The effect of green Mediterranean diet on intrahepatic fat; The DIRECT PLUS randomized controlled trial

Supplemental Material

Supplemental Methods: Exclusion criteria
Exclusion criteria were an inability to partake in physical activity (PA), a serum creatinine level≥2mg/dL, disturbed liver function, a major illness that might require hospitalization, pregnancy or lactation for women, presence of active cancer or undergoing chemotherapy either at present or in the prior three years, participation in another trial, chronic treatment with warfarin (given its interaction with vitamin K), and being implanted with a pacemaker or platinum implant (due to inability to undergo magnetic resonance imaging included in the study design).

Supplemental Methods 2: Physical activity protocol
The aerobic effort increased gradually, starting with 20 minutes of aerobic training at 65% maximum heart rate, and increased to 45-60 minutes of aerobic training at 80% of maximum heart rate. The full workout program included 45-60 minutes of aerobic training 3-4 times/week; resistance training starting with one set of weights corresponding to 60% of the maximum weight, eventually reached the use of two sets of weights corresponding to 80% of the maximum weight. The resistance training included leg extensions, leg curls, squats, lateral pull-downs, push-ups, shoulder presses, elbow flexions, triceps extensions, and bent leg sit-ups.

Supplemental Methods 3: Provided polyphenol-rich products
Walnuts [groups Mediterranean (MED), green-MED]: The main polyphenols in walnuts are ellagitannins, ellagic acid, and its derivatives [1]. Walnuts are considered to have a beneficial effect on health maintenance and disease prevention [2]. In addition, Ellagitannin found in nuts was reported to reduce waist circumference (WC), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) [3].
Green tea (group green-MED)**:** an unfermented tea produced from the leaves of *Camellia sinensis,* is prepared by drying and steaming the leaves and is a rich source of polyphenols [4]. Most of the polyphenols found in green tea are Catechins (the monomer form of flavanols), mainly epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [5,6]. Short-term (weeks long) intervention studies and meta-analyses have found an association between administrating green tea or its extracts and improvement in cardiometabolic health [7,8], weight reduction [6], and improved cognitive function [9,10].
Wolffia globosa duckweed - Mankai (group green-MED): A specific strain of *Wolffia globosa,* an aquatic plant, which can serve as a plant protein source. In Asian cuisines, *Wolffia globosa* (Mankai cultivated strain) is considered a natural food source or "vegetable meatball" [11]. Nutritionally, Mankai is characterized by high protein content (more than 45% of the dry matter) and the presence of 9 essential and 6 conditional amino acids [12]. In addition, it is a good source of omega-3 fatty acids [13]. The Mankai plant is rich in non-soluble fibers, iron, vitamins, minerals [14], and polyphenols, including catechins, caffeic acid, apigenin, quercetin, naringenin, and kaempferol [15,16]. Mankai provides bioavailable essential amino acids [12], iron [17], vitamin B12 [18], and has beneficial effects on postprandial and fasting glycemic control [19]. We guided the participants to prepare a green Mankai shake with additional ingredients, which also were part of the diet regimen (fruits,

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walnuts, or vegetables) each evening. The green protein shake partially substituted for dinner, replacing beef/poultry protein sources.

Supplemental Methods 4: Lifestyle sessions and motivation techniques
The lifestyle interventions included 90-minute nutritional and PA sessions in the workplace with multidisciplinary guidance (physicians, clinical dietitians, and fitness instructors). These sessions were held every week during the first month, once a month, over the following five months, and every other month until the 18th month. All the lifestyle educational programs were provided at the same intensity to all three groups. To keep the participants motivated, text messages with relevant information for each assigned intervention group were sent on fixed time intervals. In addition, a website listing all nutritional and PA information needed by the participants to continue with the intervention was accessible to the participants according to their intervention group.

Supplemental Methods 5: H-MRS by Magnetic Resonance scanner
In order to quantify and follow intrahepatic (IHF%) changes, we used H-MRS, a reliable tool for liver fat quantification [20]. Localized, single-voxel proton spectra were acquired using a 3.0T magnetic resonance scanner (Philips Ingenia, Best, The Netherlands). The measurements were taken from the right frontal lobe of the liver, with a location determined individually for each subject using a surface, receive-only phased-array coil. Spectra with and without water suppression were acquired using the single-voxel stimulated echo acquisition mode (STEAM) with the following parameters: TR=4000msec, TE=9.0msec, and TM=16.0msec. The receiver bandwidth was 2000Hz, and the number of data points was 1024. Second-order shimming was used. Four averages were taken in a single breath hold for an acquisition time of 16 sec. The voxel size varied somewhat according to anatomy but was approximately 50(AP) × 45(RL) × 54(FH) mm. Water suppression was achieved using the MOIST (Multiple Optimizations Insensitive Suppression Train) sequence consisting of four phase-modulated T1 and B1 insensitive pulses with a 50Hz window. Data analyzed using Mnova software (Mestrelab Research, Santiago de Compostela, Spain) by an experienced physicist blinded to the intervention groups, who also performed visual quality control of fitted spectra. The total hepatic fat fraction in the image was determined as the ratio between the sum of the area under all fat divided by the sum of the area under all fat and water peaks[21]. Inter-class reliability was tested between two different technicians and resulted in an average measure of r=0.99 ($p<0.001$). Intra-class reliability was tested among all baseline scans and resulted in an average measure of r=0.96 ($p<0.001$). Liver fat color images were produced using PRIDE software (by Philips).

