Expression of inflammation-related miRNAs in white blood cells from subjects with metabolic syndrome after 8 wk of following a Mediterranean diet–based weight loss program

José Luiz Marques-Rocha B.Sc. a,b,c, Fermin I. Milagro Ph.D. b,c,d, Maria Luisa Mansego Ph.D. b,c,d, Maria Angeles Zulet Ph.D. b,c,d, Josefa Bressan Ph.D. a, J. Alfredo Martinez M.D., Ph.D. b,c,d,*

aDepartment of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Brazil
bDepartment of Nutrition, Food Science and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain
cCenter for Nutrition Research, University of Navarra, Pamplona, Spain
dCIBERobn, Physiopathology of Obesity, Carlos III Institute, Madrid, Spain

A B S T R A C T

Objectives: The aim of this study was to evaluate the influence of a dietary strategy for weight loss (the RESMENA [reduction of metabolic syndrome in Navarra, Spain] diet) on the expression of inflammation-related microRNAs (miRNAs) and genes in white blood cells (WBC) from individuals with metabolic syndrome (MetS).

Methods: The clinical, anthropometric, and biochemical characteristics of 40 individuals with MetS (20 men and 20 women; age: 48.84 ± 10.02 y; body mass index: 35.41 ± 4.42 kg/m²) were evaluated before and after an 8-wk hypocaloric diet based on the Mediterranean dietary pattern. Nutrient intake was assessed with a food frequency questionnaire and 48-h weighed food records. Total RNA was isolated from WBC and the expression of some inflammation-related miRNAs and mRNAs (IL-6, TNF-α, ICAM-1, IL-18, SERPINE1, VCAM-1, GAPDH) was assessed by quantitative polymerase chain reaction.

Results: The RESMENA nutritional intervention improved most anthropometric and biochemical features. The expression of miR-155-3p was decreased in WBC, whereas Let-7b was strongly upregulated as a consequence of the dietary treatment. However, they were not correlated with the expression of the proinflammatory genes in the same cells. The changes in the expression of let-7b, miR-125b, miR-130a, miR-132-3p, and miR-422b were significantly associated with changes in diet quality when assessed by the Healthy Eating Index. Moreover, low consumption of lipids and saturated fat (g/d) were associated with higher expression of let-7b after the nutritional intervention.

Conclusions: The Mediterranean-based nutritional intervention was able to induce changes in the expression of let-7b and miR-155-3p in WBC from patients with MetS after 8 wk. Moreover, the quality of the diet has an important effect on the miRNAs expression changes. These results should be highlighted because these miRNAs have been associated with inflammatory gene regulation and important human diseases.

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Introduction

Metabolic syndrome (MetS) is a complex disorder defined by a cluster of interconnected cardiometabolic alterations, including hypertension, insulin resistance, dyslipidemia, and abdominal obesity [1]. Individuals exhibiting combinations of these metabolic disturbances have a substantial synergistic cardiovascular risk greater than the sum associated with each abnormality [2]. Furthermore, the pathologic enlargement of the adipose tissue in obesity leads to an elevated production of proinflammatory mediators [3]. These altered signals mediate multiple processes, which predispose to diabetes mellitus, hepatic steatosis, atherosclerosis, plaque rupture, and atherothrombosis [1]. However, to date, the available information is controversial and does not necessarily imply a causal role neither putatively involve epigenetic mechanisms.

Data obtained from functional genomic methodologies indicate that several hundred genes participate in the inflammatory response and that their coordinated expression is tightly controlled [4]. Hence, microRNAs (miRNAs) are small noncoding RNAs that have the ability to control multiple genes, establishing an orchestrated network governing remote processes through intertwined pathways [5]. Several studies have highlighted the significance of miRNAs in maintaining metabolic homeostasis, and the regulation of these miRNAs could serve as a potential therapeutic target for metabolic disorders [6]. For example, the inflammatory toll-like receptor/nuclear factor-xB-related miR-181a is downregulated in monocytes of obese patients, which is associated with a higher number of MetS components and with coronary artery disease [7]. In this sense, miRNA expression contributes to regulate body weight, sometimes in relation to inflammation regulation [8]. Thus, some of these transcripts could be used as prognostic biomarkers of response to hypocaloric diets.

