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Health benefits of a five-day at-home modified fasting program: a randomised controlled trial

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Abstract

Background Fasting is one of the most cost-effective methods to improve cardiometabolic health. We tested a 5-day hypocaloric (~600 kcal/day) and ketogenic, modified fasting program (MFP) in a two-arm randomised controlled trial, where sixty-four healthy subjects were randomised to MFP or control group.

Methods We randomly assigned 64 participants to a group receiving the MFP or to a group of participants who were told to continue with their usual eating behaviour and lifestyle (control group). The changes in blood pressure and body weight were considered as primary endpoints. Secondary outcomes included ketosis, glucose and lipid metabolism, inflammatory markers, antioxidant capacity and well-being. Biological pathways and metabolic processes were explored with nuclear magnetic resonance blood metabolomics and gut metagenomics analyses. Outcomes were assessed at baseline, end of the MFP, after food reintroduction, and one month later.

Results MFP participants ($n=32$) experienced weight loss compared to controls (-0.52 ± 0.03 kg vs. -0.03 ± 0.02 kg, $p<0.001$). Changes in blood pressure caused by the MFP were non-significant at the end of the fasting period. However, blood pressure was significantly reduced following food reintroduction (systolic: -0.56 ± 0.12 mmHg vs. -0.16 ± 0.12 mmHg, $p<0.05$ and diastolic: -0.36 ± 0.08 mmHg vs. -0.01 ± 0.08 mmHg, $p<0.01$). Serum biochemistry showed the MFP reduced glucose levels and coagulation factors. The MFP also significantly increased physical well-being. Blood metabolomics revealed a significant decrease in chronic inflammation markers. Shotgun metagenomics of the gut microbiome showed significant changes in relative abundance of 11 bacterial species and in the genomic repertoire of 52 carbohydrate-active enzymes (CAZymes), reflecting an increase in families metabolising host-derived glycan substrates. None of these differences in gut microbiome and blood metabolome were shown to be statistically different from the control group one month after the intervention. Comparing MFP effects with a previous cohort's 5-day prolonged fasting showed similar metabolic changes.

Françoise Wilhelmi de Toledo is deceased; this manuscript is dedicated to her memory.

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Conclusions This MFP is safe and transiently improves cardiometabolic health and physical well-being in healthy individuals.

Clinical trial registration This trial was prospectively registered at ClinicalTrials.gov (NCT05821660) on 6 April 2023 prior to the start of patient recruitment.

Keywords Fasting, Cardiometabolic health, FMD, Gut microbiome, Weight loss, Metabolomics

Background

Throughout history, humans and free living animals have regularly undergone long periods of fasting in response to limited food availability, during several days or weeks according to seasonal cycles [1], and several hours while they sleep in alignment with circadian rhythms [2]. In current times, the disruption in life rhythmicity brought by the western lifestyle and the occurrence of "chronic fasting deficiency"—when individuals do not respect physiologic fasting periods (e.g. eating at night or skipping longer circannual fasting periods)—can be hypothesised to contribute to the development of cardiometabolic diseases, along with other factors such as poor nutrition, exposure to toxic pollutants, decreased sleep duration and sedentarism [3]. Conversely, calorie restriction and fasting are known to extend lifespan and health span in various animal models [1, 4].

Fasting strategies vary widely in terms of duration and frequency, as well as in caloric intake and macronutrient profiles [5]. These can range from daily time-restricted eating, which reduces eating windows thus allowing fasting periods each day [6, 7], to extended fasting periods lasting up to 21 days or more [8, 9]. Retaining the health benefits of fasting is often difficult once subjects return to their adipogenic environment [10]. Incorporating fasting strategies in everyday life under online supervision could improve compliance and increase the range of applications of fasting interventions and thus promote healthy longevity in the general population [11, 12].

We previously reported the safety, well-being and metabolic improvements in a cohort of 1422 patients undergoing prolonged fasting programme (PF) with the Buchinger Wilhelmi protocol (200–250 kcal daily as fruit juice, vegetable soup and honey) lasting between 4 and 21 days under medical supervision and moderate physical activity [8]. Whether effects of fasting persist depends largely on the diet during the period of food reintroduction and the adherence to a healthy lifestyle post-intervention, as demonstrated in the case of rheumatoid arthritis [13]. This underscores the necessity for new, easily accessible interventions that can be routinely implemented in daily life. To address this need, a five-day, 600-calorie ketogenic diet with restricted protein and carbohydrate intake was designed to mimic a 5-day period of prolonged fasting (PF) in an at-home setting [8]. This program is therefore referred to as a modified fasting program (MFP), as it preserves key metabolic features of prolonged fasting while allowing limited caloric intake and safe implementation at home.

Mechanisms of fasting-induced health benefits are well understood. At the molecular level, the restriction of glucose and proteins causes the inactivation of nutrient-sensing pathways, which in return modulate adaptive cellular responses such as DNA repair, autophagy, apoptosis, and oxidative stress defence [14]. These mechanisms are recognized hallmarks of ageing which can partially explain the healthy longevity-enhancing effects of fasting [15]. In addition, the switch from food-derived glucose to endogenous energy substrates, mainly fatty acids, triggers the production of ketone bodies which, beyond energy provision, signals fasting adaptations, reducing oxidative stress and inflammation [4]. The ability of the body to switch between different fuel sources, such as carbohydrates and fats, is known as metabolic flexibility and can be trained by regular fasting bouts [16, 17].

Our two-arm randomised controlled trial (RCT) aimed to explore the effects of this MFP on cardiometabolic risk factors, well-being, gut microbiome composition and functional potential as well as the feasibility of this MFP at home. In order to understand whether the effects of our MFP can induce hallmarks of fasting metabolism, we performed blood biochemistry, blood metabolomics and a gut metagenomics analysis.

Methods

Trial design

This study was designed as a RCT with two parallel study arms and was conducted in accordance with the principles of the Declaration of Helsinki and the standards of Good Clinical Practice. The protocol received approval from the Ethics Committee of the Medical Association of Baden-Württemberg, Stuttgart, Germany (approval number: F-2023-014) and was prospectively registered at ClinicalTrials.gov (NCT05821660). A total of 64 subjects were randomised either to five days of MFP followed by four days of food reintroduction ($n = 32$) or to their usual diet ($n = 32$; Fig. 1B). Enrolment occurred between 30 May and 28 June 2023. The study visits were carried out on an outpatient ambulatory basis between 5 June and 17 August 2023 at the Buchinger Wilhelmi Clinic in Überlingen (Baden-Württemberg, Germany). The study is completed and no longer ongoing.

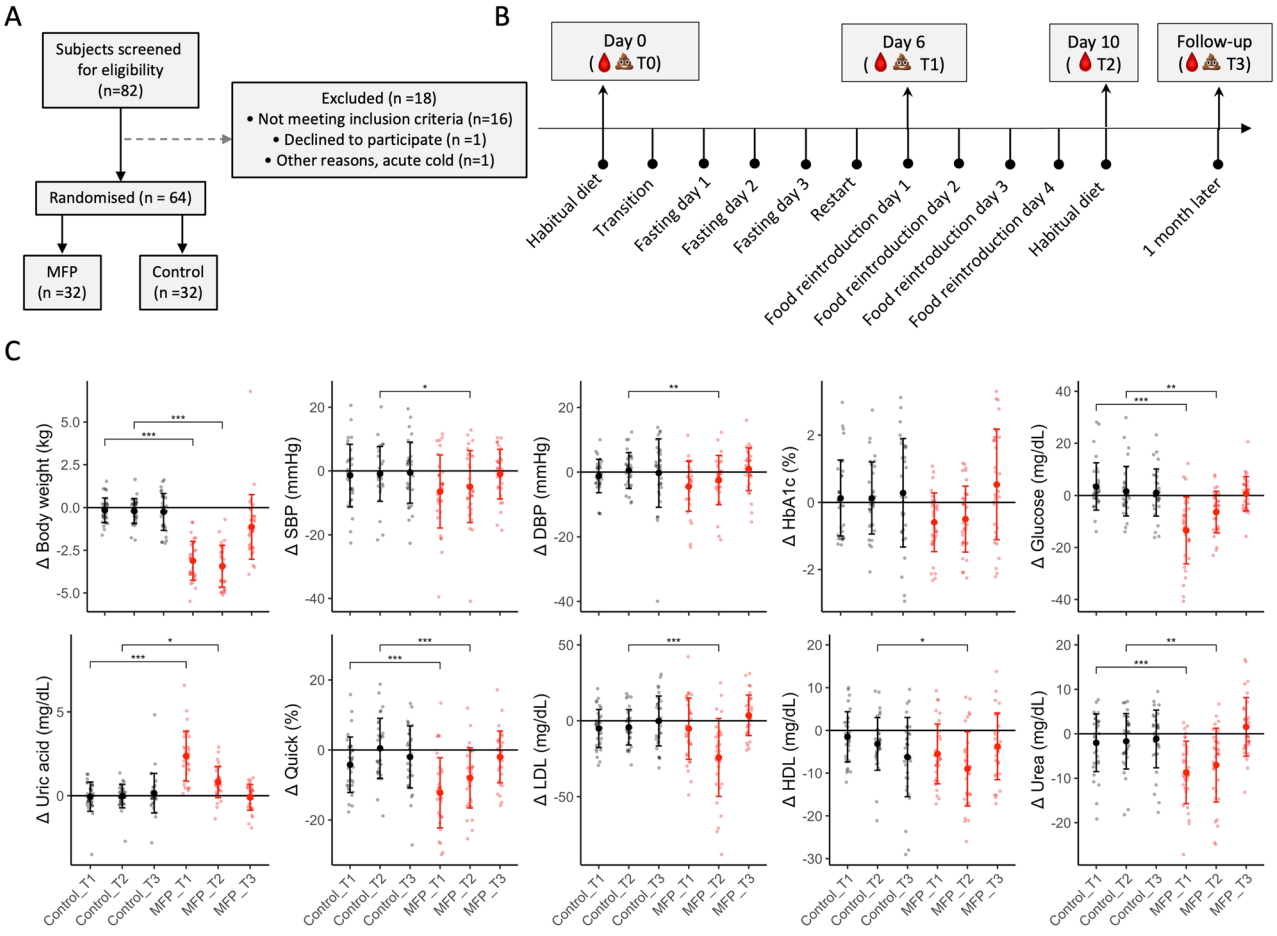

Fig. 1 Metabolic effects of a 5-day at-home MFP compared to a control group maintaining usual lifestyle. **A** Diagram of the recruitment procedure. **B** Study design with repeated sampling of blood and faecal samples. **C** Effects of the MFP (red) in comparison to the control group (black) at the end of the MFP period (T1), after 5 days food reintroduction (T2), or a month later (T3), for major parameters reflecting the switch to the fasting metabolism, presented as changes in the mean ± SD together with individual differences for each study individual relative to baseline (T0). The p-value indicates the statistical significance in a linear-mixed model where the effects are compared to the control group at a given timepoint with the ID of participants as a random effect (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