Supplemental Methods 6: Further laboratory methodology, anthropometric measurements, lifestyle, plasma polyphenol assessments, and risk scores calculations
Anthropometric parameters and laboratory methodology
Measurements were taken at baseline, after 6 and 18 months of intervention. Height was measured to the nearest millimeter using a standard wall-mounted stadiometer. Bodyweight was measured without shoes to the nearest 0.1kg. WC was measured halfway between the last rib and the iliac crest to the nearest millimetre by standard procedures using an anthropometric measuring tape. Two blood pressure (BP) measurements and resting pulse were recorded after resting, using an automatic BP monitor (Accutorr-4, Datascope) and calculated as the mean of the two measurements taken. Blood samples were obtained at 8:00 AM after a 12-hour fast. The samples were centrifuged and stored at -80°C. Serum total cholesterol (TC; Coefficient-of-variation (CV), 1.3%), High-density lipoprotein cholesterol (HDL-c), LDL-c, and TG (CV, 2.1%) were determined enzymatically with a Cobas-6000

automatic analyzer (Roche). Plasma levels of high-sensitivity C-reactive protein (hsCRP) were measured by ELISA (DiaMed; CV, 1.9%). Plasma glucose levels were measured by Roche GLUC3 (hexokinase method). Plasma insulin levels were measured with an enzyme immunometric assay (Immulite automated analyzer, Diagnostic Products; CV, 2.5%). The homeostatic model of insulin resistance (HOMA IR) was calculated as follows: insulin(μIU/ml)×glucose(mg/dl)/405 [22]. All biochemical analyses were performed at the University of Leipzig, Germany.

Assessment of nutritional intake and lifestyle habits

Self-reported food frequency questionnaires were administered through a computer at baseline, after 6 months, and at the end of the trial [23,24], which included intake assessment of provided items. We followed overall changes in the intake of specific food groups, as described previously [25] and further used lifestyle and validated PA questionnaire [26]. PA intensity levels were measured using metabolic equivalent (MET) units [27].

Plasma polyphenols metabolites:

The determination of polyphenol metabolites was performed according to the method of Vrhovsek et al [28] with some modifications. Briefly, a previously developed targeted metabolomic method was performed with an ultra-performance liquid chromatographic system coupled to a tandem mass spectrometry system with electrospray ionization (UHPLC-ESI-MS/MS). Before injection, samples were thawed at 4 °C. Sample preparation was performed using an Ostro™ Pass-through 96-well plate to remove phospholipids and proteins (Waters, Milford, MA, USA). An Ostro™ 96-well plate was fixed on top of a 96-well collection plate. 50 μl of plasma were pipetted into the wells, followed by the addition of 1% formic acid in acetonitrile (3:1 solvent/sample). The mixture was then quickly shaken for 5 minutes to promote protein precipitation. Vacuum (15 in. (∼381 mm) Hg) was then applied to the Ostro plate through a vacuum manifold, filtering out the nonphospholipid plasma components. This step was repeated twice to ensure protein precipitation. Then, samples were dried under nitrogen and reconstituted in 100 μl of methanol: water (1:1, v/v), containing hippuric acid D5 (1 μg/ml) as an external standard. Samples were finally transferred to LC vials and injected (2 μL) into the UHPLC–MS/MS system. All solvents were kept at 4 °C prior to their use, and all procedures were carried out in a cold room, assuming that a 4 °C extraction temperature and the relatively short extraction time (10 min) may be favorable for avoiding biological sample degradation and reducing the risk of metabolite precipitation. Quality control (QC) samples were also prepared prior to analysis by pooling a small fraction of all the individual analyzed samples. Data processing was performed using Waters MassLynx 4.1 (Waters, Milford, CT, USA) and TargetLynx software (Waters, Milford, CT, USA). Details of the liquid chromatography and mass spectrometry are described in Vrhovsek et al[28] and Gasperotti et al [29]. The analysis was performed at the Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, Trento. Italy.

Fecal samples collection and 16s rRNA sequencing

Fecal samples were collected at baseline and 18 months at the study site, immediately frozen to –20°C for 1-3 days, then transferred to –80°C pending DNA extraction. Following extraction, samples were sequenced on a MiSeq platform following amplification of V3-V4 hypervariable region of the 16S rRNA gene using the primer set 341F/806R, and processed by the DADA2 pipeline. Rare OTUs (< 3% prevalence of all samples) were filtered out. Samples of participants prescribed antibiotic therapy 2 months prior to randomization and samples with less than 103 reads were excluded from the analysis. Analysis was performed at the Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, Trento. Italy.