The aim of this study was to evaluate the effect of a weight loss strategy based on the Mediterranean dietary pattern (the RESMENA diet) on anthropometric measurements, biochemical markers, and the expression of some inflammation-related genes and miRNAs in white blood cells (WBC) of individuals with MetS. We hypothesized that a hypocaloric pattern, as well as the improvement of the overall diet quality components designed to reduce MetS features, could have a positive effect on the expression of genes and miRNAs related to the inflammatory state. We sought to further explore the possible epigenetic underlying mechanisms and the putative interactions with the diet.

Methods

Study population

Forty white individuals (20 men and 20 women) with a body mass index (BMI) of 33.41 ± 4.42 kg/m², aged 48 ± 10 years, and diagnosed with MetS according to the International Diabetes Federation cutoffs [9] were enrolled in this study. The inclusion and exclusion criteria have been previously reported [10], but it should be pointed out that individuals with presence of psychiatric disturbances, eating disorders, chronic diseases related to the metabolism of nutrients, major body weight changes in the previous 3 mo, and difficulties in changing food habits were excluded. Volunteers were recruited through local newspaper advertisements and the database of Department of Nutrition, Food Science and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain. Calculations were based on findings of previous studies [10,11]. The study protocol was performed in accordance with the ethical guidelines of the Declaration of Helsinki, and was approved by the Research Ethics Committee of the University of Navarra (ref. 065/2009). All individuals provided written informed consent also approved by the same research ethics committee.

Study protocol

The research is based on a subsample of the RESMENA-S study [10], a controlled intervention study that aimed to reduce MetS features based on energy restriction over 6 mo [10,11] and with components of the Mediterranean diet to specifically combat MetS features. The complete project is registered at www.clinicaltrials.gov (NCT01087086) and can be accessed elsewhere [10]. The diet of the RESMENA study prescribed an energy restriction of 30% applied to the total energy requirements of each patient. Resting metabolic rate was calculated using the Harris–Benedict equation, in which the Wilkens-adjusted weight was applied [12]. The physical activity factor was considered to calculate the total energy requirements according to the Food and Nutrition Board, National Research Council: Recommended Dietary Allowances [13]. Fasting blood samples, habitual dietary intake, and body composition were measured at baseline and at the end point of the 8-wk intervention period following standardized protocols, as published elsewhere [10,12].

Diet and dietary assessments

Participants were provided a 7-d menu plan as previously described [14]. The plan was composed of seven meals per day, including breakfast, lunch, dinner, two snacks in the morning, and two more snacks in the afternoon. Some characteristics of the RESMENA diet were a moderately high protein intake and the expression of some selected genes and miRNAs related to the inflammatory state. We sought to further explore the possible epigenetic underly- ing mechanisms and the putative interactions with the diet.
necrosis factor (TNF-α) and high-sensitivity C-reactive protein (hs-CRP). German subjects were selected using ELISA kits and an automated analyzer system (Triturus, Grifols, Barcelona, Spain). In our laboratory, the inter- and intra-assay variability were <10% for all analytical determinations.

**RNA extraction, miRNA, and mRNA quantitative PCR**

At baseline and at the end of the study, total RNA was extracted from WBC using Trizol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNA were determined at 260/280 nm using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). For the miRNA analysis, 20 ng of total RNA was reverse-transcribed using the TaqMan MicroRNA Reverse Transcription kit (Life Technologies, Foster City, CA, USA) according to the manufacturer's protocol and the miRNA-specific reverse-transcription primers provided with the TaqMan MicroRNA Assay (Life Technologies), as shown in Table 1. The miRNA-specific cDNA was amplified in triplicate with the TaqMan Universal polymerase chain reaction (PCR) master mix and the respective specific probe provided in the TaqMan MicroRNA Assay (Life Technologies).

