Online Supplementary material

Supplementary material 1: The CENTRAL trial

Study participants

The CENTRAL trial (ClinicalTrials.gov Identifier: NCT01530724) was conducted from October 2012 through April 2014 at an isolated research center workplace in Israel with a monitored provided lunch (1). The study was approved and monitored by the human subjects committee of Soroka Medical Center. All participants provided written informed consent. During the 18-month study period, 278 adults with abdominal obesity (75%) or dyslipidemia were randomly assigned to one of two equally hypocaloric diets, a low-fat (LF) diet or a Mediterranean/low-carbohydrate (MED/LC) diet. Exclusion criteria were the presence of impaired liver function or elevated serum creatinine or active cancer, pregnancy or lactation, highly physically active or unable to take part in PA, or participation in another trial.

Both diets aimed to achieve moderate, long-term weight loss with restricted trans-fats and refined carbohydrates, as well as increased vegetables. The achievement goal of the LF diet was to reduce fat intake (30% of energy from fat, with up to 10% of saturated fat and 300 mg/day of cholesterol) and to increase dietary fibers. The MED/LC diet was rich in vegetables and legumes and low in red meat, with poultry and fish replacing beef and lamb, and its goal was to achieve carbohydrate intake<40 g/day during the first 2 months, and thereafter a gradual increase ≤70 g/day, and increased protein and fat intake. The MED/LC diet group was also provided 28 g/day of walnuts starting from the third month. After 6 months, 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 the last 12 months of the intervention. Study investigators assessing outcomes were blinded to the group assignments. As previously reported, a total of 86% of the study participants completed the 18-month trial with good adherence.

Measurements of adiposity and body fat distribution

Weight and height were measured without shoes; waist circumference was measured halfway between the last rib and the iliac crest to the nearest millimeter using an anthropometric measuring tape. Abdominal adipose tissue area (VAT, deep subcutaneous adipose tissue [DSAT], superficial subcutaneous adipose tissue [SSAT]) and ectopic fat accumulation in the liver were assessed by whole-body MRI using a 3-Tesla magnet (Ingenia 3.0 T, Philips Healthcare, Best, the Netherlands) (1). Total adipose tissue (TAT) was calculated as the sum of VAT, DSAT, and SSAT. Intrahepatic fat percentage was assessed as the percentage of fat of the liver as a whole, using a standard method (2).

Measurements of glucose and insulin

Fasting blood samples were collected at baseline and at 18 months of the interventions and were stored at –80°C. Fasting plasma glucose was measured by Roche GLUC3 (hexokinase method), and plasma insulin was measured with an enzyme immunometric assay. Biochemical analyses were performed at the laboratories of the University of Leipzig, Germany. To test whether circulating miR-375-3p may regulate the insulin metabolism when escalating insulin demand (such as in pre-diabetes), we tested associations of miR-375-3p with fasting insulin and insulin

resistance (HOMA-IR) considering the presence of impaired fasting glucose (IFG: 100–<126 mg/dl) or pre-diabetes (IFG or elevated HbA1c (5.7%–<6.5%)) (3).

References:

1. Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, Tsaban G, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Komy O, Wolak A, Chassidim Y, Golan R, Avni-Hassid H, Bilitzky A, Sarusi B, Goshen E, Shemesh E, Henkin Y, Stumvoll M, Blüher M, Thiery J, Ceglarek U, Rudich A, Stampfer MJ, Shai I. Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. Circulation 2018;137:1143-1157
2. Gepner Y, Shelef I, Komy O, Cohen N, Schwarzfuchs D, Bril N, Rein M, Serfaty D, Kenigsbuch S, Zelicha H, Yaskolka Meir A, Tene L, Bilitzky A, Tsaban G, Chassidim Y, Sarusy B, Ceglarek U, Thiery J, Stumvoll M, Blüher M, Stampfer MJ, Rudich A, Shai I. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content. J Hepatol 2019;71:379-388
3. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care 2021;44:S15-S33