Dietary intervention

The MFP used was the Buchinger Wilhelmi FASTING BOX (Buchinger Wilhelmi Development und Holding GmbH, Germany) which includes ready-made meal replacements to be consumed over a five-day period. The ingredients are organic, plant-based products. The 24 items include vegetable soups as the main supplement, high-quality oils (linseed and algae oil) rich in omega 3 polyunsaturated fatty acids, chickpea puree, apple puree, honey and cashew nuts. The MFP provides on average 623 kcal per day, consisting of 9.8 g (6.2% of caloric intake) of proteins, 41.5 g (26.6%) of carbohydrates, and 44.7 g (64.7%) of fat (Additional file 1: Table S1). The aim of the MFP regimen is to achieve a steady reduction in calorie intake while limiting carbohydrate consumption to less than 50 g and proteins to less than ~10 g per day. The MFP includes a food supplement containing Tricalcium citrate, potassium citrate, magnesium citrate, zinc citrate trihydrate, sodium chloride. Furthermore, herbal teas, soup essences and herbal extracts are included as refreshment options, along with the recommendation to drink at least 2.5 l non-caloric, caffeine-free beverages daily. The consumption of water and herbal tea is not restricted. A detailed meal plan describes the recommended daily food intake and reflects the energy, fat, carbohydrate and protein content (Additional file 1: Table S23).

The 5-day program begins with a transition day. This is the only day when a morning serving is provided. On the following four MFP days, subjects skip the morning meal inducing a time-restricted eating pattern. The entire MFP programme is gluten-free and contains only a very restricted diversity of food items. The MFP was followed by four days of gradual food reintroduction from 800 to 1600 kcal per day. Half of the intervention group received a standard food reintroduction (Additional file

1: Table S24) ($n = 16$) and half received a ketogenic food reintroduction ($n = 16$) (Additional file 1: Table S25). The food was freshly cooked, organic, plant-based meals to take home after their daily measurements. Subjects in the control group were advised to stick to their usual diet.

Participants

Participants were recruited from the local area of Überlingen, Germany. Information about the study was offered in social media and per mail. An online questionnaire was used to recruit volunteers. If they appeared eligible, potential candidates were invited to an online presentation of the study. They were then invited to an assessment with the study physician, who checked the inclusion and exclusion criteria and asked for written informed consent. Women and men between 18 and 80 years were eligible. The following criteria led to exclusion: (1) intake of antibiotics up to 2 months prior the study; (2) medicated high blood pressure; (3) diagnosed hyperuricemia; (4) diagnosed diabetes mellitus type I and II; (5) diagnosed kidney stone; (6) active malignant diseases; (7) known substance addiction; (8) pregnancy or breastfeeding; (9) diagnosed with cachexia, anorexia, nervosa, advanced kidney, liver or cerebrovascular insufficiency; (10) inability to sign the informed consent and (11) participation in another study. The participants received no fee for their study participation.

Adherence

Adherence to the intervention was assessed primarily through daily face-to-face consultations with the participants during their daily clinical visits to ensure compliance with dietary guidelines. During these visits, participants reported their adherence directly to the research team. Additionally, adherence was indirectly validated by measuring ketone bodies in urine using ketone test strips, as participants adhering to the fasting program were expected to enter ketosis starting from the second day of the intervention. Adherence refers to completion of the intervention and study procedures. It does not imply perfect compliance with all dietary instructions.

Measurements

After a thorough physical examination by the study physician participants were randomised to either the intervention or control group. In order to facilitate the conduction of the study by the clinical centre, the study was conducted in two phases, each comprising 32 participants from both the control and intervention groups, with a one-month interval between them. For eleven consecutive days, the participants were examined daily in the morning. Four main visits were conducted: T0 at baseline (BL) before the start of the intervention, T1 in the morning of the 7th day after the five-day MFP (end MFP), T2 in the morning of the 11th day after the subsequent four-days of food reintroduction and T3 at follow-up one month later.

Endpoints

The changes from T0 to T1 in blood pressure and body weights are considered as primary endpoints. Changes in lipid and glucose metabolism, inflammatory parameters, total antioxidant capacity, and well-being were also analysed as secondary endpoints using linear mixed models with adjusted p-values < 0.05 being considered significant. All other parameters are considered as exploratory endpoints. All endpoints were assessed at the four main time points. In addition, body weight and blood pressure were monitored daily during the intervention period.

Clinical examination

Clinical parameters were measured daily by trained research assistants. Body weight was evaluated with participants lightly dressed, utilising a Seca 704 (Seca, Hamburg, Germany). Height was measured using the same device. Blood pressure and heart rate were measured twice on the non-dominant arm while seated, after a resting period, using a boso Carat professional (Bosch und Sohn GmbH u. Co. KG, Jungingen, Germany). Waist circumference was determined using a tape, which was placed halfway between the lowest rib and the iliac crest (openmindz GmbH, Heidelberg, Germany). Acetoacetic acid was self-measured twice per day in the first morning and last evening urine using Ketostix (Bayer, Leverkusen, Germany).

Blood parameters

Blood samples were collected at all four main visits (baseline, end of fasting, end of food reintroduction period, follow-up) by trained medical assistants after participants had fasted overnight (Fig. 1B). Lipid, glucose, kidney, liver and inflammatory parameters were determined as described in a previous study [18]. The antioxidative capacity of water-soluble substances (ACU) and lipid-soluble substances (ACL) was determined by photochemiluminiscence in serum and lipidperoxide by photometry in EDTA plasma (MVZ Labor Ravensburg, Germany). TNF-α was measured in serum by chemiluminiscence immunoassay (MVZ Labor Ravensburg, Germany).

Questionnaires

Self-reported data was documented by completing online questionnaires in the Buchinger Wilhelmi Amplius app. The participants received instructions and further information via this app to enable them to participate in this programme remotely. Adverse events were recorded in a report form by study physicians.

Participants reported the following outcomes on all four main visits (T0-T3): The Well-being Index World Health Organization 5 (WHO-5) [19], International Physical Activity Questionnaire (IPAQ) - short form, Pittsburgh Sleep Quality Index (PSQI) [20], Short healthy eating index (sHEI) [21], and Questionnaire of highly processed food consumption (sQ-HPF) [22]. Furthermore, lifestyle habits such as smoking, and alcohol consumption were documented. Stress level was rated on a visual scale from 0 (none) to 10 (extreme), and energy level from 0 (weak) to 10 (full of energy). Alcohol consumption was recorded as glasses of beer (0.5 l; 2 units of alcohol), wine (0.25 l; 2 units of alcohol) or spirits (0.02 l; 1 unit of alcohol) per week and added up to the number of drinks per week.

Additionally, participants self-reported daily their emotional and physical well-being as well as energy level on visual scales from 0 (very bad) to 10 (excellent). Sleep disturbances (0, none to 10, very strong) and sleep quality (0, calm to 10, restless) were reported. Further, participants rated whether they had cravings, felt bloated or cold (0; strongly disagree to 10; strongly agree). Symptoms such as fatigue, muscle weakness, back pain, headache, hunger, anxiety and digestive disorders on visual scales from 0 (none) to 10 (maximum) were evaluated. Self-perceived physical activity was recorded on a visual scale from 0 (inactive) to 10 (very active) and the actual activity time was captured in hours per day.

Faecal microbiota shotgun metagenomics

Three faecal samples were collected using EasySampler Stool Collection Kit (GP Medical Devices, Holstebro, Denmark) at T0, T1 and T3. Samples were stabilised by DNA/RNA ShieldTM Reagent, Zymo Research Europe GmbH, Freiburg, Germany and stored at -20 °C. The samples were processed and analysed by CeGaT, Tübingen, Germany.

Genomic DNA samples were profiled with shotgun metagenomic sequencing. Sequencing libraries were prepared with the Illumina preparation kit (M) Tagmentation (Illumina, San Diego, CA) with 100 ng genomic DNA following the manufacturer's protocol. Q30 value of sequencing was > 90.25%. The final library was sequenced on the NovaSeq 6000® (Illumina, San Diego, CA) platform.

Metagenomics shotgun sequencing data processing was performed as described in Ducarmon et al. [23]. Raw reads underwent quality filtering using bbduk (v38.93): (1) low-quality trimming (qtrim = rl, trimq = 3), (2) discarding low-quality reads (maq = 25), (3) adapter removal, and (4) length filtering (ml = 45). Taxonomic profiling used mOTUs (v3.1), while functional profiling filtered out human genome reads via Kraken2 (v2.1.2) against hg38, then mapped to a reduced GMGC human gut catalogue. Profiling of carbohydrate-active enzymes was done using Cayman [24].

Statistical analysis of metagenomic data was done in the statistical programming language R. Alpha-diversity was assessed using in phyloseq. Differences between baseline and other timepoints were statistically evaluated using a linear mixed model (LMM). Beta-diversity was determined using Bray-Curtis dissimilarity. Differential abundance analysis was done using LMM in SIAMCAT (v2.5.1), with participant-specific random effects. Features with < 10% prevalence were excluded. Pseudocounts varied and were 1e-4 for taxonomic analysis or 0.01 RPKM for CAZy. FDR correction was applied to p-values, with significance defined as adjusted $p < 0.05$.

Functional analyses in gut metagenome were done as described in Ducarmon et al. [23]. Gene-set enrichment analysis (GSEA) using fgsea identified higher-level CAZy substrates.