To perform the miRNA analyses, 2 µg of total RNA was reverse-transcribed using the high-capacity complementary DNA (cDNA) reverse transcription (RT) kit (Life Technologies) according to the manufacturer’s protocol. cDNA was amplified in triplicate with the TaqMan Universal PCR master mix and the respective specific probe provided in the TaqMan Gene Expression Assays (Life Technologies; Table 1). The analyzed miRNAs and genes were selected on the basis of previous studies strongly supporting their possible involvement in inflammatory pathways related to MetS features.

**Statistical analyses**

A group size of 40 was estimated to be necessary to obtain a significant (P < 0.05) difference in the reduction of waist circumference of 4.3 ± 6.8 cm with a power of 80% [10]. Only those individuals who completed the study were analyzed. The results are expressed as mean ± SD. Normality distributions of the measured variables were determined according to the Shapiro–Wilk test. Differences between the beginning and the end of the intervention period were analyzed by a paired t test. Spearman correlation and linear regression analyses were applied to assess the potential relationships and associations among diet, biochemical, and anthropometrical components with miRNA and mRNA variations. Analyses were carried out using SPSS 15.1 software for Windows (SPSS Inc, Chicago, IL, USA). P < 0.05 was considered statistically significant.

**Table 1**

References of the specific PCR primers/probes used for the measurement of the miRNAs and mRNAs

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>TaqMan MicroRNA Assay reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Let-7b</td>
<td>002619</td>
</tr>
<tr>
<td>miR-125b</td>
<td>000449</td>
</tr>
<tr>
<td>miR-130a</td>
<td>000454</td>
</tr>
<tr>
<td>miR-132-3p</td>
<td>000457</td>
</tr>
<tr>
<td>miR-146a</td>
<td>000468</td>
</tr>
<tr>
<td>miR-155-3p</td>
<td>002287</td>
</tr>
<tr>
<td>miR-223-5p</td>
<td>002098</td>
</tr>
<tr>
<td>miR-422b</td>
<td>001314</td>
</tr>
<tr>
<td>miR-4772- p</td>
<td>464414</td>
</tr>
<tr>
<td>GAPDH</td>
<td>001006</td>
</tr>
</tbody>
</table>

**Results**

All clinical, laboratory, and dietary characteristics of study participants at the beginning and at the end of the dietary intervention are shown in Table 2. After 8 wk, the RESMENA diet improved most anthropometric and biochemical markers included in this study (Table 2). There was a significant reduction of all variables related to body weight, such as BMI, waist circumference, and fat mass (P < 0.05). There was also a significant reduction of TC, triacylglycerols, glucose, and insulin levels and, consequently, of the HOMA index (P < 0.05). Moreover, the circulating levels of MDA and PAI-1 were lower when compared with the levels at baseline (P < 0.05).

**Table 2**

Anthropometric, biochemical, and dietary characteristics of the participants at baseline and after 8 wk on the RESMENA Diet

