Ramadan diurnal intermittent fasting modulates SOD2, TFAM, Nrf2, and sirtuins (SIRT1, SIRT3) gene expressions in subjects with overweight and obesity

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ABSTRACT

Aim: A growing body of evidence supports the impact of intermittent fasting on normalizing body metabolism and lowering oxidative stress and inflammation. Mounting evidence confirms that oxidative stress and chronic inflammation trigger the way for the development of metabolic diseases, such as diabetes. This research was conducted to evaluate the impact of Ramadan intermittent fasting (RIF) on the expression of cellular metabolism (SIRT1 and SIRT3) and antioxidant genes (TFAM, SOD2, and Nrf2).

Methods: Fifty-six (34 males and 22 females) overweight and obese subjects and six healthy body weight controls were recruited and monitored before and after Ramadan.

Results: Results showed that the relative gene expressions in obese subjects in comparison to counterpart expressions of controls for the antioxidant genes (TFAM, SOD2, and Nrf2) were significantly increased at the end of Ramadan, with percent increments of 90.5%, 54.1%, and 111.5% for the three genes, respectively. However, the metabolism-controlling gene (SIRT3) showed a highly significant (P < 0.001) downregulation accompanied with a trend for reduction in SIRT1 gene at the end of Ramadan month, with percent decrements of 61.8% and 10.4%, respectively. Binary regression analysis revealed significant positive correlation (P < 0.001) between high energy intake (>2000 Kcal/day vs. <2000 Kcal/day) and expressions of SOD2 and TFAM (r = 0.84 and r = 0.9, respectively).
1. Introduction

Intermittent fasting (IF) encompasses eating pattern in which individuals go extended periods with little or no caloric intake, with intervening feeding periods regularly. Different forms of IF, including alternate-day fasting, periodic fasting, and time-restricted feeding, in healthy and overweight human subjects, demonstrated effectiveness in reducing body weight and improving multiple health indicators including reductions in cardio-metabolic risk factors [1,2].

Oxidative stress can be defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidative defense system in the body, which ends with overwhelming the capacity of human body's antioxidant defense system [3,4]. Oxidative stress has been recognized as a principal etiological factor in a wide range of chronic human ailments [5]. Moreover, the prevailing oxidative and inflammatory conditions constitute significant risk factors for the development of several pathologies, such as diabetes [6].

Sirtuin 1 (SIRT1), a conserved histone deacetylase with widespread effects on cellular metabolism, mitochondrial bioenergetics, and aging, has been found to regulate several genes [7,8]. These genes were correlated to aging and to increase the expression of genes that act as endogenous ROS scavengers, protecting the cells from further ROS insult. Mitochondria are believed to play a central role in these events, as mitochondria are the primary site of endogenous ROS production, and mitochondrial transcription factors are upregulated with fasting and caloric restriction [9]. In turn, dietary modifications, such as caloric restriction, have been found to promote a transient state of oxidative stress, leading to adaptive protective responses, which in the long run protect cells from future ROS harms [10].

SIRT3 is the most well described of the mitochondrial sirtuins and is located primarily in the mitochondrial matrix. SIRT3, a deacetylase enzyme that affects the lysine residue and is involved in the regulation of the proteomic and biochemical activities in the mitochondria. Calorie restriction, fasting, and exercise training have all been shown to increase SIRT3 levels in different tissues [11]. In contrast, a recent study reported a reduction in SIRT3 protein in skeletal muscle of fasted mice [12].

Nuclear factor erythroid 2 related factor 2 (Nrf2) is considered a fundamental factor in resistance to oxidative stress over the last decade [13]. Superoxide dismutase 2 (SOD2) is one of three forms of SOD, located in the mitochondria and plays a significant role in ROS attenuation. SOD2 is the primary mitochondrial scavenging enzyme that converts superoxide to hydrogen peroxide, which is finally converted to water by catalase [14]. Nrf2 has been involved in maintaining mitochondrial redox homeostasis by activating mitochondrial antioxidant enzymes such as SOD2 [15]. Mitochondrial transcription factor A (TFAM) is involved in the transcriptional control of mitochondrial DNA (mtDNA) [16]. Recent reports showed that TFAM protects against diseases with oxidative stress [17] and maintains the mitochondrial organelle genome [18]. Thus, any intervention causing an increase in TFAM levels, and therefore mtDNA stability and enhanced mitochondrial biogenesis [19].

