Food security status is a method used to differentiate food secure and food insecure experience. Throughout our lives, nutritious food and lifestyle are closely related with most lifestyle-associated illness. This study investigated young adults in both groups to determine molecular changes on gene expression of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ plays an important role in adipocyte differentiation, fatty acids, and insulin sensitivity. Increase of PPARγ expression help to improve metabolic indices in dysregulated metabolism associated with obesity, diabetes, and cardiovascular disease. There are no significant differences (P>0.05) of PPARγ expression and BMI for both groups. However, expression of PPARγ is detected in earlier amplification for food insecure group. Mean of BMI (20.70±3.025) is also slightly higher in food insecure group than food secure. Conclusively, there are some effects on expression of PPARγ and BMI based on food security status.

Keywords: Food security status, food insecure, PPARγ, BMI

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1.0 INTRODUCTION

Food security affects a number of unsuspecting individuals worldwide. While logically one might assume food security is closely associated with poor countries, those in high income countries are also affected. This includes low-income households in the United Kingdom (29%), New Zealand (20%), Canada (15%), United States (11%) and Australia (5%). This also meant that huge resources have been spent to attenuate the problem. Despite the efforts, a huge number of people are still unable to obtain adequate nutritious food [1]. According to Food and Agriculture Organization of the United Nations (FAO), food security is “a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. Based on the definition, four food security dimensions can be identified: 1) food availability; 2) economic and physical access to food; 3) food utilization and 4) stability over time” [2]. For those experiencing food insecurity, this meant limited access to adequate and nutritious food to ensure a healthy life [3]. Due to that, those experiencing food insecurity seldom obtain adequate daily nutrition, for example, low fruit and vegetable intake which leads to micronutrient deficiencies and malnutrition. Food insecurity has also been associated with adult obesity, type-2 diabetes, HIV infection, poor academic development, and poor mental health such as stress and depression[1].

In Malaysia, obesity, hypertension, hypercholesterolemia and diabetes among adult increases within 5 years from 14%, 32.2%, 20.7% and 11.6% (NHMS 2006) to 15.%, 32.7%, 35.1% and 15.2 (NHMS, 2011) respectively[4]. These are health conditions that disturb the metabolic syndrome (MetS). Evidence have shown that MetS increased with the development of cardiovascular disease (CV)
and type-2 diabetes [5]. Additionally, central obesity is also associated with CV and MetS with abdominal obesity found also to increase risk of CVD [6]. Not only that, but increasing storage of fatty acids in an expanded adipose tissue mass have also been associated with insulin resistance in peripheral tissue such as skeletal muscle and liver [7].

In adipose tissues, large intestine and macrophages; high peroxisome proliferator-activated receptor gamma (PPARγ) expression can be found and is regulated by diet. [8]–[10]. PPARγ is one member of the nuclear receptor superfamily and a ligand-dependent transcription factor that was originally identified by virtue of its role in adipocyte differentiation [11]. This transcription factor directly regulates the expression of several genes participating in fatty acid uptake, lipid storage and synthesis, systemic energy homeostasis and glucose metabolism. It is also a target of anti-diabetic and plays a significant role in adipogenesis [9].

Since past research tend to focus more on healing and overcoming adverse health outcome rather than how to prevent or delay the development of critical illness, this study aim is to measure PPARγ expression and BMI based on food security status among young adults in a university. This study focuses on PPARγ because it is easily affected by nutrition and due to the decrease detection of adipose tissue during fasting[12]. BMI is also selected since nutrient is commonly stored in the form of fat[13].

2.0 MATERIAL AND METHOD

2.1 Participants

This study includes students from all departments in Universiti Teknologi MARA (UiTM) Puncak Alam, namely, Health Sciences, Pharmacy, Hotel and Tourism Management, Foundation of Basic Science, Art and Design and Business and Office Management. All participants were between the ages of 18 – 25 years old and were categorized as either food secure or food insecure based on Adult Food Security Survey Module (AFSSM) after taking into account all inclusion and exclusion criteria. Inclusion criteria include being free from non-chronic diseases, especially one of that affect nutritional status, and not pregnant. This is to avoid bias in participant’s nutrient profile and micronutrient level that could occur due to hormonal changes and demand of the nutrient needs of a pregnant woman and her fetus.