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline Mean ± SD</th>
<th>8 wk Mean ± SD</th>
<th>P value&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric measurements (N = 40)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>99.7 ± 16.5</td>
<td>92.6 ± 15.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.4 ± 4.4</td>
<td>32.8 ± 4.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.9 ± 12.1</td>
<td>101.2 ± 10.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.96 ± 0.10</td>
<td>0.92 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DXA total fat (%)</td>
<td>42.6 ± 6.0</td>
<td>40.1 ± 6.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DXA gynoid fat mass (kg)</td>
<td>7.96 ± 1.74</td>
<td>7.33 ± 1.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DXA android fat mass (kg)</td>
<td>3.93 ± 0.94</td>
<td>3.72 ± 0.87</td>
<td>0.02</td>
</tr>
<tr>
<td>DXA trunkal fat mass (kg)</td>
<td>26.73 ± 5.18</td>
<td>25.92 ± 5.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood pressure (N = 40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>147.39 ± 20.87</td>
<td>134.10 ± 16.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84.34 ± 9.01</td>
<td>76.69 ± 9.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Biochemical parameters (N = 40)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>219 ± 45</td>
<td>202 ± 45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>43 ± 10</td>
<td>41 ± 9</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>137 ± 39</td>
<td>131 ± 39</td>
<td>0.29</td>
</tr>
<tr>
<td>OX-LDL (U/L)</td>
<td>44.3 ± 18.5</td>
<td>40.2 ± 13.9</td>
<td>0.11</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>196 ± 124</td>
<td>150 ± 100</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>124 ± 38</td>
<td>109 ± 25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>14.18 ± 8.12</td>
<td>8.95 ± 5.97</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HOMA index</td>
<td>4.46 ± 3.01</td>
<td>2.49 ± 1.82</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.82 ± 1.38</td>
<td>5.94 ± 1.06</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Inflammatory markers (N = 40)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>0.84 ± 0.36</td>
<td>0.74 ± 0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.30 ± 3.45</td>
<td>3.53 ± 3.75</td>
<td>0.71</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.63 ± 1.73</td>
<td>2.76 ± 1.58</td>
<td>0.53</td>
</tr>
<tr>
<td>PAI-1 (µg/ml)</td>
<td>157 ± 127</td>
<td>144 ± 152</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.69 ± 0.50</td>
<td>0.88 ± 0.94</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Dietary characteristics (n = 35)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>2255 ± 287</td>
<td>1353 ± 559</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Meal frequency (meals/d)</td>
<td>5.35 ± 1.30</td>
<td>6.47 ± 1.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proteins (% TCV/d)</td>
<td>17.1 ± 2.8</td>
<td>24.5 ± 2.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lipids (% TCV/d)</td>
<td>44.4 ± 6.0</td>
<td>37.8 ± 7.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CHO (% TCV/d)</td>
<td>37.9 ± 5.9</td>
<td>37.4 ± 6.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>18.3 ± 10.2</td>
<td>20.2 ± 8.5</td>
<td>0.07</td>
</tr>
<tr>
<td>GL (U/d)</td>
<td>663 ± 217</td>
<td>332 ± 124</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GL (U/d)</td>
<td>112 ± 43</td>
<td>53 ± 26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Saturated fatty acids (g/d)</td>
<td>34.3 ± 13.2</td>
<td>16.1 ± 5.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ω-3 fatty acids (g/d)</td>
<td>0.33 ± 0.16</td>
<td>0.34 ± 0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>TAC (mmol/d)</td>
<td>8.32 ± 4.74</td>
<td>13.89 ± 5.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Healthy Eating Index (U)</td>
<td>55.9 ± 11.8</td>
<td>71.4 ± 10.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; DXA, dual-energy x-ray absorptiometry; GL, glycemic load; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; GL, glycemic index; LDL-C, low-density lipoprotein cholesterol; MDA: malondialdehyde; ox-LDL, oxidized low-density lipoprotein cholesterol; RESMENA, reduction of metabolic syndrome in Navarra, Spain; SBP, systolic blood pressure; TG, triacylglycerols; TCV, total caloric value.

Bolded P values indicate statistically significant (less than 0.05).

<sup>+</sup> P value: comparison by paired samples statistics from baseline (Student’s t test).
The dietary characteristics of the participants changed in accordance with the recommendations provided by the RESMENA diet. There was a lower intake of energy, lipids, and saturated fatty acids (P < 0.05). On the other hand, meal frequency (meals/day), protein intake, and TAC of the diet were higher (P < 0.05) at the end of the nutritional intervention (Table 2). These changes subsequently improved (P < 0.05) the HEI.