Supplementary material 2: Measurements of circulating miR-375-3p

In this study, a total of 227 samples at baseline and 157 samples at 18 months after the intervention were eligible with sufficient quality to proceed to the next-generation sequencing to quantify circulating miR-375-3p levels. Briefly, we extracted total serum RNA using the RNeasy Serum/Plasma Advanced kit (Qiagen, Hilden Germany). We used up to 20 ng of total serum RNA in the small RNA protocol of the NEBNext® Small RNA Library Prep Set for Illumina according to instructions of the manufacturer (NEB, Ipswich, MA USA). The barcoded libraries between 140 and 165 bp were purified and quantified using the Library Quantification Kit-Illumina/Universal (KAPA Biosystems, Wilmington, MA). In addition, the size distribution of the miRNA libraries was visualized on a Fragment Analyzer (Agilent, Santa Clara, CA, USA). A pool of up to 100 libraries was used for cluster generation at a concentration of 1.5 pM followed by sequencing of 75 bp using an Illumina NextSeq 550 sequencer at the sequencing core facility of the University Leipzig (Faculty of Medicine) employing version 2.5 flowcell and chemistry according to the instructions of the manufacturer (Illumina, San Diego, CA, USA). Demultiplexing of raw reads, adapter trimming and quality filtering was conducted using Illumina bcl2fastq conversion (v2.20.0) and cutadapt software (v1.18). Mapping against the human reference genome (hg38) and miRbase reference sequences (v22) were conducted using Bowtie2. Read counts were calculated with the Rsamtools R Bioconductor package; DESeq2 normalized data were used for analyses.

Circulating levels of miR-375-3p were measured at baseline and 18 months. Data on miR-375-3p were log-transformed to improve data normality. Changes in miR-375-3p from baseline to 18 months after the interventions were calculated after the log transformation. Fold changes (% changes) were also calculated for a better understanding of the magnitude of the changes.

Supplemental Results:

STable 1: Characteristics of the total study participants

Variables	N	Data
miR-375-3p*	222	4.6 (0.6)
Men	222	197 [88.7%]
Age, y	222	47.7 (9.4)
Alcohol intake, g/day	221	2 (0, 5)
Body mass index, kg/m^2	222	30.8 (3.8)
Waist circumference, cm	222	106.4 (9.3)
Abdominal adipose tissue area, cm^2		
Total adipose tissue	222	531.9 (146.6)
Visceral adipose tissue	222	174.9 (67.5)
Deep subcutaneous adipose tissue	222	215.3 (69.3)
Superficial subcutaneous adipose tissue	222	141.7 (60.7)
Intrahepatic fat, %	221	10.1 (10.2)
Fasting glucose, mg/dL	222	107.3 (19.7)
Fasting insulin	221	17.1 (10.6)
HOMA-IR	221	4.6 (3.3)

Data are mean (SD) or N [%]. *Data after log-transformation.

HOMA-IR, Homeostatic Model Assessment for Insulin Resistance

STable 2: Associations of miR-375-3p levels with adiposity measurements at baseline

Outcomes	β (SE)	*P* value
Body mass index, kg/m^2	–0.86 (0.25)	<0.001
Waist circumference, cm	–2.00 (0.59)	<0.001
Abdominal adipose tissue area, cm^2		
Total adipose tissue, TAT	–29.5 (9.6)	0.002
Visceral adipose tissue, VAT	–7.0 (3.7)	0.059
Deep subcutaneous adipose tissue, DSAT	–11.9 (4.6)	0.011
Superficial subcutaneous adipose tissue, SSAT	–10.7 (3.5)	0.003
Intrahepatic fat, %	–1.0 (0.7)	0.15

β (SE) per 1 SD increment of log-transformed miR-375-3p at baseline after adjusting for age and sex using the general linear model.

STable 3: Associations of circulating miR-375 with fasting insulin and insulin resistance (HOMR-IR) according to glycemic status

		Glycemic status by glucose*		Glycemic status by glucose and HbA1c**	
Outcomes		Normal fasting glucose (n=88)	Impaired fasting glucose (n=110)	Normoglycemia (n=79)	Pre-diabetes (n=129)
Log-Fasting insulin	β (SE)	0.02 (0.05)	–0.11 (0.05)	0.04 (0.05)	–0.1 (0.05)
	P value	0.72	0.037	0.39	0.039
Log-HOMA-IR	β (SE)	0.02 (0.05)	–0.12 (0.05)	0.05 (0.05)	–0.1 (0.05)
	P value	0.69	0.027	0.36	0.035

HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

Association of miR-375-3p with fasting insulin or HOMA-IR were also tested after stratifying participants according to the glycemic status.

*Normal fasting glucose: <100 mg/dl; Impaired fasting glucose: 100-<126 mg/dl.

**Normoglycemia: fasting glucose <100 mg/dl and HbA1c <5.7%; Pre-diabetes: fasting glucose 100-<126 mg/dl or HbA1c 5.7%-<6.5%. β (SE) per 1 SD increment of miR-375 for the respective outcome after adjusting for age and sex.