Meanwhile, exclusion criteria included participants with chronic diseases such as cardiovascular disease, hypercholesterolemia and a family history of hypercholesterolemia or other health-related illness that can also affect nutritional status. Pool of participants were contacted during a health seminar where pamphlets were distributed to visitors. Those who volunteered were selected for the study based on the exclusion and inclusion criteria, yielding 124 participants from 236 volunteers, although the sample size calculation was 128 participants. Anthropometric measurements (height and weight) of participants were also measured.

2.2 Blood Samples Collection

Volunteer participants were required to fast within 8 to 12 hours prior to blood collection procedures. Phlebotomist and nurses were hired for the blood collection procedures. Identification (ID) number and one small biohazard bag containing one ethylene-diamine-tetra-acetic (EDTA) tube was given to each participant to avoid human error.

Approximately three mL to the EDTA tube. The blood samples in EDTA was centrifuged at 3300 rpm at room temperature for 10 minutes. The red blood cells (RBCs) was deposited at the bottom layer and plasma forms the top layer, while the buffy coat containing white blood cells will form a white intermediate layer on the top of the red blood cells. In the next step, supernatant, which consist of plasma, was removed. Then, buffy-coat was gently removed by using pipet to 1.5mL Eppendorf tube.

This buffy-coat usually contains a trace of RBCs, which can affect molecular assays. Red cell lysis was removed via centrifuge again to isolate buffy coat. Complete RBCs lysis was done by centrifuge to isolate RBCs-free buffy-coat. The buffy coat was kept in – 80 °C freezer until testing.

2.3 Genotyping

RNA extraction was extracted by using QIAamp RNA Blood Mini Kit (Qiagen, Germany), and the process was carried out as per the manufacturer’s instruction. RNA extraction was proceed to Reverse Transcription reaction was performed by using QuantiTect® Reverse Transcription according to the manufacturer’s instruction. This step is needed to convert RNA into cDNA prior real-time PCR (RT-qPCR) procedures. cDNA concentration was measured by using bioPhotometer before proceeding the gene expression in RT-qPCR. Primer set for PPARγ gene was exclusively designed from the Homo sapiens PPAR gamma mRNA for peroxisome proliferative activated receptor gamma, complete genome. The genome was obtained from the website of National Center for Biotechnology Information (NCBI) via http://www.ncbi.nlm.nih.gov/ with the accession number AB565476.1. To ensure an appropriate primer set was chosen, the qPCR product was gene sequencing. Forward primer for PPARγ is 5′-AAAGGCTTCATGCAAGGGAG-3′ and reverse is 5′-CACAGCAAACCTCAACTTTGG-3′. Whereas internal control which is GAPDH is 5′-AGGCCACATCGTCAGACAC-3′(Forward) and 5′-GCCCAATACGGACCAAATCC-3′(Reverse) described by [14] and β-actin 5′-AACTGGAACGGTGAAGGTGAC-3′(Forward) and 5′-TGTTGACCTTGGGAGAGGACTG-3′(Reverse) described by [15].
2.4 Statistical Analysis

All data were analyzed using Statistical Package for Social Science (SPSS) program version 21. The significance difference between means was established by independent t-test. All data are presented as mean ± standard deviation (SD). Values of p<0.05 were denoted as statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Prevalence of Food Security Status

The U.S. Adult Food Security Survey Module (AFSSM) is a subset of the US Household Food Security Survey Module (HFSSM) and was used to access the food security status. This module contains ten questions that addresses conditions and behaviours for the previous 12 months. The response from each questions such as “Yes”, “Often”, “Sometimes”, “Almost every month” and “Some months but not every month” were given a score and coded as an affirmative responses, and it will differentiate into four food security categories (Table 1). There are high food security, marginal food security, low food security and very low food security. This four categories were collapsed into two categories which is food secure (high food security+ marginal food security) and food insecure (low food security + very low food security), prior to statistical analysis. From this two categories, food secure percentage (56.5%) slightly higher than food insecure (43.5%).

Food insecurity experience usually occurs in poverty or rural areas. According to [17], the population that lives below poverty line, has a bigger household size (number of family members), low educational level, more children and school children, mothers become the food security status, issue and challenge in Malaysia. Another study showed that 82.3% of the household with 40.8% of child hunger, 24.9% of household showed food insecure and 19.5% for individual food insecure experience [18]. From a definition of food insecure is inadequate of nutritious food, for those who are living under low income families, they tend to get an energy dense food rather than nutrient dense food because of the different price. Nutrient dense food is more expensive than energy dense food.

Therefore, high consumption of energy dense food will have a later on overweight and obesity which can cause in adverse health outcome [19]. Gene expression of PPARγ was investigated in molecular part based on food security status.

