ABSTRACT

Objective: The study was conducted to determine the changes of lipid profile and total antioxidant status (TAS) before, during and after Ramadan fasting among staffs in Institute of Higher Education, UniSZA. Methods: A total of 46 subjects were recruited (31% males and 69% females) ranging from the age of 25 to 40 years old. Information about socio-demographic data was obtained via questionnaire. Lipid profile and TAS were determined using Olympus AU-400 analyser machine and Cayman’s Antioxidant Assay kit respectively. Results: The changes were detected in TC value, TG value, HDL-C value, and LDL-C value before, during and after Ramadan fasting. The TC value has shown significant changes at T2, T3 and T4 (p< 0.05). However, TG value was significantly changed only at T3 (p = 0.31). There was no significant difference in HDL-C value except the value was recorded to increase significantly at T3 (p=0.22). In contrast, significant changes in LDL-C was noted at T4 (p =0.01). TAS reading has shown no significant changes towards these three distinctive periods. However, there was significant difference of TAS value between male and female at T1 (p = 0.03). Conclusions: Ramadan fasting provides various information in evaluating lipid profile. This is hoped to help health professionals to construct strategic dietary intake during Ramadan.

KEY WORDS: Total Antioxidant Status, Lipid Profiles, Ramadan and Fasting.
INTRODUCTION

Fasting in Ramadan is one of the Muslim pillars. It is an obligation that every Muslim must satisfy. Submitters (Muslims) are abstained from eating and drinking starting from dawn until sunset. It also precludes sexual intercourse during this period of time as commanded by God in the Quran (Holy Quran). Ramadan fasting is classified as an intermittent fasting since it is performed during daytime rather than while asleep at night [1]. The fasting time period varies over the globe in relations to different geographic position as well as the diversification of seasons. The length of the time usually between 12 to 19 hours daily [2] and around 11 to 18 hours in tropical countries [3].

Ramadan month occurs eleven days earlier every year and it may fall in any of the four seasons in the following years [4]. The first stage of fasting is the post absorptive period, where the nutrient ingestion begins after the last meal and later absorption process will take place in the small intestine. However, the timing of nutrient ingestion depends on the size and the composition of the meal. Normally, it can be as soon as 3 to 4 hours or last within 7 to 8 hours. During the period of fasting, the body metabolism is altered due to irregularities of eating time.

The majority of Muslims typically consume two meals per day during Ramadan fasting which is one immediately after sunset (iftar) and the other just before dawn (sahur). Hence, both the frequency and quantity of food intake are usually reduced during Ramadan [5]. In parallel, the expected caloric intake would be reduced during this month of Ramadan, which results in body weight reduction [6]. However, it does not only affect the eating pattern solely but also involves the amount and type of food consumed at night when compared to other months throughout the year [7]. There is an established fact towards any given nutrient ingested at an unusual time can induce metabolic effects [8]. In regards, this will affect many metabolic processes in human body [9]. Till this date, there were a few studies on the metabolic changes during Ramadan fasting especially in Malaysia.

The serum lipid profile in healthy fasting individuals is considerably altered during Ramadan. This condition is due to the impact of fasting towards body metabolism in order to maintain its equilibrium. A number of studies on the effects of Ramadan fasting on blood lipids have recorded variety of results for each study. Serum cholesterol may decrease in the first days of fasting [10] and rise to pre-fasting values [11].
MATERIALS AND METHODS

Sample size required for the study was determined based on convenience sampling. This cohort study was conducted at Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, Terengganu between July – October 2012. A total of 46 UniSZA staff consisted of 14 males and 32 females were recruited with mean age of 33.04 ± 4.57 years old. The nominal inclusion criteria was subjects aged 25 to 40 years old, Muslim, healthy without any metabolic syndrome complication, fasting at least 21 days of Ramadan month. Meanwhile the exclusion criteria were recently exposed to medical or diagnostic radiation, taking any supplements and antibiotics, surgery, pregnancy and childbirth within 2 months, use of corticosteroid and receiving contraceptive medication. The study protocol was approved by Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC) (UniSZA.N/1/628-1(3)). Subjects were selected based on the exclusion and inclusion criteria. Interviews were performed using structured questionnaire including information on demographic data which consisted eight questions including gender, age, race, marital status, education level, occupation, monthly income, and category of residential.

