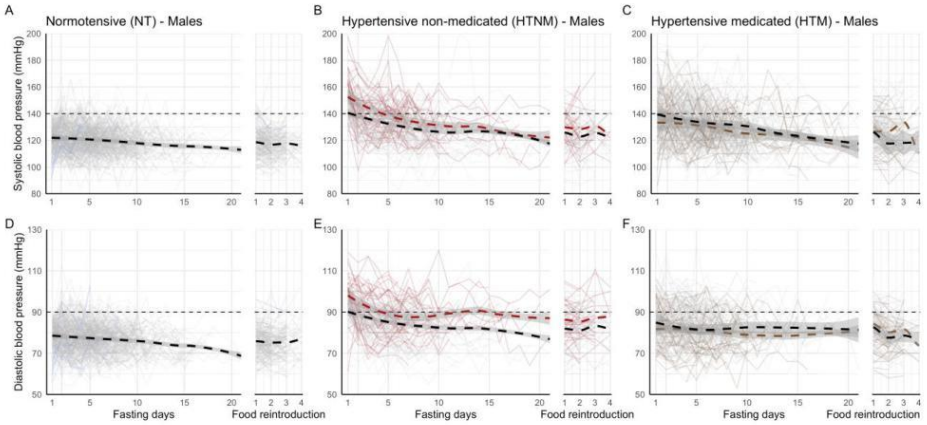



**Figure S2. Systolic and diastolic blood pressure during long-term fasting in male individuals.** The BP in normal (A, SBP ; D, DBP), hypertensive (B, SBP ; E, DBP) and medicated subjects (C, SBP ; F, DBP) is presented during the first 20 days of fast and the consecutive refeeding. Individual changes in BP are displayed as grey lines. Smoothed conditional means are plotted as dashed lines to illustrate global trends. A colour code is used to highlight subgroups consisting of 5 males with low blood pressure (<100/60 mmHg, in blue in panels A and D, no smoothed conditional means presented because of the low number of. individuals), 36 males with very high BP (>160/100 mmHg, in red in panels B and E), as well as 58 males who stopped their antihypertensive treatment (in brown in panels E and F).

BP indicates blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

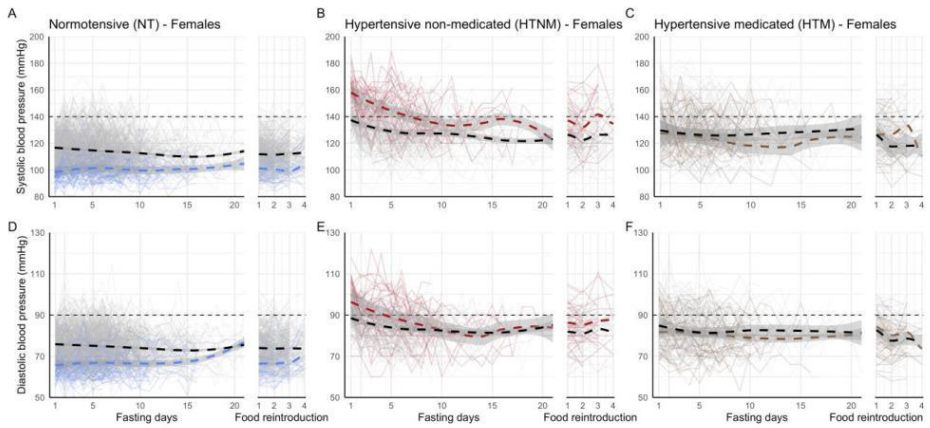



**Figure S3. Systolic and diastolic blood pressure during long-term fasting in female individuals.** The BP in normal (A, SBP ; D, DBP), hypertensive (B, SBP ; E, DBP) and medicated subjects (C, SBP ; F, DBP) is presented during the first 20 days of fast and the consecutive refeeding. Individual changes in BP are displayed as grey lines. Smoothed conditional means are plotted as dashed lines to illustrate global trends. A colour code is used to highlight subgroups consisting of 69 subjects with low blood pressure (<100/60 mmHg, in blue in panels A and D), 40 females with very high BP (>160/100 mmHg, in red in panels B and E), as well as 31 females who stopped their antihypertensive treatment (in brown in panels E and F).

