Lesser yam induces pgc-1α protein expression on brown adipose and skeletal muscle tissues in diabetic rats.

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Abstract

Background: Low expression of PGC-1α in Diabetes Mellitus (DM) is closely related to impaired function of mitochondria. Secondary metabolites from fermented lesser yam can increase the expression of PGC-1α protein.

Aim: This study evaluated the effect of lesser yam flour on Pgc-1α expression of brown adipose and skeletal muscle in diabetic rats.

Method: Twenty-five male Wistar rats, divided into 5 groups: 1) normal rats, 2) diabetic rats, 3) diabetic rats with 1.25 g, 4) diabetic rats with 2.5 g, and 5) diabetic rats with lesser yam flour 5 g per day. Diabetic rats were conducted by inducing of nicotinamide and streptozotocin via intraperitoneal injection. Blood glucose levels were measured before and after administration of the lesser yam flour. After 4 weeks of treatment, the brown adipose and the skeletal muscle tissues were taken under anesthesia, and the expression of Pgc-1α protein was analysed by immunohistochemistry method.

Results: Blood glucose levels were significantly reduced in the group of lesser yam compared to diabetic control group (P=0.001). The expression of Pgc-1α protein is highly expressed in both brown adipose and skeletal muscle tissues compared to diabetic control group (P=0.162; P=0.004), respectively.

Conclusion: Lesser yam may improve mitochondrial function through increased the expression of Pgc-1α protein.

Keywords: Lesser yam, Diabetes, Pgc-1α expression, Brown adipose, Skeletal muscle.

Accepted on January 9, 2017
starch is a type of starch that is resistant to digestive enzymes in the gut [14]. Previous study showed that the beneficial effects of resistant starch in improving metabolic syndrome [15]. Lesser yam (Dioscorea esculenta) is one of resistant starch sources, Marsono [16] reported that lesser yam containing resistant starch 10.4 mg/g dry basis. Therefore, the aim of this study was to evaluate the impact of lesser yam flour in Pgc-1α expression in both brown adipose and skeletal muscle in diabetic rats.

Materials and Methods

Preparation of lesser yam flour
Lesser yam flour was prepared according to Richana et al. [17]. In brief, lesser yams were purchased from the local market, then peeled, washed and cut into small pieces. The lesser yams then dried in an oven (50°C for 24 h) and mashed. The lesser yam flour was stored in vacuum bags before use.

Animals and diets
A total of twenty-five male Wistar rats (8 weeks old, ±180-200 g) were obtained from the Unit of Animal Development for Research, Institut Pertanian Bogor (IPB). Rats were individually housed in a temperature and humidity chamber controlled with good lighting (12 h light/12 h dark). During the acclimatization for 7 days, the rats were fed a standard diet (Table 1).

Table 1. Composition of animal diet.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Standard diet (%)</th>
<th>Modification diet lesser yam flour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>DL-Metionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>61</td>
<td>52.67</td>
</tr>
<tr>
<td>Vitamin Mix AIN 93</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral Mix AIN 93</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lesser yam</td>
<td>8.33</td>
<td>16.67</td>
</tr>
</tbody>
</table>

Rats were divided into 5 groups: normal rats (K1), diabetic rats (K2), diabetic rats+lesser yam flour 1.25 g (P1: 1.25 g), diabetic rats+lesser yam flour 2.50 g (P2: 2.5 g), and diabetic rats+lesser yam flour 5.0 g (P3: 5 g). Diabetes was conducted in rats according to Broca et al. [18]. In brief, rats were injected with 65 mg/kg BW streptozotocin (Nacalai Tesque, Japan), 15 minute after being injected with 120 mg/kg BW nicotinamide (Sigma Aldrich, USA). Diabetic was confirmed by plasma glucose after 5 days induction. In this study, rats were considered in diabetic condition if their fasting blood glucose >140 mg/dL. Animal diet was derived from the standard diet by substituting corn flour with lesser yam flour (Table 1). All animals were treated for 4 weeks. Blood was drawn from orbital sinus of the animals before and after treatment with anesthesia. At the end of the treatment, the animals were sacrificed to collect the BAT from interscapular, and skeletal muscle from gastrocnemius. The tissues were fixed in 10% of formalin and embedded in paraffin before analysis. This study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Universitas Gadjah Mada.

Biochemical analysis
Plasma glucose was analysed using a commercial kit (Diasys, Holzheim, Germany), according to the manufacturers’ protocol.

