Research Article
Genetic and Metabolic Diseases

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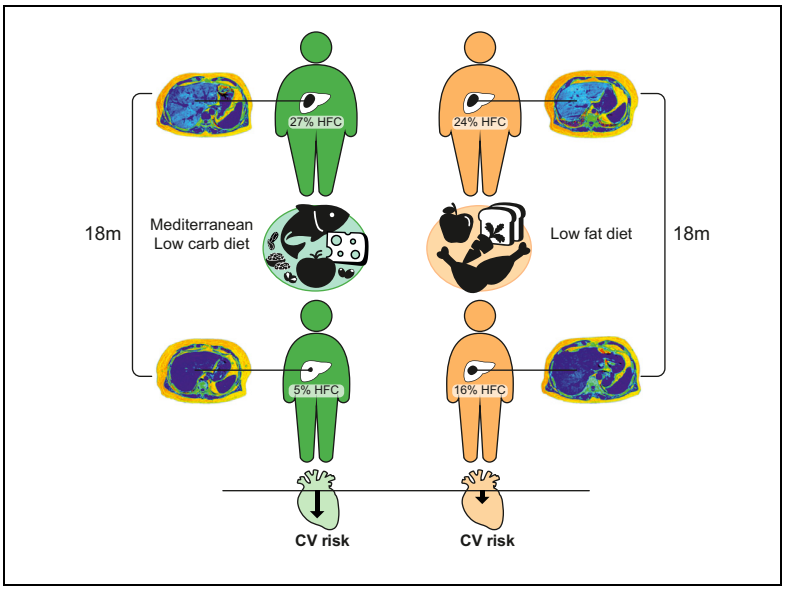
Graphical abstract



Highlights

- A Mediterranean and low carbohydrate diet decreases hepatic fat more than a low-fat diet, beyond visceral fat changes.
- Decreases in hepatic fat are independently associated with specific improved parameters.
- The beneficial effect of a Mediterranean diet over a low-fat diet is mainly mediated by decreases in hepatic fat.

Authors

Yftach Gepner, Ilan Shelef, Oded Komy, ..., Meir J. Stampfer, Assaf Rudich, Iris Shai

Correspondence

irish@bgu.ac.il
(I. Shai)

Lay summary

High hepatic fat content is associated with metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease. In the CENTRAL 18-month intervention trial, a Mediterranean/low-carbohydrate diet induced a greater decrease in hepatic fat content than a low-fat diet, conferring beneficial health effects that were beyond the favorable effects of visceral fat loss.

http://dx.doi.org/10.1016/j.jhep.2019.04.013
 J. Hepatol. 2019, 71, 379–388

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The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content

Yftach Gepner[1,2,†], Ilan Shelef[3,†], Oded Komy[1], Noa Cohen[1], Dan Schwarzfuchs[3,4], Nitzan Bril[1], Michal Rein[1], Dana Serfaty[1], Shira Kenigsbuch[1], Hila Zelicha[1], Anat Yaskolka Meir[1], Lilac Tene[1], Avital Bilitzky[1], Gal Tsaban[1], Yoash Chassidim[3], Benjamin Sarusy[4], Uta Ceglarek[5], Joachim Thiery[5], Michael Stumvoll[5], Matthias Blüher[5], Meir J. Stampfer[6], Assaf Rudich[1], Iris Shai[1,*]

[1]*Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel;* [2]*Department of Epidemiology and Preventive Medicine, School of Public Health, Sackler Faculty of Medicine and Sylvan Adams Sports Institute, Tel Aviv University, Israel;* [3]*Soroka University Medical Center, Beer-Sheva, Israel;* [4]*Nuclear Research Center-Negev, Dimona, Israel;* [5]*Department of Medicine, University of Leipzig, Germany;* [6]*Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA, USA*

See Editorial, pages 249–251

Background & Aim: It is unclear if a reduction in hepatic fat content (HFC) is a major mediator of the cardiometabolic benefit of lifestyle intervention, and whether it has prognostic significance beyond the loss of visceral adipose tissue (VAT). In the present sub-study, we hypothesized that HFC loss in response to dietary interventions induces specific beneficial effects independently of VAT changes.

Methods: In an 18-month weight-loss trial, 278 participants with abdominal obesity/dyslipidemia were randomized to low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC + 28 g walnuts/day) diets with/without moderate physical activity. HFC and abdominal fat-depots were measured using magnetic resonance imaging at baseline, after 6 (sub-study, n = 158) and 18 months.

Results: Of 278 participants (mean HFC 10.2% [range: 0.01%–50.4%]), the retention rate was 86.3%. The %HFC substantially decreased after 6 months (−6.6% absolute units [−41% relatively]) and 18 months (−4.0% absolute units [−29% relatively]; p <0.001 *vs.* baseline). Reductions of HFC were associated with decreases in VAT beyond weight loss. After controlling for VAT loss, decreased %HFC remained independently associated with reductions in serum gamma glutamyltransferase and alanine aminotransferase, circulating chemerin, and glycated hemoglobin (p <0.05). While the reduction in HFC was similar between physical activity groups, MED/LC induced a greater %HFC decrease (p = 0.036) and greater improvements in cardiometabolic risk parameters (p <0.05) than the LF diet, even after controlling for VAT changes. Yet, the greater improvements in cardiometabolic risk parameters induced by MED/LC were all markedly attenuated when controlling for HFC changes.

Conclusions: %HFC is substantially reduced by diet-induced moderate weight loss and is more effectively reduced by the MED/LC diet than the LF diet, independently of VAT changes. The beneficial effects of the MED/LC diet on specific cardiometabolic parameters appear to be mediated more by decreases in %HFC than VAT loss.

Lay summary: High hepatic fat content is associated with metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease. In the CENTRAL 18-month intervention trial, a Mediterranean/low-carbohydrate diet induced a greater decrease in hepatic fat content than a low-fat diet, conferring beneficial health effects that were beyond the favorable effects of visceral fat loss.

ClinicalTrials.gov Identifier: NCT01530724.