Serum metabolomics

Serum metabolomics was performed by lifespin GmbH (Regensburg, Germany). Sample preparation was done from 350 µl of serum mixed with 350 µl of aqueous buffer. The buffer consists of H_2O p.A., 0.1 g/l NaN_3, 0.067 mol/l Na_2HPO_4, 0.033 mol/l NaH_2PO_4 (pH: 7.15 ± 0.05), 5% D_2O field-lock substance and an internal standard (6 mM pyrazine) for quantification. From this mixture, 600 µl were transferred into a 5 mm Bruker NMR tube and sealed with barcode-labelled lids. The final NMR samples were stored after preparation at refrigerator temperature until measurement, not more than 24 h. NMR measurement was performed on a Bruker AVANCE NEO 600 MHz (measuring method 1D 1 H noesygppr1d_d20, NS = 16, T=310 K).

The spectra obtained were Fourier transformed using TopSpin software (version 4.2.0, Bruker Biospin, Germany). All spectra were automatically phased and subjected to baseline correction. Subsequently, the spectra were analyzed using the proprietary lifespin Profiler software (version 1.4_Blood) and a quantitative metabolite list was generated.

Statistical analysis

Based on previously collected data for changes in systolic blood pressure [25], we calculated that a sample size of 32 participants per arm is required to detect a decrease in 1 mm/hg per day with three timepoints and an effect size of 0.33 with 90% power and an alpha error probability of 0.05 with an ANOVA test including repeated measurements (G*Power version 3.1.9.7). Randomisation was performed with R, with a custom script splitting individuals into two groups randomly. No stratification or blocking procedure was applied. Group assignment was determined solely by the random allocation sequence.

All available data were analyzed without excluding participants based on adherence which was 100%. Missing data were not imputed and were simply excluded from the analysis. Given the limited extent of missingness, no changes to the statistical strategy were necessary.

Statistical significance for the primary endpoints was assessed using linear mixed models, with participant ID included as a random effect to account for repeated measures (*value ~ C(timepoint)*C(group), groups = df["id"]*). The reported p-values correspond to between-group comparisons, evaluating the treatment effect relative to the control group, rather than within-group changes over time. For the primary endpoints (body weight and blood pressure; Additional file 1: Table S2), p-values were adjusted using the Bonferroni post-hoc test, while the Benjamini–Hochberg procedure was applied to all other clinical, blood, lifestyle, and well-being parameters to control the false discovery rate (Additional file 1: Table S2-S5). Comparisons of changes from T0 to T1 stratified by risk factors were analyzed using the Mann–Whitney U test to account for small sample size and non-normal distribution of some variables. Baseline comparisons between two groups were performed using either the T-test, Welch's test, or the Mann–Whitney U test, depending on normality (assessed using the Shapiro–Wilk test) and homoscedasticity (assessed using Levene's test). This strategy was used for all the baseline comparison in order to keep simplicity and coherence.

For the metabolomics, samples with > 50% missing values per metabolite were excluded. Remaining missing values were imputed by replacing them with one-fifth of the minimum positive value in each metabolite column. Data were normalized to the median metabolite intensity within each sample and subsequently $\log_{10}$-transformed to approximate a normal distribution before statistical analyses.

Extraction of 32 closest neighbors of our participants (Additional file 1: Table S26), from subjects of our study with a cohort of 1422, was done using the NearestNeighbors class from the module neighbors of sklearn (1.3.2). The distance computed was the Euclidean, based on the following normalized parameters: BMI (kg/m^2), age, SBP, waist circumference, triglycerides (Additional file 1: Table S22). All statistics and tables were generated with python using the packages statsmodels (0.14.0) and scipy.stats (1.11.3). Differences were calculated using unrounded values to maintain accuracy, with rounded values presented for clarity. P-values less than 0.05 were considered to be statistically significant.

Results

Study participation

A total of 82 healthy subjects were screened for eligibility between March 2023 and August 2023. Sixteen subjects were excluded due to limited availability during the trial ($n = 10$), drug intake ($n = 4$), recent antibiotic use ($n = 1$), and pregnancy ($n = 1$). Two subjects declined participation due to loss of motivation and an acute cold. We randomly assigned 64 participants to a group receiving the MFP or to a group of participants who were told to continue with their usual eating behaviour and lifestyle (control group). Recruitment, randomization and follow-up participation are summarised in the Consolidated Standards of Reporting Trials (CONSORT) diagram (Fig. 1A) together with a timeline of the programme phases (Fig. 1B).

The 22 men (34.4%) and 42 women (65.6%) who participated in this RCT were mostly Caucasian (62 out of 64), non-smokers and moderate alcohol consumers (Additional file 1: Table S1). There were no significant differences in baseline characteristics between the MFP and control group with respect to age, sex, body weight, BMI, blood pressure and waist circumference (Table 1, S1 and S2).

Adverse events

We observed transient adverse events. A woman experienced brief nausea and vomiting on the second day of the MFP. Three participants working together in the same workplace developed symptoms on day 2 of the study, including a woman (control group) who reported nausea, vomiting, headache, dizziness and increased body temperature, a woman (MFP group) who reported dizziness, and a man (MFP group) who reported dizziness and nausea. The latter person took medication (metamizol dipyrone 500 mg for 3 days). All continued their participation. An environmental cause for these adverse events cannot be excluded, especially given that symptoms occurred in one participant of the control group. No clinically significant constipation or diarrhoea was reported.

Improved metabolic health in MFP as compared to controls

Participants in the MFP group experienced a variety of statistically significant improvements in cardiometabolic health in comparison to controls (Table 1). Body weight was decreased by 0.55 ± 0.02 kg compared to controls (-0.03 ± 0.02 kg, $p < 0.001$) by MFP. BMI, waist circumference and heart rate were significantly reduced (each $p < 0.001$). Changes in systolic blood pressure (-0.75 ± 0.23 mmHg vs. -0.03 ± 0.23 mmHg, $p = 0.08$) and diastolic blood pressure (-0.67 ± 0.16 mmHg vs. -0.21 ± 0.17 mmHg, $p = 0.13$) indicated a downward trend toward reduction, although these differences between groups did not reach significance (Fig. 1C; Table 1). Ketone bodies measured in urine with ketone test stripes showed that participants in the MFP group entered ketosis rapidly from day 2 onwards (Table 1). Blood glucose significantly decreased in the MFP group compared to controls (-0.75 ± 0.10 mmol/L

Table 1 Daily changes in clinical biomarkers and cardiometabolic risk factors during the 5-day MFP, food reintroduction, and follow-up one month later compared to controls

	T0			T1-T0			T2-T0			T3-T0		
	CON	MFP	Δ	CON	MFP	Δ	CON	MFP	Δ	CON	MFP	Δ
Body weight [kg]	70.10 ± 11.46	73.95 ± 13.77	3.85 ± 3.17	-0.03 ± 0.02	-0.55 ± 0.02	0.52 ± 0.03***	-0.02 ± 0.01	-0.36 ± 0.01	0.34 ± 0.02***	-0.25 ± 0.27	-1.13 ± 0.27	0.88 ± 0.39
BMI [kg/m²]	23.81 ± 3.01	25.35 ± 2.67	1.54 ± 0.71	-0.01 ± 0.01	-0.19 ± 0.01	0.18 ± 0.01***	-0.01 ± 0.00	-0.12 ± 0.00	0.12 ± 0.01***	-0.09 ± 0.09	-0.42 ± 0.09	0.33 ± 0.13*
Waist [cm]	82.30 ± 9.66	82.85 ± 9.44	0.55 ± 2.41	-0.05 ± 0.06	-0.41 ± 0.06	0.36 ± 0.08***	-0.07 ± 0.03	-0.38 ± 0.03	0.31 ± 0.04***	-2.05 ± 0.50	-1.92 ± 0.51	-0.13 ± 0.71
DBP [mmHg]	81.41 ± 8.75	85.06 ± 11.44	3.66 ± 2.55	-0.21 ± 0.17	-0.67 ± 0.16	0.47 ± 0.23	-0.01 ± 0.08	-0.36 ± 0.08	0.35 ± 0.12**	-0.28 ± 1.55	1.11 ± 1.55	-1.39 ± 2.20
SBP [mmHg]	128.95 ± 13.47	132.70 ± 15.90	3.75 ± 3.68	-0.03 ± 0.23	-0.75 ± 0.23	0.72 ± 0.32	-0.16 ± 0.12	-0.56 ± 0.12	0.40 ± 0.16*	-0.52 ± 1.53	-0.80 ± 1.53	0.28 ± 2.17
Resting heart rate [beats/min]	67.38 ± 10.86	67.58 ± 11.53	0.20 ± 2.80	0.16 ± 0.21	0.22 ± 0.21	-0.06 ± 0.29***	-0.05 ± 0.11	-0.16 ± 0.11	0.11 ± 0.16***	-1.06 ± 1.95	-1.66 ± 1.95	0.59 ± 2.76
Ketonuria morning [mg/dL]	1.25 ± 2.20	1.29 ± 3.87	0.04 ± 0.81	-0.07 ± 0.52	8.18 ± 0.51	-8.25 ± 0.73***	0.01 ± 0.74	-6.73 ± 0.73	6.74 ± 1.04***	-0.00 ± 0.86	-0.18 ± 0.84	0.18 ± 1.20
Ketonuria evening [mg/dL]	1.07 ± 2.09	0.78 ± 1.84	-0.29 ± 0.51	-0.08 ± 0.45	4.37 ± 0.43	-4.45 ± 0.63***	0.03 ± 0.52	-3.27 ± 0.52	3.30 ± 0.74***	0.89 ± 1.15	0.15 ± 1.12	0.75 ± 1.60

The baseline column presents group comparisons at T0 using independent t-tests, Welch's t-tests, or Mann–Whitney U tests depending on normality and variance assumptions. The subsequent columns reflect the effects at the end of the MFP (T1-T0), after food reintroduction (T2-T0), and at the one-month follow-up (T3-T0), respectively, expressed as the difference in change from baseline between the MFP and control groups. All other p-values are derived from a linear-mixed model including timepoints considered as continuous variable, their interaction with group (MFP vs. CON) and individuals as random effects. The models assess group-by-timepoint interactions, comparing the MFP group to the control (CON) group at each subsequent timepoint relative to baseline.