Pgc-1α expression in both Brown adipose tissue and skeletal muscle
Around 3 μm paraformaldehyde-fixed tissues with de-parafined were committed by xylol I, II, III and then rehydrated with absolute alcohol, 95% and 70% for 3 minute. Streptavidin-peroxydase was inactivated by 1% hydrogen peroxide in methanol for 15 min at 4°C. The tissues were added a specific antibody or primary antibody (bs-1832R; 1:200 dilution) and incubated overnight at 4°C. The secondary antibody was added and incubated for 30 minutes at room temperature. This study used diaminobenzydine as the chromogen, and hematoxylin meyer as a counterstain and mounting using E-Z mount. The expression of Pgc-1α protein was signed with the brown color in the nucleus of tissues (skeletal and brown adipose). The expression of Pgc-1α was calculated as follows: expression (%)=brown nucleus cell (Pgc-1α)/total cell × 100%.

Statistical analysis
All values are presented as mean ± Standard Deviation (SD). One-way ANOVA was used to analyse differences in levels of plasma glucose and the expression of Pgc-1α protein in both BAT and skeletal muscle between groups. Paired t-test was used to analyse differences in levels of plasma glucose before and after administration of lesser yam flour. Correlation between the plasma glucose levels and the expression of Pgc-1α protein in BAT and skeletal muscle examined by Pearson correlation. Differences were considered statistically significant at p<0.05.

Results
Plasma glucose levels
After 4 weeks of administration of the lesser yam flour, the fasting plasma glucose of the diabetic rats was reduced, and the largest decreased of it were found in the diabetic rats with
lesser yam flour 2.5 g. The decreased of the fasting plasma glucose also occur in the diabetic control rats, but it was smallest than it in those with the lesser yam flour. The decreased percentage of the fasting plasma glucose is shown in Table 2.

Table 2. Plasma glucose levels before and after administration lesser yam flour.

<table>
<thead>
<tr>
<th></th>
<th>Before administration (mg/dL)</th>
<th>After administration (mg/dL)</th>
<th>Reduction (%)</th>
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</thead>
<tbody>
<tr>
<td>K1</td>
<td>81.32 ± 12.78a</td>
<td>94.90 ± 13.76a</td>
<td>16.70%</td>
</tr>
<tr>
<td>K2</td>
<td>146.22 ± 30.90p</td>
<td>125.67 ± 9.15a</td>
<td>-14.05%</td>
</tr>
<tr>
<td>P1</td>
<td>152.33 ± 20.69p</td>
<td>79.77 ± 9.07a,b</td>
<td>-47.63%</td>
</tr>
<tr>
<td>P2</td>
<td>152.11 ± 24.30p</td>
<td>67.14 ± 4.98a,b</td>
<td>-55.86%</td>
</tr>
<tr>
<td>P3</td>
<td>148.81 ± 4.90p</td>
<td>90.16 ± 35.63a,b</td>
<td>-39.41%</td>
</tr>
</tbody>
</table>

K1: normal control group; K2: diabetic control group; P1: diabetic rats with 1.25 g of lesser yam flour; P2: diabetic rats with 2.5 g of lesser yam flour; P3: diabetic rats with 5 g of lesser yam flour. Value presented as mean ± standard deviation (n=5). a and b indicate p<0.05 according to one way ANOVA test followed by Tukey HSD test. a, b Indicate no difference between K1 group, K2 group, and all treatment groups, but significantly difference between K2 and treatment groups.

Figure 1. Expression of Pgc-1α in BAT. Pgc-1α protein expression was marked by red arrow, while blue arrow showed no Pgc-1α protein expression.

Figure 2. Expression of Pgc-1α in skeletal muscle. Pgc-1α protein expression was marked by pink arrow, while blue arrow showed no Pgc-1α protein expression.

Table 3. Correlation between plasma glucose level and Pgc-1α expression in BAT and skeletal muscle.

<table>
<thead>
<tr>
<th></th>
<th>BAT</th>
<th>Skeletal muscle</th>
</tr>
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<tbody>
<tr>
<td>Plasma glucose</td>
<td>Pearson correlation (r)</td>
<td>-0.356</td>
</tr>
<tr>
<td>level</td>
<td>p</td>
<td>0.07</td>
</tr>
</tbody>
</table>

The expression of Pgc-1α in both BAT and skeletal muscle of the diabetic control rats and those with lesser yam flour be shown in Figures 1 and 2. The expression of Pgc-1α in the nucleus cell is marked by brown color. The lowest expression of Pgc-1α protein in BAT was found in the diabetic rats control (K2), while the highest expression was found in the diabetic rats with lesser yam flour 2.5 g (P2), even though they did not differ significantly (p=0.162) (Figure 3). Similar to the expression of Pgc-1α protein in BAT, the lowest expression of Pgc-1α protein in skeletal muscle was found in the diabetic rats control, whereas the highest expression was found in the diabetic rats with lesser yam flour 2.5 g (P2) (p=0.004) (Figure 4). The correlation between the plasma glucose levels and the

Expression of Pgc-1α in Brown adipose tissue and skeletal muscle
expression of Pgc-1α protein in both BAT and skeletal muscle is shown in Table 3. In present study, insignificantly negative correlation between plasma glucose levels seen after 4 weeks of treatment (r=-0.356; p=0.070) for BAT and (r=-0.418; p=0.037) for skeletal muscle.