BP indicates blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

## 4. Lebenslauf

„Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.“

## 5. Komplette Publikationsliste

Françoise Wilhelmi de Toledo*, **Franziska Grundler***, Audrey Bergouignan, Stefan Drinda, Andreas Michalsen. Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects.
*PLoS ONE*, 2019. (* Authors contributed equally)
DOI: 10.1371/journal.pone.0209353; PMID: 30601864; Impact Factor: 2,7

Stefan Drinda, **Franziska Grundler**, Thomas Neumann, Thomas Lehmann, Nico Steckhan, Andreas Michalsen, Françoise Wilhelmi de Toledo. Effects of Periodic Fasting on Fatty Liver Index—A Prospective Observational Study.
*Nutrients*, 2019.
DOI: 10.3390/nu11112601; PMID: 31671589; Impact Factor: 4,5

Robin Mesnage*, **Franziska Grundler***, Andreas Schwiertz, Yvon Le Maho, Françoise Wilhelmi de Toledo. Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting.
*Journal of Nutritional Science*, 2019. (* Authors contributed equally)
DOI: 10.1017/jns.2019.33; PMID: 31798864; Impact Factor: -

Françoise Wilhelmi de Toledo, **Franziska Grundler**, Cesare R. Sirtori, Massimiliano Ruscica. Unravelling the health effects of fasting: a long road from obesity treatment to healthy life span increase and improved cognition.
*Annals of Medicine*, 2020.
DOI: 10.1080/07853890.2020.1770849; PMID: 32519900; Impact Factor: 3,2

Françoise Wilhelmi de Toledo*, **Franziska Grundler***, Nikolaos Goutzourelas, Fotios Tekos, Eleni Vassi, Robin Mesnage, Demetrios Kouretas. Influence of Long-Term Fasting on Blood Redox Status in Humans.
*Antioxidants*, 2020. (* Authors contributed equally)
DOI: 10.3390/antiox9060496; PMID: 32517172; Impact Factor: 5,0

**Franziska Grundler***, Robin Mesnage*, Nikolaos Goutzourelas, Fotios Tekos, Sotiria Makri, Michel Brack, Demetrios Kouretas, Françoise Wilhelmi de Toledo. Interplay between oxidative damage, the redox status, and metabolic biomarkers during long-term fasting.
*Food and Chemical Toxicology*, 2020. (*Authors contributed equally)
DOI: 10.1016/j.fct.2020.111701; PMID: 32858131; Impact Factor: 4,7

**Franziska Grundler**, Robin Mesnage, Andreas Michalsen, Françoise Wilhelmi de Toledo. Blood Pressure Changes in 1610 Subjects With and Without Antihypertensive Medication During Long-Term Fasting.
*Journal of the American Heart Association*, 2020.
DOI: 10.1161/JAHA.120.018649; PMID: 33222606; Impact Factor: 4,6

Stylianos Ravanidis, **Franziska Grundler**, Françoise Wilhelmi de Toledo, Evangelos Dimitriou, Fotios Tekos, Zoi Skaperda, Demetrios Kouretas, and Epaminondas Doxakis. Fasting-mediated metabolic and toxicity reprogramming impacts circulating microRNA levels in humans.
*Food and Chemical Toxicology*, forthcoming 2021.
DOI: 10.1016/j.fct.2021.112187; Impact Factor: 4,7

## 6. Danksagung

Mein besonders herzlicher Dank gilt zu allererst Frau Dr. med. Françoise Wilhelmi de Toledo für die Möglichkeit diese Dissertation bei Buchinger Wilhelmi erstellen zu dürfen und ihre vertrauensvolle und kontinuierliche fachliche Unterstützung während meiner gesamten Publikationspromotion.

Zudem richtet sich mein außerordentlicher Dank an Herrn Prof. Dr. med. Andreas Michalsen, der durch die Übernahme der Doktorvaterschaft diese Arbeit großzügiger Weise in Kooperation mit dem Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie der Medizinischen Fakultät Charité – Universitätsmedizin Berlin, ermöglichte.

Besonders bedanke ich mich bei Dr. Robin Mesnage und Dr. med. Stefan Drinda für die gute Zusammenarbeit und stete Hilfsbereitschaft. Außerdem möchte ich mich bei Prof. Gilles-Éric Séralini und Prof. Massimiliano Ruscica bedanken, die mich auf meinem Weg begleitet haben und von denen ich viel lernen durfte.

Mein Dank gilt darüber hinaus allen Co-Autoren, die zur Entstehung der Publikationen beigetragen haben.

Ein ganz großes Dankeschön richtet sich an das gesamte Buchinger Wilhelmi Team, ohne das die Durchführung der Observationsstudie mit fast 2000 Teilnehmenden nicht möglich gewesen wäre.