STable 4: Associations of changes in circulating miR-375-3p induced by lifestyle interventions with the improvements in body adiposity outcomes in models adjusting for baseline and changes in fasting glucose

		Model 1 (Fasting glucose)		Model 2 (Fasting insulin)		Model 3 (HOMA-IR)	
Outcomes	N	β (SE)	*P*	β (SE)	P	β (SE)	P
ΔWeight, kg	144	-1.27 (0.52)	0.016	-1.28 (0.5)	0.012	-1.27 (0.51)	0.013
ΔWaist circumference, cm	136	-0.87 (0.6)	0.149	-0.91 (0.58)	0.116	-0.89 (0.58)	0.127
Adipose tissue area, cm^2							
ΔTotal adipose tissue, TAT	138	-13.02 (6.17)	0.037	-12.19 (5.9)	0.041	-11.65 (5.93)	0.052
ΔSuperficial subcutaneous adipose tissue, SSAT	138	-0.65 (1.71)	0.71	-0.77 (1.69)	0.65	-0.71 (1.7)	0.68
ΔDeep subcutaneous adipose tissue, DSAT	138	-7.79 (2.35)	0.0012	-7.84 (2.27)	0.0008	-7.8 (2.3)	0.0009
ΔVisceral adipose tissue, VAT	138	-6.71 (3.2)	0.038	-6.12 (3.17)	0.056	-5.78 (3.16)	0.07
ΔIntrahepatic fat, %	135	-1.28 (0.52)	0.016	-1.2 (0.51)	0.021	-1.17 (0.51)	0.025

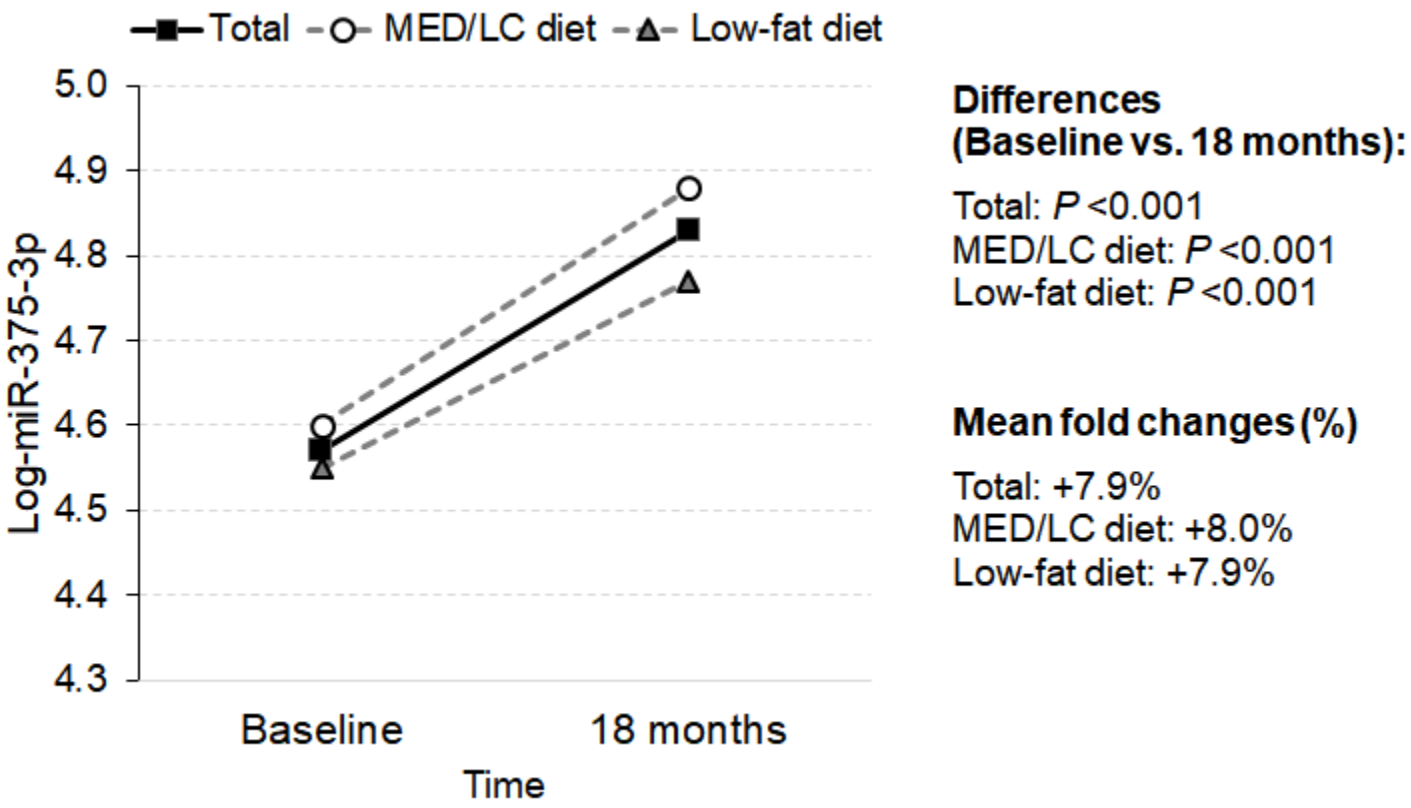
β (SE) per 1 SD increase in miR-375 changes (% change from baseline to 18 months) for the respective outcome.

