

RESEARCH ARTICLE

# Intramyocellular triacylglycerol accumulation across weight loss strategies; Sub-study of the CENTRAL trial

Yftach Gepner<sup>1,2\*</sup>, Ilan Shelef<sup>3</sup>, Dan Schwarzfuchs<sup>3</sup>, Noa Cohen<sup>1</sup>, Nitzan Bril<sup>1</sup>, Michal Rein<sup>1</sup>, Gal Tsaban<sup>1</sup>, Hila Zelicha<sup>1</sup>, Anat Yaskolka Meir<sup>1</sup>, Lilac Tene<sup>1</sup>, Benjamin Sarusy<sup>4</sup>, Philip Rosen<sup>3</sup>, Jay R. Hoffman<sup>2</sup>, Jeffrey R. Stout<sup>2</sup>, Joachim Thiery<sup>5</sup>, Uta Ceglarek<sup>5</sup>, Michael Stumvoll<sup>5</sup>, Matthias Blüher<sup>5</sup>, Meir J. Stampfer<sup>6</sup>, Iris Shai<sup>1</sup>

**1** Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel, **2** Institute of Exercise Physiology and Wellness, Sport and Exercise Science; University of Central Florida, Orlando, FL, United States of America, **3** Soroka University Medical Center, Beer-Sheva, Israel, **4** Nuclear Research Center Negev, Dimona, Israel, **5** Department of Medicine, University of Leipzig, Leipzig, Germany, **6** Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA, United States of America

\* [gepner@bgu.ac.il](mailto:gepner@bgu.ac.il)



**OPEN ACCESS**

**Citation:** Gepner Y, Shelef I, Schwarzfuchs D, Cohen N, Bril N, Rein M, et al. (2017) Intramyocellular triacylglycerol accumulation across weight loss strategies; Sub-study of the CENTRAL trial. PLoS ONE 12(11): e0188431. <https://doi.org/10.1371/journal.pone.0188431>

**Editor:** Gordon Fisher, University of Alabama at Birmingham, UNITED STATES

**Received:** April 28, 2017

**Accepted:** November 6, 2017

**Published:** November 30, 2017

**Copyright:** © 2017 Gepner et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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

**Funding:** This work was supported by The Deutsche Forschungsgemeinschaft (DFG): SFB1052; the Deutsche Forschungsgemeinschaft, Obesity Mechanisms (SFB 1052, A01 to MS, B01 to MB, and B08 to IS), Israel Science Foundation (ISF), Israel Ministry of Science and Technology (grant # 3-13604), and the Dr. Robert C. and Veronica Atkins Research Foundation.

## Abstract

### Background

Intramyocellular triacylglycerol (IMTG) is utilized as metabolic fuel during exercise and is linked to insulin resistance, but the long-term effect of weight loss strategies on IMTG among participants with abdominal fat, remain unclear.

### Methods

In an 18-month trial, sedentary participants with abdominal fat/dyslipidemia were randomized to either a low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC) diet (including 28g·day<sup>-1</sup> of walnuts). After 6-months, the participants were re-randomized to moderate intense physical activity (PA+) or non-physical activity (PA-). Magnetic resonance imaging (MRI) was used to quantify changes of IMTG, abdominal sub-depots, hepatic and intermuscular fats.

### Results

Across the 277 participants [86% men, age = 48 years, body-mass-index (BMI) = 31 kg/m<sup>2</sup>, visceral fat = 33%] 86% completed the 18-m trial. At baseline, women had higher IMTG than men (3.4% vs. 2.3%, p<0.001) and increased IMTG was associated with aging and higher BMI, visceral and intermuscular fats, HbA1c%, HDL-c and leptin(p<0.05), but not with intra-hepatic fat. After 18 month of intervention and a -3 kg mean weight loss, participants significantly increased IMTG by 25%, with a distinct effect in the MED/LC<sup>PA+</sup> group as compared to the other intervention groups (57% vs. 9.5–18.5%, p<0.05). Changes in IMTG were associated with visceral and intermuscular fat, metabolic syndrome, insulin and leptin (p<0.05 for all), however, these associations did not remain after adjustment for visceral fat changes.

**Competing interests:** The authors have declared that no competing interests exist.

## Conclusions

Lifestyle strategies differentially affect IMTG accumulation; combination of exercise with decreased carbohydrate/increased unsaturated fat proportion intake greatly increase IMTG. Our findings suggest that increased IMTG during diet-induced moderate weight loss may not be directly related to cardiometabolic risk.

## Trial registration

ClinicalTrials.gov [NCT01530724](https://clinicaltrials.gov/ct2/show/study/NCT01530724)

## Introduction

Intramyocellular triacylglycerol (IMTG) represents ~1% to 2% of the total fat stores within the body[1] and is used as a substrate source during exercise at low to moderate intensities[1–3]. The accumulation of IMTG is significantly greater in women compared to BMI matched men[4,5]. Furthermore, IMTG has been demonstrated to be correlated with BMI, insulin resistance[6] and with central abdominal fat[7] in sedentary obese subjects who are not physically active. Interestingly, highly trained athletes exhibit similar, if not greater concentrations of IMTG, than obese or type 2 diabetics (“the athlete’s paradox”)[8,9]. Improvements in insulin sensitivity with exercise or calorie restriction and weight loss in sedentary overweight humans is associated with reduction in intra-abdominal fat but not in IMTG[10,11].

IMTG plays an important role as an oxidative substrate during and following physical activity (PA)[2,3]. Some studies have demonstrated that acute post exercise training, IMTG is reduced by 20–30% during the recovery period while muscle glycogen was replenishing[12]. In addition, some studies have reported that high-fat diets (50% to 60%) can increase IMTG content[13–15], while others have reported that a high-fat diet may decrease IMTG following low calorie-induced weight loss[16]. Furthermore, combining endurance exercise training with the consumption of a high-fat diet has been shown to increase in IMTG content[17–19]. In addition, other investigations have suggested that saturated fatty acid composition may have a greater effect on IMTG and in the development of skeletal muscle insulin resistance than total fat intake[20,21].

Although weight loss and exercise intervention can both decrease pathogenic fat[22,23] and improve insulin sensitivity[24,25], these interventions may have different effects on IMTG. The physiological importance of IMTG beyond its relationship with abdominal adiposity remains unclear. Furthermore, to our knowledge limited research has examined the chronic effects of weight-loss from different diet and exercise strategies on IMTG. Thus, the purpose of this study was to examine the IMTG response to diets with or without moderate PA, and to assess the association between changes of IMTG with changes in cardiometabolic risk parameters.