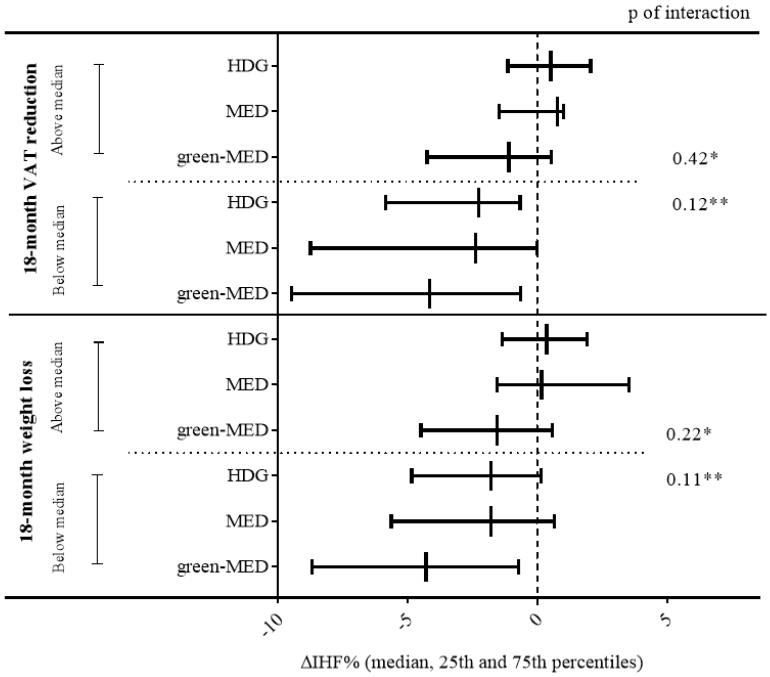
Supplemental Methods 7: Sample size calculations
We based the sample size calculation on the outcomes of a previous trial that resulted in a significant reduction in liver fat [30]: 6.7±6.1% reduction in the intervention group (increasing energy expenditure and reducing caloric intake) vs. 2.1±6.4% reduction in the control group (encouraged to reduce carbohydrate and fat intake and to engage in physical activity) with a 4.6 difference, pooled variance of 39.085. Calculation for the sample size needed for this trial, with a 5% α and a 90% power, suggested 39 participants in each intervention group. Considering a 14% expected dropout rate (based on our previous CENTRAL trial [31]), in order to detect differences between intervention groups, we needed a number of 45 participants in each group, and ultimately recruited a number of 98 participants per group (~90 in each group with a valid MRI scan). Sample size calculations were performed using Winpepi software, version 11.6.

Supplemental Methods 8: Microbiome statistical analysis
Microbiome composition was assessed based on relative abundance. For composition change, a change matrix was generated by calculating the log2 ratio between 18m and baseline, for each taxa and each individual as follows: log2 (18m relative abundance/baseline relative abundance). Dissimilarity between samples was measured by the UniFrac distance. Associations between gut microbiome composition and IHF, and IHF% change was assessed by permutational multivariate analysis of variance (PERMANOVA) with the adonis function (R "vegan" package), and by comparing principle coordinate scores across IHF% tertiles. The assess the association between microbiome composition, lifestyle intervention and IHF%, the principle coordinate vector most highly correlated with IHF% was chosen. The mediation analysis was performed by employing the meditation analysis suggested by Imai et al. [32] by the 'mediate' package in R (https://cran.r-project.org/web/packages/mediation/mediation.pdf). Lifestyle intervention was considered a ranked variable, taking into account the gradual increase of polyphenols, and gradual decrease in red and processed meat across the groups.
For per-taxa analysis, we first aggregated all fetures to the genus level, and performed quality control filtering for taxonomic and functional features before including them in the subsequent analyses. To be qualified for downstream analyses, a taxonomic feature needed to be detected at a minimum relative abundance of 0.01% in at least 5% of samples. This analysis yielded 180 microbial species that met the criteria. We employed the R package MaAsLin 2 1.0.0 to perform per-feature tests taking into account the compositionality of the data and multiple testing (https://huttenhower.sph.harvard.edu/maaslin2). At first, features were selected by their association with ln transformed IHF at baseline. In the second phase features were selected by a model taking into account time, IHF and time*IHF interaction as fixed effects, with each individual as random effect. In the third phase, features were selected by a model taking into account group, time and time*group interaction as fixed effects, with each individual as random effect. All high-dimensional tests were corrected for multiple hypothesis testing by controlling the false discovery rate (FDR) using the Benjamini-Hochberg method with a target rate of 0.25 for q values estimated from the per-feature tests.

Supplemental Results 1: Further information regarding the adherence to the intervention
Among the green-MED group, the 18-month daily green tea consumption weighted average (accounting for the reported consumption after 6 months and the reported consumption after additional 12 months) was 2.8±1.6/day (median=2.7 cups/day, range 0-8.7) and the weekly consumption weighted average of Mankai was 2.6±1.8/week (median=2.3 shakes/week, range 0-7). Serum folate change was correlated with higher weekly Mankai intake ($r=0.41$, $p<0.001$), but not with daily green tea intake ($r=0.08$, $p=0.53$). In addition, the green-MED

and MED groups also similarly reduced their carbohydrates consumption (-27.9%±34.0 and -29.8%±31.3 respectively, over 18 months; p=0.72) as compared to the HDG group (p=0.03 vs. green-MED and p=0.01 vs. MED). Both MED groups also had similar daily walnuts amount (p=0.36) and monthly frequency of consumption (p=0.52) and were higher as compared with the HDG group (p≤0.001 HDG vs. the two MED groups).