Ramadan intermittent fasting (RIF) is considered a unique model of intermittent diurnal fasting, as food and fluid intake, becomes exclusive at the nocturnal time without restriction on the type or amount of food intake for one month yearly [20]. Many physiological changes occur during RIF [21–28]. Recent systematic review and meta-analysis unraveled that RIF is associated with reductions in important inflammatory and oxidative stress markers after completing diurnal RIF [29]. Up to the best of our knowledge, none of the previous research investigated the change of metabolism-controlling and antioxidant defense systems gene expression concerning RIF. Further, there is a paucity of clinical studies exploring the effect of IF and RIF in particular, on protective cellular responses in human subjects. Based on previous studies, we hypothesized that the RIF could incur beneficial effects on expression of genes related to aging and metabolism, namely metabolism-controlling and aging genes sirtuins 1 and 3 (SIRT1 and SIRT3) and the antioxidative stress genes (TFAM, SOD2, and Nrf2).

2. Methods

2.1. Ramadan fasting and research design

The prospective study design was conducted during Ramadan from June to July of the lunar calendar in 2016. Data were collected on the baseline (T1, one week before the month of Ramadan), and after completing 28 or 29 or 30 consecutive days of the fasting month. During the fasting month of Ramadan, individuals refrain from all oral intakes (including food and water) from dawn to sunset. The daily fasting hours were about 15 h. Each subject served as self-control by comparing her/his values before and at the end of Ramadan. No particular dietary regimens or recommendations were given to the participants during any stage of the study, and all the subjects were asked to pursue their physical exercise patterns during the non-fasting period. According to the Islamic regulations, females are exempted from observing Ramadan fasting during their menstrual period; thus the intervention period for the female subjects was less than males, and ranged from

Conclusion: Results suggest that RIF ameliorates the genetic expression of antioxidant and anti-inflammatory, and metabolic regulatory genes. Thus, RIF presumably may entail a protective impact against oxidative stress and its adverse metabolic-related derangements in non-diabetic obese patients.
23 to 25 days, whereas the intervention period for the men lasted from 28 to 30 days.

2.2. Subjects

The study protocol was designed and conducted per the Declaration of Helsinki and was approved by the Research Ethics Committee of the University of Sharjah, United Arab Emirates (Reference no: REC-16-05-11-01). All enrolled subjects (56 in total) were provided with an information sheet describing the research plan, objectives, and requirements of involvement. The subjects were recruited using personal communication, social media, and institutional e-mails and attended at the University Hospital of Sharjah (UHS) for screening, investigations, and signing the informed consent. Subjects of both genders who were a healthy weight and overweight/obesity (BMI > 25 kg/m²), willing to fast during Ramadan and participate in the study were asked to sign the consent form. Subjects with a history of diabetes or cardiovascular disease, on regular medications, following a weight-reducing diet or had a history of bariatric surgery within the last nine months of both genders who were a healthy weight and overweight/obesity (BMI > 25 kg/m²), willing to fast during Ramadan and participate in the study were asked to sign the consent form. Subjects with a history of diabetes or cardiovascular disease, on regular medications, following a weight-reducing diet or had a history of bariatric surgery within the last nine months before Ramadan, being a pregnant woman or perimenopausal woman were excluded from the study. Six subjects with healthy body weight were recruited as controls.

2.3. Anthropometric assessment

Anthropometric measurements were taken at the two time points (before and after RIF). Body weight, fat mass and percent, fat-free mass, and total body water were measured using direct segmental multi-frequency bioelectrical impedance analysis (DSM-BIA; TANITA, MC-980, Tokyo/Japan). The visceral fat rating was measured by DSM-BIA machine, and the value (from 0 to 100) was converted into a visceral fat surface area by multiplying the obtained value by 10, as per the instructions of the manufacturer. Height was measured using a fixed stadiometer to the nearest 0.1 cm. BMI was calculated as kg/m² accordingly. Waist and hip circumferences were measured to the nearest 0.01 m using a non-stretchable measuring tape (Seca, Hamburg/Germany), and waist: hip ratio (WHR) was calculated after that.