## Materials and methods

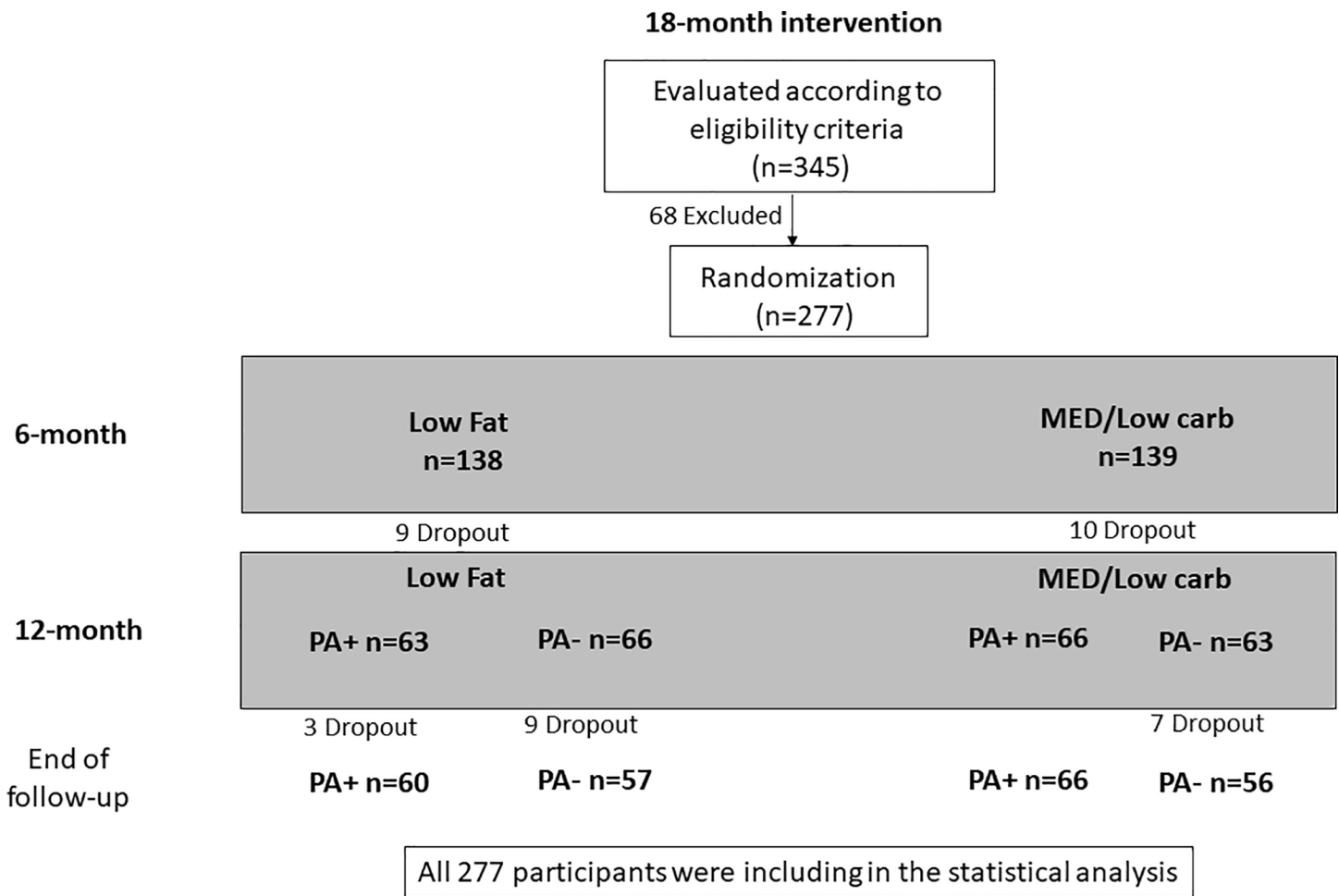
### Study population

This is a sub-study of the CENTRAL randomized controlled trial (ClinicalTrials.gov identifier: NCT01530724, [S1 Table](#)) aimed to assess whether different diet and exercise interventions could preferentially induce the loss of visceral fat in patients with central adiposity (primary endpoint), with changes in other fat depots, including IMTG. The trial involved 277 participants and was conducted between October 2012 and April 2014 at the Nuclear Research

Center Negev (Dimona, Israel), a workplace with a dedicated cafeteria and an on-site medical clinic. Inclusion criteria were: abdominal obesity [waist circumference >102cm (40 inches) for men and >88cm (35 inches) for women], or serum triglycerides (TG)>150 mg·dL<sup>-1</sup> and high-density-lipoprotein cholesterol (HDL-c) <40 mg·dL<sup>-1</sup> for men and <50 mg·dL<sup>-1</sup> for women. Exclusion criteria were: serum creatinine ≥ 2mg·dl<sup>-1</sup>; impaired liver function (≥ threefold the upper level of ALT and AST), active cancer, pregnancy or lactation, highly physically active (>3 h·week<sup>-1</sup>) or unable to take part in PA, or participation in another trial. The study protocol was approved by the Medical Ethics Board and the Helsinki Committee of Soroka University Medical Center (S1 Appendix). All participants provided written informed consent and received no financial compensation or gifts.

### Randomization and interventions

After completion of baseline measures, participants were randomly assigned, without stratification, to one of two equally hypocaloric diets: low-fat diet (LF, n = 138) and Mediterranean/low-carbohydrate/ diet (MED/LC, n = 139). After 6 months of dietary intervention, each diet intervention group was further randomized into PA groups (LF<sup>PA+</sup>, MED/LC<sup>PA+</sup>) or non-PA groups (LF<sup>PA-</sup>, MED/LC<sup>PA-</sup>), (Fig 1).



**Fig 1. Flow chart of the 18-months study intervention.** Intention to treat analysis was performed for all participants. PA+, Physical activity. PA-, Non-Physical activity.