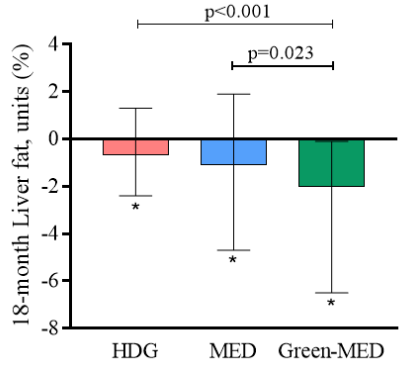
Supplemental Figure 1: Subgroup analysis of IHF% change by the degree of weight loss/VAT reduction



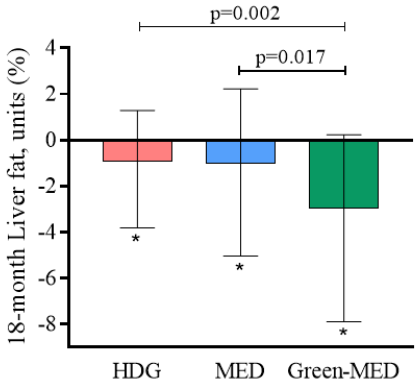
Date presented as medians, 25th and 75th percentiles of intrahepatic fat. No interactions between the degree of either weight loss or visceral adipose tissue reduction with either MED or green-MED diets were observed. Weight loss and visceral adipose tissue reductions categories are presented as above/below sex-specific median value of 18-month change. Models adjusted to age and baseline IHF. * p of interaction MED diet with weight/VAT degree of change. ** p of interaction green-MED diet with weight/VAT degree of change.

Supplemental Figure 2: A comparison between the per-protocol changes and intention to treat techniques for the changes in intrahepatic fat (IHF)

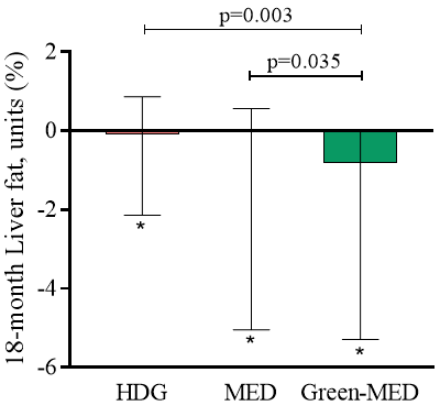
(a) Per-protocol analysis



(b) Multiple imputation (MI)

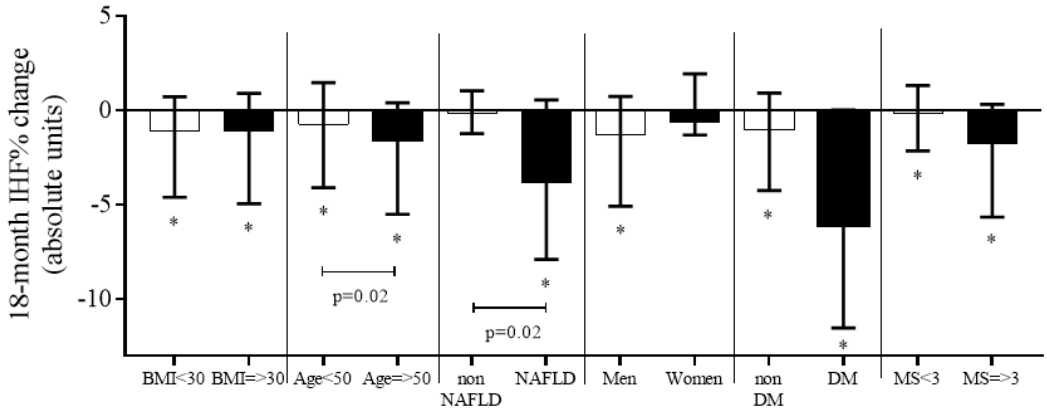


(c) Last observation carried forward (LOCF)



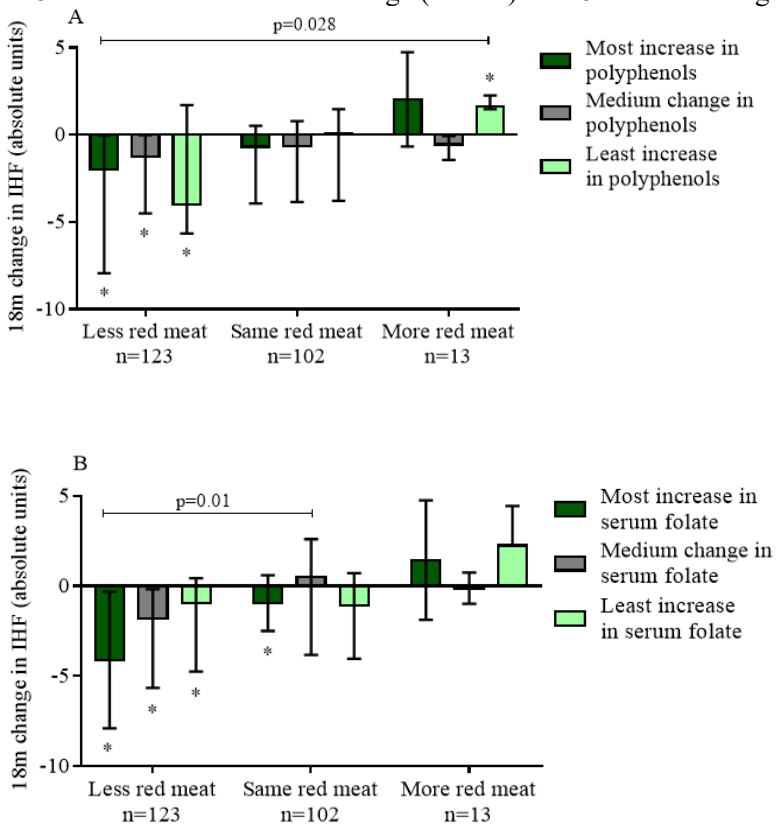
Date presented as medians, 25th and 75th percentiles of intrahepatic fat. Comparing the per-protocol results to 2 intention to treat techniques (multiple imputation and last observation carried forward) showed that while the significant differences between the intervention groups remained, the effect size (measured as the 18-month change in intrahepatic fat) was reduced, as compared to the per protocol-analysis. This is in accordance with previously published regarding the conservative nature and potential bias of the last observation carried forward technique [33].