Introduction

Beyond total body fat content, fat distribution, both within adipose tissue depots and in ectopic fat deposits, is increasingly being shown to determine obesity-related health impact.[1,2] Visceral adipose tissue (VAT), due to its unique anatomical location, releases free fatty acids (FFAs) and adipokines to the liver via the portal vein. Previous studies have demonstrated the inter-relationship between VAT and hepatic fat content (HFC), and indeed, increases in HFC were associated with similar metabolic abnormalities as observed for increases in VAT.[3,4] In addition, reductions in VAT and HFC are increasingly thought to mediate the beneficial cardiometabolic outcomes of weight loss.[1,5] Though closely associated with HFC, VAT and HFC may uniquely associate with specific effects and be linked independently with risk factors of cardiometabolic disease.[6] Interestingly, data from recent studies found that HFC was more strongly associated with obesity's metabolic complications than VAT,[7] including the deterioration of glucose tolerance,[8] possibly by mediating the link between obesity and metabolic dysfunc-

Keywords: Hepatic fat content; Visceral adipose tissue; Diet; Lifestyle; Clinical trial; Low carbohydrate; Cardiovascular risk; NAFLD.
Received 11 September 2018; received in revised form 26 March 2019; accepted 17 April 2019; available online 8 May 2019

* Corresponding author. Address: The S. Daniel Abraham International Center for Health and Nutrition, Department of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel. Tel.: +972 8 647 7449/3; fax: +972 8 647 7637/8.
E-mail address: irish@bgu.ac.il (I. Shai).
† Equal contribution.

tion.[9,10] Most recently, the decrease in HFC was associated with diabetes remission.[11]

Diet plays an important role in the accumulation of HFC and VAT.[12] Several short-[6,13] and long-term[14,15] dietary interventions have suggested that Mediterranean and low-carb diets had favorable effects on VAT and HFC accumulation, but also on glycemic status and lipid biomarkers. Others found no differences between HFC changes induced by diets with different amounts of carbohydrate.[16] The effect of specific long-term lifestyle interventions on HFC and its association with the dynamics of cardiometabolic risk, beyond VAT loss, remain unclear. Notably, recent guidelines for decreasing HFC do not suggest a particular lifestyle strategy, but only endorse weight loss as a general recommendation.[17]

In the present sub-study, we hypothesized that, similarly hypocaloric, low-fat (LF) and Mediterranean/low-carbohydrate diets differ in their capacity to induce HFC loss, which mediates the improvements in cardiometabolic parameters independently of the impact of accompanying decreases in VAT.

Materials and methods

The CENTRAL trial (ClinicalTrials.gov Identifier: NCT01530724) was an 18-month randomized controlled trial.[18] In the first randomization, participants were randomly assigned to 1 of 2 calorie-restricted diets for the entire study period: an LF diet[19] (n = 139) or a Mediterranean/low-carbohydrate (MED/LC) diet[15] (n = 139). In the second randomization, 6 months after initiating the dietary intervention, each diet group was further randomized into added physical activity (PA) groups (LF^{PA+}, MED/LC^{PA+}) or no added PA groups (LF^{PA-}, MED/LC^{PA-}) for another 12 months of intervention (diets were continued throughout, according to the first randomized dietary assignment). This 2-stage study design was based on our previous results from the DIRECT study,[15] in which the dietary weight loss was maximal by 6 months (“rapid weight loss phase”). Participants were randomized after all had been recruited, in 1 phase, and after their strata characteristics were defined.[18] The intervention was conducted at a facility with a workplace medical clinic and monitored cafeteria.

Eligibility and study design

Inclusion criteria were: abdominal obesity (waist circumference [WC] >40 inches [102 cm] for men and >35 inches [88 cm] for women), or participants with triglycerides ≥150 mg/dl and high-density lipoprotein (HDL) <40 mg/dl for men and <50 mg/dl for women. Candidates were excluded if: they had serum creatinine ≥2 mg/dl, impaired liver function (≥3-fold the upper level of alanine aminotransferase [ALT] and aspartate aminotransferase), or active cancer; they were pregnant or lactating women, highly physically active (>3 h/week) or unable to take part in PA, or if they had participated in another trial. The study protocol was approved by the Medical Ethics Board and the Helsinki Committee of the Soroka University Medical Center. All participants provided written informed consent and received no financial compensation or gifts.

Diet intervention

Both diets aimed for a moderate, long-term, weight loss with restricted intake of trans-fats and refined carbohydrates, and an increased intake of vegetables. Lunch was provided exclusively by the workplace cafeteria during the working week, and a dietitian worked closely with the kitchen staff to adjust the diets to the specific groups. The 18-month dietary intervention included a 90-min nutritional session in the workplace with clinical dietitians every week during the first month of the intervention, and every month thereafter. Participants were trained to adhere to their specific diets during the entire week. For the LF diet, the aim was to limit total fat intake to 30% of calories, with up to 10% of saturated fat, and no more than 300 mg/day of cholesterol, and to increase dietary fiber. Participants were counseled to consume whole grains, vegetables, fruits, and legumes and to limit their consumption of additional fats, sweets, and high-fat snacks. The MED/LC diet combined the Mediterranean and low-carbohydrate diets described in our previous weight-loss trial (the DIRECT trial).[15] The diet restricted carbohydrate intake to less than 40 g/day in the first 2 months (induction phase), and thereafter a gradual increase up to 70 g/day, and increased protein and fat intake, according to the MED diet. The MED/LC diet was rich in vegetables and legumes and low in red meat, with poultry and fish replacing beef and lamb. This group was provided 28 g of walnuts/day (160 kcal/84% fat, mostly polyunsaturated fatty acids [omega-3 α-linolenic acid]) starting from the third month after the induction phase.

Physical activity intervention

At the second randomization (after 6 months of dietary intervention), participants assigned to added PA received a free supervised gym membership for the following 12 months. The exercise intervention included monthly educational workshops, and 1 h of exercise, 3 times a week. Participants were guided to engage in 45 min of aerobic training at 80% of maximum heart rate and 15 min of resistance training at 80% of the 1-repetition maximum of the weight.

Magnetic resonance imaging outcomes

Magnetic resonance imaging (MRI) was performed using a 3-Tesla magnet (Ingenia 3.0 T, Philips Healthcare, Best, the Netherlands) at baseline, after 6 (only for 157 of participants, randomly selected) and 18 months. The scanner utilized a 3D modified DIXON (mDIXON) imaging technique without gaps (2 mm thickness and 2 mm of spacing).[18] The percentage of HFC was assessed in a defined area of 2,000 mm^2, using a regions of interest (ROI) method[20] based on measurements of tissue signals (fat/fat + water) using the Fat Ratio Calculation PRIDE software from Philips Medical Systems. We analyzed the liver in 2-dimensional 3 cm intervals, referring to each image as a “slice”. The number of ROIs in each slice was determined proportionally to the image area. We divided each slice into quarters, and chose ROIs in each of the four-quarters in order to represent the entire liver. We determined the mean percentage of fat for each slice and quarter, and then calculated the mean percentage of fat of the liver as a whole. Fat quantification was performed blinded to time-point and treatment group. The reliability of measurements between technicians was measured in 28 images. Inter-observer correlation (2 independent observers) was r = 0.99, p <0.001 and intra-observer correlation was r = 0.98, p <0.0001. Abdominal fat was quantified using MATLAB-based semi-automatic software that was written in-house.[21,22] Three slices were selected from an intra-vertebral space of L5-S1, L4-L5 and L2-L3 and a continuous line was drawn over the superficialis fascia to differentiate between the deep subcutaneous adipose tissue (SAT) and superficial SAT. Mean VAT, deep SAT and superficial SAT were calculated from the 3 axial slices.