3.2 PPARγ Primer Design And Sequencing Analysis

PPARγ primer was designed using the NCBI website and RT-qPCR products generated in this study were sequenced on both primers to verify the identity of PPARγ in human. The obtained sequences were subjected to BLAST using the NCBI BLAST program available at http://blast.ncbi.nlm.nih.gov/Blast.cgi. All the sequences were MEGABLAST separately with high percentage of similarity (95%) to the reference sequences in the database.

3.3 Gene Expression of PPARγ and BMI

Amplification of PPARγ is shown in Figure 1. The red colour line showed that there are no amplification occurring in no reverse transcriptase control (NRT) and no template control (NTC). Meanwhile, for the PPARγ in orange colour, housekeeping gene act as an internal control GAPDH (blue) and β-actin (green).

![Figure 1](image)

**Table 1** Affirmative Score of Food Security Status

<table>
<thead>
<tr>
<th>Affirmative responses</th>
<th>Food security category</th>
<th>Food security status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>High security</td>
<td>Food secure</td>
</tr>
<tr>
<td>1-2</td>
<td>Marginal security</td>
<td>Food</td>
</tr>
<tr>
<td>3-5</td>
<td>Low security</td>
<td>Food insecure</td>
</tr>
<tr>
<td>&gt;5</td>
<td>Very low security</td>
<td>food</td>
</tr>
</tbody>
</table>

Adapted from [16]

PPARγ were analysed by normalized expression. From the data, 20 samples were excluded, and 104 samples were chosen for data analysis. An independent-sample t-test was conducted to compare Cq value on gene expression of PPARγ for food security status. There is no significant difference in score for food secure (32.54, ± 3.06) and food insecure (32.32, ± 3.02; t[104] = 0.35, p = 0.72) [two-tailed]. The magnitude of the differences on the means (mean differences = 0.22, 95% CI: - 0.985 to 1.415] was very small (eta squared = 0.001). Based on the mean, food secure showed a higher mean value than food insecure. However, this does not mean PPARγ expression is higher in food secure group. This is due to the results from amplification cycle (Figure 1). Amplification of food insecure group expresses earlier than food secure group. On the other hand, RT-qPCR showed that a relative normalized expression (RNE) of food insecure group (1.188) is slightly higher than food secure group.
secure group (1.000) (Figure 2). BMI was included to determine whether it has some effect on PPARγ expression for both group (Table 2).

**Figure 2** Gene Expression of PPARγ based on food security status among young adults divided into two groups which is food secure (Pink colour) and food insecure (Blue colour) by RT-qPCR

**Table 2** Gene Expression of PPARγ and BMI

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Food Secure Mean ± SD</th>
<th>Food Insecure Mean ± SD</th>
<th>Confidence Interval (CI) Lower</th>
<th>Confidence Interval (CI) Upper</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of PPARγ</td>
<td>32.54 ± 3.068</td>
<td>32.32 ± 3.022</td>
<td>-0.985</td>
<td>1.415</td>
<td>0.992</td>
</tr>
<tr>
<td>BMI</td>
<td>20.36 ± 2.686</td>
<td>20.70 ± 3.025</td>
<td>-1.459</td>
<td>0.771</td>
<td>0.702</td>
</tr>
</tbody>
</table>

Nutrition, such as macronutrient element (fat, carbohydrate, protein) or some specific micronutrient elements (vitamin, mineral, water), when taken in sufficient quantities, can trigger the resistance to fight against infections. Additionally, these micro and macronutrients can assist in carrying out important functions for the immune system [20]. In contrast, insufficient intake of key nutrients or even overconsumption of certain food can cause nutrient-related diseases and metabolic disorders. This can even be influenced by our genetic background, resulting in diseases such as obesity and diet related anemia [21], [22]. In order to allow the public to better understand their daily nutritional needs, a dietary guideline has been provided by the World Health Organization. This guideline provides information that includes dietary recommendation to prevent onset of diseases and to promote optimal health for individuals, especially those at high risk of developing pathological conditions such as obesity, hypertension and diabetes [23]. It is widely known that certain key micro and macronutrients plays an important role in metabolic pathways, energetic homeostasis and the alteration of crucial gene expressions. In such cases, gene expression is influenced by nutrients through the main responsible members of the transcription factor superfamily. One of the members of the transcriptional factor superfamily is PPARγ.