<https://doi.org/10.1371/journal.pone.0188431.g001>

## Diet intervention

Both diets were aimed at achieving an energy intake of 1500/kcal/day for women and 1800/kcal/day for men, restricting intake of *trans*-fats, refined carbohydrates, and emphasizing the consumption of vegetables. Lunch was provided exclusively by the workplace cafeteria during the work week. A dietitian worked closely with the kitchen staff to adjust the meals to the specific diet groups[26]. The 18-month diet intervention included a 90-minute nutritional session in the workplace with clinical dietitians every week during the first month of intervention, and every month thereafter. To maintain equal intensity of treatment, the workshop format and the quality of the materials were similar across the diet groups, except for instructions and materials specific to each dietary strategy. The LF diet, limited total fat intake to 30% of daily caloric intake, with up to 10% being saturated fat, and no more than 300 mg of cholesterol per day. An additional goal was to increase dietary fiber consumption. 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.[26] The diet restricted carbohydrate intake to less than 40 g/day in the first two 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 also provided 28 g of walnuts/day [160 Kcal/84% fat, mostly PUFA (omega-3  $\alpha$ -linolenic acid)] starting from the third month.

## Physical activity intervention

Participants randomized to the PA intervention groups at the 6-month time point received a free supervised gym membership for the following 12 months. The gym was located away from the workplace and the intervention included monthly 60-minute educational workshops, and training group sessions at the gym, directed by a certified fitness instructor, who was blinded to the assigned diets of the participants. The exercise program included three sessions/week of mostly aerobic training. In the first month participants started with 20 minutes of aerobic training at 65% maximum heart rate and 10 minutes of resistance training. Exercise was gradually increased to 45 minutes of aerobic training at 80% of maximum heart rate and 15 minutes of resistance training. The resistance training increased from one set using 60% of the participants' maximum strength (1RM) to two sets at 80% of the 1RM. Exercises included leg extension, leg curl, elbow flexion, triceps extension, lateral pull-down, lower back extension and bent knee sit-ups. The latter exercise used the participant's body mass only.

## MRI acquisition and image analysis

All participants underwent whole body MRI imaging. Scans were performed using a 3-Tesla magnet (Intera, Philips, Medical Systems, Netherlands). The MRI scanner utilized a 3D modified DIXON (mDIXON) imaging technique without gaps (2 mm thickness and 2 mm of spacing), a fast-low-angle shot (FLASH) sequence with a multi-echo two-excitation pulse sequence for phase-sensitive encoding of fat and water signals (TR 3.6 ms; TE 1, 1.19 ms; TE2 2.3 ms; FOV 520×440×80mm; 2×1.4×1 mm voxel size). Four images of the phantoms were generated, including in-phase, out-phase, fat phase and water phase. A breath-hold technique was used to prevent motion artifacts when the chest and abdomen were scanned. In all simultaneous fat depots quantification and comparisons, observers were blinded to time point and group treatment. We estimated the measurement error by evaluating a phantom included in the MRI acquisition. Utilizing the same software used to assess adipose tissue depot area, the mean  $\pm$  SEM phantom area was 1.4693 $\pm$ 0.0046 cm<sup>2</sup>, corresponding to the < 3% error reported in the

literature.[27] All fat depots were assessed by one or two raters. The Inter-observer and intra-observer correlations were  $> 0.96$  ( $p < 0.001$ ) for all measured fat storage pools, and  $0.95$ ,  $p < 0.001$  for pericardial fat volume.

**Intramyocellular triacylglycerol.** The IMTG was assessed by utilizing the region of interest (ROI) technique[28]. This method is based on comparison of tissue density (Fat/Fat+Water) in the selected regions. Using semi-automatic PRIDE software from Philips Medical Systems, we analyzed the middle hip 2D image in the central area of four muscles: rectus femoris, vastus lateralis, adductor magnus and semitendinosus. Mean percentage of IMTG was calculated by using all the values of each ROI.

**Abdominal fat sub-depots.** The quantification of the three sub-depots in the abdomen [superficial subcutaneous adipose tissue (superficial-SAT), deep-SAT and visceral (VAT)] was assessed by using a MATLAB-based program. The MRI scan allows visualizing the fascia superficialis as a fine black line, and to divide superficial-SAT from deep-SAT we drew a continuous line over the fascia superficialis. We selected the specified fat mass area, by mean of three slices L2-L3, L5-L4 and L5-S1, using semiautomatic method software, and quantified the fat mass regions[29,30].

**Hepatic fat.** We quantified the percentage of hepatic fat using PRIDE software (Philips Medical Systems). We calculated mean percentage from four 2D slices (3cm intervals divided into quarters) by utilizing the region of interest (ROI) approach, which is based on measurements of tissue densities (fat/fat+water) using the Fat Ratio Calculation.[31] 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 in the liver as a whole.

**Femoral intermuscular adipose tissue.** Femoral intermuscular fat was quantified from a single 2D fat-phase axial slice from the mid-thigh of the right leg, from the femoral head to the medial and lateral condyle. Our semi-automatic MATLAB-based program was applied to distinguish between adipose and lean tissues and to calculate the area ( $\text{cm}^2$ ) of femoral intermuscular adipose tissue[32].

## Anthropometric measurements

Height was measured to the nearest millimeter by using a standard wall-mounted stadiometer. Waist circumference (WC) was measured to the nearest millimeter with an anthropometric measuring tape; the measurement was made half-way between the last rib and the iliac crest. Body weight was measured monthly without shoes to the nearest 0.1kg.

## Blood measures

Resting blood samples were obtained prior to each testing session. All blood samples were obtained following a 15-min equilibration period. Each participant's blood samples were obtained at the same time of day during each session following an overnight fast. Fasting blood samples were stored at  $-80^\circ\text{C}$ . Fasting plasma glucose (FPG) was measured by Roche GLUC 3 (hexokinase method). Plasma insulin was measured with the use of an enzyme immuno-metric assay [Immulite automated analyzer, Diagnostic Products, coefficient of variation (CV) = 2.5%]. Serum total cholesterol (CV = 1.3%), high-density-lipoprotein cholesterol (HDL-c), low-density-lipoprotein (LDL) cholesterol, and triglycerides (CV = 2.1%) were determined enzymatically with a Cobas 6000 automatic analyzer (Roche). Plasma leptin levels were assessed by ELISA (Mediagnost), with a CV of 2.4%. All biochemical analyses were performed at the University of Leipzig, Germany.

## Dietary and exercise compliance

Adherence to the diet was assessed via a self-administered validated electronic 127 item food-frequency questionnaire (FFQ). [33,34] Adherence to exercise was followed by an electronic self-reported validated PA questionnaire [35] and by electronically monitoring entry to the gym. Text messages were sent to update participants and to motivate adherence to the diets on specific occasions (such as before and after holidays). By using electronic questionnaires, [26] the completeness of the data was ensured by prompting the participants when a question was not answered or when an answer was not within a logical range. Symptoms, adverse effects, quality of life, and medication usage were also followed electronically.