Clinical, metabolic and anthropometric outcomes

Height (±0.1 cm) was measured using a standard wall-mounted stadiometer. Body weight (±0.1 kg) was measured without shoes. WC (±0.1 cm) was measured half-way between the last rib and the iliac crest. Fasting blood samples were centrifuged and stored at −80 °C. All biomarkers were assayed in the Leipzig University laboratories, Germany. Fasting plasma glucose was measured by Roche GLUC 3 (hexokinase method). Plasma insulin was measured with an enzyme immunometric assay (IMMULITE® automated analyzer, Diagnostic Products, coefficient of variation [CV] = 2.5%). Serum total cholesterol (CV = 1.3%), HDL, low-density-lipoprotein, and triglycerides (CV = 2.1%) were determined enzymatically with a Cobas® 6000 automatic analyzer (Roche). Homeostasis model assessment of insulin resistance (HOMA-IR) were calculated using HOMA Calculator v2.2.3. Chemerin serum concentrations were determined using a commercially available ELISA kit (Human Chemerin ELISA, Biovendor, Heidelberg, Germany) according to the manufacturer's instructions.

Electronic questionnaires

Adherence to the dietary and PA interventions was evaluated using a validated electronic food-frequency and activity questionnaire (FFQ)[23] as published in our previews publication.[18] The FFQ contains 127 food items, 17 of them with 3 portion-size pictures, and lifestyle and PA questions, as well as symptoms, adverse-effects, quality of life, medication usage, and safety at baseline and after 6 and 18 months of intervention. The electronic questionnaires were self-administered and helped to ensure completeness of the data by prompting the participant when a question was not answered, and it permitted rapid automated reporting to the group dietitians.

Statistical analysis

The primary outcome of the key CENTRAL study,[18] as defined in clinicaltrials.gov, was change in body fat composition. In this sub-study, we aimed to address the influence of HFC reduction by lifestyle interventions, on improvement of cardiometabolic markers, beyond VAT loss. For the 18-month time point, we performed intention-to-treat analyses, including all 278 participants, by imputing the missing observations for all adipose tissues for 38 individuals by the multiple imputation technique.[24] No imputation has been performed for adipose tissue at 6-month time point (only 57%, randomly selected). For missing body weight data, we used the last observation carried forward. To characterize the entire study population, quantitative variables were expressed as means and standard deviations. All p values were 2-sided and p values <0.05 were considered statistically significant. Analysis of variance with a covariance test was used to assess changes in nutrient intakes between the dietary strategies. %HFC was ln-transformed at each time point, and the delta was calculated accordingly, allowing us to generate a normal distribution. At baseline, the association (p of trend) between %HFC and cardiometabolic risk parameters across sex-specific quintiles of VAT was tested, in order to estimate baseline relationships between these 2 main parameters of interest, using univariate linear regression (Table 1). Pearson's correlation coefficient was used between continuous variables. We used multivariate linear regression models to assess changes between diet groups in the dynamics of %HFC after 6 and 18 months, adjusted for age, sex, ln-transformed %HFC at baseline, WC (cm) at baseline, and VAT changes in order to identify independent intervention effects. The association between 18-month changes in %HFC and the dynamics of cardiometabolic risk parameters was tested by linear regression models adjusted for age, sex and the 4 intervention groups. Next, in separate models, we further adjusted for body weight or VAT changes. We calculated cardiovascular risk using 3 different scores: The Framingham risk score,[25] Systematic COronary Risk Evaluation (SCORE),[26] and the American College of Cardiology/American Heart Association (ACC/AHA)-pooled cohort equations.[27] The data were analyzed by SPSS software Version 23.

Results

Baseline characteristics

At baseline, participants (mean age = 48 years, 89% men, body mass index [BMI] = 30.8 ± 3.8 kg/m^2) had 10.2% HFC (median 6.38%), widely ranging between 0.01% and 50.4%. Of the 278 participants, 53% had non-alcoholic fatty liver disease (NAFLD) (HFC above 5%), 40% met the criteria for the metabolic syndrome, 75% had abnormal WC and 11% were diabetic. Few participants used medications chronically (anti-platelet = 7%, anti-hypertensive = 8%, lipid-lowering = 12%, oral glycemic-control = 3% and insulin treatment = 1%), with minor changes during the intervention that were similar between groups. Characteristics of the CENTRAL study population across intervention groups are shown in Table 1. There were no significant differences at baseline between the intervention groups in demographic variables, energy intake and consumption of macronutrients, blood markers, HFC, or abdominal fat sub-depots, but only in VAT area in females.

Dynamics of HFC throughout the intervention

In the entire cohort, HFC substantially decreased after 6 months (−6.6% absolute units [−41% relatively]) and 18 months (−4.0% absolute units [−29% relatively]) (p <0.001 *vs.* baseline), along with moderate body weight loss (−5.8% and −3.1% after 6 and 18 months, respectively). The 18-month retention rate was 86.3%. Decreased %HFC directly correlated with loss in all 3 layers of abdominal sub-depots after 6 and 18 months, when the models were adjusted for age and sex (p <0.001 for all). However, when the models were further adjusted for weight loss, decreased %HFC remained associated only with reduction of VAT at 6 months (β = 0.232; 95% CI 0.13–0.34) and 18 months (β = 0.155; 95% CI 0.04–0.31), but not with deep SAT or superficial SAT changes at 6 and 18 months (p >0.54 for all). After controlling for VAT changes, MED/LC diet tended to decrease %HFC more than LF diet after 6 months (MED/LC: −7.3 ± 9.2% *vs.* LF: −5.8 ± 7.2% [absolute units], p = 0.079 between diets), a differential effect between the dietary intervention groups that became significant at 18 months of intervention (MED/LC: −4.2 ± 7.1% *vs.* LF: −3.8 ± 6.7% [absolute units], p = 0.036 between diets). Furthermore, the advantageous effect of MED/LC on HFC reduction over LF diet was significant even in patients without NAFLD (HFC ≤5%, p = 0.037), as in patients with NAFLD (HFC >5%, p = 0.014). No significant differences were observed between the PA groups (p = 0.32) for HFC changes after 18 months, with or without adjustment for VAT changes. The changes in HFC over 18 months of intervention across different subgroups of the cohort are shown in Fig. 1. Higher HFC at baseline was found, as expected, in males (10.7% *vs.* 5.8%, p = 0.001), in participants with BMI ≥30 (12.7% *vs.* 7.0%, p <0.001) and in those with VAT >30% at baseline (11.5% *vs.* 8.0%, p = 0.007). The relative reductions of %HFC induced by the intervention were higher in males

Table 1. Baseline characteristics of the CENTRAL study population across the 4 intervention groups, n = 278.