2.4. Dietary intake assessment

Dietary intake was assessed by 24-h recalls, collected on three consecutive days (one weekend day and two weekdays) at the two time points, by trained nutritionists. Two-dimensional food models were used to help subjects approximate portion sizes. Dietary intakes of calories and macronutrients (carbohydrates, proteins, fats, fibers, and water) were estimated using the Food Processor software (version 10.6 ESHA Research, Salem, OR/USA).

2.5. Physical activity level

General physical activity level was assessed using the Dietary Reference Intakes classification [30]. In this classification, the activity level is considered sedentary if subject spends most of the day time in living activities without additional physical exercise. Low active level is considered when the subject spends the day time on living activities plus 30–60 min per day of moderate intensity exercises. The active level is considered when day time is spent on living activities plus at least 60 min per day of moderate intensity exercise. Very active level is considered when the subject spends day time on living activities plus at least 120 min per day of moderate intensity exercises or 60 min of vigorous exercise [30].

2.6. Blood sampling and blood pressure measuring

Ten milliliters of the venous blood sample was collected from each participant after completing at least eight hours of fasting at both time points; the baseline which was before RIF and at the testing point was at the end of the fourth week of Ramadan. The samples were collected between 11 am and 1 pm for the two time points, in order to eliminate the effect of timing and dietary intake on the measured biochemical parameters and to ensure the consistency in the duration of fasting at both time points. Collected blood samples were divided into two aliquots. The first aliquot was centrifuged at 2500 rpm for 15 min within an hour of collection, and the serum was aliquoted, cored, and stored at −80 °C until being used for biochemical analysis. The second aliquot was used for RNA extraction, as explained below. Blood pressure was measured before blood sampling using a digital blood pressure monitor (GE, USA) with subjects in erected seated position after a 5-min resting period.

2.7. Glucose homeostasis markers

Serum glucose was assessed using the fully automated clinical chemistry analyzer ‘Adalis’ (Pchem1, Rome/Italy), according to their recommended kits and protocols. Fasting insulin, insulin-like growth factor-1 (IGF-1), were measured by an enzyme-linked immunosorbent assay (ELISA; Elabscience, USA). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as (fasting glucose × fasting insulin)/405. Insulin sensitivity was assessed by using the quantitative insulin sensitivity check index (QUICKI). All the measurements were performed in triplicate.

2.8. RNA extraction, reverse transcription, qPCR

RNA was extracted using the column-based, Total RNA Purification kit (Norgen, Canada) and reverse transcribed to cDNA by the TruScript Reverse Transcriptase kit (Norgen, Canada), according to the manufacturer’s instructions. qPCR reaction was performed at the volume of 20 μL, including 100 ng of cDNA with QuantiTect SYBR Green PCR mixture (Qiagen, Germany). The cycling conditions included initial activation of the polymerase for 15 min at 95 °C, followed by 45 cycles of 15-s denaturation at 94 °C, annealing at 55 °C for 30 s followed by extension at 72 °C for 30 s. The forward and reverse primers used in the study were summarized in Table 1. For each sample, the expression of each gene was normalized to the housekeeping gene glyceraldehyde 3 phosphate dehydrogenase (GAPDH) at the same time point. The data were compared to a pool of four healthy subjects with normal BMI (18.5–24.9) at each time point. The relative expression was shown as fold change according to Livak and Schmittgen.
Fifty-six (34 males and 22 females, mean age of 35.72 y) subjects were planned for enrollment. The statistical analysis and sample size calculation were described before [31].