Discussion
The decreased of the fasting plasma glucose in the diabetic rats after administration of the lesser yam flour for 4 weeks showed that the lesser yam flour has antihyperglycemic. These results are consistent with previous studies; Harijono et al. [19] reported that the administration of water-soluble polysaccharides of lesser yam can lower fasting plasma glucose levels of hyperglycaemic rats. Lesser yam is a good source of dietary fiber and resistant starch [16]. Treatment of dietary fiber and resistant starch in the diabetic subjects were able to reduce plasma glucose levels [20,21]. It is also known that dietary fiber is able to control blood glucose levels through several mechanisms such as slowing of gastric emptying and absorption of nutrients, including glucose [22]. However, in this study, the diabetic rats that received 5 g of lesser yam flour had glucose levels higher than those receiving 1.25 g and 2.5 g of lesser yam flour. This might be due to a high intake of dietary fiber or resistant starch may supply energy through fermentation by colonic bacteria [23]. Resistant starch is one type of starch that resistant to enzymatically degradation in the gut [14], while inulin can be slowly digested [22]. Both carbohydrates can be fermented by colonic bacteria to produce SCFAs, especially butyrate [24]. A previous study showed that administration of sodium butyrate could increase expression of Pgc-1α in BAT and skeletal muscle. Butyrate is able to induce the expression of Pgc-1α due to inhibition of Histone Deacetyl (HDAC) activity. Inhibition of HDAC activates Histone Acetylation (HAT), and opens chromatin DNA for transcription initiation and elongation of Pgc-1α mRNA [13].

In this study, the expression of Pgc-1α protein in both BAT and skeletal muscle in the diabetic rats with the lesser yam flour is higher than in those without the lesser yam flour. It shows that the reduced of the fasting plasma glucose may through increased of the expression of Pgc-1α protein. The expression of Pgc-1α in several organs, both BAT and skeletal muscle, represents mitochondrial function in both animal and human model [25]. Recent study suggested that mitochondrial function was closely related to homeostasis of glucose and dysfunction of mitochondrial that found in T2DM and insulin resistance condition [1]. Impaired of mitochondrial function lead to decreased oxidative capacity [26]. Previous report showed in T2DM rats was found decreased of expression of cytochrome C-oxidase (COX), an enzyme that play a role in production Adenosine Triphosphate (ATP), thus result in decreased ATP production [27]. Under a condition of chronic low energy due to impairment mitochondrial respiration, the insulin-independent glucose transport system may possibly upregulated; result in accumulation of glucose. Increased glucose load is accompanied by a reduction in aerobic glucose oxidation, can lead to excess glucose. Excess glucose can be streamed to glucosamine pathway that leads to insulin resistance. Under hyperinsulinemic condition, glucosamine can cause downregulation of genes that involve in Oxidative Phosphorylation (OXPHOS), including Pgc-1α [8]. Previous research has shown that expression of Pgc-1α decreased in the diabetic subject [28]. Our study also shows that increased the expression of Pgc-1α in both BAT and skeletal muscle are consistent with a decrease in plasma glucose levels, especially in the diabetic rats with lesser yam flour 2.5 g. This may be due to an increase in mitochondrial function in both BAT and skeletal muscle. This is supported by previous study showing an increase in mitochondrial function accompanied by up regulation of Pgc-1α expression [13,29]. Improved of mitochondrial function, especially in the skeletal muscles, can improve insulin sensitivity which mediated by increasing of insulin sensitivity in the skeletal muscle [30,31]. It is showed that lesser yam has hypoglycaemic effect through increased the expression of Pgc-1α, especially in skeletal muscle. The limitations of this study are no measuring SCFAs profile. In conclusion, the lesser yam improves mitochondrial function in the diabetic rats, which characterized by increased expression of Pgc-1α, thus reducing the fasting glucose levels.

Acknowledgement
This research was supported by funding from the Community Fund, Faculty of Medicine, Universitas Gadjah Mada. We also admit to Dianandha Septiana Rubi.

References


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