	Intervention groups				
	Low-fat diet without physical activity (n = 76)	Low-fat diet with physical activity (n = 63)[#]	Mediterranean/low-carbohydrate without physical activity (n = 73)	Mediterranean/low-carbohydrate with physical activity (n = 66)[#]	All (n = 278)
Hepatic fat content (%)	10.8 ± 10.3	9.2 ± 9.0	10.1 ± 10.8	10.5 ± 11.3	10.2 ± 10.4
NAFLD patient (>5%), %	57	56	48	52	53
Age (yr)	49.3 ± 9.3	47.2 ± 9.0	47.0 ± 8.9	47.9 ± 9.8	47.8 ± 9.3
Male, %	84	92	85	95	89
Weight (kg)	90.6 ± 14.1	91.5 ± 12.8	91.6 ± 14.5	92.2 ± 11.9	91.4 ± 13.4
Waist circumference (cm)	105.6 ± 9.4	106.9 ± 8.5	106.4 ± 11.6	108.0 ± 8.5	106.7 ± 9.6
BMI (kg/m^2)	31.1 ± 3.9	30.4 ± 3.5	31.0 ± 4.5	31.0 ± 3.3	30.8 ± 3.8
Systolic pressure (mmHg)	125 ± 16	122 ± 13	124 ± 18	126 ± 16	124 ± 16
Diastolic pressure (mmHg)	79 ± 11	78 ± 10	81 ± 12	82 ± 11	80 ± 11
Fasting blood biomarkers					
Glucose (mg/dl)	106.4 ± 17.1	106.7 ± 18.2	107.4 ± 18.3	108.8 ± 18.3	107.3 ± 23.6
HOMA-IR	4.4 ± 2.6	4.7 ± 3.4	4.7 ± 3.8	4.5 ± 3.8	4.6 ± 2.7
Triglycerides (mg/dl)	71.8 ± 41.4	78.7 ± 44.4	73.5 ± 41.9	66.5 ± 41.9	72.6 ± 36.6
LDL (mg/dl)	123.2 ± 33.7	124.5 ± 29.9	120.6 ± 34.1	121.1 ± 34.1	122.3 ± 27.1
HDL (mg/dl)*					
Male	41.8 ± 11.7	41.2 ± 10.7	40.8 ± 9.0	42.8 ± 8.4	41.6 ± 10.0
Female	54.3 ± 15.2	58.1 ± 24.8	51.3 ± 15.8	63.6 ± 7.3	54.8 ± 16.4
Chol/HDL ratio	5.03 ± 1.90	5.23 ± 1.65	4.95 ± 1.46	4.76 ± 1.46	4.99 ± 1.58
ALP (IU/L)	73.8 ± 17.1	72.5 ± 22.4	70.9 ± 21.6	66.7 ± 21.6	71.0 ± 19.3
ALT (U/L)	25.3 ± 13.5	29.0 ± 24.7	25.8 ± 12.4	28.8 ± 12.4	27.1 ± 14.3
GGT (U/L)	25.8 ± 14.3	32.9 ± 24.9	26.9 ± 15.3	28.1 ± 15.3	28.4 ± 18.7
Chemerin (ng/ml)	189.5 ± 22.7	185.7 ± 19.0	185.7 ± 22.4	193.2 ± 22.4	188.5 ± 24.4
Leptin (mg/dl)*					
Male	11.1 ± 7.6	11.8 ± 6.2	11.3 ± 9.0	13.6 ± 7.9	11.9 ± 7.8
Female	41.1 ± 23.0	22.3 ± 6.1	33.7 ± 25.8	30.8 ± 1.3	34.4 ± 21.6
Adiponectin (mg/dl)	9.9 ± 9.4	10.0 ± 9.0	9.6 ± 8.3	13.2 ± 8.3	10.6 ± 12.4
Abdominal fat sub-depots					
Visceral fat (cm^2)*					
Male	180.0 ± 74.2	189.2 ± 62.2	168.0 ± 57.5	189.7 ± 61.4	181.6 ± 64.4
Female	159.7 ± 53.8	92.7 ± 37.9	118.2 ± 67.8	69.7 ± 27.6	125.4 ± 61.7^
Deep-SAT (cm^2)*					
Male	209.5 ± 70.4	219.9 ± 71.8	220.7 ± 87.3	223.0 ± 71.0	218.2 ± 75.2
Female	211.1 ± 52.5	177.2 ± 39.1	213.2 ± 91.7	166.8 ± 33.9	200.1 ± 66.3
Superficial-SAT (cm^2)*					
Male	130.4 ± 58.2	135.6 ± 50.0	133.9 ± 55.6	131.5 ± 47.1	132.8 ± 52.7
Female	224.3 ± 79.1	184.3 ± 44.5	243.0 ± 104.5	179.0 ± 34.2	220.1 ± 82.8

Values in the table are means ± SD. ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; Chol, total cholesterol; GGT, gamma glutamyl-transferase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model of insulin resistance; LDL, low-density lipoprotein; SAT, subcutaneous adipose tissue. One-way ANOVA test was used to assessed differences between groups at baseline.

* p <0.05 between gender groups.

^ p <0.05 between intervention groups.

[#] After 6-months of dietary the intervention (19 dropout), each diet group was further randomized into added physical activity groups or diet only for the last 12-months of intervention.

and in patients with BMI ≥30 or VAT ≥30%, even after controlling for 18-month VAT changes. Interestingly, in a model adjusted for weight loss, the beneficial effect of MED/LC diet over the LF diet was more apparent among males (p = 0.016) and in participants with VAT over 30% at baseline (p = 0.018), but similarly in both BMI groups. We further performed sensitivity analyses among the participants that completed both 6 and 18 months of MRI-body fat measurements (*i.e.*, 6-month sub-study), and a similar pattern was observed (data not shown). Similar results were also found when excluding participants using insulin.