2.9. Statistical analysis and sample size calculation

The primary outcome measure was the change in genetic expressions of the five genes (SIRT1, SIRT3, SOD2, TFAM, Nrf2), between the two time points. We estimated that 51 subjects would provide 80% power to detect a significant difference of 5% in genetic expression between baseline (pre-fasting) and post-fasting using a two-tailed paired-samples t-test with α = 0.05. With an expected dropout rate of 10%, 56 subjects were planned for enrollment. The statistical analyses were done using Stata statistical software for statistical analysis (v.13.1 Stata Corp. USA). Tests for normality were included in the model. The variables are expressed as the mean ± standard deviation (SD). Two-tailed Paired sample t-tests were used to compare within-subject changes from baseline (pre-fasting) to post-fasting time points. Binary logistic regression was calculated considering genetic expression as dependent variables, and sex (male vs. female), caloric intake (high, >2000 Kcal vs. low, <2000 Kcal), waist circumference as dependent variables, and sex (male vs. female), caloric intake (high, >2000 Kcal vs. low, <2000 Kcal), waist circumference as independent variables. We recoded the waist circumference variable as high waist circumference or low waist circumference as per the corresponding sex of the participant. The following criteria were used: High, ≥102 cm vs. Normal <102 cm for men and High, ≥88 cm vs. Normal <88 cm for women. In logistic regressions, the odds ratio, 95% confidence interval (CI) were calculated, and result were considered significant (P < 0.05) decreases in total cholesterol, triglycerides, and, unexpectedly, HDL cholesterol were observed (Table 4).

Changes in dietary intake are shown in Table 5. Significant (P < 0.05) increases were reported in the dietary intake of total carbohydrates, total sugars, total water, and fluid during Ramadan in comparison with the pre-fasting intakes, while the total caloric intake did not significantly change.

3. Results

Fifty-six (34 males and 22 females, mean age of 35.72 y ± 12.35) overweight or obese subjects (BMI = 30.74 ± 3.60 kg/m²) and six healthy body weight controls (mean age of 29.8 ± 14.0 y, BMI = 21.4 ± 2.20 kg/m²) were recruited and monitored before and after fasting the whole month of Ramadan. Basic and anthropometric characteristics of the participants are shown in Table 2. Body weight and composition, and blood pressure changes between pre- and post-Ramadan fasting are shown in Table 3. By the end of Ramadan fasting month, body weight, BMI, fat mass, and visceral fat tissue area were significantly (P < 0.05) decreased when compared to pre-fasting levels. Total body water, waist circumference, and systolic blood pressure were also significantly reduced (P < 0.05) at the end of the fasting month (Table 3).

4. Discussion

The current work is the first study conducted to examine the impact of RIF on metabolism and oxidative stress -controlling genes, and was designed principally to investigate the impact of RIF on the genetic expression of metabolic and cellular regulator genes (SIRT1 and SIRT3) along with antioxidant defense enzyme system genes (TFAM, SOD2 and Nrf2). SIRT1 and SIRT3 have been studied for its role in caloric restriction, maintenance of metabolic homeostasis, and the prevention of diabetes research and clinical practice 155 (2019) 107801
We assessed the expression of these genes in obese subjects upon intermittent fasting, as these genes have been implicated from previous reports in mediating diet-induced benefits on metabolism, oxidative stress and inflammation [4,10,13,34–36]. Only a single published study examined the impact of RIF on genetic expression conducted by Ajabnoor and colleagues [37] who examined the impact of RIF on genes related to circadian rhythm control.

Considering that physical activity is one of the influential factors on gene expression [38], this factor is minimized in the current work. During Ramadan, fasting subjects are doing their daily jobs and routine works during the day fasting hours, while spending the night hours in eating and performing the ritual and social activities. Thus, fasting subjects tend to be less active during the fasting month in comparison to the non-fasting periods [39].

Sirtuins (SIRT) are epigenetic and metabolic regulators. Mounting evidence supports that sirtuins family (SIRT1–7) guard homeostasis by sensing bioenergy needs and responding by making alterations in the cell nutrients, and play a critical role in restoring homeostasis during stress responses [40]. Because sirtuins integrate metabolism, bioenergetics, and immunity during inflammation, the expression of sirtuins becomes more pronounced upon acute/chronic inflammation. This fact could also explain the decreased expression

