Changes in Circulating miR-375-3p and Improvements in Visceral and Hepatic Fat Contents in Response to Lifestyle Interventions: The CENTRAL Trial

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OBJECTIVE

To investigate whether changes in circulating levels of pancreatic islet–related miRNA-375 (miR-375) are related to improved visceral and intrahepatic fat accumulation.

RESEARCH DESIGN AND METHODS

This study included adults with abdominal obesity from an 18-month weight loss lifestyle intervention trial. Circulating miR-375-3p was measured at baseline and 18 months. MRI was performed ($n = 139$) to assess 18-month changes in abdominal and intrahepatic fat depots.

RESULTS

Circulating miR-375-3p was related to fasting insulin and insulin resistance in participants with prediabetes. After the interventions, there was a significant increase of miR-375-3p ($P < 0.001$). Greater increase in miR-375-3p was associated with greater reductions of visceral ($P = 0.024$) and deep subcutaneous ($P < 0.001$) adipose tissues and intrahepatic fat content ($P = 0.012$).

CONCLUSIONS

Increases in circulating miR-375-3p were associated with visceral and intrahepatic fat reduction. Changes in circulating pancreatic islet–related miR-375-3p may be linked to improved diabetogenic fat depots during weight loss lifestyle interventions.

miRNAs are important posttranscriptional regulators of gene expressions; circulating miRNAs have been emerging biomarkers that coordinate whole-body metabolism through intercellular communications (1). Pancreatic islet–specific miRNA-375 (miR-375) regulates insulin secretion and has an essential role in pancreatic β-cell compensation when escalating insulin demand, such as in insulin resistance and obesity (2,3). In animal studies, miR-375 was a key factor in establishing adequate β-cell mass and function (4), and circulating miR-375 was a marker of β-cell death and involved in the development of diabetes (5). Insulin resistance and hyperinsulinemia are closely linked to visceral and intrahepatic fat depots (6). Recent studies

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suggest that circulating miR-375 may be related to regulating obesity, metabolic syndrome, fatty liver disease, and hepatic inflammation (7–11).

Nonetheless, whether temporal changes in circulating miR-375 are related to decreases in visceral and intrahepatic fat depots in adults with abdominal obesity remains to be clarified. We investigated associations of changes in circulating miR-375 with the long-term changes in visceral and ectopic fat accumulations in response to lifestyle interventions over 18 months.

RESEARCH DESIGN AND METHODS

This study included participants of an 18-month lifestyle intervention trial, CENTRAL (clinical trial reg. no. NCT01530724, clinicaltrials.gov) (12), which was conducted at an isolated research center workplace in Israel (Supplementary Material 1). The study was approved and monitored by the human subjects committee of Soroka Medical Center. All participants provided written informed consent. Adults (n = 278) with abdominal obesity (75%) or dyslipidemia were randomly assigned to one of two equally hypocaloric diets, a low-fat diet or a Mediterranean/low-carbohydrate diet, during the 18-month study period. After 6 months, each diet group was further randomized into one of two groups: added physical activity or no added physical activity. The study outcomes were abdominal adipose tissue area (visceral adipose tissue [VAT], deep subcutaneous [DSAT], and superficial subcutaneous [SSAT] adipose tissue) and ectopic fat accumulation (including intrahepatic fat) measured by whole-body MRI. Of the total participants, 86% completed the trial; baseline characteristics (such as VAT, fasting glucose, or insulin [Supplementary Table 1]) were not significantly different between participants with and those without data at 18 months ($P > 0.05$ for all).

Circulating levels of miR-375 (i.e., mature sequence miR-375-3p) were measured at baseline (n = 227) and 18 months (n = 157) (Supplementary Material 2). After exclusion of a few (n = 5) outliers of miR-375-3p, this study included 222 participants with data on baseline miR-375-3p and 152 participants with data on 18-month changes in miR-375-3p. Data were log transformed to improve data distribution before calculation of the changes. Changes in body fat distribution (particularly VAT) and intrahepatic fat were assessed as the primary and secondary outcomes, respectively. General linear models were performed to calculate β (SE) per 1-SD increase in miR-375-3p (fold change) for the outcomes. We assessed any nonlinear relations using the quadratic polynomial model by including linear and quadratic terms of the miRNA exposure variable in the model. Missing data were excluded in performing each analysis. Statistical analyses were performed with SAS (SAS Institute). $P < 0.05$ was considered a statistically significant level.

RESULTS

At baseline, higher circulating levels of miR-375-3p were related to lower degrees of VAT (P = 0.059), DSAT (P = 0.011), and SSAT (P = 0.003) (Supplementary Table 2). Higher miR-375-3p was also associated with lower degrees of insulin resistance and hyperinsulinemia in participants with impaired fasting glucose (100 to <126 mg/dL) or prediabetes (impaired fasting glucose or elevated HbA_{1c}) (Supplementary Table 3).