Association between %HFC loss and nutritional intake changes

Overall, during the intervention, participants significantly decreased their energy intake after 6 and 18 months (p <0.001 *vs.* baseline), but similarly across diet groups (total calorie intake [−26% *vs.* −22% in the MED/LC diet *vs.* LF diet, respectively, p = 0.18]). Changes in the intake of marco- and micro-nutrients compared to baseline are shown in Fig. 2. After 18 months of intervention the MED/LC diet greatly decreased intake of carbohydrate and trans-fat, while the LF diet decreased the intake of total fat, monounsaturated fat and cholesterol, and tended to decrease polyunsaturated and saturated fats (p <0.05 for all, Fig. 2A). In addition, the MED/LC diet increased the consumption of nuts (p <0.05, Fig. 2B). Decrease of HFC after 18 months correlated with decreased carbohydrate intake (r = 0.175, p = 0.009), and with increased fat intake (r = −0.217, p = 0.001), as proportions of total calorie intake.

Association between HFC and cardiometabolic risk parameters

In the entire group, significant improvements in cardiometabolic markers were observed after 18 months of

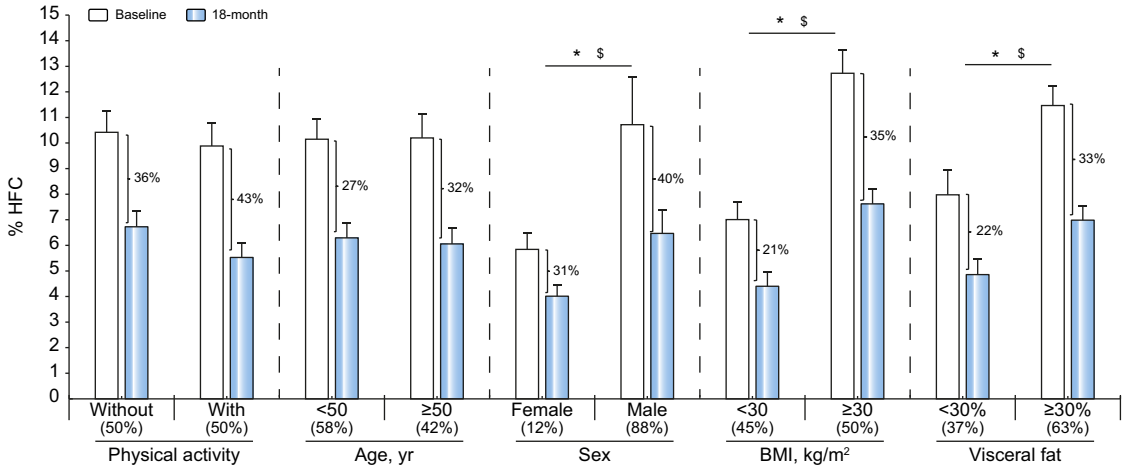

Fig. 1. Hepatic fat content at baseline and after 18 months of intervention by subgroups. Bar values in the fig. are means ± SE of HFC, at baseline and after 18-month of intervention. Numbers represent relative changes. Data were analyzed using a repeated measures ANOVA across all subgroups. *p <0.05 for models without adjustment. $^{\$}p$ <0.05 for models adjusted for visceral adipose tissue changes. Males and groups with higher BMI and visceral fat at baseline show larger decreases in HFC for both models. BMI, body mass index; HFC, hepatic fat content.

intervention, including decreases in total cholesterol/HDL ratio by −0.3 (4.4%), gamma-glutamyl transferase (GGT) by 12.4 IU/L (8.2%) and fasting circulating insulin levels by −3.3 μU/ml (12.2%), (p <0.001 for all *vs.* baseline). We examined the association between 18-month dynamics of HFC with 18-month changes in hepatic and cardiometabolic parameters using multivariate regression models (Fig. 3). In models adjusted for age, sex and intervention group, decreased HFC was associated with decreased GGT (β = 0.443; 95% CI 0.32–0.56), ALT (β = 0.253; 95% CI 0.12–0.39), cholesterol/HDL ratio (β = 0.226, 95% CI 0.09–0.35), triglyceride/HDL ratio (β = 0.209, 95% CI 0.03–0.30) and chemerin (β = 0.393, 95% CI 0.26–0.52). To assess the contribution of decreased HFC independent of VAT, the model was further adjusted for VAT changes. Associations between reductions in HFC and lipid parameters were attenuated by adjusting for VAT changes. However, reduction of HFC remained significantly and independently associated with reduced GGT (β = 0.330; 95% CI 0.24–0.42), ALT (β = 0.189; 95% CI 0.04–0.35) and chemerin (β = 0.382; 95% CI 0.23–0.53) even after controlling for VAT changes. When data was stratified to HFC within and above normal range (*i.e.* <5% and ≥5%, respectively), similar associations between groups were found with GGT, chemerin, and cholesterol/HDL ratio (p <0.05 for all). However, while ALT and % glycated hemoglobin (%HbA1c) were associated with HFC loss only for the ≥5% HFC group, insulin levels were associated with HFC in the <5% HFC group (Fig. S1).

To compare the impact of losses of HFC, VAT and total weight on improvement in cardiometabolic parameters induced by MED/LC versus LF, we determined how adjustment for those parameters attenuated the differences between the 2 dietary interventions. Compared to the LF diet, MED/LC diet induced a greater increase in HDL (3.3 ± 7.5 *vs.* 5.6 ± 7.1 mg/dl), and a more pronounced decrease in diastolic blood pressure (1.2 ± 10.1 *vs.* −1.9 ± 7.5 mmHg), triglycerides (−3.4 ± 43.7 *vs.* −10.8 ± 28.0 mg/dl), triglyceride/HDL ratio (−0.15 ± 0.4 *vs.* −0.23 ± 0.4) and cardiovascular risk by the 3 different scores: Framingham (−0.27 ± 2.2 *vs.* −0.81 ± 1.9), SCORE (−0.16 ± 1.4