Model 1 (fasting glucose): adjusting for the same covariates in the main table (age, sex, intervention groups, baseline BMI (for outcomes of ΔTAT, ΔSSAT, ΔDSAT, ΔVAT, and ΔIntrahepatic fat), baseline miR-375, the respective outcome trait at baseline), fasting glucose at baseline, and concurrent changes in fasting glucose.

Model 2 (fasting insulin): adjusting for the same covariates in the main table, fasting insulin (log-transformed) at baseline, and concurrent changes in fasting insulin (log-transformed).

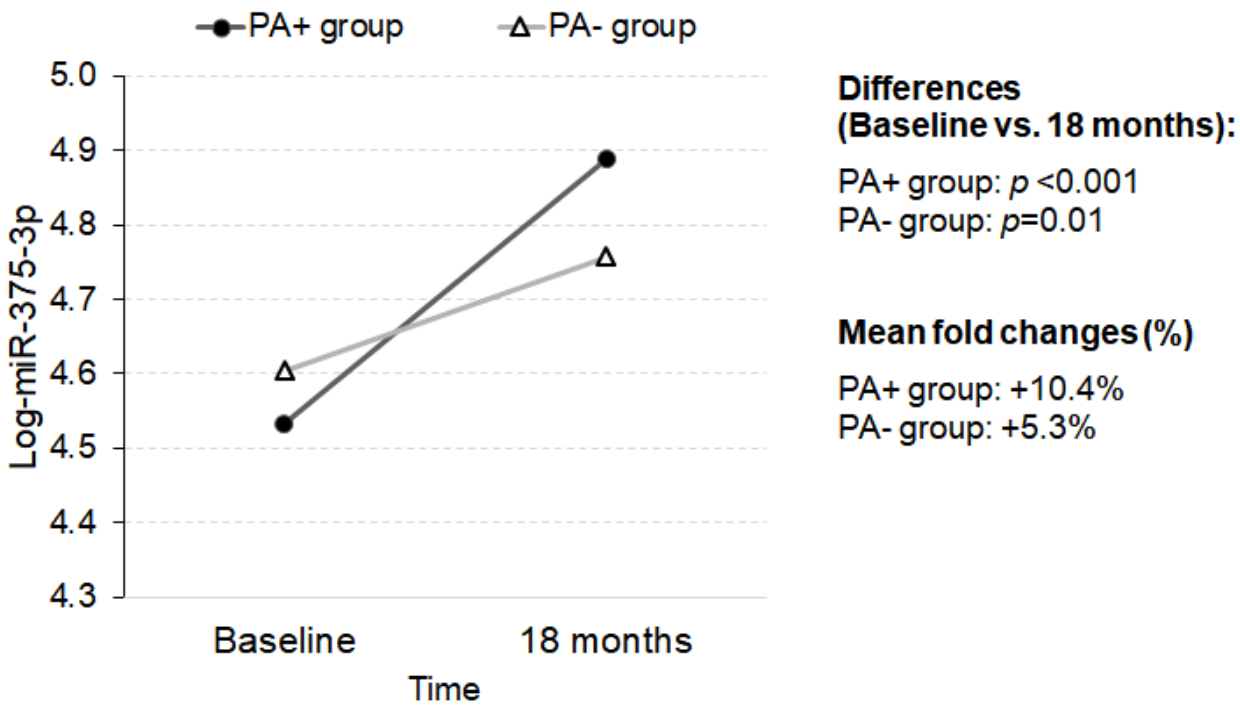
Model 3 (HOMA-IR): adjusting for the same covariates in the main table, HOMA-IR (log-transformed) at baseline, and concurrent changes in HOMA-IR (log-transformed).

SFigure 1: Changes in circulating miR-375 levels from baseline to 18 months in total participants, Mediterranean/low-carbohydrate (MED/LC) diet group, or low-fat (LF) diet group.