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Table 2 – Basic and anthropometric characteristics of the study subjects according to sex variable.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males (n = 34)</th>
<th>Females (n = 22)</th>
<th>Significance between males and females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>93.16 ± 12.23</td>
<td>83.58 ± 16.89</td>
<td>*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.74 ± 3.60</td>
<td>31.18 ± 7.11</td>
<td>NS</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>25.69 ± 7.97</td>
<td>29.80 ± 11.35</td>
<td>NS</td>
</tr>
<tr>
<td>BFP (%)</td>
<td>27.10 ± 5.43</td>
<td>35.67 ± 6.11</td>
<td>NS</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>66.90 ± 6.64</td>
<td>51.37 ± 8.89</td>
<td>*</td>
</tr>
<tr>
<td>MM (kg)</td>
<td>63.58 ± 6.33</td>
<td>48.78 ± 8.45</td>
<td>*</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>47.83 ± 4.74</td>
<td>36.84 ± 6.31</td>
<td>*</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>237.18 ± 84.98</td>
<td>283.27 ± 113.98</td>
<td>*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>97.41 ± 10.95</td>
<td>91.27 ± 15.77</td>
<td>NS</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>104.97 ± 8.40</td>
<td>107.37 ± 13.29</td>
<td>*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93 ± 0.07</td>
<td>0.85 ± 0.07</td>
<td>*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127.24 ± 8.96</td>
<td>117.05 ± 11.86</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.94 ± 9.89</td>
<td>64.86 ± 7.56</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P* value: Independent samples *t*-test comparing baseline variables between men and women.

BMI, Body mass index; BFP, Body fat percent; DBP, Diastolic blood pressure; FM, Fat mass; FFM, Fat-free mass; HC, Hip circumference; MM, Muscle mass; SBP, Systolic blood pressure; TBW, Total body water; VFA, Visceral fat area measured by DSM-BIA; WC, Waist circumference; WHR, Waist: hip ratio.

* *P* < 0.05, significant difference.

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Table 3 – Difference in anthropometric variables between pre- and post-RIF (n = 56).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ramadan</th>
<th>After Ramadan</th>
<th>Significance as compared to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>89.39 ± 14.87</td>
<td>88.24 ± 14.56</td>
<td>**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.91 ± 5.21</td>
<td>30.45 ± 5.09</td>
<td>*</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>27.31 ± 9.56</td>
<td>26.09 ± 9.24</td>
<td>*</td>
</tr>
<tr>
<td>BFP (%)</td>
<td>30.47 ± 7.06</td>
<td>29.64 ± 7.11</td>
<td>*</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>60.80 ± 10.73</td>
<td>60.39 ± 10.58</td>
<td>NS</td>
</tr>
<tr>
<td>MM (kg)</td>
<td>57.77 ± 10.22</td>
<td>57.37 ± 10.08</td>
<td>NS</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>43.51 ± 7.62</td>
<td>43.23 ± 7.52</td>
<td>NS</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>104.96 ± 71.17</td>
<td>99.14 ± 69.02</td>
<td>*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>95.00 ± 13.04</td>
<td>91.94 ± 11.37</td>
<td>*</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>105.91 ± 10.54</td>
<td>104.65 ± 10.42</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.89 ± 0.08</td>
<td>0.89 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123.24 ± 11.27</td>
<td>119.02 ± 9.46</td>
<td>*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70.98 ± 10.25</td>
<td>69.05 ± 9.25</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Paired* *t*-test comparing end of RIF (T2) with pre-fasting baseline (T1).

BMI, Body mass index; BFP, Body fat percent; DBP, Diastolic blood pressure; FM, Fat mass; FFM, Fat-free mass; HC, Hip circumference; MM, Muscle mass; SBP, Systolic blood pressure; TBW, Total body water; VFA, Visceral fat area measured by DSM-BIA; WC, Waist circumference; WHR, Waist: hip ratio.

* *P* < 0.05, significant difference.

** *P* < 0.001, highly significant difference.
of sirtuins (SIRT1 and SIRT3) upon intermittent fasting of Ramadan where inflammatory markers are modulated while anti-inflammatory markers are augmented.