Additionally, when discussing metabolic syndromes, PPARγ is considered a good candidate gene [22], [23]. For the purpose of this research, data analysis focus more on how PPARγ is expressed in young adult population based on their food security status. PPARγ acts as metabolic nuclear sensors in different cell type such as adipocytes, fibroblast, and myocytes [22]. Therefore, expression and activation of PPARγ are required for adipogenic factor, and no transcriptional regulator was detected for adipocytes differentiation [24]. From the data presented, the expression of PPARγ (Figure 2) is higher in food insecure. Meanwhile, the mean value of BMI (Table 2) is also slightly higher in food insecure group (M = 20.70) than food secure group (M = 20.36). The data supported the findings made in a previous study which stated that concentration of PPARγ can increase with increasing levels of weight, BMI, fat mass, free fat mass (FFM) and trunk fat among participants [25].

Apart from that, another research studied the regulation of the PPARγ expression under in vivo and in vitro conditions. In a study by Vidal-Puig et al., [as cited in [26] expression of PPARγ in the subcutaneous adipose tissue of thin and obese individuals was investigated. The results from the research also supported the findings of other studies, in which adipose tissue of obese people presents an increased amount of PPARγ2/ PPARγ1 mRNA. Furthermore, a connection was also found with BMI value and data showed that PPARγ2 expression is reduced by the consumption of low-calorie diet. In contrast to earlier findings, however, PPARγ1 mRNA levels in the abdominal subcutaneous adipose tissue did not correlate with BMI among obese individuals.

Therefore, this study surmised that molecular mechanisms may lead to obesity by means of the activation of different PPARγ isoforms. Furthermore, in this case, it seems that the isoform 2 is the most active in adipogenesis. It might be influenced due to the fact that PPARγ2 isoform expression is limited to adipose tissue. In addition, it is a more potent transcriptional activator and is regulated in response to nutrient...
intake and obesity, while PPARγ1 isoform is expressed in nearly all cells [27]. On the other hand, a study by Vaccaro et al., [as cited in [28] stated that the Ala carriers from Pro12Ala polymorphism of PPARγ had higher BMI, waist circumference, and fat mass than non-carriers. However, they are more resistant to weight gain and metabolic deterioration when exposed to a high fat intake.

PPARγ is an important requirement and it also acts as an important regulator of adipose tissue development in adipocyte differentiation and for the maintenance of differentiated adipocytes, fatty acid synthesis and insulin sensitivity of major glucose-utilizing tissue [29]. Development of obesity, type-2 diabetes, atherosclerosis and other disease condition was linked with dysregulation of PPARγ. In order to improve its activity, agonist had been used to promote stimulation [30]. Obesity and other disease conditions like type-2 diabetes mellitus and cardiovascular disease is involved in dysregulated metabolism, and is implicated to the abnormalities of PPARγ. Upregulated PPARγ has been reported to improve metabolic indices in type-2 diabetes patients and other conditions while the regulation of PPARγ has been shown to stimulate anti-obesity effects [31].

Normally, research would focus more on developed diseases rather than early stages before disease manifestation. This is because in early stages, main homeostatic parameters such as blood test remain within the physiological range making it difficult to notice presence of problems. Therefore, conditions on level of advancement are sometimes overlooked and gain little notice since physical manifestation of the disease would show up in a matter of time. However, should early risk factors are identified prior to the manifestation of diseases, the cost of treatment can be reduced and the onset of the diseases could delayed and even ultimately prevented. For example, atherosclerosis can occur at young age, during childhood and adolescence. Identification of the age-related risk factors of atherosclerosis at an early stage can help with improvement in managing cardiovascular disease even at a young age [32].

Similarly, non-adipose tissue is protected by adipose PPARγ against excessive lipid overload and to maintain normal organ function such as liver and skeletal muscle. Therefore, activated PPARγ in adipocytes will ensure a balanced and adequate secretion of adipokines (adiponectin and leptin) for mediators of insulin action in peripheral tissue, thus maintaining the insulin sensitivity of the whole body [33]. PPARγ is a potent function modulator not only found in adipose tissue but also in endothelial cells and vascular smooth muscle cells. In endothelial cells, it regulates targets relevant to inflammation and atherosclerosis [27]. Another objective, inflammatory marker and lipid profile [34], e-selectin parameter for endothelial dysfunction was investigated based on food security status.

4.0 CONCLUSION

This study concluded that the expression of PPARγ is slightly higher in food insecure groups. Therefore, they must be taught to be aware of the potential problem to prevent an abnormality expression in our body. Based on the gravity of collected data, food security status will be a major problem if our body did not get adequate quality and quantity of nutrition.

Acknowledgement

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