Abbreviations: *BMI* Body mass index, *DBP* Diastolic blood pressure, *SBP* Systolic blood pressure

For primary endpoints (#), p-values were adjusted using the Bonferroni post-hoc test. For all other parameters, the Benjamini–Hochberg procedure was applied to control the false discovery rate

Significance levels are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Baseline values are presented as mean ± SD. Changes from baseline are presented as model-based estimated mean ± standard error

T0: Baseline; T1: Day 6 or end of MFP; T2: Day 10 or end of food reintroduction; T3: 1 month follow-up.

vs. + 0.19 ± 0.10 mmol/L, $p < 0.001$), reflecting the known improvement of glycaemic control caused by fasting (Table 2).

Participants in the MFP group exhibited characteristic features of fasting-related kidney metabolism and coagulation changes (Table 2), including an increase in uric acid compared to controls (+ 140.15 ± 11.79 µmol/L vs. – 3.53 ± 11.79 µmol/L, $p < 0.001$) and a slight increase in creatinine (+ 4.31 ± 1.01 µmol/L vs. – 0.33 ± 1.01 µmol/L, $p = 0.008$). Urea decreased significantly by 1.45 ± 0.20 mmol/L compared to controls (– 0.34 ± 0.20 mmol/L, $p < 0.001$) by MFP. Coagulation parameters also changed, with increases in INR (+ 0.09 ± 0.01 vs. + 0.03 ± 0.01, $p < 0.001$) and PTT (+ 1.41 ± 0.27 s vs. + 0.41 ± 0.27 s, $p = 0.039$), reflecting mild anticoagulative effects characteristic of the fasting metabolism (Table 2). The increase in uric acid levels is another hallmark of fasting metabolism which has been associated with an increased antioxidative capacity and reduced lipid peroxidation in previous fasting studies [26]. The liver enzymes GOT ($p < 0.001$) and GPT ($p = 0.039$) also showed a typical fasting-induced increase in the MFP group. Furthermore, hematological shifts were observed at the end of the intervention, with erythrocyte counts being significantly higher in the MFP group compared to controls ($p = 0.033$), whereas red cell distribution width (RDW) was significantly lower ($p = 0.039$).

This MFP programme also includes a food reintroduction phase (Fig. 1B), which consists of a stepwise reintroduction of different food items, gradually increasing the number of calories (800 to 1600 kcal per day during 4 days). Most of the effects observed at the end of the MFP were stable or continuously improving after the 4 days of food reintroduction (Tables 1 and 2), including further reductions in body weight, BMI and waist circumference. Additionally, systolic (– 0.56 ± 0.12 mmHg vs. – 0.16 ± 0.12 mmHg; $p = 0.046$) and diastolic blood pressure (– 0.36 ± 0.08 mmHg vs. – 0.01 ± 0.08 mmHg; $p = 0.007$) decreased significantly in the MFP group. TC (– 0.74 ± 0.08 mmol/L vs. – 0.23 ± 0.08 mmol/L, $p < 0.001$), LDL-C (– 0.63 ± 0.09 mmol/L vs. – 0.11 ± 0.09 mmol/L, $p < 0.001$) and non-HDL-C (– 0.50 ± 0.08 mmol/L vs. – 0.15 ± 0.08 mmol/L, $p = 0.008$) and HDL-C (– 0.23 ± 0.04 mmol/L vs. – 0.08 ± 0.04 mmol/L, $p = 0.012$) levels were significantly reduced in the MFP group compared to controls after food reintroduction (Table 2), while they were only non-significantly reduced at the end of the MFP, suggesting that the MFP can have lipid metabolism-normalising effects detectable mainly after food reintroduction.

Supplementary tables provide all the results of clinical measurements (Additional file 1: Table S2), blood analyses (Additional file 1: Table S3), and questionnaires measured at the 4 main timepoints (Additional file 1:

Table 2 Biomarker or risk factor changes measured after the 5-day MFP, food reintroduction, and follow-up one month later compared to controls

	T0			T1-T0			T2-T0			T3-T0		
	CON	MFP	Δ	CON	MFP	Δ	CON	MFP	Δ	CON	MFP	Δ
Glycemic control												
Serum glucose [mmol/l]	4.71 ± 0.55	4.75 ± 0.39	0.05 ± 0.12	0.19 ± 0.10	-0.75 ± 0.10	0.94 ± 0.13***	0.09 ± 0.10	-0.35 ± 0.10	0.44 ± 0.13**	0.06 ± 0.10	0.04 ± 0.10	0.03 ± 0.13
HbA1c [mmol/mol]	36.28 ± 3.70	35.00 ± 2.62	-1.28 ± 0.80	0.12 ± 0.23	-0.59 ± 0.23	0.72 ± 0.33	0.12 ± 0.23	-0.50 ± 0.23	0.62 ± 0.33	0.28 ± 0.23	0.53 ± 0.23	-0.25 ± 0.33
Insulin [mIU/l]	6.91 ± 3.73	6.50 ± 4.48	-0.41 ± 1.03	0.16 ± 1.51	-3.44 ± 1.51	3.6 ± 2.1	-0.56 ± 1.51	-2.00 ± 1.51	1.44 ± 2.14	2.75 ± 1.51	-0.06 ± 1.51	2.81 ± 2.14
Lipids												
Total cholesterol [mmol/l]	4.98 ± 0.84	4.98 ± 1.18	0.00 ± 0.26	-0.20 ± 0.08	-0.25 ± 0.08	0.04 ± 0.12	-0.23 ± 0.08	-0.74 ± 0.08	0.51 ± 0.12***	-0.06 ± 0.08	0.17 ± 0.08	-0.24 ± 0.12
LDL-C [mmol/l]	3.18 ± 1.00	3.19 ± 1.07	0.01 ± 0.26	-0.13 ± 0.09	-0.14 ± 0.09	0.01 ± 0.12	-0.11 ± 0.09	-0.63 ± 0.09	0.52 ± 0.12***	-0.00 ± 0.09	0.09 ± 0.09	-0.10 ± 0.12
HDL-C [mmol/l]	1.75 ± 0.44	1.79 ± 0.51	0.03 ± 0.12	-0.04 ± 0.04	-0.14 ± 0.04	0.10 ± 0.05	-0.08 ± 0.04	-0.23 ± 0.04	0.15 ± 0.05*	-0.16 ± 0.04	-0.10 ± 0.04	-0.07 ± 0.05
Non-HDL-C [mmol/l]	3.22 ± 0.97	3.19 ± 1.10	-0.04 ± 0.26	-0.17 ± 0.08	-0.10 ± 0.08	-0.07 ± 0.11	-0.15 ± 0.08	-0.50 ± 0.08	0.35 ± 0.11**	0.10 ± 0.08	0.28 ± 0.08	-0.18 ± 0.11
LDL/HDL [ratio]	1.99 ± 0.91	1.97 ± 0.98	-0.02 ± 0.24	-0.05 ± 0.06	0.08 ± 0.06	-0.13 ± 0.09	0.02 ± 0.06	-0.15 ± 0.06	0.17 ± 0.09	0.18 ± 0.06	0.15 ± 0.06	0.03 ± 0.09
TG [mmol/l]	1.02 ± 0.45	1.00 ± 0.56	-0.023 ± 0.13	-0.10 ± 0.07	-0.09 ± 0.07	-0.01 ± 0.10	-0.11 ± 0.07	-0.12 ± 0.07	0.01 ± 0.10	0.00 ± 0.07	0.14 ± 0.07	-0.14 ± 0.10
Electrolytes												
Calcium [mmol/l]	2.32 ± 0.08	2.33 ± 0.10	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	-0.01 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	-0.00 ± 0.02
Magnesium [mmol/l]	0.84 ± 0.05	0.84 ± 0.06	0.00 ± 0.01	-0.01 ± 0.01	0.00 ± 0.01	-0.02 ± 0.01	-0.00 ± 0.01	-0.03 ± 0.01	0.03 ± 0.01	-0.00 ± 0.01	-0.02 ± 0.01	0.01 ± 0.01
Potassium [mmol/l]	4.25 ± 0.23	4.34 ± 0.45	0.08 ± 0.09	-0.11 ± 0.07	-0.14 ± 0.07	0.03 ± 0.09	0.08 ± 0.07	-0.05 ± 0.07	0.13 ± 0.09	-0.01 ± 0.07	0.00 ± 0.07	-0.02 ± 0.09
Sodium [mmol/l]	139.44 ± 2.11	139.78 ± 2.62	0.34 ± 0.60	-0.63 ± 0.44	-1.44 ± 0.44	0.81 ± 0.63	0.62 ± 0.44	-0.69 ± 0.44	1.31 ± 0.63	0.66 ± 0.44	-0.03 ± 0.44	0.69 ± 0.63
Kidney function												
Creatinine [µmol/l]	67.50 ± 10.35	69.22 ± 12.56	1.71 ± 2.88	-0.33 ± 1.01	4.31 ± 1.01	-4.64 ± 1.43**	1.13 ± 1.01	0.83 ± 1.01	0.30 ± 1.43	-0.58 ± 1.01	-2.04 ± 1.01	1.46 ± 1.43
GFR [ml/min]	103.59 ± 13.67	99.56 ± 17.37	-4.03 ± 3.91	0.31 ± 1.71	-2.56 ± 1.71	2.88 ± 2.42	-1.59 ± 1.71	1.47 ± 1.71	-3.06 ± 2.42	0.19 ± 1.71	4.63 ± 1.71	-4.44 ± 2.42
Urea [mmol/l]	5.34 ± 1.43	4.97 ± 1.25	-0.37 ± 0.34	-0.34 ± 0.20	-1.45 ± 0.20	1.11 ± 0.28***	-0.28 ± 0.20	-1.17 ± 0.20	0.89 ± 0.28**	-0.19 ± 0.20	0.26 ± 0.20	-0.45 ± 0.28
Uric acid [µmol/l]	276.58 ± 79.23	292.75 ± 71.60	16.17 ± 18.88	-3.53 ± 11.79	140.15 ± 11.79	-143.68 ± 16.67***	-1.49 ± 11.8	48.1 ± 11.79	-49.63 ± 16.67*	8.74 ± 11.79	-6.13 ± 11.79	14.87 ± 16.67
Inflammation and coagulation												