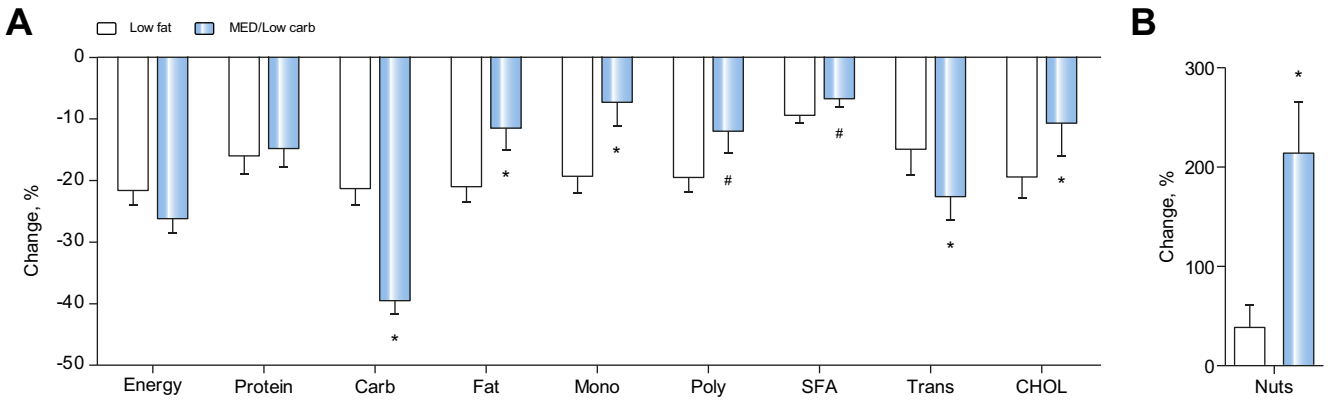

Fig. 2. Changes from baseline of the intake of energy and macro- and micro-nutrients between the diet intervention groups. Changes from baseline between the diet intervention groups in macro- and micro-nutrients. The Mediterranean/low-carbohydrate (MED/low-carb) diet more greatly decreased the intake of (A) carbohydrate, trans-fat and more greatly increased (B) nut consumption. (A) The low-fat diet more greatly decreased the intake of total fat, monounsaturated fat and cholesterol and tended to decrease polyunsaturated and saturated fat more. Analysis of variance with a covariance (ANCOVA) test was used. *p <0.05, $^{\#}p$ <0.1. Carb, carbohydrate; Chol, cholesterol; Mono, monounsaturated fat; Poly, polyunsaturated fat; SFA, saturated fatty acids; Trans, trans-fat.

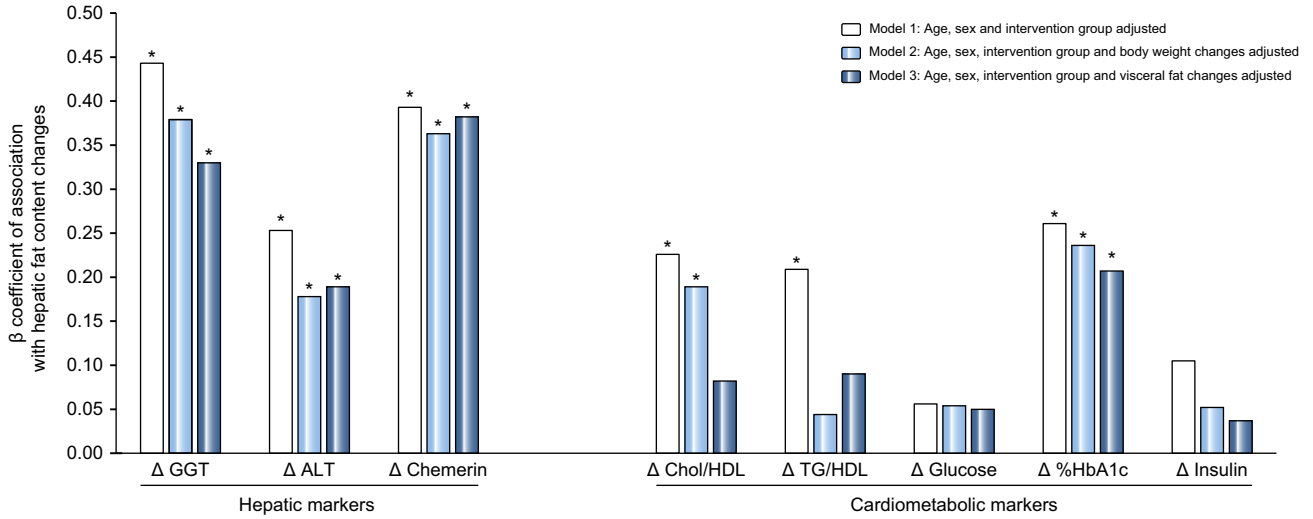

Fig. 3. Hepatic and cardiometabolic markers associated with 18 months hepatic fat content loss. Values are the β coefficients of the associations between 18-month changes in hepatic fat content and the indicated parameters at the X-axis. Multivariate model adjusted for age, sex and intervention groups (model 1); age, sex, intervention groups and body weight change (model 2); age, sex, intervention groups and visceral fat change (model 3). *p <0.05. %HbA1c, % glycated hemoglobin; ALT, alanine aminotransferase; Chol/HDL, total cholesterol to high-density lipoprotein ratio; GGT, gamma-glutamyl transferase; TG/HDL, triglyceride to high-density lipoprotein ratio.

vs. −0.50 ± 1.2), and ACC/AHA score (−0.39 ± 2.7 *vs.* −1.13 ± 2.5), (p <0.05 for all, Fig. 4). These differences remained significant when body weight and VAT changes were added to the multivariate model (Fig. 4). However, after adjustment for HFC changes, differences between diets were significantly attenuated, particularly the changes in triglycerides, triglyceride/HDL ratio and in the cardiovascular risk scores. When data were stratified for within and above normal HFC at baseline, the beneficial effect of the MED/LC diet over the LF in reducing cardiovascular risk scores became insignificant, possibly due to the lower power of the analysis. Nevertheless, similar trends were noted in both the normal HFC and abnormal HFC subgroups (Figs. S2A and S2B, respectively). There was no significant effect by the PA intervention on improvement of cardiometabolic parameters or cardiovascular risk.

Discussion

In this long-term lifestyle intervention trial, the MED/LC induced a significantly greater decrease in HFC than the LF diet, even after accounting for the differences in VAT loss. The impact of HFC reduction is highlighted by associated improvements in GGT, ALT, chemerin and HbA1c, which remained significant after adjustment for total weight loss or VAT change. In addition, the MED/LC diet was superior to the LF diet in decreasing cardiometabolic risk, a difference that was attenuated when adjusting for the decrease in HFC, but not following adjustment for weight or VAT.