The analysis of the expression of proinflammatory genes in WBC showed no relevant changes (P > 0.05) as a result of the 8-wk nutritional intervention (Table 3). However, the expression of miR-155-3p decreased (P = 0.007), whereas let-7b was strongly upregulated (P = 0.002) as a consequence of the dietary intervention (Table 3). However, they were not correlated with the expression of the proinflammatory genes analyzed. Interestingly, the expression of let-7b was highly correlated (P < 0.05) with the other miRNAs analyzed, both at baseline (miR-130a, miR-132-3p, miR-146a, miR-155-3p, miR-223-5p, miR-422b, and miR-4772-3p) or after the RESMENA diet (miR-125b, miR-130a, miR-146a, miR-223-5p, miR-422b, and miR-4772-3p; Table 4).

The changes (8 wk versus baseline) in the miRNA expression levels were statistically associated with the changes in the diet quality, when assessed by the HEI. The improvement of HEI positively correlated with the changes in the expression of some miRNAs (Table 5) such as let-7b (r² = 0.478; P = 0.008), miR-125b (r² = 0.359; P = 0.047), miR-130a (r² = 0.546; P = 0.001), miR-132-3p (r² = 0.551; P = 0.001), and miR-422b (r² = 0.374; P = 0.042). Some of these significant correlations were found even after adjustment for energy restriction, sex, and age: let-7b (β = 0.425; P = 0.035), miR-130a (β = 0.469; P = 0.009), and miR-132-3p (β = 0.543; P = 0.003; Table 6). Furthermore, the miR-155 expression was associated with changes in the HEI and weight loss in a regression model adjusted for sex and age (β = 0.577; P = 0.014 and β = 0.740; P = 0.003, respectively; Table 6).

### Discussion

In the present study, the effects of a novel dietary strategy on the expression levels of miRNAs and genes related to the inflammatory process in WBC were reported. To our knowledge, this is a pioneer study in patients with MetS evaluating the effects of an energy-restricted intervention based on Mediterranean diet principles, including a modified macronutrient distribution and increased meal frequency, as well as the

### Table 3

<table>
<thead>
<tr>
<th>Relative expression (fold change) of some miRNAs and genes (mRNAs) at baseline and after 8 wk on the RESMENA diet</th>
<th>Baseline Mean ± SD</th>
<th>8-wk Mean ± SD</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>miRNAs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Let-7b</td>
<td>1.04 ± 0.63</td>
<td>1.51 ± 0.90</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-155-3p</td>
<td>0.94 ± 0.48</td>
<td>0.51 ± 0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>miR-146a</td>
<td>0.54 ± 0.23</td>
<td>0.58 ± 0.25</td>
<td>0.83</td>
</tr>
<tr>
<td>miR-132-3p</td>
<td>0.68 ± 0.40</td>
<td>0.59 ± 0.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-223-5p</td>
<td>0.82 ± 0.50</td>
<td>0.89 ± 0.47</td>
<td>0.26</td>
</tr>
<tr>
<td>miR-422b</td>
<td>0.70 ± 0.43</td>
<td>0.74 ± 0.36</td>
<td>0.13</td>
</tr>
<tr>
<td>miR-4772-3p</td>
<td>0.26 ± 0.59</td>
<td>0.78 ± 0.27</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.62 ± 0.47</td>
<td>1.05 ± 0.86</td>
<td>0.20</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.01 ± 0.86</td>
<td>1.53 ± 1.25</td>
<td>0.08</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>2.21 ± 1.79</td>
<td>1.92 ± 1.64</td>
<td>0.66</td>
</tr>
<tr>
<td>IL-18</td>
<td>2.30 ± 2.16</td>
<td>1.54 ± 1.32</td>
<td>0.21</td>
</tr>
<tr>
<td>SERPINE1</td>
<td>5.59 ± 4.15</td>
<td>4.74 ± 2.96</td>
<td>0.91</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>5.21 ± 4.62</td>
<td>3.02 ± 2.32</td>
<td>0.30</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; TNF, tumor necrosis factor; ICAM, intercellular adhesion molecule; PCR, polymerase chain reaction; RESMENA, reduction of metabolic syndrome in Navarra, Spain

Bolded P values indicate statistically significant (less than 0.05).