SIRT3 is one of the sirtuins localize primarily in the mitochondria and is one of the major mitochondrial deacetylase enzymes involved in the activation of fatty acid breakdown upon prolonged fasting. In the current study, the expression of SIRT1 gene showed an insignificant reduction at the end of RIF, while SIRT3 behaved differently and significantly reduced at the end of the month. This finding could be

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**Table 4 – Glucose homeostasis before and at the end of Ramadan (n = 56).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ramadan</th>
<th>After Ramadan</th>
<th>Significance as compared to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>95.94</td>
<td>21.52</td>
<td>98.01</td>
</tr>
<tr>
<td>Fasting insulin (ng/ml)</td>
<td>14.5</td>
<td>14.18</td>
<td>19.3</td>
</tr>
<tr>
<td>Insulin resistance (HOMA-IR)</td>
<td>1.24</td>
<td>1.09</td>
<td>1.58</td>
</tr>
<tr>
<td>QUICKI Insulin sensitivity</td>
<td>0.35</td>
<td>0.06</td>
<td>0.33</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>1.07</td>
<td>0.79</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*P < 0.001.
Paired t-test comparing end of RIF (T2) with pre-fasting baseline (T1).
QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, Insulin-like growth factor-1.

* P < 0.05.
** P < 0.001.

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**Table 5 – Dietary intakes before and at the end of Ramadan. (n = 56).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ramadan</th>
<th>After Ramadan</th>
<th>Significance as compared to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>1760.87</td>
<td>477.29</td>
<td>1920.79</td>
</tr>
<tr>
<td>Fat calories (kcal/day)</td>
<td>604.79</td>
<td>208.86</td>
<td>614.42</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>69.74</td>
<td>17.02</td>
<td>74.52</td>
</tr>
<tr>
<td>Total carbohydrates (g/d)</td>
<td>225.49</td>
<td>70.76</td>
<td>258.86</td>
</tr>
<tr>
<td>Total sugars (g/d)</td>
<td>77.50</td>
<td>39.91</td>
<td>102.42</td>
</tr>
<tr>
<td>Total fats (g/d)</td>
<td>67.39</td>
<td>23.21</td>
<td>68.26</td>
</tr>
<tr>
<td>Total water (ml/day)</td>
<td>1102.12</td>
<td>721.13</td>
<td>1824.91</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>14.22</td>
<td>6.40</td>
<td>15.59</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>7.11</td>
<td>3.04</td>
<td>9.87</td>
</tr>
</tbody>
</table>

Significant at P < 0.05, Paired t-test comparing end of RIF (T2) with pre-fasting baseline (T1).
MUFA, Monounsaturated fat; PUFA, Polyunsaturated fat.
* P < 0.05, significant difference.
** P < 0.001, highly significant difference.

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**Fig. 1 – Relative gene expression in obese subjects in comparison to counterpart expressions of controls for SIRT1 at the end of Ramadan fasting month in comparison to pre-fasting level using qPCR.**

**Fig. 2 – Relative gene expression in obese subjects in comparison to counterpart expressions of controls for SIRT3 at the end of Ramadan fasting month in comparison to pre-fasting level using qPCR. * Significantly different at P < 0.001.**
explained by the lack of significant difference in total energy and fat intake along with the excessive simple sugar intake during feeding night hours of Ramadan month reported in the current work. SIRT1 and SIRT3 were reported to be overexpressed upon prolonged fasting and excessive caloric restriction [41,42], which is not the case in Ramadan. RIF is considered as a partial or short-term intermittent fasting or time-restricted feeding that ranges from 12 to 17 h/day, with the rest of night hours available for eating without restriction [1].

The lack of significant changes in glucose homeostasis markers (serum insulin and HOMA-IR) is consistent with the lack of significant changes in SIRT1, which is involved in the regulation of glucose homeostasis and insulin resistance [43]. Evidence shows that SIRT1 counters insulin resistance and increased SIRT1 expression and activation of elevating insulin secretion. The lack of insulin, as in SIRT1-deficient mice, shows blunted insulin response to glucose stimulation [44,45]. From a mechanistic point of view, SIRT1 triggers insulin secretion from pancreatic β-cells by suppressing the expression of uncoupling protein (UCP)-2 [46]. This relationship was further supported in the animal models of diabetes when SIRT1 activation improved insulin sensitivity and increased energy expenditure [47,48].

On the other hand, both SIRT1 and SIRT3 gene are involved in triggering fatty acid oxidation upon fasting, which contradicts with the increased fatty acid oxidation and significant reduction of body and visceral fat upon Ramadan fasting. This discrepancy could be explained by the impact of other genetic and hormonal regulations in triggering fatty acid oxidation rather than sirtuins. Expression of the SIRT3 gene is highly responsive for the prevailing nutrient availability of the cell. Fasting, calorie restriction, and exercise training have all been reported to trigger the expression of SIRT3 in different tissues [11,49,50]. However, a recent study revealed a reduction in SIRT3 in skeletal muscle during fasting [51].