Our study has several notable limitations. The small number of women (12%) limits our confidence in applying conclusions to females (although expected differences between males and females in various laboratory and fat distribution parameters were detectable). Information regarding adherence to the diets is based on questionnaires and attendance to the diet sessions. However, validated[23] questionnaires were used in order to ensure the highest level of accuracy as possible. In addition, since this study did not include histological tissue analyses, we were unable to trace changes in inflammatory processes in the liver and/or hepatocellular damage (beyond aminotransferase levels). The accuracy of quantifying liver fat in patients with HFC within the normal range (<5%) had been questioned. However, several recent publications evaluated the accuracy of hepatic proton density fat fraction (PDFF) measurements using MRI, and found that MRI-PDFF is an accurate non-invasive method for quantifying HFC even within the range below 5%.[28,29]. Participants in this trial, although overweight or obese, were relatively healthy (low rate of chronic medicine and only 11% were diabetic), therefore, it may be difficult to extend conclusions to individuals with more advanced liver disease. The strengths of the study include the following: all participants started the study simultaneously (1-phase study design); the use of the 3-T MRI scan; we treated HFC as a continuous variable, enabling us to define the amount of fat even within the normal range (<5%); the relatively long duration of the study and the high rate of adherence.

Despite the moderate weight loss in this study (−3 kg), we observed a highly discernable decrease in HFC that was greater in response to MED/LC diet than to LF diet. Previous studies focused on weight loss as a key factor in reducing HFC and its comorbidities in obese individuals.[30,31] Therefore, it is not surprising that the guidelines of the American Association for the Study of Liver Diseases[32] suggest weight loss through general nutritional care as a first-line intervention for NAFLD. However, the weight-loss phase mostly occurs during the first 6 months, followed by a weight regain phase, as we have previously shown.[15] Adipose tissue expansion during weight gain may result in a decreased insulin response and, thus, increased lipolysis and FFA production, which support hepatic lipid accumulation.[33] Some recent studies[34,35] have shown long-term reduction of HFC and improvement in liver markers despite weight gain after dietary weight loss. Bozzetto *et al.*[13,36] showed that an isocaloric diet enriched in monounsaturated fatty acids results in a reduction in HFC (by increasing fat oxidation), independent of weight change. In addition, the beneficial effect of different dietary strategies has been demonstrated in previous studies suggesting significant reductions in HFC with minor to

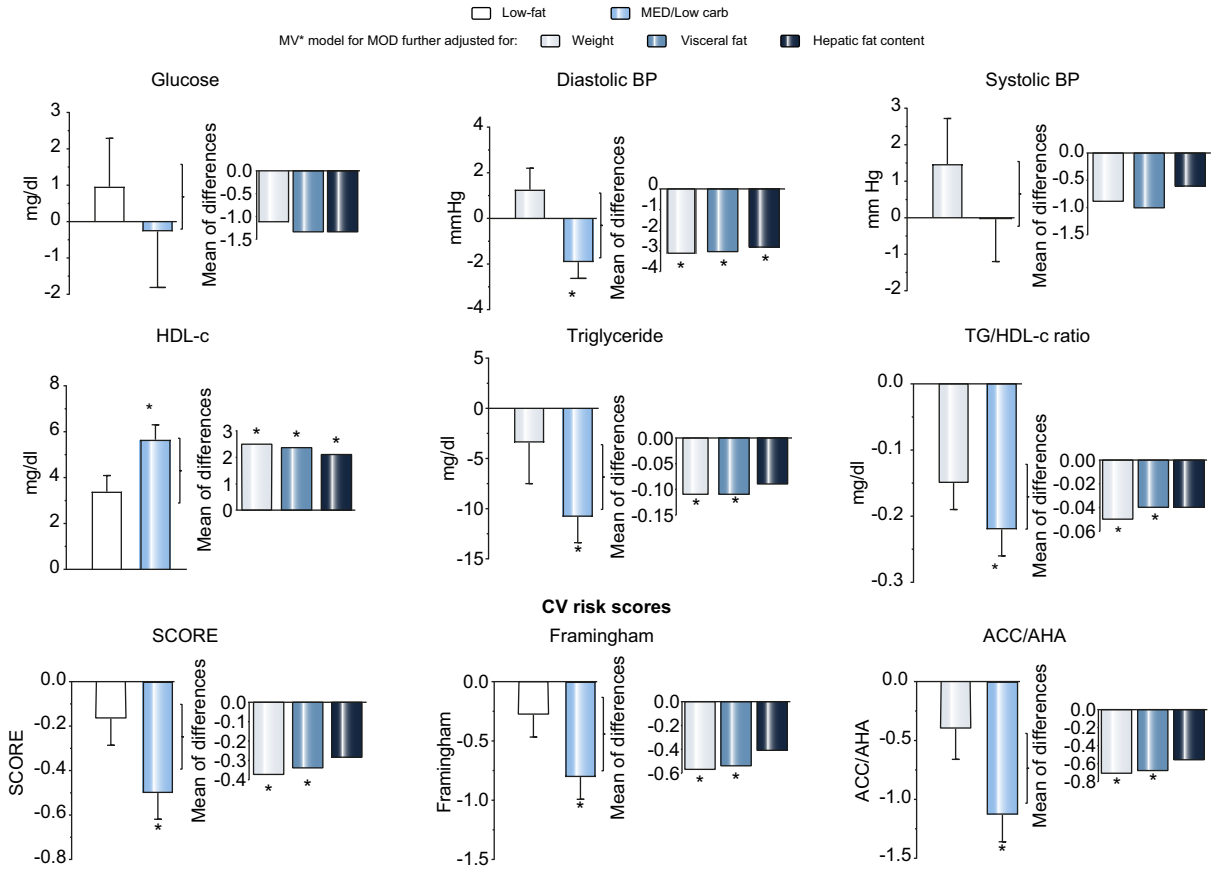

Fig. 4. Effect of dietary strategies on cardiometabolic state beyond changes of weight, visceral and hepatic fat content. Values in the figure are means ± SE. The statistical analysis was performed by multivariate general linear regression models adjusted for age, sex, and baseline abdominal obesity. *p <0.05. The MOD model was further adjusted for 18-month weight changes (grey); 18-month visceral fat changes (green); 18-month hepatic fat changes (turquoise). Intention-to-treat analyses, including all 278 participants by multiple imputation technique. After 18 month of intervention, 38 participants dropped out and had incomplete sets of observations (86.3% adherence). Cardiovascular risk scores include the Framingham risk score, Systematic Coronary Risk Evaluation (SCORE), and the American College of Cardiology/American Heart Association (ACC/AHA). BP, blood pressure; CV, cardiovascular; MOD, mean-of-differences; TG/HDL, triglyceride to high-density lipoprotein ratio.

moderate weight loss,[37] but this was not observed in larger, more recent studies.[38] Nevertheless, data are sparse regarding the effect of specific long-term dietary interventions on HFC, beyond VAT loss. In a randomized study,[39] 170 overweight or obese individuals were randomly assigned to either reduced fat or reduced carbohydrate, calorie-restricted diets for 6 months, and found similar beneficial effects of the 2 intervention arms on HFC reduction.