* P value: comparison by paired samples after log transformation (Student’s t test).

miRNA and mRNA expression were measured by quantitative real-time PCR and normalized by RNU48 and GAPDH, respectively.

### Table 4

<table>
<thead>
<tr>
<th>Spearman correlations between the relative expression of Let-7b and other miRNAs* analyzed at baseline and after 8 wk on the RESMENA diet</th>
<th>Baseline</th>
<th>8 wk</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Let-7b</td>
<td>miR-125b</td>
<td>0.290</td>
<td>0.09</td>
<td>0.360</td>
</tr>
<tr>
<td>miR-130a</td>
<td>0.702</td>
<td>&lt;0.01</td>
<td>0.636</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-132-3p</td>
<td>0.002</td>
<td>0.99</td>
<td>0.025</td>
<td>0.88</td>
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<tr>
<td>miR-146a</td>
<td>0.049</td>
<td>0.77</td>
<td>0.425</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-155-3p</td>
<td>-0.435</td>
<td>0.03</td>
<td>-0.159</td>
<td>0.41</td>
</tr>
<tr>
<td>miR-223-5p</td>
<td>0.605</td>
<td>&lt;0.01</td>
<td>0.674</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-422b</td>
<td>0.441</td>
<td>&lt;0.01</td>
<td>0.404</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>miR-4772-3p</td>
<td>0.380</td>
<td>0.02</td>
<td>0.632</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; RESMENA, reduction of metabolic syndrome in Navarra, Spain

Bolded P values indicate statistically significant (less than 0.05).

* miRNAs were measured by quantitative real-time PCR and normalized by RNU48.

### Table 5

<table>
<thead>
<tr>
<th>Spearman correlations between changes in the healthy eating index and changes in the relative expression of the miRNAs and genes analyzed†</th>
<th>Baseline Mean ± SD</th>
<th>8-wk Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Δ healthy eating Index (N = 35)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Let-7b</td>
<td>0.478</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Δ miR-125b</td>
<td>0.359</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Δ miR-130a</td>
<td>0.546</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Δ miR-132-3p</td>
<td>0.551</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Δ miR-146a</td>
<td>-0.026</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Δ miR-155-3p</td>
<td>0.414</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Δ miR-223-5p</td>
<td>0.023</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Δ miR-422b</td>
<td>0.374</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Δ miR-4772-3p</td>
<td>-0.238</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Δ IL-6</td>
<td>-0.258</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Δ TNF-α</td>
<td>-0.059</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Δ ICAM-1</td>
<td>0.058</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Δ IL-18</td>
<td>0.153</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Δ SERPINE1</td>
<td>0.020</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Δ VCAM-1</td>
<td>-0.049</td>
<td>0.81</td>
<td></td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ICAM, intercellular adhesion molecule; IL, interleukin; PCR, polymerase chain reaction; SERPINE, serine protease inhibitor member E; VCAM, vascular cell adhesion molecule

Bolded P values indicate statistically significant (less than 0.05).

† Changes (8 wk vs baseline) in mRNA and gene expression were measured by quantitative real-time PCR and normalized by RNU48 and GAPDH, respectively.

† P < 0.05.
presence of bioactive ingredients, such as fiber and beneficial fatty acids, and controlling glycemic index and GL, dietary TAC, and HEI score.

Metabolic diseases are characterized by the failure of regulatory genes or enzymes to effectively orchestrate specific pathways involved in the control of many biological processes [2]. In addition to the classical regulators of metabolic homeostasis, recent discoveries have evidenced the putative remarkable role of miRNAs in the post-transcriptional regulation of a number of genes, and their involvement in many pathologic states, such as the MetS features [22]. However, results from human studies are still unclear and more research is needed to find links between miRNAs and the pathways involved.