## Statistical analysis

The primary aim of the main CENTRAL study was change in body fat distribution over 18 months of intervention, and the main primary specific endpoint was VAT. The priori hypothesis was that VAT could be differentially altered by lifestyle intervention strategies. The sample size was estimated based on findings from a previous 14-week intervention study, in which 33 postmenopausal obese women (57yr, 92kg, 36% body fat) were randomized to one of three interventions: diet alone, exercise alone, and diet and exercise group and significant relative change in VAT of 12.8% ( $P < 0.05$ ) was found. Thus, the minimum detectable effect after 18 months for the primary VAT between the intervention groups was estimated as 3.57cm<sup>2</sup>, and for an alpha = 5% and power = 80%, 250 participants were required (calculated using Winpepi software). We increased our sample size from 250 (planned protocol) to 278 participants in order to reach significant differences in other sub-studies analysis.

The aim of this sub-study was changes in IMTG over 18 months of intervention, and the secondary was the associations between IMTG to cardiometabolic biomarkers. From one MRI scan at baseline we were unable to analyze IMTG, therefore, the sample size for this sub-study was 277 participants. A post-hoc power calculation analysis for this sub-study was based on 0.47% differences in IMTG deltas between the LF<sup>PA-</sup> and the MED/LC<sup>PA+</sup> groups with 1.18 and 1.14 standards deviations, yield power of 92.9% between intervention groups (calculated using Winpepi software).

We calculated mean  $\pm$  standard deviation of IMTG percentages and all adipose tissue. We performed intention-to-treat analysis, including all 277 participants, by imputing the missing observations for all adipose tissues for 38 individuals by the multiple imputation technique, wherein the following predictors were used in the imputation model: age, gender, baseline weight, baseline BMI and waist circumference at the end of the intervention. For missing data of body weight, we used the last observation carried forward. Pearson correlations were used to assess selected bivariate relationships at baseline. To test the effect between intervention groups differences on IMTG changes, we performed multivariate linear regression models, using dummy variables of the intervention strategies and adjusting gender, age and visceral adipose tissue changes. Paired sample t-tests were used to assess changes from baseline to 18-months within each intervention group. To test the association between IMTG and selected cardiometabolic parameters at baseline and over the intervention, we used a linear regression model adjusted for the interventions groups and to VAT changes. Statistical analysis was performed with IBM SPSS version 23. A criterion alpha level of  $p \leq 0.05$  was used to determine statistical significance.

## Results

### Baseline

Baseline characteristics of the participants across intervention groups are shown in [Table 1](#). The participants (age = 47.9  $\pm$  9.3 y, 86.4% males, BMI = 30.6  $\pm$  3.9) had on average across all

**Table 1. Characteristics of the study population across intervention groups.**

	Low-Fat PA-	Low-Fat PA+	MED/Low-Carb PA-	MED/Low-Carb PA+	All (n = 277)	P between groups
Age, y	49.5±9.2	47.2±9.0	47.0±8.8	47.8±9.8	47.9±9.3	0.33
Waist circumference, cm	105±9.5	106±8.5	106±11	108.0±8.5	106.0±10	0.51
BMI, kg/h <sup>2</sup>	31.1±3.9	30.3±3.4	30.9±4.4	30.99±3.3	30.6±3.9	0.68
Blood pressure, mmHg						
Systolic	125±16	122±13	124±18	126±16	124±16	0.49
Diastolic	79±11	78±10	81±12	82±11	80±11	0.18
Adipose depots						
IMTG, %	2.7±1.8	2.3±1.4	2.4±1.5	2.1±1.3	2.4±1.6	0.13
Visceral AT, cm <sup>2</sup>	177±71	181±65	160.5±61	184.3±65	175±66	0.14
Deep SAT, cm <sup>2</sup>	208±67	216±70	219±87	220±70	216±74	0.78
Superficial SAT, cm <sup>2</sup>	144±70	139±51	150±75	133±47	142±63	0.45
Intra Hepatic fat, %	10.8±10	9.2±9.0	10.0±10	10.4±11	10.2±10	0.82
IMAT, cm <sup>2</sup>	10.4±5.4	10.1±4.8	8.74±4.1	9.23±3.5	9.6±4.5	0.093
Blood biomarkers						
HDL cholesterol, mg/dl	43.8±13	42.5±12	42.4±10	43.7±9.3	43.1±12	0.82
LDL cholesterol, mg/dl	123±33	124±29	120±34	121±27	122±43	0.88
Triglyceride, mg/dl	71.7±41	78.7±44	73.5±41	66.4±36	72.6±41	0.41
Glucose, mg/dl	106±17	106±18	107±18	108±23	107±19	0.91
HbA1c, %	5.54±0.5	5.52±0.4	5.54±0.4	5.58±0.5	5.5±0.5	0.91
HOMA-IR	4.42±2.6	4.71±3.4	4.74±3.8	4.48±2.6	4.6±3.2	0.92
Leptin, ng/mL	16.0±16	12.6±6.8	14.7±15	14.4±8.6	14.5±12	0.47

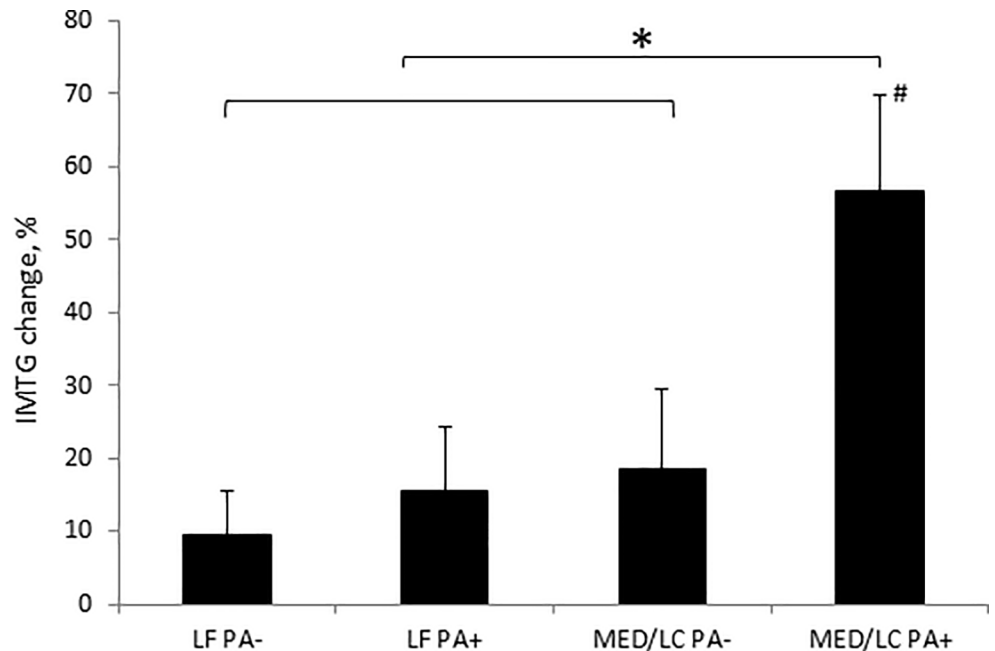
BMI: body mass index; SAT, subcutaneous adipose tissue; IMTG: Intramyocellular triacylglycerol IMAT: intermuscular adipose tissue. Values in the Table are means ± standard deviation.