Supplemental Figure 3: Subgroup analysis of differences in 18-month IHF% change (completers).



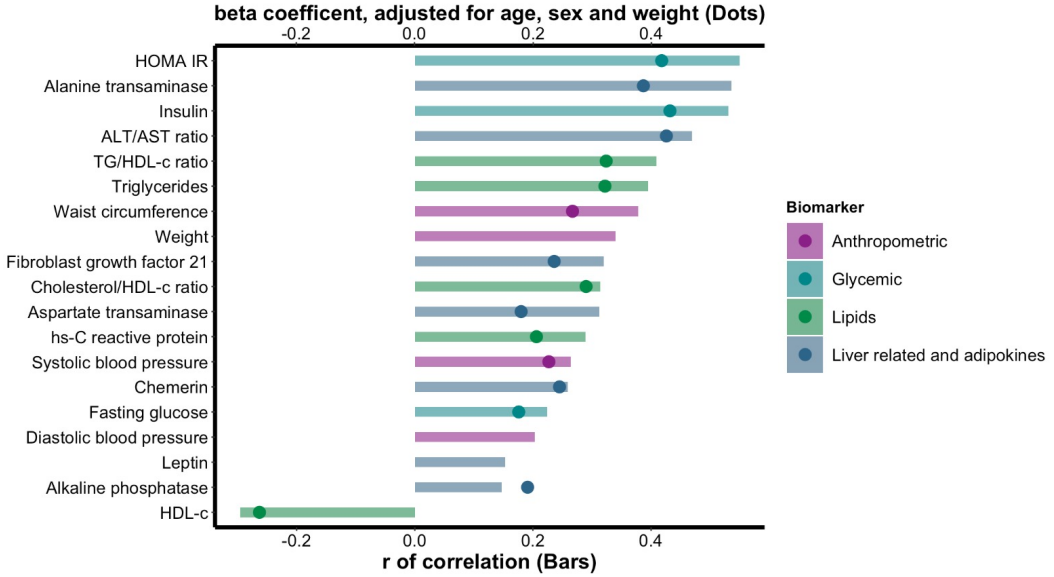
Date presented as medians, 25th and 75th percentiles of 18-month change in IHF. Generalized linear models adjusted for baseline IHF%, 18-month weight change, and intervention group (BMI subgroup differences are adjusted for baseline IHF% and intervention group). The presence of DM was defined for participants with baseline fasting plasma glucose levels≥126mg/dL or hemoglobin-A1c levels≥6.5% or if regularly treated with oral antihyperglycemic medications or exogenous insulin. MS criteria were assessed based on the National Cholesterol Education Program Adult Treatment Panel III criteria. BMI, body mass index; DM, diabetes mellitus; IHF, intrahepatic fat. MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease. * denotes significant difference within-group at $p<0.05$ level.

Supplemental Figure 4: (a) Red meat consumption change at T18 vs. 18-month total polyphenol change (tertiles) vs. 18m IHF% change. **(b)** Red meat consumption change at T18 vs. 18-month serum folic acid change (tertiles) vs. 18m IHF% change.