The main results of the current study were the expression changes of miRNA-155 and let-7b, especially for the latter. Let-7 family was, after lin-4, the second miRNAs found and has nine members, namely, let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, and miR-98. All members are believed to exert similar functions because they share a common seed region (nucleotides 7g, let-7i, and miR-98). All members are believed to exert similar functions because they share a common seed region (nucleotides 7g, let-7i, and miR-98.

To evaluate the quality of the diet, some indices or scores have been developed, such as the HEI, the Alternate HEI, or the Diet Quality Index and derivatives [16]. Most of these tools take into consideration the Mediterranean diet guidelines, widely recognized as a healthy pattern [36]. They consist of a single score that results from computing different components, such as foods, food groups, or a combination of foods and nutrients. In this context, the HEI score was selected because it takes into account macro- and micronutrient intake, as well as food variety. The improvement of HEI score due to the 8-wk RESMENA intervention was related to changes in the expression of let-7b, miR-125, miR-130, miR-132, and miR-422. Additionally, the consumption of total and saturated fat inversely correlated with the HEI and publications have provided new insights into the protective properties of let-7b in preventing the endothelial dysfunction associated with cardiovascular disease [26]. Circulating let-7b levels were lower in patients with acute myocardial infarction than in healthy adults [27]. Additionally, overexpression of let-7b promoted nitric oxide production [26], which is considered a key factor in vascular protective actions including vasodilatation. Although this study did not imply a direct action, we found that blood pressure was lower at the end of the dietary intervention period.

Global knockdown of the let-7 family with an anti-miR was sufficient to prevent and treat impaired glucose tolerance in mice with diet-induced obesity, at least in part by improving insulin sensitivity in liver and muscle [23]. In the same study, anti-miR treatment of mice on a high-fat diet also resulted in increased lean and muscle mass, but not increased fat mass, and prevented ectopic fat deposition in the liver. These findings demonstrate that let-7 regulates multiple aspects of glucose metabolism and suggests anti-miR-induced let-7 knockdown as a potential treatment for type 2 diabetes.

In this trial, the expression of miR-155 was lower at the end of the nutritional intervention and was associated with the quality of the diet and with the weight loss by regression analysis. These results suggest that miR-155 could be a prognostic marker of obesity, as advanced by previous studies. In this context, some authors have shown that the expression of miR-155 was higher in diet-induced obese rats than in control animals [28]. In the same study, a 30% calorie restriction led to significant reduction in the expression of miR-155. Recent findings identified miR-155 as a central apigenin-regulated miRNA in inflammation and provided evidence of the underlying mechanism by which apigenin and diets rich in apigenin, a flavonoid abundant in parsley and celery, contribute to restore homeostasis [29]. Additionally, quercetin decreased lipopolysaccharide (LPS)-induced expression of miR-155 in macrophages [30], highlighting the benefits of dietary interventions as a strategy to restore proper immune function in vivo.

Overexpression of miR-155 has been reported in lung, breast, and colon tumors [31] and has been positively associated with intestinal inflammation and ulcerative colitis [32]. Furthermore, in vivo studies have found that transgenic mice overexpressing miR-155 in B-cell lineage (Eμ-miR-155) produced more TNF-α when challenged with LPS and were hypersensitive to LPS/galactosamine–induced septic shock with respect to their normal counterparts [33]. Interestingly, miR-155 targets the lipid phosphatase SHIP1 [34], an important signal for macrophage activation. The exposure of cultured macrophages to LPS leads to upregulation of miR-155, which targets the CCAAT/enhancer binding protein β (C/EBPβ) mRNA, implicated in the regulation of proinflammatory cytokines during macrophage activation and the acute-phase response [35].