Our results reveal that reductions in liver markers and chemerin are associated with a decrease in HFC, potentially at least partially independent of the VAT-liver axis. Thus, clinically, tracking changes in those biomarkers may reveal changes in HFC that are currently difficult to track directly by imaging or to estimate by other means: liver biopsy is still considered the current clinical gold standard in this regard,[2] but there is an urgent need for non-invasive blood biomarkers that can be relied on. A particularly difficult challenge is to find independent biomarkers that reflect the dynamics of HFC, even beyond VAT. Liver enzyme ALT is the biomarker most commonly used to assess HFC content and liver injury. However, several studies[40,41] have shown that ALT does not necessarily correlate strongly with HFC or with the severity of liver damage. In a cross-sectional study[40] in which 31% of participants had elevated HFC (assessed by MRI), 79% of those had normal levels of ALT. These highlight the urgent need for novel biomarkers indicating HFC. GGT is frequently elevated in patients with NAFLD,[41] possibly because increased fat in the liver may induce hepatocellular damage that leads to increased GGT synthesis.[42] Moreover, histological improvement of the liver was associated with reductions in GGT concentrations with weight loss.[43] Yet, GGT has been included only as part of a group of biomarkers used to predict increased HFC[44] and not as an independent biomarker. In the present study, mean GGT and ALT levels at baseline were in the normal range. Therefore, it is possible that considering changes (deltas) in these parameters may be of greater clinical impact than the absolute values in a cross-sectional setting, even within the normal range of these parameters.

Chemerin[45] is an "adipo-hepatokine" which was found to be associated with obesity and impaired cardiometabolic state.[46] In a prospective study[47] levels of chemerin were found to be directly correlated with severity of NAFLD in obese patients. Previously we reported[48] that chemerin dynamics tightly correspond to changes in body weight in the DIRECT trial,[15] decreasing during the weight-loss phase and stabilized or increased during the weight maintenance/regain phase. Nonetheless, the

link between chemerin and the dynamics of HFC over time has yet to be demonstrated. Notably, the dynamic range of total chemerin levels is low, potentially limiting its use as a biomarker. Possibly, considering different isoforms of this adipo-hepatokine could enhance its sensitivity and specificity as a biomarker for HFC dynamics.

Our analyses suggest that HFC changes, rather than VAT changes, may play a particular role in mediating the greater beneficial effects of MED/LC over the LF dietary intervention. After adjusting for HFC changes, the differences in the association of the diets with improvements in lipid profile and in the cardiovascular risk scores became statistically insignificant. We did not observe such attenuation when controlling for weight or VAT loss. Recent long-term dietary interventions and meta-analyses have shed light on the ability of low-carbohydrate and Mediterranean[14,15,49,50] diets to serve as alternatives to traditional LF diets in inducing weight loss and improved cardiometabolic profile. A previous cross-sectional analysis has also suggested that HFC has a stronger association with obesity-related cardiometabolic risk than VAT does.[8] Moreover, another study that matched subgroups of patients with similar VAT but different HFC, suggested that increased HFC was cross-sectionally associated with insulin resistance.[7] Our current randomized trial results strengthen the notion, and provide evidence, supporting the unique impact of HFC on such risk, showing that these associations occur in response to intervention and are not merely cross-sectional observations. Our findings are also in line with results from mechanistic, fat transplantation studies in mice, in which mesenteric (portally drained), but not parietal peritoneal (systemically drained via the vena cava) transplantation induced worse metabolic outcome.[51,52] However, in humans, conflicting results were obtained on the putative metabolic benefit of omentectomy (surgical VAT reduction) during bariatric surgery.[53] Thus, although HFC partially reflects a downstream consequence of increased VAT, our results strengthen the notion that HFC mechanistically contributes to cardiometabolic risk independently of VAT. Moreover, they highlight the potential value of interventions specifically targeting the hepatic manifestations of obesity, such as LC/MED diet, in diminishing health risks associated with obesity.

The amount of HFC accumulation depends, among other things, on an interaction between hepatic FA uptake, derived from plasma FFAs released from triglyceride hydrolysis in adipose tissue and circulating triglycerides, and *de novo* lipogenesis (DNL)[54]. It has been demonstrated that an LF, high-carbohydrate diet significantly increased hepatic DNL compared to an isocaloric high-fat, low-carbohydrate diet.[55] Moreover, it is well-established that excessive consumption of sugar, and fructose in particular, leads to dietary carbons channeling directly to the liver, supporting DNL.[56] These mechanisms may also explain the superiority of the MED/LC diet, including a daily intake of walnuts, over the LF diet, regarding the reduction in HFC. Thus, our study highlights the specific potential of MED/LC as a particular dietary strategy to treat NAFLD.

In summary, this sub-study demonstrates how different weight-loss strategies may induce favorable dynamics of HFC and consequently improve cardiometabolic risk. We suggest that improvements in specific easily tracked blood biomarkers and cardiovascular risk were associated with a decrease in HFC, beyond the loss of VAT. Thus, rather than focusing on weight loss only, our findings suggest that an LC/MED dietary intervention may be used as a specific approach for the management of NAFLD.

Financial support

This work was supported by grants from: The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 209933838 – SFB 1052. The Israel Science Foundation (ISF), Israel Ministry of Science and Technology (grant # 3-13604), and the Dr. Robert C. and Veronica Atkins Research Foundation. The foundations were not involved in any stage of the design, conduct, or analysis of the study and had no access to the study results before publication.

Conflict of interest

Authors have no conflict of interest to disclose. All authors had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

IS, AR, MJS, YH, DS, YG and IS designed research; YG, NC, NB, MR, DS, SK, LT, HZ, AYM, OK, GT and AB conducted research; DF UC, MS, MB, and JT analyzed blood and urine samples; DD provided scientific consultation; NC, OK, YH and YG analyzed imaging; YG analyzed the data; YG, AR and IS wrote the paper; IS had primary responsibility for final content.

Acknowledgements

We thank the CENTRAL participants for their significant contribution. We thank California Walnut Commission for kindly supplying the walnuts. We thank Osnat Tangi-Rosental, Dr Rachel Golan, Eyal Goshen, Dr. Rafi Gonen, Dr. Lena Novak, Victor Haddad, Roman Tsirkin, David Shushan, Shula Witkow, Liz Shabtay, Philip Rosen, Julia Kovsan, Hadar Cohen, and Dr. Moti Salti for their valuable contributions to this study.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.04.013.

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Author names in bold designate shared co-first authorship

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