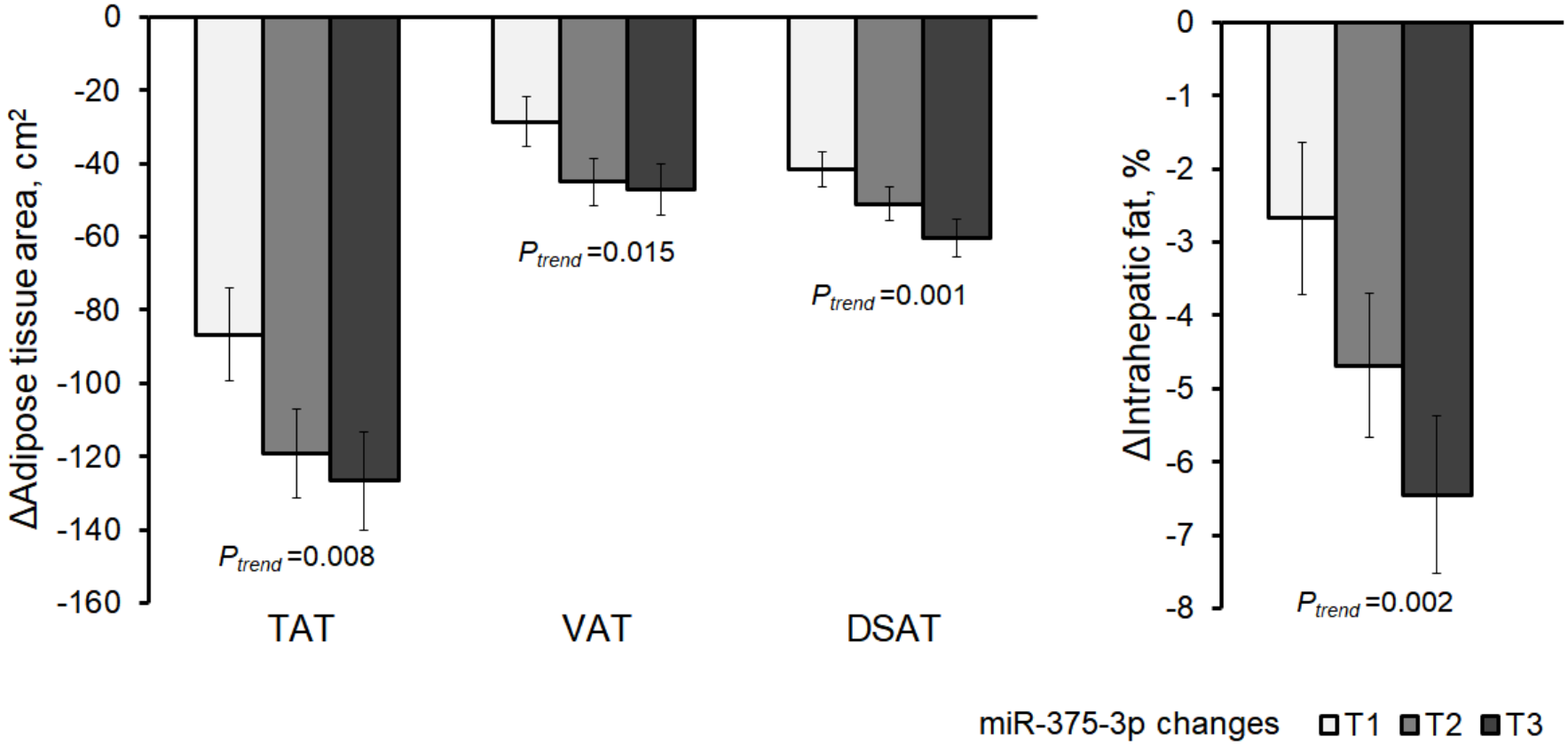
P values were calculated by the paired t-test for differences in miR-375 levels (baseline vs. 18 months)

SFigure 2: Changes in circulating miR-375 levels from baseline to 18 months in PA+ or PA- intervention group



P values were calculated by the paired t-test for differences in miR-375 levels (baseline vs. 18 months)

SFigure 3: Changes in total (TAT), visceral (VAT), deep subcutaneous adipose tissue (DSAT), and intrahepatic fat over 18 months according to tertile (T) categories of miR-375 changes.



The higher tertile group included participants with larger increases in miR-375-3p. Mean (SD) miR-375-3p changes (% change from baseline to 18 months) across the tertile (T) groups: T1 (lowest tertile): –7.8% (5.8%), T2: 6.3% (3.9%), T3 (highest tertile): 25.3% (9.4%)