Blood Sampling Analysis

A total of 5mL venous blood samples were obtained from subjects to determine lipid profile and plasma was prepared by centrifugation 30 minutes at room temperature. Blood is an important role in this data collection because it transports and redistributes antioxidants to every part of the body [12, 13]. On the other hand, plasma antioxidants status is the result of the interaction of many different compounds and systemic metabolic interaction [14]. The plasma sample for total antioxidants, and lipid profile were stored at –20°C prior to analysis.

Lipid Profiles

Concentrations of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein (LDL-C) in plasma were analyzed colorimetrically using kit determination by using Olympus AU-400 analyser machine.

Total Antioxidant Status

The antioxidant level was determined by using Cayman’s Antioxidant Assay kits to measure the total antioxidant capacity of blood serum. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS’ (2,2’-Azino-di-[3-ethylbenzthiazolin sulphonate]) to ABTS by metmyoglobin. The amount of ABTS produced can be monitored by reading the absorbance at 750 nm. Under the reaction conditions used, the antioxidants in
the sample cause suppression of the absorbance at 750 nm to a degree which is proportional
to their concentration. The capacity of the antioxidants in the sample to prevent ABTS
oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and is
quantified as milimolar Trolox equivalents [15]. The antioxidant concentration of the sample
was calculated by using the equation obtained from the linear regression of the standard curve
by substituting the average absorbance value for each sample into the equation. While, lipid
profile test were measured spectrophotometrically using commercially prepared reagents and
lipid profile was analysed by using Olympus AU-400 analyser machine.

Statistical Analysis
Data obtained from demographic data, anthropometry assessment and biochemical analysis
were analysed using Statistical Package for The Social Science (SPSS) software, version
17.0. A Student’s t-test was used to determine the mean difference between, total cholesterol,
triglyceride, HDL- Cholesterol, LDL- Cholesterol and total antioxidant status. Descriptive
statistical was performed to obtained mean and standard deviation. The level of significance
was set at 0.05.

RESULTS AND DISCUSSION
A total of 46 subjects consisted of 31% males and 69% females were involved in this study.
The mean age group were 33.04± 4.57. All subjects were Malay and three-quarters of them
were reported married. Only one male and six females were recorded bachelors. Among the
46 subjects, 19 (41.3%) of them had normal BMI (comprising 2 males and 17 females), 18
(39.1%) of them were overweight (comprising 8 males and 10 females) and 9 (19.6%) of
them were obese (comprising 4 males and 5 females). There were no subjects in the
underweight category. The mean age for males was 34.3 ± 2.8 and for females was 32.5 ± 5.1.

Lipid Profiles
Table 1 shows that Ramadan fasting was associated with the decrease of total cholesterol
(TC) level during the first week (T2) of fasting (4.77 ± 1.27). However, there was a slight
increase of TC level during the third week (T3) of Ramadan (5.63 ± 0.87), while TC level
measured one month after Ramadan (T4) had shown to be reduced (4.26 ± 1.21). The
decreasing of TC until one month after Ramadan shows similar result with the study
performed by Adlouni et al. [2]. In contrast, few studies have reported an increase in the TC
value [14, 18].
The diet pattern within two weeks in Ramadan did not have any effect on the lipids and lipoproteins parameters but fasting for one month had altered the plasma lipid and lipoprotein profile significantly. One month after Ramadan, the lipids and lipoprotein remain at the same values as observed on the last day of Ramadan [18].