Table 2 (continued)

	T0			T1-T0			T2-T0			T3-T0		
	CON	**MFP**	**Δ**	**CON**	**MFP**	**Δ**	**CON**	**MFP**	**Δ**	**CON**	**MFP**	**Δ**
ESR [mm/h]	4.19 ± 3.40	3.19 ± 2.91	-1.00 ± 0.79	0.19 ± 0.81	0.41 ± 0.81	-0.22 ± 1.15	-0.75 ± 0.81	-0.75 ± 0.81	0.00 ± 1.15	1.72 ± 0.81	0.84 ± 0.81	0.88 ± 1.15
INR	1.00 ± 0.05	0.99 ± 0.07	-0.01 ± 0.02	0.03 ± 0.01	0.09 ± 0.01	-0.06 ± 0.01***	-0.00 ± 0.01	0.05 ± 0.01	-0.06 ± 0.01***	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.01
PTT [sec]	30.53 ± 2.29	31.44 ± 2.91	0.91 ± 0.65	0.41 ± 0.27	1.41 ± 0.27	-1.00 ± 0.38*	0.16 ± 0.27	1.72 ± 0.27	-1.56 ± 0.38***	-0.16 ± 0.27	-0.28 ± 0.27	0.13 ± 0.38
Quick [%]	100.69 ± 8.25	102.31 ± 10.77	1.62 ± 2.40	-4.20 ± 1.39	-12.25 ± 1.39	8.03 ± 1.96***	0.47 ± 1.39	-8.00 ± 1.39	8.47 ± 1.96***	-2.00 ± 1.39	-2.00 ± 1.39	0 ± 1.96
CRP [mg/l]	1.54 ± 1.46	1.23 ± 1.94	-0.31 ± 0.43	-0.34 ± 0.73	0.48 ± 0.73	-0.82 ± 1.04	-0.52 ± 0.73	0.10 ± 0.73	-0.62 ± 1.04	1.48 ± 0.73	-0.12 ± 0.73	1.60 ± 1.04
TNF-α [pg/ml]	5.75 ± 1.42	5.84 ± 1.63	0.10 ± 0.38	0.25 ± 0.27	-0.04 ± 0.27	0.29 ± 0.38	-0.08 ± 0.27	-0.22 ± 0.27	0.13 ± 0.38	0.87 ± 0.27	-0.19 ± 0.27	1.07 ± 0.38
Liver function												
γ-GT (U/l)	20.81 ± 14.81	19.00 ± 10.82	-1.81 ± 3.24	-1.22 ± 1.00	-2.69 ± 1.0	1.47 ± 1.42	-2.53 ± 1.00	-4.88 ± 1.00	2.34 ± 1.42	-1.00 ± 1.00	2.03 ± 1.00	-3.03 ± 1.42
AP (U/l)	64.31 ± 20.25	64.19 ± 22.66	-0.12 ± 5.37	-2.28 ± 1.242	-4.03 ± 1.242	1.75 ± 1.76	-2.31 ± 1.24	-7.38 ± 1.24	5.06 ± 1.76 *	1.38 ± 1.2 4	-1.22 ± 1.24	2.60 ± 1.76
GOT (U/l)	17.78 ± 6.79	16.91 ± 5.40	-0.88 ± 1.53	-0.56 ± 1.70	9.78 ± 1.70	-10.34 ± 2.40***	-1.25 ± 1.70	2.75 ± 1.70	-4.00 ± 2.40	1.19 ± 1.70	1.72 ± 1.70	-0.53 ± 2.40
GPT (U/l)	23.88 ± 9.90	21.97 ± 5.73	-1.91 ± 2.02	-1.28 ± 1.51	4.28 ± 1.51	-5.56 ± 2.14*	-2.47 ± 1.51	2.88 ± 1.51	-5.34 ± 2.14*	-0.06 ± 1.51	-1.03 ± 1.51	0.97 ± 2.14
Miscellaneous												
ACL [µmol/l]	42.19 ± 14.80	38.31 ± 10.94	-3.88 ± 3.25	-3.53 ± 2.00	-2.69 ± 2.00	-0.84 ± 2.82	-3.03 ± 2.00	-4.88 ± 2.00	1.84 ± 2.82	-2.37 ± 2.02	0.38 ± 2.00	-2.75 ± 2.84
ACU [µmol/l]	82.41 ± 26.51	80.16 ± 23.41	-2.225 ± 6.25	6.44 ± 4.74	-8.56 ± 4.74	15.00 ± 6.70	-2.69 ± 4.74	0.28 ± 4.74	-2.97 ± 6.70	-0.76 ± 4.78	-10.72 ± 4.74	9.96 ± 6.73
Plasma lipid peroxides [µmol/l]	410.72 ± 245.35	386.81 ± 197.58	- 23.91 ± 55.69	- 18.41 ± 23.54	-63.63 ± 23.54	45.22 ± 33.29	- 81.31 ± 23.54	- 52.00 ± 23.54	-29.31 ± 33.29	- 43.33 ± 23.79	- 17.47 ± 23.54	- 25.86 ± 33.47
TSH [mE/l]	2.37 ± 1.58	2.32 ± 1.13	-0.05 ± 0.34	-0.40 ± 0.18	-0.67 ± 0.18	0.27 ± 0.25	-0.35 ± 0.18	-0.58 ± 0.18	0.23 ± 0.25	0.07 ± 0.18	-0.28 ± 0.18	0.35 ± 0.25
Hematology												
Erythrocytes [10^6/µl]	4.57 ± 0.33	4.71 ± 0.40	0.15 ± 0.09	-0.09 ± 0.04	0.05 ± 0.04	-0.14 ± 0.05*	-0.10 ± 0.04	-0.11 ± 0.04	0.01 ± 0.00	-0.07 ± 0.04	-0.05 ± 0.04	-0.02 ± 0.05
Hematocrit [%]	40.87 ± 3.00	41.19 ± 3.17	0.33 ± 0.77	-0.96 ± 0.35	0.06 ± 0.35	-1.02 ± 0.50	-1.23 ± 0.35	-1.40 ± 0.35	0.17 ± 0.50	-0.87 ± 0.36	-0.04 ± 0.35	-0.82 ± 0.50
Hemoglobin [g/dl]	13.70 ± 1.04	14.01 ± 1.19	0.31 ± 0.28	-0.15 ± 0.10	0.17 ± 0.10	-0.33 ± 0.14	-0.21 ± 0.10	-0.31 ± 0.10	0.11 ± 0.14	-0.07 ± 0.10	-0.09 ± 0.10	0.02 ± 0.14
Leukocytes [10^3/µl]	6.37 ± 1.46	5.72 ± 2.23	-0.65 ± 0.47	-0.55 ± 0.26	-0.75 ± 0.26	0.21 ± 0.37	-0.63 ± 0.26	-1.12 ± 0.26	0.50 ± 0.37	-0.00 ± 0.27	-0.19 ± 0.26	0.19 ± 0.37
MCH [pg]	30.03 ± 1.43	29.77 ± 1.60	-0.26 ± 0.38	0.27 ± 0.09	0.08 ± 0.09	0.19 ± 0.13	0.25 ± 0.09	0.05 ± 0.09	0.20 ± 0.13	0.32 ± 0.09	0.15 ± 0.09	0.17 ± 0.13
MCHC [g/dl]	33.54 ± 1.06	34.01 ± 1.04	0.47 ± 0.26	0.39 ± 0.18	0.37 ± 0.18	0.02 ± 0.25	0.52 ± 0.18	0.40 ± 0.18	0.12 ± 0.25	0.55 ± 0.18	-0.16 ± 0.18	0.70 ± 0.25
MCV [fl]	89.58 ± 3.64	87.60 ± 4.83	-1.98 ± 1.07	-0.29 ± 0.33	-0.78 ± 0.33	0.49 ± 0.46	-0.67 ± 0.33	-0.95 ± 0.33	0.28 ± 0.46	-0.47 ± 0.33	0.79 ± 0.33	-1.26 ± 0.46
MPV [fl]	10.67 ± 0.68	10.78 ± 0.70	0.11 ± 0.19	-0.03 ± 0.11	0.04 ± 0.11	-0.06 ± 0.15	0.12 ± 0.10	0.21 ± 0.11	-0.09 ± 0.15	0.17 ± 0.11	-0.06 ± 0.11	0.23 ± 0.15
P-LCR [%]	30.41 ± 5.52	30.59 ± 4.72	0.18 ± 1.40	-0.40 ± 0.55	1.04 ± 0.53	-1.44 ± 0.76	-0.29 ± 0.53	2.59 ± 0.55	-2.88 ± 0.76**	0.70 ± 0.54	0.02 ± 0.54	0.68 ± 0.76
PDW [fl]	12.63 ± 1.36	12.63 ± 1.24	0.00 ± 0.36	-0.21 ± 0.20	0.24 ± 0.20	-0.45 ± 0.28	-0.08 ± 0.19	0.56 ± 0.20	-0.64 ± 0.28	0.12 ± 0.20	-0.07 ± 0.20	0.19 ± 0.28

Table S4) or daily during the intervention (Additional file 1: Table S5) comparing the MFP and control groups. BMI-adjusted comparisons are presented in Tables S6 (clinical), S7 (blood), and S8 and S9 (questionnaires). Subgroup analyses were also made to explore differences by sex (Tables S10, S11, S12 and S13).

To explore the effects of two food reintroduction strategies after fasting, participants were evenly divided: half received a ketogenic diet, and the other half received a non-ketogenic diet. Limited differences were observed between the ketogenic and standard diet groups (Tables S14, S15, S16 and S17). Both groups continued to excrete urinary ketone bodies consistently. Notably, the increase in PTT seen during the modified fasting program (MFP) persisted and was amplified after ketogenic food reintroduction but not with the standard diet ($p = 0.003$). Conversely, the decrease in urea levels observed during MFP was further amplified with standard food reintroduction but not with the ketogenic diet ($p < 0.001$). Altogether, these findings can suggest that reintroducing food with a ketogenic diet can prolong the health benefits of fasting by sustaining ketosis.