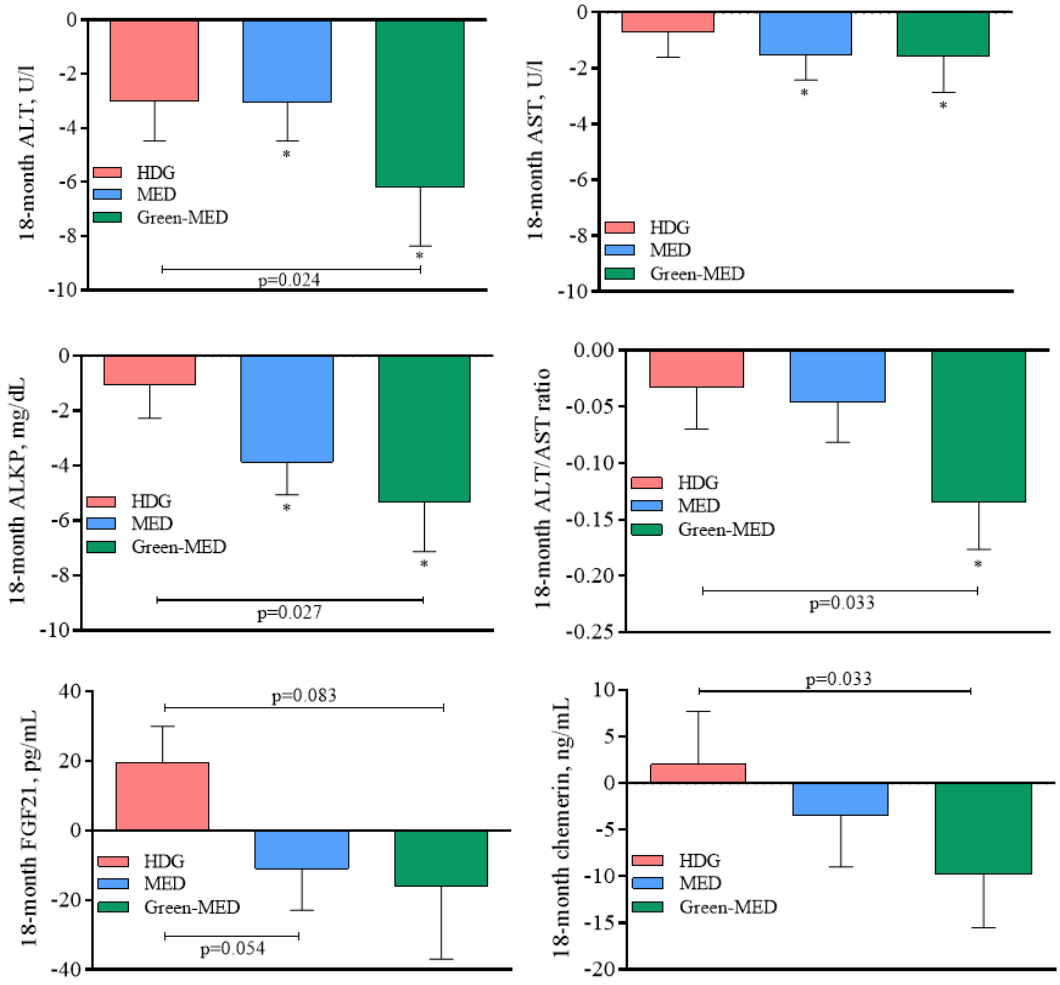
Date presented as medians, 25th and 75th percentiles of 18-month change in IHF. (a) Reductions in IHF% were significantly higher (-4.5±6.9% absolute units) in participants who both reduced red meat consumption and had the greatest increase in plasma polyphenols, as compared with participants who ate more red meat and had the least change in plasma polyphenols (2.11±1.4% absolute units; p=0.028 between groups). No interactions between tertiles of plasma polyphenol change and red meat change were observed. (b) IHF% reduction was greater among participants who reduced red meat and increased serum folate (-4.7±6.6%), as compared with participants who did not change red meat intake and had at least a moderate change in serum folate level (-1.8±7.5%). No interactions between tertiles of tertiles of serum folate change and red meat change. *significant within group, T0 vs. T18. Between-group p values are corrected for multiple comparisons. IHF, intrahepatic fat.

Supplemental Figure 5: Associations of intrahepatic fat with cardiometabolic markers



At baseline, IHF% levels were correlated with anthropometric parameters, blood pressure, glycemic, lipid, and liver related markers (presented as bars, $p<0.05$ for all). When adjusted for baseline body weight, age, and sex (presented as dots on the bars), higher IHF% remained significantly associated with higher WC, systolic BP, fasting blood glucose, insulin, HOMA-IR, TG, TG/HDL-c ratio, TC/HDL-c ratio, hs-CRP, ALKP, ALT, AST, ALT/AST ratio, FGF21 and chemerin ($p<0.05$ for all). IHF% was found to be inversely associated with HDL-c ($p<0.001$). AST, aspartate transaminase; ALT, alanine transaminase; BP, blood pressure; HDLc, high density lipoprotein cholesterol; HOMA IR, Homeostatic Model Assessment of Insulin Resistance; IHF, intrahepatic fat; TG, triglycerides.

Supplemental Figure 6: 18-month change in liver related blood biomarkers, across intervention groups – for participants with valid MRI scans.



Between group p-values are adjusted for age, sex, and baseline level of the biomarker in interest, using General Linear Model. * significant within group as compared with baseline levels ($p<0.05$). AST, aspartate transaminase; ALT, alanine transaminase; ALKP, alkaline phosphatase; HDG, healthy dietary guidance; MED, Mediterranean.