In contrast, the expression or activity of SIRT3 has been shown to decrease in high fat fed rodents and human subjects with metabolic syndrome [52–54]. In the current work, there was a clear trend toward increased consumption of total fats during Ramadan night hours, which may help in explaining the significant reduction in SIRT3 gene expression upon Ramadan fasting. It is noteworthy to indicate that caloric restriction is different from fasting; the former entails a significant reduction in caloric intake by about 20–40% of the total daily intake, while the latter entails a continuous or intermittent abstinence from eating or drinking for specific periods of time, with different health and metabolic implications are reported for the two types of dietary restriction [55,56].

Furthermore, overexpression of antioxidant defense system genes (SOD2, TFAM, and Nrf2) implying their role in countering the increased oxidative stress generated by the nutritional stresses faced upon fasting. Such pattern of expression could explain, at the genetic level, the reported reduction in inflammatory and oxidative stress markers at the end of RIF recently revised and meta-analyzed by Faris and colleagues [29]. This overexpression of the three antioxidant stress genes entails a kind of loop feedback mechanisms that hinder the need for SIRT3 to be upregulated,
providing that SIRT3 is well known for its ability to eliminate ROS and to prevent the development of cancerous cells or apoptosis [57]. As a connection between SIRT3 and SOD2 genes, the latter is a downstream mediator of the former, and protects nuclear and mtDNA as well as other cellular macromolecules from ROS-related damage [14]. The discrepancy between the downregulation of SIRT3 and the upregulation of its downstream mediator SOD2 in the current study could be explained by the negative feedback mechanism that renders SIRT3 less demanded in the presence of sufficient amount of the antioxidant enzyme SOD2 in body tissues. The significance of SOD2 in counteracting the damaging effect of ROS generated by mitochondria upon cellular respiration is confirmed in the current study by the binary logistic regression analysis. No significant correlations were reported between genetic expressions of the five tested genes and any of the independent variables (sex, waist circumference, caloric intake and BMI) except for SOD2 and TFAM toward caloric intake, where SOD2 and TFAM expressions were significantly ($P < 0.05$) and directly associated with increased caloric intake (>2000 Kcal/day vs. <2000 Kcal per day) among fasting subjects during Ramadan. This finding is similar to a previous report indicated that overnutrition and excessive caloric intake are associated with increased expression of TFAM, SOD2, and Nrf2 [317], along with IGF-1, were critically important in the antioxidant response [58] and TFAM [59]. Providing that mounting evidence confirms that oxidative stress and chronic inflammation trigger the way for the development of metabolic diseases, such as diabetes, and prevailing oxidative and inflammatory conditions constitute major risk factors for the development of a number of pathologies such as diabetes [6]; it can be postulated that such overexpression for the aforementioned anti-oxidative stress genes during RIF presumably may entail a kind of short-term protection against the development of diabetes in obese subjects.

The upregulation of the antioxidant defense enzyme genes (TFAM, SOD2, and Nrf2) augments the anti-inflammatory status of the fasting subjects, and hence makes the expression of sirtuins less demanded. Accumulating body of evidence supports an overall protective effect of SIRT1 activation on the chronic inflammation associated with atherosclerosis; thus the expression of this gene becomes less demanding in our healthy subjects free of cardiovascular diseases and not exposed to caloric restriction [60–62].

It has been proposed that fasting state, such as intermittent fasting practiced during Ramadan month, includes the elevation in free fatty acids and ketone bodies which in turn may serve to impose damage upon the metabolically active mitochondria within the neurons of the fasting organism. However, this damage can be corrected by several mechanisms, including upregulation of the antioxidant defense genes and enhanced mtDNA repair [63].