We investigated if the MFP-induced effects persisted after a month (Tables 1 and 2). While BMI remained significantly lower than baseline ($p = 0.038$), body weight showed a continuing downward trend without reaching statistical significance. Blood pressure returned to pre-intervention levels. All other parameters in these healthy subjects, which reflected beneficial changes in metabolism caused by the MFP intervention, returned to baseline levels.

Exploratory post-hoc analyses on individuals at risk of chronic diseases

We evaluated whether the MFP caused even larger benefits for the health of individuals with risk factors of cardiometabolic disease (Additional file 1: Table S18). Subjects with BMI > 25 kg/m^2 showed a stronger reduction in BMI than those with BMI < 25 ($p = 0.006$). Systolic and diastolic blood pressure were reduced by 0.94 ± 8.7 mmHg and 0.31 ± 6.37 mmHg in healthy subjects, respectively, but by 9.32 ± 11.8 mmHg and 7.31 ± 7.34 mmHg in at-risk individuals. This reached statistical significance for diastolic ($p = 0.009$) but not systolic ($p = 0.08$) blood pressure. Cholesterol and LDL levels also significantly decreased in participants with elevated baseline values: cholesterol > 200 mg/dl dropped by 19.7 mg/dl ($p = 0.02$), and LDL > 130 mg/dL decreased by 16.8 mg/dl ($p = 0.02$).

Well-being, symptoms and lifestyle

Participants answered daily questionnaires to assess their well-being, possible side effects, and lifestyle factors throughout the study. Those undergoing the MFP markedly enhanced physical activity ($p = 0.015$). Quality of life evaluated using the WHO-5 index was reported lower in the MFP group at the end of the intervention ($p = 0.030$), and energy levels were lower compared to controls ($p < 0.001$). All changes were fully reversible following food reintroduction (Additional file 1: Table S4). It is likely that these initial symptoms were linked to concerns about undergoing the fasting experience. Hunger or sleep quality was not different between the two groups. Note that these findings are based on self-reported measures, which may limit their robustness.

Serum metabolomics reflects remodelling of energy metabolism

NMR metabolomics revealed that the MFP caused marked metabolic changes, which persisted during the food reintroduction and returned to baseline after a month (Fig. 2 and Additional file 1: Table S19). We observed that concentrations of 24 metabolites were significantly decreased in the MFP in comparison to the control group, and that 9 metabolites were increased. In line with expectations from metabolic switching, strong increases were observed for the ketone bodies acetoacetic acid and 3-hydroxybutyric acid after the MFP. Isopropanol was also strongly increased. This could be explained by its synthesis as a byproduct of acetone as observed in cases of diabetic ketoacidosis [27]. Strongly decreased metabolites include amino acids (i.e., proline, alanine, glutamine, glycine, methionine, lysine, serine, threonine, histidine). Other notably decreased metabolites include glycoprotein acetyls A, a known biomarker of chronic inflammation [28], even if other classical inflammatory markers such as TNF-α showed limited changes (Table 2). Altogether, this serum metabolomics analysis reflected a strong metabolic remodelling caused by the fasting intervention, but also a return to baseline after a month.

Shotgun metagenomics reveals foraging of host-derived substances

Changes in the gut microbiota were investigated by performing shotgun metagenomics on participants' faecal samples. Both alpha diversity analysed with the Shannon index (Fig. 3A) and beta diversity analysed with Bray-Curtis dissimilarity remained unchanged (Fig. 3B). Limited changes were observed with only 11 species having their relative abundance statistically significantly changed at T1 of MFP versus T1 of controls (as assessed by mOTUs, Fig. 3C and Additional file 1: Table S20). No changes were observed at T3 compared to T0, indicating that the effects on the gut microbiota were transitory. *Akkermansia Muciniphilia* which is commonly altered in fasting states was not affected.

Changes in energy metabolism in the gut microbiota were even clearer (Additional file 1: Table S21). We analyzed substrate preference derived from CAZyme

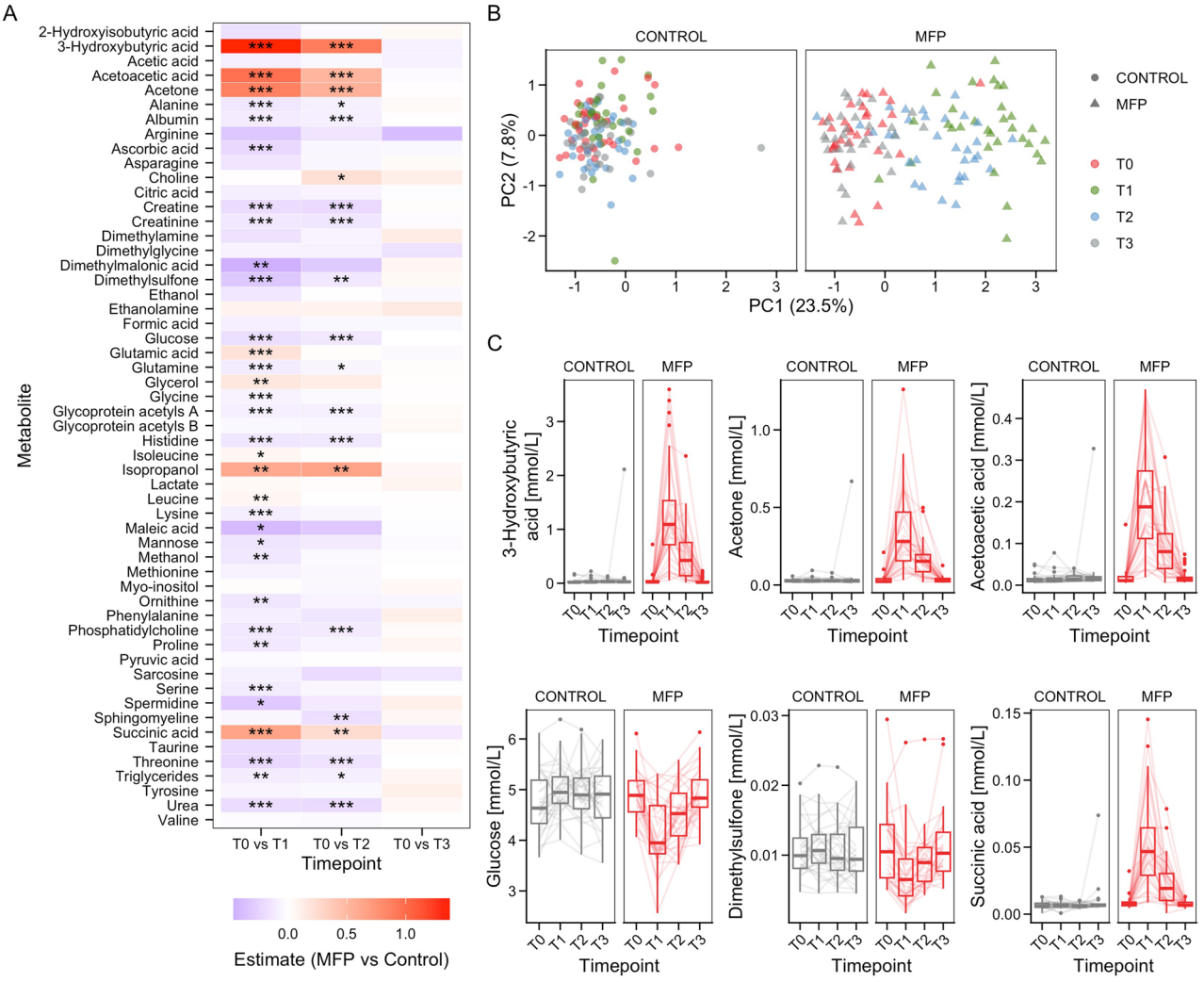

Fig. 2 Serum metabolomics analysis of the effects of the MFP on serum metabolite composition. **A** Heatmap showing the changes in the levels of metabolites between the MFP group and the control group at different timepoints. The colour scale shows the estimate of the difference between the MFP group and the control group in a linear mixed model with individuals as random effects. The p-value resulting from these models was adjusted for multiple comparisons using the Benjamini-Hochberg procedure (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). **B** Principal Component Analysis (PCA) shows the structure of the variance between these groups and timepoints. The PCA was done for all samples together, but the grouping of samples are visualised facetted for the sake of clarity. **C** Time trajectory for the abundance of 6 metabolites with the lowest p-values

abundances quantified from metagenomes [24]. A total of 52 CAZymes had their abundance significantly changed at T1 of MFP versus T1 of controls, but none were persistently changed at T3. This reflected a transient reduction in the abundance of CAZymes that metabolise dietary fibre concomitant with an increase in CAZymes involved in host-derived glycosaminoglycan foraging. Gene-set enrichment analysis (GSEA) to evaluate substrate enrichments suggested that the gut microbiome switched metabolism and started foraging host-derived substances during the MFP intervention (Fig. 3H).

Comparison with 5 days of prolonged fasting

We compared the effects of the MFP to the effects of 5 days of PF according to the Buchinger Wilhelmi protocol (75–250 kcal/day). In order to allow for fair comparisons between groups, we matched each of the 32 participants from the MFP group to individuals with similar sex, age, blood pressure, triglycerides, BMI, waist circumference from a total of 400 patients who performed 5 days of fasting in the Buchinger Wilhelmi clinic in 2016 [8]. Effect sizes of the MFP or 5 days of fasting were comparable for most clinical parameters (Fig. 4A and Additional file 1: Table S22). Effects of the MFP were slightly more pronounced than 5 days of prolonged fasting for the changes in the anticoagulative effects and the glucose levels. The latter is related to the glucose content of the diet, higher in PF according to the Buchinger protocol (56 g/day) than in MFP (32.8 g/day). Altogether, this shows that the MFP has comparable beneficial metabolic effects to PF.