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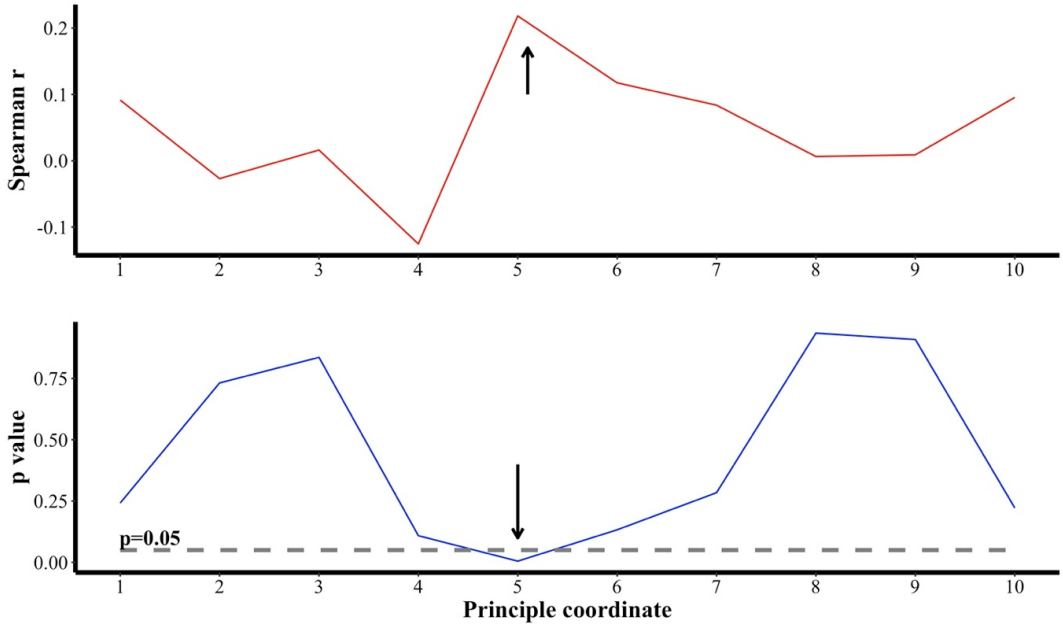
Supplemental Figure 7: Association between the top 10 principle coordinates (based on UniFrac distance of the log2 change matrix) and IHF change (Ln transformed).



Spearman correlation between IHF change and top 10 principle coordinates (x axis). Top panel shows the correlation coefficient (red line) and bottom panel shows p value (blue line). Arrows point to the most highly correlated principal coordinate (#5; r=0.25,p=0.001).

Supplemental Table 1: Outline of the lifestyle interventions

	HDG	MED	Green-MED
Physical activity	18 months free gym membership 45-60 minutes of aerobic training + resistance training, 3-4 times/week.		
Lifestyle group sessions	18-months group sessions in the workplace, weekly for the first month, and monthly thereafter.		
General dietary guidance	Limit dietary cholesterol, trans-fat, saturated-fat, sugars, and salt and increase intake of vegetables		
Energy, kcal/day	Guidelines for a healthy MED diet with no specific recipes or calorie restriction	1500-1800 kcal/day for men, 1200-1400 kcal/day for women	
Total fat, % of daily consumption		~40% mainly PUFA and MUFA	
Carbohydrates, gr/day		Less than 40 gr/day in the first 2 months with increased gradual intake for up to 80 gr/day	
Specific recommendations		Less/Avoid red and processed meats. Reduced poultry intake	
Polyphenols, mg/day		+440 mg/day [source: provided walnuts (28g/day]	+1240 mg/day [source: provided walnuts (28 g/day), green tea (3-4 cups/day), Wolffia globosa duckweed (Mankai) shake (100 g/day frozen cubes)]

Supplemental Table 2: Lifestyle habits and medications, DIRECT PLUS population, baseline

	HDG (n=98)	**MED (n=98)**	**Green-MED (n=98)**	**All (n=294)**	***p* between groups[1]**
Smoking, %	19.4	13.3	16.3	16.3	0.51
Shift workers, %	22.4	23.4	23.4	23.1	0.98
PA intensity[2], METs/week	18.4 (11,41)	29.1 (16,50)	24.4 (13,38)	23.5 (12,42)	0.08
Alcohol[2], servings/day	0.2 (0.05,0.03)	0.2 (0.05,0.3)	0.2 (0.05,0.3)	0.16 (0.05,0.3)	0.75
Chronic Pharmacotherapy					
Anti-hypertensive, %	14.3	11.2	16.3	13.9	0.59
Cholesterol lowering, %	11.2	8.2	14.3	11.2	0.40
Anti-platelet, %	7.1	3.1	9.2	6.5	0.21
Exogenous insulin, %	1.0	1.0	3.1	1.7	0.27
Oral anti-hyperglycemic, %	6.1	4.1	8.2	6.1	0.49

[1] according to the chi-square test, except for MET and alcohol intake (assessed by Kruskal-Wallis test). [2] Median (25th and 75th percentiles). HDG, healthy dietary guidelines; MED, Mediterranean.

Supplemental Table 3: Baseline and 18-month changes in reported dietary intake across intervention groups

	HDG	MED	Green-MED	p between groups
Macronutrients				
Energy				
Energy at baseline (kcal/day)	2193±1180.7	2200.2±1119.3	2065.9±955.8	0.63
Energy change from baseline (kcal/day)	-336±1046	-666±1021	-544±975	0.73
change from baseline, %	-11.6±42.9	-23±27.6	-20±32.0	0.17
Total carbohydrates				
% of energy at baseline	45.3±7.0	44.4±8.5	46.3±7.6	0.24
Change in g/d from baseline, %	-14.0±36.4	-29.8±31.3	-27.9±34.0	**0.003**
Protein				
% of energy at baseline	20.6±4.0	20.9±4.6	19.9±3.9	0.29
% change out of total energy intake	-5.8±58.3	-12.8±38.4	-14.3±40.2	0.6
Total fat				
% of energy at baseline	34.5±4.5	35.2±4.7	34.5±5.23	0.45
Change in g/d from baseline, %	-7.1±58.5	-15.7±32.0	-10.9±38.4	0.84
Baseline intake of food items, g/day[1]				
Red meat	37.8±31.5	41.8±31.3	40.6±38.7	0.58
Processed meat	13.2±16.6	12.7±12.4	12.3±11.6	0.99
Fish	24.0±22.2	26.6±35.8	19.8±12.7	0.65
Poultry	173.7±138.8	180.4±132.1	152.0±117.7	0.22
Eggs	33.2±28.0	32.0±25.3	33.9±25.9	0.88
Dairy	292.8±250.8	354.7±330.6	286.3±231.0	0.27
Tea (any type)	295.4±380.9	339.5±347.8	327.6±400.4	0.39
Nuts	7.8±9.3	8.6±12.9	8.1±8.6	0.6
Food change frequency[2]				
Red meat				
More	5.9	6.7	3.8	**0.012**
Same	52.9	46.7	28.2	
Less	41.2	46.7	67.9	
Processed meat				
More	0	0	0	