Table 1: Lipid profile before, during and after Ramadan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(T1) mean ± SD</th>
<th>(T2) mean ± SD</th>
<th>(T3) mean ± SD</th>
<th>(T4) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>5.17 ± 1.12</td>
<td>4.77 ± 1.27*</td>
<td>5.63 ± 0.87*</td>
<td>4.26 ± 1.21*</td>
</tr>
<tr>
<td>TG</td>
<td>1.57 ± 0.92</td>
<td>1.40 ± 1.00</td>
<td>1.23 ± 0.83*</td>
<td>1.56 ± 0.93</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.12 ± 0.30</td>
<td>1.13 ± 0.31</td>
<td>1.27 ± 0.26*</td>
<td>1.03 ± 0.23</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.67 ± 0.98</td>
<td>3.40 ± 1.03</td>
<td>3.75 ± 0.93</td>
<td>2.93 ± 0.90*</td>
</tr>
</tbody>
</table>

T1: Before Ramadan; T2: 1st week of Ramadan; T3: 3rd week of Ramadan; T4: One month after Ramadan. *Significantly different from baseline T1 at $P<0.05$, n = 46

There were also several studies that observed no changes in cholesterol level during Ramadan [19, 20]. However, triglyceride (TG) value was significantly decreased at T2 and T3. This results was supported by Afrasiabi et al. [25] and Furuncuoglu et al. [28], explaining that the reduction of TG value during Ramadan fasting was the consequence of hypocaloric diet associated to intense physical exercise during fasting.

Besides that, El Ati et al. [27] stated that even though the protein and fat intake were increased during Ramadan, there were no changes recorded towards total energy when measured before and during Ramadan even though carbohydrate intake was reduced. Increased value of TG was recorded at T4 (one month after Ramadan) when comparing to T1 and similar result was documented in previous study conducted by Gumaa et al. [11]. This might due to high carbohydrate consumption after Ramadan. Furthermore, the increasing value of TG with high sucrose after Ramadan was observed in another study by Albrink and Ullrich [26].

Increased value of high density lipoprotein cholesterol (HDL-C) in T2 and T3 were observed but only the latter was significant. While at T4 the HDL-C value was decreased but it was not significant. During Ramadan, Muslims normally will increase their physical activities via religious pursuits such as extensive extra congregational prayers every night throughout this fasting month. These prayers include; ‘Tarawih’ that is performed approximately 1 to 2 hours...
after breaking fast. There is unlimited number of non-obligatory ‘Nafl’ prayers; and ‘Tahajud’ that is performed after midnight especially within the last 10 days of Ramadan, may, arguably, constitute adequate level of moderate physical activities. In this study, the HDL-C value showed only small changes which were contradictory as reported by Furuncuoglu et al. [28]. However, from the previous study done by Ibrahim et al. [29] and Lamri-Senhadj et al.[32] beneficial effects of Ramadan fasting on lipid profile were recorded by significant increase of HDL level. According to Mansi [34], despite the high-fat intake during Ramadan, this favourable effect was also seen on lipid profile. The decrease of low density lipoprotein cholesterol (LDL-C) value at T2 was not significant. At T3, the LDL-C value was increased but was not significant. At T4, the LDL-C value was significantly decreased.

The result of this study also showed that there was a significant reduction in LDL-C value after Ramadan which was similar to the study done by Kamal [31]. Significant reduction in LDL-C value was due to the fact that Muslims tend to consume more fried foods during Ramadan. Maislos et al.[33] also reported that there were no changes in the total-cholesterol and LDL-cholesterol, although they observed a significant increase in HDL-cholesterol.

**Total Antioxidant Status**

Table 2 shows the changes of TAS value before Ramadan (T1), 1st week of Ramadan (T2), 3rd week of Ramadan (T3), and one month after Ramadan (T4). The TAS value was decreased at T2 as compared to baseline T1, but was not significant ($p = 0.47$). During fourth week of Ramadan, the TAS reading was increased from T2 but slightly lower than baseline T1 and was found insignificant ($p = 0.58$). The increasing of TAS value at T4 which was taken one month after Ramadan might be due to high intake of fresh vegetables and fruits with high natural sources of antioxidants.