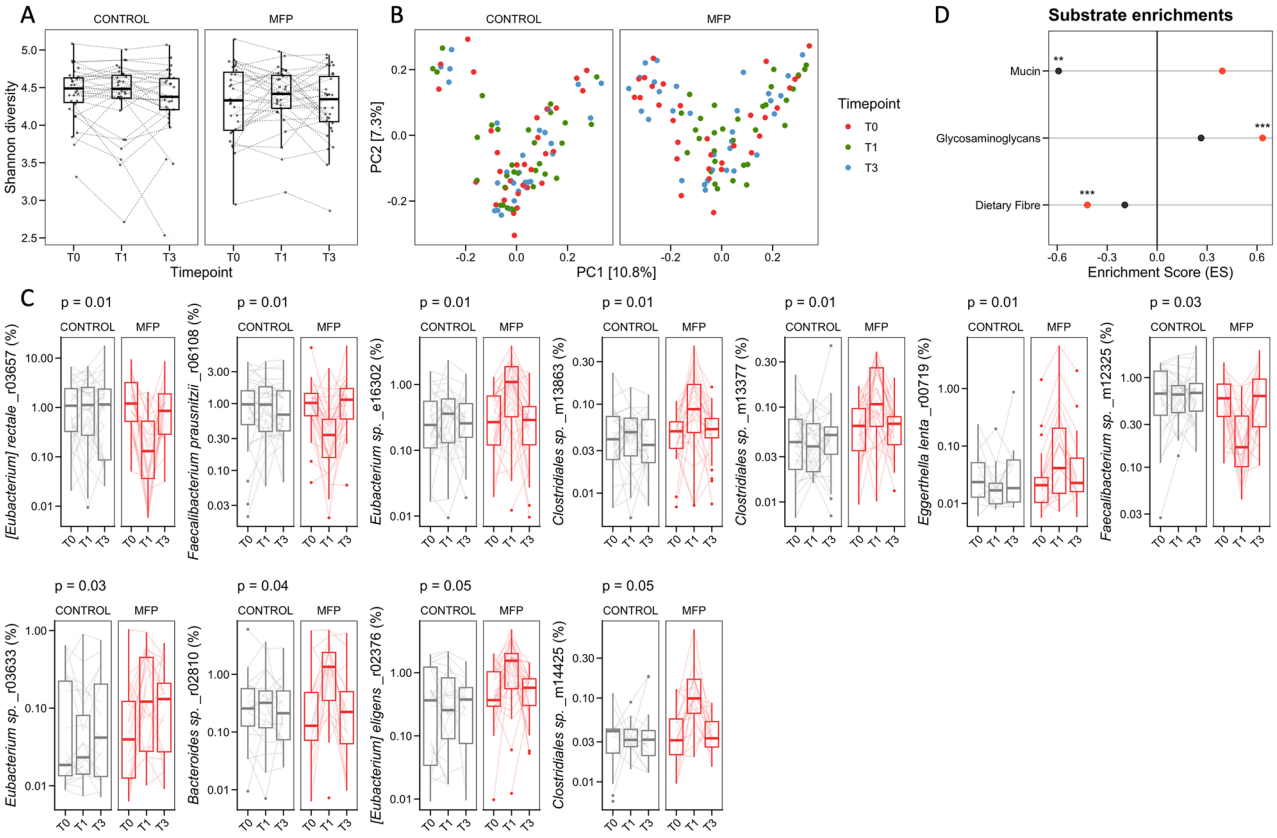

Fig. 3 Changes in faecal microbiota composition and function. **A** Alpha diversity was computed as Shannon diversity. **B** The Principal Coordinate Analysis (PCoA) of microbial communities is based on Bray-Curtis dissimilarities, coloured by timepoints and faceted by study arms. **C** Individual changes in abundance for the 11 bacteria species with the p-values < 0.05 in the evaluation of the effect of the MFP (T1 vs. T0). **D** All CAZyme families were additionally subjected to gene-set enrichment analysis (GSEA) to evaluate substrate enrichments (p-values are indicated as start symbols, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Generalized fold changes were calculated using linear mixed models (LMMs) with random intercepts for the individuals

We compared the directionality of the changes in gut microbiota composition with the changes caused by PF we observed in another study [23]. The changes induced during PF are comparable to those induced by the MFP (Fig. 4B), as shown by the statistically significant correlation between generalized fold changes for each mOTU measured in both interventions (Fig. 4D). The changes in CAZy families were highly correlated (Fig. 4E) and thus comparable to those observed during PF (Fig. 4B). Gene-set enrichment analysis (GSEA) suggested that the gut microbiome switched metabolism in a comparable way to what is observed during PF (Fig. 4F).

Discussion

Large research efforts are currently undertaken to find pharmacological and non-pharmacological interventions for maintaining body weight and blood pressure within recommended limits, in order to reduce the high incidence of chronic metabolic diseases such as type 2 diabetes or coronary heart disease [29]. The possibility to modulate cardiovascular risk factors triggers the need for proactive healthcare policy planning [30]. The MFP evaluated in the present RCT demonstrates that short fasting periods performed at home may serve as a feasible approach to transiently reduce body weight and improve the cardiometabolic profile in healthy individuals. Although statistically significant, changes were small in this predominantly healthy population and do not allow conclusions about potential benefits in individuals with chronic disease.

Compared to pharmacological approaches for weight loss and metabolic normalisation, such as GLP-1 agonists [31], fasting strategies generally present with only mild side effects, particularly when conducted under professional supervision, either in specialised institutions or for shorter periods through online platforms, as is the case of MFP [8]. Unlike GLP-1 agonists, which are associated with significant costs [32], non-pharmacological strategies like MFP empowers individuals to explore and manage on their own dietary strategies enhancing a sense of self-efficacy and safety [33]. The alternance of MFP with a normal diet triggers well-being and diminishes cravings often associated with continuous caloric restriction. MFP offers health benefits and is compatible with daily life

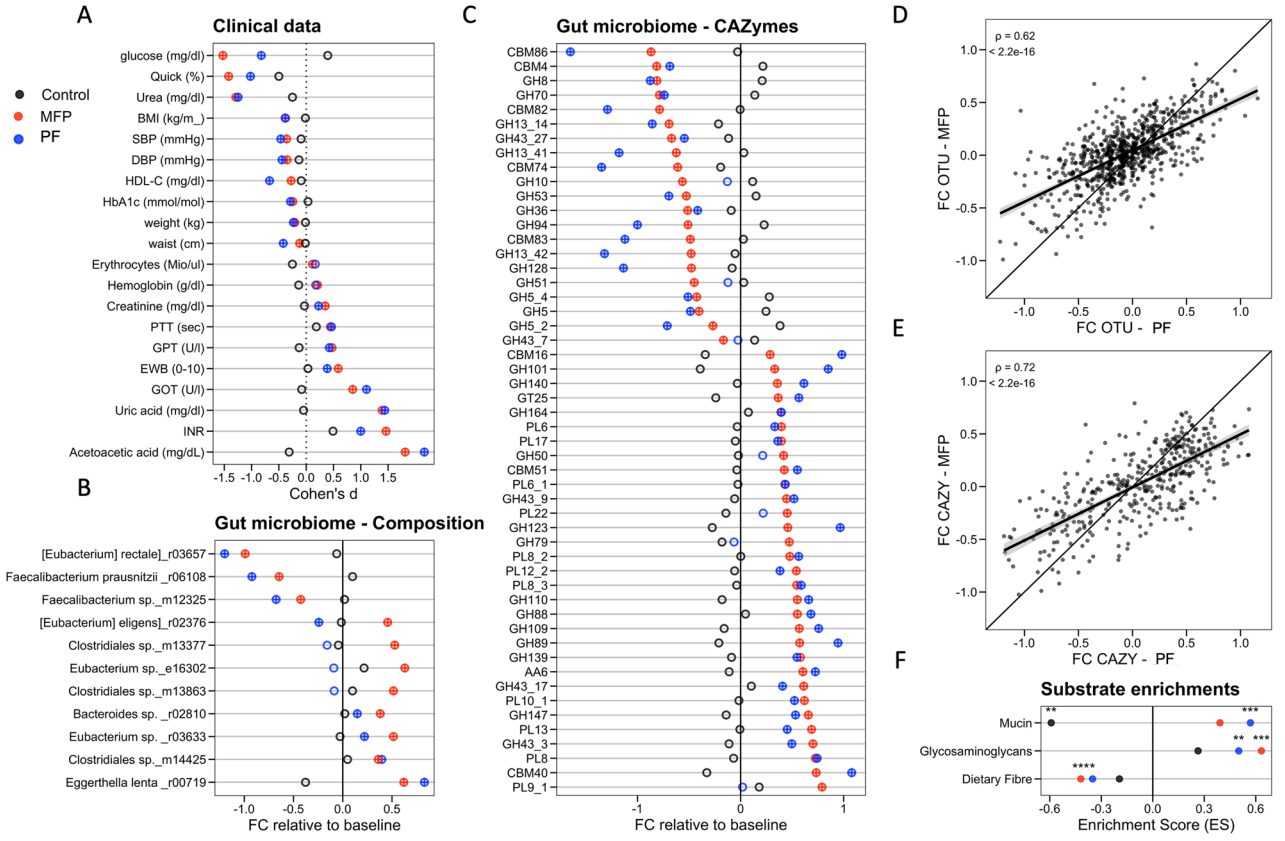

Fig. 4 Metabolic changes caused by the MFP were comparable to those of a 5-day prolonged fasting. **A** Comparison of the effect size estimated from the effects of 5-day MFP in comparison to the control group at T1 for parameters showing statistically significant changes in their levels at T1. These effects are also compared to 5 days of prolonged fasting in 32 individuals matched based on their age, sex, body weight and triglyceride levels out of 400 participants who fasted according to the Buchinger Wilhelmi fasting programme (250 kcal/day) in 2016. **B** Dot plot shows fold changes in differential abundance in microbiota composition before and after the MFP intervention in comparison to PF in another study. **C** Differential CAZyme family abundance in comparison to PF in another study. **D** Correlations between fold changes in relative abundance for microbiota composition. **E** Correlations between fold changes for CAZyme family abundance. **F** All CAZyme families were additionally subjected to gene-set enrichment analysis (GSEA) to evaluate substrate enrichments (p-values are indicated as start symbols, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Parameters are ordered by MFP effect sizes. Statistical significance is indicated with crossed circles ($p < 0.05$)

[34]. Short and periodically repeated interventions also prevents from developing with its deleterious effects on the immune system and wound healing [35]. Nevertheless, the risk of falling back into old lifestyle habits that led to poor metabolic health cannot be fully eliminated.