Silencing SIRT1 and SIRT3 in H9C2 cell line was found to induce no changes in the expression of TFAM and NRF1, while found a reduction in the activity of TFAM in SIRT3 silenced cells rather than in SIRT1 silenced cells. Thus, it was found that silencing SIRT3 increases the acetylation of TFAM and reduces its DNA binding activity [64]. This could explain the current findings of a simultaneous reduction in SIRT3 and increment in TFAM genetic expressions in the current study. Further, overexpression of TFAM in the current work of one-month intermittent fasting with ad libitum night eating is consistent with the overexpression reported after six months of 25% caloric restriction in healthy overweight subjects [65]. Interestingly, a similar trend was observed for SIRT1, where six-month caloric restriction failed to induce SIRT1 protein expression, suggesting that additional factors may regulate SIRT1 content during caloric restriction.

Nrf2 is an important mechanistic link between the stress response-induced antioxidant gene expression and cell survival. Much of the protective antioxidant response of Nrf2 is mediated through upregulation and activation of the Nrf2 gene. Nrf2 regulates a coordinated transcriptional program that maintains cellular redox homeostasis and protects the cell from oxidative injury. Nrf2 regulates several enzymes that are important in the antioxidant response [66]. These include “direct” response enzymes such as SOD2, or “indirect” enzymes such as heme oxygenase-1, glutathione, and thioredoxin generating enzymes [67]. Thus, it becomes logic that SOD2 is upregulated concomitantly with the upregulation of the Nrf2 gene upon RIF and could be explained by the direct effect of Nrf2 on SOD2 expression and activity.

Insulin-like growth factor (IGF)-1 is synthesized by almost all tissues and is an essential mediator of cell growth, differentiation, and transformation. Activation of the insulin/IGF-1 signaling pathway has been associated with increased levels of oxidative stress and subsequently increased levels of pathogenesis for cancer and atherosclerosis [68]. Interestingly, caloric restriction has been reported as one of the main factors that hinder the activation of this pathway [69]. The significant reduction in IGF-1 in the current study is a reflection of the significant activation for the antioxidative stress genes (TFAM, SOD2, and Nrf2), and incur a positive transient protective impact on fasting people against oxidative stress and subsequent pathological conditions. Sophisticated and complex interactive pathways for the impact of dietary restrictions on mitochondrial function and aging concerning the studied sirtuins (SIRT1 and SIRT3) and antioxidative stress genes (TFAM, SOD2, and Nrf2), along with IGF-1, were critically revised by Ruetenik and Barrientos [69].

One possible argument was that increased expression of antioxidative stress genes (TFAM, SOD2, and Nrf2) during Ramadan month implies that RIF is accompanying with increased levels of ROS production, and thus may entail adverse effects of oxidative stress on fasting people. Ristow and Schmeisser defended this argument in their review “Extending life span by increasing oxidative stress” [70]. The authors reported that several longevity-promoting interventions, such as caloric restriction and intermittent fasting, may converge by causing activation of mitochondrial oxygen consumption to promote the increased formation of ROS. These dietary interventions may serve as molecular signals to exert downstream effects to ultimately induce endogenous defense mechanisms culminating in increased stress resistance and longevity, an adaptive response more specifically named mitohormesis or mitochondrial hormesis [70].

In conclusion, the current work showed that RIF might entail a short-term protective impact against oxidative stress in obese subjects, and may help in delaying the development of diabetes and other metabolic derangements associated with increased oxidative stress and low-grade inflammation.
The current work entails more than one limitation that should be considered when discussing the current findings. As an observational prospective study, causality cannot be inferred in the current work, and undetected confounding factors could be implicated in the upregulation or downregulation of the tested genes upon Ramadan fasting month. Other confounding factors that may be implicated include changes in circadian rhythm and sleep pattern that have been reported to affect the expression of some genes [37]. Although the physical exercise was controlled by asking the subjects to maintain their habitual physical exercise pattern during Ramadan month, providing that the vast majority (96%) of the recruited subjects were sedentary to inactive in comparison to before Ramadan; more precise and subjective measurements have to be applied in measuring physical exercise levels.

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Author contribution

MF contributed to the conception and design of the work; MF, MM, and AS participated in the acquisition, analysis, and interpretation of data for the work; MF contributed to drafting the work; HJ contributed to statistical analysis; SA, NS, and RH contributed to critically revising the manuscript and suggested further tests. All authors were involved in writing the paper and had final approval of the submitted and published version.

Declaration of Competing Interest

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Appendix A. Supplementary material

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REFERENCES