<https://doi.org/10.1371/journal.pone.0188431.t001>

four muscles 2.4 ± 1.6% IMTG. Specifically, IMTG in the rectus femoris averaged 0.7 ± 1.2%, in the vastus lateralis 1.7 ± 2.1%, in the adductor magnus 2.8 ± 2.2% and in the semitendinosus 4.5 ± 3.4%. The mean percentage of each abdominal fat tissue compartment in the entire population was: superficial-SAT = 27%, deep-SAT = 40% and VAT = 33%. Females had a greater amount (p < 0.001) of IMTG 3.4 ± 1.9% than men 2.3 ± 1.5%. No statistical differences were found at baseline between the intervention groups for IMTG or any other parameters. At baseline, IMTG was positively associated with age (r = 0.18, p = 0.001), waist circumference (r = 0.17, p = 0.014), BMI (r = 0.18, p = 0.002), HDL-c (r = 0.15, p = 0.014) and area of abdominal sub-adipose tissue: superficial-SAT (r = 0.22, p < 0.001), deep-SAT (r = 0.16, p < 0.001), VAT (r = 0.14, p = 0.007) and IMAT (r = 0.54, p < 0.001). No significant association was observed between IMTG to hepatic fat (r = -0.08, p = 0.15) and metabolic syndrome criteria (r = -0.02, p = 0.77). Only 19% of the participants were regularly taking prescription medications, with similar changes noted during the intervention for all groups.

### Adherence

Following the 18-month intervention the retention rate of participants was 86%, with similar demographic and metabolic profiles between completers and non-completers. At baseline, there were no significant differences in the metabolic equivalent (MET) between the PA groups or in consumption of energy or macronutrients between the LF and MED/LC diet groups. However, during the intervention the PA+ groups significantly increased their MET as compared with the PA-groups (19.0 MET·week<sup>-1</sup> vs. 2.1 MET·week<sup>-1</sup>; p = 0.009). According



**Fig 2. Effect of dietary strategies with or without physical activity on intramyocellular triacylglycerol over 18 months of intervention.** Values in the Figure are means  $\pm$  standard errors. Multivariate linear model adjusted for age, sex and visceral fat changes. \* $p < 0.05$ , Mediterranean/ Low-carbohydrate diet with physical activity significantly increased intramyocellular triacylglycerol as compared to each of the other intervention groups. #  $p < 0.05$ , paired t-test was used to test changes over time. LFPA-: Low fat diet non-physical activity; LFPA+: Low fat diet with physical activity; MED/LC PA-: Mediterranean/low-carbohydrate/ diet non-physical activity; MED/LC PA+: Mediterranean/low-carbohydrate/ diet with physical activity.

<https://doi.org/10.1371/journal.pone.0188431.g002>

to the self-reported FFQ, participants adhered to the dietary guidelines for the group that they were randomized (S1 Fig).

### 18-months changes

Following the 18-month intervention a significant decrease was found, for all participants combined, in the change in body weight ( $-3.0 \pm 5.5$  kg,  $p < 0.001$ ) and in insulin-sensitivity parameters, such as HbA1c ( $-0.05 \pm 0.31\%$ ,  $p = 0.022$ ) and HOMA-IR ( $-0.9 \pm 2.4$ ,  $p < 0.001$ ). IMTG was increased by 0.2% [(95%CI 0.04 to 0.35), relative change 25%,  $p = 0.008$ ] for the entire study population. Upon further examination, elevations in IMTG was significant only in the MED/LC<sup>PA+</sup> group [0.56% (95%CI 0.21 to 0.91), relative change 56%,  $p = 0.002$ ], while no significant changes were found in the other intervention groups [(ranges = 0.03–0.12%, relative change range 9.7% to 18.5%,  $p > 0.39$ ), (Fig 2)]. The change observed in the MED/LC<sup>PA+</sup> combination group was significantly greater than each of the other groups ( $p = 0.001$ ) in multivariate model adjusted for age, sex and 18-month visceral fat changes, (Fig 2). Similarly, we found that the MED/LC diet further increased IMTG by 24% (95% CI = 4.82 to 43.87) than the LF diet, and the PA+ groups increased IMTG by 25% (95% CI = 4.34 to 44.6) compared to the PA- groups.

### Association with body fat, metabolic syndrome and selected biomarkers

The association between changes of IMTG, body fat, metabolic syndrome and selected biomarkers changes can be observed in Table 2. In controlling for intervention groups, an



**Table 2. Associations between 18-month changes of intramyocellular triacylglycerol and body fat, metabolic syndrome and selected biomarkers.**

	Adjusted for intervention group		Adjusted for intervention group and 18m visceral fat changes	
	$\Delta\beta$	p	$\beta$	p
18-month changes				
$\Delta$ Visceral fat	0.167	0.013	—	—
$\Delta$ IMAT	0.572	<0.001	0.0.542	<0.001
$\Delta$ Intra Hepatic fat	0.113	0.099	0.042	0.500
$\Delta$ Metabolic syndrome parameters	-0.058	0.398	0.025	0.528
Blood biomarkers				
$\Delta$ Cholesterol	0.179	0.009	0.122	0.072
$\Delta$ LDL-c	0.194	0.005	0.155	0.025
$\Delta$ HDL-c	0.017	0.809	0.102	0.125
$\Delta$ Triglyceride	0.110	0.111	0.003	0.960
$\Delta$ Insulin	0.143	0.039	0.108	0.125
$\Delta$ HbA1c	0.151	0.027	0.102	0.110
$\Delta$ HOMA-IR	0.109	0.117	0.075	0.291
$\Delta$ Leptin	0.020	0.774	-0.030	0.673

Linear regression adjusted for the 4 intervention groups and further for visceral fat changes. The amount and direction by which changes in IMTG (intramyocellular triacylglycerol) was associated with changes in selected parameters represented by  $\beta$  standardized coefficient. IMAT: intermuscular adipose tissue.