Same	37.6	34.7	28.2	0.43
Less	62.4	65.3	71.8	
Fish				
More	63.5	69.3	74.4	0.55
Same	35.3	28.0	24.4	
Less	1.2	2.7	1.3	
Poultry				
More	27.1	41.3	26.9	**0.002**
Same	61.2	52.0	46.2	
Less	11.8	6.7	26.9	
Eggs and dairy				
More	15.3	36.0	35.9	**0.02**
Same	72.9	56.0	53.8	
Less	11.8	8.0	10.3	
Green Tea				
More	31.8	37.3	82.1	**<0.001**
Same	58.8	53.3	15.4	
Less	9.4	9.2	2.6	
Nuts				
More	32.9	60.0	56.4	**<0.001**
Same	61.2	30.7	28.2	
Less	5.9	9.3	15.4	

Data are means ± Standard deviations for continuous parameters and percentage for categorical parameters. [1] Data extracted from reported food intake, not part of a recipe. [2] Self-reported as compared with the last food change questionnaire.

Supplemental Table 4: Weekly walnut consumption and frequency (reported, time 18):

	HDG	MED	Green-MED	p between groups[1]	p between MED groups[1]
Amount of consumption					
Few	53	16	11	<0.001	0.36
28 g/day	23	46	56		
Bunch	8	11	9		
Frequency of consumption					
less than once a month or never	25	7	6		
1-3 times/month	21	14	8		
1-2 times/week	19	12	10	<0.001	0.52
3-4 times/week	8	8	14		
5-6 times/week	4	13	12		
every day	3	12	19		
2-3 times/day	4	6	7		
4-5 times/day	0	1	0		

[1] Test by Chi-Square test. HDG, healthy dietary guidelines; MED, Mediterranean.

Supplemental Table 5: Genus level bacteria associated with IHF, IHF change and lifestyle intervention.

Baseline associations	Genus Level bacteria	Coefficient	Standard error	p value	q value
IHF	Fournierella	-0.0003709	7.65E-05	2.33E-06	0.00041326
IHF	Anaerosporobacter	-0.0002198	6.36E-05	0.00064969	0.05041708
IHF	Lachnospiraceae_UCG-003	-0.0001376	4.07E-05	0.00085453	0.05041708
IHF	Ruminococcaceae_UCG-009	-5.34E-05	1.77E-05	0.00276759	0.12246594
IHF	Ruminococcaceae_UCG-014	-0.0043576	0.00155184	0.00542229	0.19194916
IHF	Erysipelotrichaceae_UCG-003	0.00154705	0.00059427	0.00984842	0.21805458
IHF	Ruminococcaceae_UCG-008	-0.0001195	4.59E-05	0.00985557	0.21805458
IHF	Ruminococcaceae_UCG-010	-0.0008363	0.0003195	0.00945633	0.21805458
IHF	Ruminococcaceae_UCG-002	-0.0040148	0.00158245	0.01185459	0.23314025
18-month change					
IHF	Erysipelotrichaceae_UCG-003	0.04734104	0.01450464	0.00119285	0.01610349
IHF	Fournierella	-0.0058954	0.00173597	0.00075311	0.01610349
IHF	Anaerosporobacter	-0.006328	0.00226281	0.00541248	0.04517776
Time*IHF interaction	Fournierella	0.00466757	0.00185459	0.01254249	0.05707647
Time*IHF interaction	Ruminococcaceae_UCG-008	-0.0090038	0.0038452	0.01982263	0.07645871
IHF	Ruminococcaceae_UCG-010	-0.0079535	0.00435396	0.06847972	0.16808659
IHF	Lachnospiraceae_UCG-003	-0.0033651	0.00179764	0.06193927	0.16808659
Time*IHF interaction	Lachnospiraceae_UCG-003	0.0043736	0.00223972	0.05182528	0.16808659
Time*IHF interaction	Anaerosporobacter	0.00495938	0.00278814	0.07676383	0.17271863
Time*IHF interaction	Ruminococcaceae_UCG-009	0.00576984	0.00336781	0.08760905	0.18195725
IHF	Ruminococcaceae_UCG-014	-0.0216027	0.01302554	0.09799635	0.18899296
Change by Lifestyle intervention					
time*Green-MED interaction	Fournierella	-0.0298969	0.01024317	0.00389043	0.15561715

time*MED interaction	Ruminococcaceae_UCG-014	0.10581789	0.04643635	0.02362381	0.18899047
time*Green-MED interaction	Ruminococcaceae_UCG-014	0.09732996	0.04785686	0.04316678	0.21583388

IHF, intrahepatic fat; MED, Mediterranean.

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