Perceived energy levels followed a time-dependent pattern during the intervention. A mild decrease was observed at the end of the fasting period, consistent with the early adaptation phase to caloric restriction and the metabolic switch toward fat oxidation. These transient effects were not sustained, and energy levels returned to baseline after food reintroduction. This pattern is in line with the expected physiological response to fasting, where an initial phase of fatigue may precede stabilisation as ketosis becomes established [8].

The mechanisms through which the MFP intervention may confer health benefits seem to be multifaceted and deeply interconnected. The loss of body weight by itself could explain a decrease in chronic inflammation [36]. The clinical and metabolic changes caused by the MFP were comparable to those observed after PF (i.e. total of 5 days of fasting at 250 kcal/day) in one of our previous studies [1]. These comparisons should be interpreted with caution, as they were exploratory in nature and not designed to provide confirmatory evidence of equivalence between interventions. The changes caused by the metabolic shift into ketosis fasting are known to contribute to beneficial changes in nutrient-sensing signalling pathways [37, 38], which play crucial roles in regulating cellular processes related to metabolism, growth, autophagy, stem cell-based regeneration and ultimately longevity [14]. Therefore, by modulating these pathways, our MFP holds substantial potential for enhancing public health.

After a one-month follow-up, improvement in BMI persisted among predominantly normal-weight individuals although a tendency towards a return to baseline was observed. In contrast, many metabolic changes observed during the intervention, including increases in uric acid,

returned to baseline because they represent transient physiological adaptations to the fasting metabolism. Uric acid illustrates this duality, as it is often viewed as detrimental, yet also acts as a major circulating antioxidant and may contribute to metabolic protection during ketosis [26], although safety of MFP cannot be anticipated in individuals susceptible to gout attacks. The easy implementation makes it a promising candidate for multiple-cycle programs, potentially mirroring the enhanced outcomes seen with Fasting Mimicking Diets (FMD) [39]. In humans, three cycles of a monthly 5-day FMD in a RCT involving 100 healthy subjects resulted in weight loss of on average 2.6 ± 2.5 kg, decreased BMI, total body fat, trunk fat and reduced waist circumference [40]. It also reduced the need for medication and improved glycaemic control in primary care for type 2 diabetes [41]. A study involving 24 healthy volunteers confirmed the glucose-lowering effect of a 5-day FMD and also demonstrated reductions in IGF-1 alongside improved insulin sensitivity [42]. A recent study even showed that three cycles of FMD had the potential of slowing down markers of biological ageing [43]. Longer follow-up including different frequencies of MFP use will be needed to understand whether this MFP can be used to treat chronic diseases.

Our study shows that the MFP is an effective method to address risk factors for cardiometabolic diseases. A main strength of our study is the joint collection of patient blood markers, metagenomic and metabolomic data in a longitudinal design surveyed under randomised controlled clinical conditions. This helped us demonstrate that this MFP can exert beneficial health effects by eliciting mechanisms which are known hallmarks of the fasting metabolism. We even noted that participants in the MFP group with higher baseline values above the norm ranges experienced more significant improvements in metabolic parameters compared to those whose values were within the normal range. This was suggested in another study where metabolic markers like fasting glucose, triglycerides, CRP, and cholesterol levels, which were within the normal range at baseline, did not show significant changes after three cycles of FMD in a randomised comparison [40]. The same is true for the PF [8], showing that at-risk individuals may benefit more from the intervention. However, it should be acknowledged that our subgroup analyses, including this stratification by metabolic health status or resting energy expenditure at baseline and the comparison of ketogenic versus non-ketogenic diet during food reintroduction, are exploratory due to the limited sample sizes within each subgroup. Therefore, further studies with larger cohorts are needed to validate these preliminary observations.

Another strength of our program is that participants received educational material to improve their lifestyle including instructions to do more physical activity. It can also be seen as a limitation, because even if the control group received similar advice and could be used to differentiate the effects of the MFP from the effects of the accompaniment, it is not always practical to disentangle the contribution of the numerous factors which compose such a lifestyle intervention. In addition, symptom data was subjective and exploratory. Other limitations include the focus on individuals who were mostly metabolically healthy, making it challenging to predict the potential benefits or difficulties that might arise in patients with underlying conditions. Although adjusted analyses did not materially alter the results, the findings should be interpreted with caution and therefore, further research is needed to explore the effects of MFP in patients with metabolic conditions such as hypertension or type 2 diabetes, or fatty liver disease chronic diseases.

During the MFP, dietary fiber intake was minimal. This likely explains the transient reduction in fiber-degrading CAZymes and the increase in enzymes involved in host-derived substrate metabolism. Despite these changes, participants did not report clinically relevant constipation or diarrhoea. Overall, the available evidence suggests that changes in gut microbiota composition during fasting are modest and transient [44], as observed in this study, and therefore likely to have limited health consequences.

This new programme is in line with the need for lifestyle interventions which substantially decrease healthcare costs [45, 46]. At-home interventions are accessible to a broad population, and are compatible with individuals' daily lives, thereby increasing the likelihood for long-term adherence. This could be the case when individuals are confined at home in isolation such as during the Coronavirus-19 disease (COVID-19) pandemic [47, 48] or other reasons.

Conclusions

In conclusion, the results of this RCT show that the MFP is an effective intervention for reducing body weight and inducing a metabolic switch to ketosis. Furthermore, significant improvements in blood pressure were observed following food reintroduction, indicating that the MFP elicits beneficial effects similar to those observed in other fasting interventions. The improvements in cardiovascular and metabolic risk factors, especially in subjects with elevated baseline levels of these markers, are of significant value for non-pharmacological public health strategies.

Future investigations should prioritise the study of at-risk patient groups to understand the efficacy of MFP in reducing lifestyle-related diseases, assess long-term effects, and explore the fasting-like effects to gain insight into its broader preventive and therapeutic benefits for promoting health and longevity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13073-026-01681-3.

Additional file 1: Table S1. Study demographic data. Table S2. Changes in clinical parameters in the study participants. Table S3. Changes in biochemical parameters in the study participants. Table S4. Changes in well-being, symptoms and physical activity assessed by questionnaires at the four main timepoints. Table S5. Changes in well-being, symptoms and physical activity assessed by daily questionnaires. Table S6. Changes in clinical parameters adjusted for baseline BMI. Table S7. Changes in biochemical parameters adjusted for baseline BMI. Table S8. Changes in well-being, symptoms and physical activity assessed by questionnaires at the four main timepoints, adjusted for baseline BMI. Table S9. Changes in daily questionnaire responses adjusted for baseline BMI. Table S10. Changes in clinical parameters in MFP participants with sex considered as a covariate. Table S11. Changes in biochemical parameters in MFP participants with sex considered as a covariate. Table S12. Changes in well-being, symptoms and physical activity assessed by questionnaires at the four main timepoints in MFP participants, with sex considered as a covariate. Table S13. Changes in daily questionnaire responses in MFP participants, with sex considered as a covariate. Table S14. Changes in clinical parameters in MFP participants according to food reintroduction strategy. Table S15. Changes in biochemical parameters in MFP participants according to food reintroduction strategy. Table S16. Changes in questionnaire responses at the four main timepoints in MFP participants according to food reintroduction strategy. Table S17. Changes in daily questionnaire responses in MFP participants according to food reintroduction strategy. Table S18. Post hoc analysis of changes in risk factors among MFP participants at risk of chronic disease. Table S19. Serum metabolomics analysis of the effects of the MFP on serum metabolite composition. Table S20. Stool metagenomics analysis of the effects of the MFP on gut microbiota composition. Table S21. Stool metagenomics analysis of the effects of the MFP on gut microbiota functional potential. Table S22. Comparison of the effects of the MFP and prolonged fasting. Table S23. Meal plan during the five-day MFP and corresponding energy and macronutrient content. Table S24. Food reintroduction plan for the non-ketogenic group. Table S25. Food reintroduction plan for the ketogenic group. Table S26. Baseline comparison between MFP participants and the 32 closest matched participants from a prolonged fasting cohort. Table S27. Participant metadata, reported in a de-identified format. Table S28. Clinical data of study participants, reported in a de-identified format. Table S29. Responses to daily questions. Table S30. Responses to lifestyle questions. Table S31. Laboratory parameters. Table S32. Serum metabolite concentrations identified by NMR spectroscopy. Table S33. Gut microbiota sequencing characteristics across timepoints. Table S34. Gut microbiota composition profiles

Additional file 2: Study protocol

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Authors' contributions
FG, FWT, and RM conceptualized and designed the study. AH contributed to statistical analyses, with support from RM. SS, SH and RG performed the metabolomics analysis. QRD and GZ performed the shotgun metagenomics analysis. BMT and MRM contributed to data analyses and interpretations. RM, FG and FWT drafted the initial version manuscript. RM wrote the final version of the manuscript. All authors read and approved the final manuscript.

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Data availability
The code used to perform the statistical analysis is available on Zenodo [49]. Raw metagenomic data is available under accession number PRJEB75353 at ENA [50]. This study did not generate new unique reagents. The individual-level clinical data allowing age, sex and BMI adjusted statistical analyses are not publicly available because they contain potentially identifiable participant information. This data is available from the corresponding author upon reasonable request, subject to ethics approval, participant consent, and a data transfer agreement.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of the Medical Association of Baden-Württemberg, Stuttgart, Germany (approval number: F-2023-014). The study was registered at ClinicalTrials.gov (NCT05821660) prior to the start of patient recruitment. The study was conducted in accordance with the principles of the Declaration of Helsinki and the standards of Good Clinical Practice. Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
Authors FG, RM, AH and FWT are employees of the Buchinger Wilhelmi Development and Holding GmbH. Authors SS, SH, and RG are employees of Lifespin GmbH. The remaining authors declare no competing interests.

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