<https://doi.org/10.1371/journal.pone.0188431.t002>

increase of IMTG was associated with an increase in IMAT ( $\beta = 0.572, p < 0.001$ ), intra-abdominal obesity ( $\beta = 0.167, p = 0.013$ ), %HbA1c ( $\beta = 0.151, p = 0.027$ ), total cholesterol ( $\beta = 0.179, p = 0.009$ ) and LDL-c ( $\beta = 0.194, p = 0.009$ ). However, when the model was further adjusted for VAT changes, the associations between IMTG and the obesity parameters, abdominal fat or lipid/glycemic biomarkers were not maintained, but only with IMAT and LDL-c.

## Discussion

In this 18-month randomized control trial we assessed the long-term effect of weight loss strategies combined with moderate exercise on the dynamics of IMTG changes in 277 overweight or dyslipidemia participants. During moderate weight loss, IMTG increased compared to baseline and particularly in the MED/LC diet combined with PA. The increase in IMTG was not, however, independently associated with changes in cardiometabolic markers. Increasing the IMTG accumulation with a healthy lifestyle intervention, therefore, has more to do with energy substrate supply during times of metabolic need rather than being related to cardiometabolic risk.

There are several limitations within the current study. The exercise intensity and type of exercise (aerobic versus resistance) assessment was limited, although electronic gym entry records and the PA questioner, the ability to verify exercise adherence was limited. The reliance of self-reported dietary intake is a limitation based on the assumption that the participants were accurate and honest. Regardless, the accuracy of the self-reported dietary intake has been previously validated[36]. Another limitation is that the free fat mass and total fat mass measurements were not measured, and thus was unable to test the independent relationship between IMTG changes and cardiometabolic markers beyond total body weight, but only in adjusted for VAT changes. The small sample size of women, reflecting the workplace gender

profile, limits our ability to apply results for women. The strength of the study is highlighted by the long-term intervention, the large sample size, the high rate of adherence in an isolated workplace, the used of MRI measurement, and the parallel study design in which all participants started the interventions simultaneously.

The most important finding of this study was the differential effect between the intervention strategies on changes in IMTG. Specifically, the MED/LC diet and the PA+ group appeared to induce the greatest increase in IMTG. These results are consistent with previous studies[17,37], and may be explained by increases in mitochondrial density and intrinsic mitochondrial function in response to prolonged exercise training[38,39]. Nevertheless, the apparent increase in IMTG accumulation in the exercise activity group may represent one of the many metabolic adaptations related to short term endurance training[3,40,41], similar to the increases reported in muscle glycogen storage and mitochondrial density[5,42].

In the current trial, the MED/LC diet group experienced a 24% greater increase in IMTG than the LF diet, despite a similar decrease in body mass. It has been reported that high-fat diets may increase IMTG accumulation in both healthy[13,43] and athletics[14] individuals, with high inter-individual variation of IMTG trajectory after fat loading. Thus, IMTG accumulation may be partly involved in the mechanism of lipid content by the either quality or quantity of fat intake in the diet. Therefore, increases in IMTG following consuming a healthy hypocaloric diet, including increases in unsaturated fat (mostly PUFA) and decreases in saturated fat and carbohydrate, may explain the lack of an association between excessive fat accumulation in skeletal muscle and cardiometabolic risk markers. These findings are consistent with previous work from our team[26,44] and others[45] that reported on the cardiometabolic benefits of the MED/LC diet as compared to a LF diet.

Several studies have reported on the relationship between IMTG and insulin resistance [2,8,46]. In this study we found a significant reduction in insulin-sensitivity. Moreover, the significant increase in IMTG was associated with %HbA1c and HOMA-IR, but when VAT changes was further adjusted, the relationship was no longer maintained. Therefore, our results may provide additional evidence on the athletes' paradox', in which lifestyle modification in overweight sedentary individuals, particularly with exercise training[47,48], can improve insulin resistance, as estimated by % HbA1c and HOMA-IR, despite having higher IMTG content.

In summary, this investigation appears to be the first a long-term trial demonstrating that IMTG is differentially affected by lifestyle strategies. Interestingly, the PA or MED/LC diet promoted resulted in significant increases in IMTG accumulation, however, the combination of the two induced the greatest effect. The change of IMTG content did not alter the result in an independent association with cardiometabolic markers, suggesting that increased in IMTG during healthy lifestyle intervention may be a desirable metabolic adaptation.

## Supporting information

**S1 Fig. Changes in food group intake after 6 and 18 months of intervention across diet groups.** Each bar in the figure represents the mean changes and direction of the food group as compared to baseline. T-test was used to assess differences between diet groups, \*P < 0.05. (TIF)

**S1 Table. CONSORT checklist.**  
(DOC)

**S1 Appendix. Study protocol.**  
(DOCX)

## Acknowledgments

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, Dr. Uta Ceglarek, Dr. Michael Stumvoll, Dr. Matthias Blüher, Dr. Joachim Thiery, Dr. Amir Tirosh, Dr. Rafi Gonen, Dr. Lena Novak, Dr. Lior Zeler, Dr. Ilana Harman-Boehm, Victor Haddad, Roman Tsirkin, David Shushan, Shula Witkow, Liz Shabtay, Julia Kovshan, Hadar Cohen, Dr. Omri Orr, Dr. Moti Salti, Dr Yoash Chassidim, Shira Kenigsbuch and Oded Komy for their valuable contributions for this study.

This work was supported by grants from: The Deutsche Forschungsgemeinschaft (DFG): SFB1052; the Deutsche Forschungsgemeinschaft, Obesity Mechanisms (SFB 1052, A01 to MS, B01 to MB, and B08 to IS), 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. Authors have no conflict of interest to disclosures. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The investigators were responsible for the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and approval of the manuscript; and decision to submit the manuscript for publication.

## Author Contributions

**Data curation:** Yftach Gepner, Dan Schwarzfuchs, Noa Cohen, Nitzan Bril, Michal Rein, Gal Tsaban, Hila Zelicha, Anat Yaskolka Meir, Lilac Tene, Uta Ceglarek, Michael Stumvoll, Matthias Blüher.