**Table 2: Total antioxidant status (TAS) before, during and after Ramadan.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(T1) mean±SD</th>
<th>(T2) mean±SD</th>
<th>(T3) mean±SD</th>
<th>(T4) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS</td>
<td>1.09±0.27</td>
<td>1.06±0.32</td>
<td>1.08±0.29</td>
<td>1.11±0.34</td>
</tr>
</tbody>
</table>

T1: Before Ramadan; T2: 1st week of Ramadan; T3: 3rd week of Ramadan; T4: One month after Ramadan. Previous studies were shown to have investigated a correlation between TAS and different individual antioxidants such as uric acid as major antioxidant in humans, albumin, vitamin A and E and bilirubin [21-24]. Besides, dietary antioxidants such as
vitamins appear to be of great importance for the control of the effects of reactive oxygen species. Although vitamins in the diet are of great importance in terms of antioxidant defence, however it should be bear in mind that antioxidant vitamins are only one of many protective antioxidant pathways in addition to other endogenous free radical scavengers (albumin, urate, and bilirubin) and metal-preventive antioxidants (caeruloplasmin and transferrin) [30].

Figure 1 shows the percentage of changes recorded in TAS value before, during and after Ramadan as compared to baseline T1. The TAS value was decreased 2.7% at T2 and 0.9% at T3, while the TAS value was increased 1.83% at T4 in comparison with baseline T1. According to Temizhan et al. [20] the decrease in TAS could be a response of plasma antioxidants to elevate the production of reactive oxygen species.

Table 3 shows the TAS value changes before, during and after Ramadan among gender and residential area. There was significant different of TAS value between male and female at T1 \( (p<0.05) \), while there was no significant different TAS value at T2, T3 and T4. In line, there was also no significant different TAS value between urban and rural residential area at T1, T2, T3 and T4. Previous study revealed that the oxidative stress among female is higher than male. In parallel, the similar outcome was also observed from this study whereby the TAS value in women was lower than the opposite sex. However, confirming the association between oxidative stress and compensatory increase in TAS required concurrent measurement. Hence, the number of different antioxidants in serum has made it difficult to measure each antioxidant separately. Possible interactions among them could also make a
measurement of any individual antioxidant less representative rather than overall antioxidant status [21].

**Table 3: Total antioxidant status (TAS) value before, during and after Ramadan among gender and residential area.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Living area</th>
<th>Between male and female</th>
<th>Between urban and rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n=14</td>
<td>Female n=32</td>
<td>Urban n=21</td>
<td>Rural n=25</td>
</tr>
<tr>
<td>T1</td>
<td>1.25±0.25</td>
<td>1.01±0.24</td>
<td>1.04±0.27</td>
</tr>
<tr>
<td>T2</td>
<td>1.11±0.36</td>
<td>1.04±0.31</td>
<td>1.05±0.40</td>
</tr>
<tr>
<td>T3</td>
<td>1.18±0.39</td>
<td>1.04±0.23</td>
<td>1.06±0.28</td>
</tr>
<tr>
<td>T4</td>
<td>1.17±0.36</td>
<td>1.08±0.34</td>
<td>1.11±0.33</td>
</tr>
</tbody>
</table>

T1: Before Ramadan; T2: 1st week of Ramadan; T3: 4th week of Ramadan; T4: One month after Ramadan. Results are expressed in mean ± SD

* Significant difference p < 0.05

**CONCLUSIONS**

The findings of this study revealed that TC value had shown significant changes at T2, T3 and T4 (p < 0.05). TG value was observed with significant changes only at T3 (p < 0.05). There was no significant difference in HDL-C value except at T3 whereby it was increased significantly (p< 0.05). LDL-C value showed significant changes at T4 (p <0.05). TAS value was no significant before, during and after Ramadan. However, there was significant difference of TAS value between male and female at T1 (p = 0.03). Ramadan fasting provides a healthy benefit as seen in lipid profile, however the changes of lipid profile need to be examined together with dietary patterns. Researchers in future should expand data collection at other geographical areas and to include dietary record as additional parameter.

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