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let-7b expression. Our regression analyses also indicated that the expression of these miRNAs was more dependent on the quality of the diet than on the weight loss achieved or the energy restriction. To our knowledge, this was the first time that these associations were described in the scientific literature. It has been previously mentioned that the diet may influence the expression of some miRNAs, which has been considered useful in the assessment of nutritional status in dietary intervention studies. For example, miR-125b has been reported to regulate in a negative manner the expression of the vitamin D receptor (VDR) [37]. This functional link between VDR expression and miR-125b might explain why VDR expression is upregulated, whereas miR-125b is downregulated in distinct types of cancer as the genomic locus that contains miR-125b is deleted in several cancers [37]. On the other hand, miR-125b has been reported to post-transcriptionally regulate vitamin D3 hydroxylase (CYP24), an enzyme that catalyzes the inactivation of vitamin D; the low expression of miR-125b in cancer tissues may be a possible mechanism for explaining the high CYP24 expression in cancer tissues [38]. The influence of nutrients on the expression of other miRNAs is still unclear, being a new promising area of nutrigenomic research.

In this study, MDA and PAI-1 levels decreased significantly after the intervention. High levels of plasma MDA, a biomarker of lipid peroxidation, have been associated with type 2 diabetes [39], whereas calorie-restricted dietary strategies have been previously associated with lower MDA levels [40]. In this sense, a recent publication revealed that Let-7b overexpression inhibited reactive oxygen species production [26]. In this context, it can be hypothesized that the increased expression of let-7 at the end of the intervention may contribute to decrease reactive oxygen species production and lipid peroxidation.

PAI-1, encoded by the SERPINE1 gene, is the principal inhibitor of tissue plasminogen activator and urokinase, and therefore is an inhibitor of fibrinolysis [41]. This serine protease is produced by vascular endothelium, liver, monocytes/macrophages, platelets, and adipose tissue [42]. High plasma levels of PAI-1 have been associated with higher risk for cardiovascular diseases [43]. Furthermore, PAI-1–dependent mechanisms are also implicated in the pathogenesis of obesity, insulin resistance, and type 2 diabetes [44]. In fact, increased PAI-1 levels can be considered a component of MetS. Clinical studies have demonstrated a strong correlation between PAI-1 and BMI and fibrinolysis. As elevated levels of inflammatory cytokines could increase PAI-1

![Graphs showing correlations between changes in HEI and changes in the relative expression of let-7b and changes in the intake of lipids and saturated fat.](image-url)
expression, the link between obesity and elevated PAI-1 levels could be the low-grade inflammatory state [45]. Although the RESMENA dietary strategy was successful for weight loss and the improvement of most of the anthropometric and biochemical parameters, including PAI-1, the circulating levels of other inflammatory biomarkers, such as CRP, TNF-α, and IL-6, were not reduced.

The study has some limitations. First, gene expression varies depending on the tissue or cell type [22], and it is possible that specific miRNAs are not widely expressed in human WBC. Second, this study specifically aimed to evaluate the association between the variables included and conclusions on causality cannot be measured. Also, the number of participants in this study is not very high, but it may be proposed that type 2 errors were overcome because important statistical differences were found. In the analysis of before and after responses in a single group, confounding by measured or unmeasured factors that are potentially time varying across the time periods compared remains a critical threat. However, we used statistical methods adjusting for measured confounding factors and quantifying the potential effect of unmeasured factors on effect estimates. Finally, despite limitations of structured questionnaires of this type, the advantage of food frequency questionnaire is its feasibility for establishing long-term habitual dietary intake [46].

Conclusion

The results of this study provide evidence that the quality of a Mediterranean-based nutritional intervention was related to changes in the expression of let-7b and miR-155-3p in WBC from patients with MetS after 8 wk. These results should be highlighted because these miRNAs have been associated with important human diseases linked to MetS, such as cancer, atherogenic and adipogenic processes, and other inflammatory conditions.

Acknowledgments

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