**Formal analysis:** Dan Schwarzfuchs, Joachim Thiery, Uta Ceglarek, Michael Stumvoll, Matthias Blüher.

**Investigation:** Yftach Gepner, Ilan Shelef, Noa Cohen, Nitzan Bril, Michal Rein, Gal Tsaban, Hila Zelicha, Anat Yaskolka Meir, Lilac Tene, Iris Shai.

**Methodology:** Yftach Gepner, Ilan Shelef, Noa Cohen, Nitzan Bril, Michal Rein, Gal Tsaban, Hila Zelicha, Anat Yaskolka Meir, Meir J. Stampfer, Iris Shai.

**Resources:** Dan Schwarzfuchs, Benjamin Sarusy, Philip Rosen, Iris Shai.

**Software:** Yftach Gepner, Ilan Shelef, Benjamin Sarusy, Philip Rosen.

**Supervision:** Jay R. Hoffman, Jeffrey R. Stout, Meir J. Stampfer, Iris Shai.

**Validation:** Yftach Gepner.

**Writing – original draft:** Yftach Gepner, Ilan Shelef, Iris Shai.

**Writing – review & editing:** Yftach Gepner, Jay R. Hoffman, Jeffrey R. Stout, Iris Shai.

## References

1. van Loon LJ (2004) Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *J Appl Physiol* (1985) 97: 1170–1187. <https://doi.org/10.1152/jappphysiol.00368.2004> PMID: 15358749
2. Kiens B (2006) Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev* 86: 205–243. <https://doi.org/10.1152/physrev.00023.2004> PMID: 16371598
3. Ipavec-Levasseur S, Croci I, Choquette S, Byrne NM, Cowin G, O'Moore-Sullivan TM, et al. (2015) Effect of 1-h moderate-intensity aerobic exercise on intramyocellular lipids in obese men before and

- after a lifestyle intervention. *Appl Physiol Nutr Metab* 40: 1262–1268. <https://doi.org/10.1139/apnm-2015-0258> PMID: 26575100
4. Haugaard SB, Mu H, Vaag A, Madsbad S (2009) Intramyocellular triglyceride content in man, influence of sex, obesity and glycaemic control. *Eur J Endocrinol* 161: 57–64. <https://doi.org/10.1530/EJE-08-0931> PMID: 19417077
  5. Tarnopolsky MA, Rennie CD, Robertshaw HA, Fedak-Tarnopolsky SN, Devries MC, Hamadeh MJ. (2007) Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. *Am J Physiol Regul Integr Comp Physiol* 292: R1271–1278. <https://doi.org/10.1152/ajpregu.00472.2006> PMID: 17095651
  6. Savage DB, Petersen KF, Shulman GI (2007) Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev* 87: 507–520. <https://doi.org/10.1152/physrev.00024.2006> PMID: 17429039
  7. Kriketos AD, Furler SM, Gan SK, Poynten AM, Chisholm DJ, Campbell LV. (2003) Multiple indexes of lipid availability are independently related to whole body insulin action in healthy humans. *J Clin Endocrinol Metab* 88: 793–798. <https://doi.org/10.1210/jc.2002-020848> PMID: 12574215
  8. Goodpaster BH, He J, Watkins S, Kelley DE (2001) Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86: 5755–5761. <https://doi.org/10.1210/jcem.86.12.8075> PMID: 11739435
  9. van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA, Saris WH. (2003) Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *J Physiol* 553: 611–625. <https://doi.org/10.1113/jphysiol.2003.052431> PMID: 14514877
  10. Redman LM, Heilbronn LK, Martin CK, Alfonso A, Smith SR, Ravussin E, et al. (2007) Effect of calorie restriction with or without exercise on body composition and fat distribution. *J Clin Endocrinol Metab* 92: 865–872. <https://doi.org/10.1210/jc.2006-2184> PMID: 17200169
  11. Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, Chisholm DJ. (2003) Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. *Diabetes Care* 26: 1706–1713. PMID: 12766098
  12. Kiens B, Richter EA (1998) Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am J Physiol* 275: E332–337. PMID: 9688636
  13. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, et al. (2005) Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obes Res* 13: 2088–2094. <https://doi.org/10.1038/oby.2005.259> PMID: 16421342
  14. Tamura Y, Watada H, Igarashi Y, Nomiyama T, Onishi T, Takahashi K, et al. (2008) Short-term effects of dietary fat on intramyocellular lipid in sprinters and endurance runners. *Metabolism* 57: 373–379. <https://doi.org/10.1016/j.metabol.2007.10.013> PMID: 18249210
  15. Sakurai Y, Tamura Y, Takeno K, Kumashiro N, Sato F, Kakehi S, et al. (2011) Determinants of intramyocellular lipid accumulation after dietary fat loading in non-obese men. *J Diabetes Investig* 2: 310–317. <https://doi.org/10.1111/j.2040-1124.2010.00091.x> PMID: 24843504
  16. Dube JJ, Amati F, Toledo FG, Stefanovic-Racic M, Rossi A, Coen P, et al. (2011) Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia* 54: 1147–1156. <https://doi.org/10.1007/s00125-011-2065-0> PMID: 21327867
  17. Helge JW, Watt PW, Richter EA, Rennie MJ, Kiens B (2001) Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. *J Physiol* 537: 1009–1020. <https://doi.org/10.1111/j.1469-7793.2001.01009.x> PMID: 11744773
  18. Haus JM, Solomon TP, Lu L, Jesberger JA, Barkoukis H, Flask CA, et al. (2011) Intramyocellular lipid content and insulin sensitivity are increased following a short-term low-glycemic index diet and exercise intervention. *Am J Physiol Endocrinol Metab* 301: E511–516. <https://doi.org/10.1152/ajpendo.00221.2011> PMID: 21712533
  19. Van Proeyen K, Szlufcik K, Nielens H, Deldicque L, Van Dyck R, Ramaekers M, et al. (2011) High-fat diet overrules the effects of training on fiber-specific intramyocellular lipid utilization during exercise. *J Appl Physiol* (1985) 111: 108–116. <https://doi.org/10.1152/jappphysiol.01459.2010> PMID: 21551007
  20. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, et al. (2001) Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 44: 312–319. PMID: 11317662
  21. Chavez JA, Summers SA (2003) Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch Biochem Biophys* 419: 101–109. PMID: 14592453

22. Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD (2013) Whole body fat: content and distribution. *Prog Nucl Magn Reson Spectrosc* 73: 56–80. <https://doi.org/10.1016/j.pnmrs.2013.04.001> PMID: 23962884
23. Goodpaster BH, Delany JP, Otto AD, Kuller L, Vockley J, South-Paul JE, et al. (2010) Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults: a randomized trial. *JAMA* 304: 1795–1802. <https://doi.org/10.1001/jama.2010.1505> PMID: 20935337
24. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. (2013) The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol* 59: 138–143. <https://doi.org/10.1016/j.jhep.2013.02.012> PMID: 23485520
25. Dube JJ, Amati F, Stefanovic-Racic M, Toledo FG, Sauers SE, Goodpaster BH. (2008) Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. *Am J Physiol Endocrinol Metab* 294: E882–888. <https://doi.org/10.1152/ajpendo.00769.2007> PMID: 18319352
26. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, et al. (2008) Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med* 359: 229–241. <https://doi.org/10.1056/NEJMoa0708681> PMID: 18635428
27. Poonawalla AH, Sjoberg BP, Rehm JL, Hernando D, Hines CD, Irrazaval P, et al. (2013) Adipose tissue MRI for quantitative measurement of central obesity. *J Magn Reson Imaging* 37: 707–716. <https://doi.org/10.1002/jmri.23846> PMID: 23055365
28. Goodpaster BH, Stenger VA, Boada F, McKolanis T, Davis D, Ross R, et al. (2004) Skeletal muscle lipid concentration quantified by magnetic resonance imaging. *Am J Clin Nutr* 79: 748–754. PMID: 15113711
29. Gepner Y, Bril N, Shelef I, Schwarzfuchs D, Serfaty D, Rein M, et al. (2015) Higher visceral adiposity is associated with an enhanced early thermogenic response to carbohydrate-rich food. *Clin Nutr*.
30. Golan R, Shelef I, Rudich A, Gepner Y, Shemesh E, Chassidim Y, et al. (2012) Abdominal superficial subcutaneous fat: a putative distinct protective fat subdepot in type 2 diabetes. *Diabetes Care* 35: 640–647. <https://doi.org/10.2337/dc11-1583> PMID: 22344612
31. Schuchmann S, Weigel C, Albrecht L, Kirsch M, Lemke A, Lorenz G, et al. (2007) Non-invasive quantification of hepatic fat fraction by fast 1.0, 1.5 and 3.0 T MR imaging. *Eur J Radiol* 62: 416–422. <https://doi.org/10.1016/j.ejrad.2006.12.009> PMID: 17267159
32. Ruan XY, Gallagher D, Harris T, Albu J, Heymsfield S, Kuznia P, et al. (2007) Estimating whole body intermuscular adipose tissue from single cross-sectional magnetic resonance images. *J Appl Physiol* (1985) 102: 748–754. <https://doi.org/10.1152/jappphysiol.00304.2006> PMID: 17053107
33. Shai I, Rosner BA, Shahar DR, Vardi H, Azrad AB, Kanfi A, et al. (2005) Dietary evaluation and attenuation of relative risk: multiple comparisons between blood and urinary biomarkers, food frequency, and 24-hour recall questionnaires: the DEARR study. *J Nutr* 135: 573–579. PMID: 15735096
34. Shai I, Shahar DR, Vardi H, Fraser D (2004) Selection of food items for inclusion in a newly developed food-frequency questionnaire. *Public Health Nutr* 7: 745–749. PMID: 15369612
35. Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, et al. (1996) Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology* 7: 81–86. PMID: 8664406
36. Shai I, Vardi H, Shahar DR, Azrad AB, Fraser D (2003) Adaptation of international nutrition databases and data-entry system tools to a specific population. *Public Health Nutr* 6: 401–406. <https://doi.org/10.1079/PHN2002445> PMID: 12795829
37. Shaw CS, Shepherd SO, Wagenmakers AJ, Hansen D, Dendale P, van Loon LJ. (2012) Prolonged exercise training increases intramuscular lipid content and perilipin 2 expression in type I muscle fibers of patients with type 2 diabetes. *Am J Physiol Endocrinol Metab* 303: E1158–1165. <https://doi.org/10.1152/ajpendo.00272.2012> PMID: 22949030
38. Takeuchi K, Reue K (2009) Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *Am J Physiol Endocrinol Metab* 296: E1195–1209. <https://doi.org/10.1152/ajpendo.90958.2008> PMID: 19336658
39. Coleman RA, Lee DP (2004) Enzymes of triacylglycerol synthesis and their regulation. *Prog Lipid Res* 43: 134–176. PMID: 14654091
40. McCormack SE, McCarthy MA, Harrington SG, Farilla L, Hrovat MI, Systrom DM, et al. (2014) Effects of exercise and lifestyle modification on fitness, insulin resistance, skeletal muscle oxidative phosphorylation and intramyocellular lipid content in obese children and adolescents. *Pediatr Obes* 9: 281–291. <https://doi.org/10.1111/j.2047-6310.2013.00180.x> PMID: 23801526

41. Bajpeyi S, Reed MA, Molskness S, Newton C, Tanner CJ, McCartney JS, et al. (2012) Effect of short-term exercise training on intramyocellular lipid content. *Appl Physiol Nutr Metab* 37: 822–828. <https://doi.org/10.1139/h2012-051> PMID: 22691059
42. Toledo FG, Menshikova EV, Azuma K, Radikova Z, Kelley CA, Ritov VB, et al. (2008) Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes* 57: 987–994. <https://doi.org/10.2337/db07-1429> PMID: 18252894
43. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, et al. (2001) Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 50: 2579–2584. PMID: 11679437
44. Schwarzfuchs D, Golan R, Shai I (2012) Four-year follow-up after two-year dietary interventions. *N Engl J Med* 367: 1373–1374. <https://doi.org/10.1056/NEJMc1204792> PMID: 23034044
45. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Arós F, et al. (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 368: 1279–1290. <https://doi.org/10.1056/NEJMoa1200303> PMID: 23432189
46. Amati F, Dube JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, et al. (2011) Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes* 60: 2588–2597. <https://doi.org/10.2337/db10-1221> PMID: 21873552
47. Bell LM, Watts K, Siafarikas A, Thompson A, Ratnam N, Bulsara M, et al. (2007) Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* 92: 4230–4235. <https://doi.org/10.1210/jc.2007-0779> PMID: 17698905
48. Lee DC, Sui X, Church TS, Lavie CJ, Jackson AS, Blair SN. (2012) Changes in fitness and fatness on the development of cardiovascular disease risk factors hypertension, metabolic syndrome, and hypercholesterolemia. *J Am Coll Cardiol* 59: 665–672. <https://doi.org/10.1016/j.jacc.2011.11.013> PMID: 22322083