From baseline to 18 months after the intervention, circulating miR-375-3p levels significantly increased in participants overall ($P_{\text{paired } t \text{ test}} < 0.001$; mean fold change: increase of 7.9%) and in different intervention groups (Supplementary Figs. 1 and 2); however, there was large variability in the degrees of change across the participants (range: 30.2% decrease to 57.3% increase). Greater increase in miR-375-3p in response to the interventions was associated with larger reductions of VAT (β [SE] −7.41 [3.25] cm^2, P = 0.024), DSAT (−7.95 [2.33] cm^2, $P < 0.001$), and intrahepatic fat content (−1.33% [0.52%], P = 0.012) (Table 1). In performing the quadratic polynomial model, the quadratic term was not significant ($P > 0.05$), showing no significant presence of nonlinear relations; the linear term was significant for weight, VAT, DSAT, and intrahepatic fat changes ($P < 0.05$), consistent with the results in Table 1. The highest tertile group with the largest increases in miR-375-3p showed 1.4–1.7 times more reduction of abdominal fat depots and 2.4 times more reduction of intrahepatic fat, as compared with the lowest tertile group (Supplementary Fig. 3).

We performed several sensitivity analyses. We did not observe significant interactions between the diet or physical activity intervention groups and miR-375-3p changes in the outcomes ($P_{\text{interaction}} > 0.05$). Results were similar in using models with further adjustment for baseline and concurrent changes in glucose, insulin, or insulin resistance (Supplementary Table 4); the associations for VAT changes were slightly attenuated after adjustment for baseline and changes in insulin metabolism. Results of a sensitivity analysis of using liver-secreted adipokine retinol-binding protein 4, supported the findings of miR-375-3p changes with intrahepatic fat changes.

CONCLUSIONS

We found that increases in circulating miR-375-3p induced by the 18-month lifestyle interventions were related to improved visceral adiposity and liver fat

Table 1—Associations of changes in circulating miR-375-3p induced by lifestyle interventions with improvements in body adiposity outcomes over 18 months

Outcomes	N	β (SE)	P
ΔWeight, kg	145	−1.32 (0.52)	0.012
ΔWaist circumference, cm	136	−0.97 (0.6)	0.11
Adipose tissue area, cm^2			
ΔTotal adipose tissue	139	−13.9 (6.2)	0.026
ΔSSAT	139	−0.75 (1.7)	0.66
ΔDSAT	139	−7.95 (2.33)	<0.001
ΔVAT	139	−7.41 (3.25)	0.024
ΔIntrahepatic fat, %	136	−1.33 (0.52)	0.012

β (SE) per 1-SD increase in miR-375 changes (% change from baseline to 18 months) for the respective outcome after adjustment for age, sex, intervention groups, baseline BMI (for outcomes of Δtotal adipose tissue, ΔSSAT, ΔDSAT, ΔVAT, and Δintrahepatic fat), and baseline miR-375-3p and the respective outcome trait at baseline.

content, which were closely related to insulin resistance and type 2 diabetes. To the best of our knowledge, this is the first clinical study to address the significant associations of circulating miR-375-3p changes with improved diabetogenic ectopic fat depots in response to long-term lifestyle interventions.

Recent studies suggest regulatory effects of miR-375 on adipose tissue metabolism (9,13), adipogenic differentiation, and VAT accumulation (13,14), in line with the baseline associations between miR-375 and body fat distribution in this study. On the other hand, the intervention-induced changes in miR-375 were linked to reductions of VAT and ectopic fat depots, independently of the baseline levels of miR-375 and the respective adiposity measure. Our findings are supported by several recent studies showing that circulating miR-375 levels were changed by bariatric surgery and short-term very-low-calorie diets (8,15). A study reported that obese women showed lower levels of plasma miR-375 as compared with lean control women, and a 4-week very-low-calorie diet restored the miR-375 expression in the obese women (8). Also, miR-375 has been implicated in the pathogenesis of nonalcoholic fatty liver disease and hepatic inflammation (9–11). Adiponectin receptor 2 was found to be a target of miR-375 (9,14); we speculated that miR-375–regulated adipokines (9) might be involved as potential pathways. Further investigations with adipokines and other biomarkers are warranted for understanding of biological mechanisms. Also, we observed the significant relations between circulating miR-375-3p and insulin metabolism in people with prediabetes. In our sensitivity analyses, the associations of miR-375 with adiposity changes did not completely disappear after controlling for baseline and changes in glucose or insulin metabolism. Clinical studies are warranted to examine changes in β-cell function and insulin sensitivity (such as measured by the euglycemic clamp technique) after weight reduction for understanding of their effects on circulating miR-375-3p levels in humans with and without diabetes.

Our study has several strengths, including the repeated assessments of circulating miR-375 and ectopic fat depots with an adequate sample size, thus far the largest of its kind. Nonetheless, we could not determine the causality of the associations, as the changes in miRNA levels and outcomes were assessed concurrently. Whether the findings could be applicable to other populations needs to be further examined.

In conclusion, changes in circulating pancreatic islet–related miR-375-3p may be linked to improved visceral and ectopic fat accumulation during weight loss lifestyle interventions.

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