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Lesser yam (dioscorea esculenta) based cookies improves lipid profile in overweight/obese adults with an ad libitum diet via glucagon like peptide 1

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Introduction

The prevalence of obesity increased tremendously in the recent decades and had become a global epidemic (1) and this nutritional problem was associated with increased risk for cardiovascular diseases, hypertension and diabetes mellitus type 2 (2). One of the possible link that is currently being investigated is the role of glucagon like peptide 1 (GLP-1) on development of diabetes mellitus type 2 (DMT2) and cardiovascular diseases in obese individuals (3). GLP-1 is a gut derived hormone that previously showed to enhance glucose-stimulated insulin secretion as well as suppress food intake (4–6). A recent meta-analysis showed the effect of GLP-1 receptor agonist on improvement of lipid profile among type 2 diabetes patients (7) making this hormone a potential therapeutic alternative for cardiovascular disease prevention. Interestingly, studies showed that GLP-1 production can be enhanced by diet such as inulin-like fructan (8,9).

Lesser yam or *Dioscorea esculenta* is traditional food from several areas in Java Island Indonesia and used as one of main source of carbohydrate. Lesser yam contains inulin which is beneficial for gut microbiota because this will be the source of fermentation. There are several studies showed the hypoglycaemic effect of lesser yam in animal model (10). However, to our knowledge there were no studies investigating the effect of lesser yam on fasting blood glucose or lipid profile in human.

Therefore, this study was aimed to examine the effect of lesser yam based cookies on blood glucose and lipid profile in overweight and obese individuals. We were also examining the changes of GLP-1 concentration to explain the mechanism on the effect of prebiotics based food on metabolic improvement. Lesser yam was given as a snack such as cookies because it is more desirable to be consumed by people living in the city compared to the traditional preparation.

Methods

This is an experiment study in overweight or obese adults living in Yogyakarta, Indonesia selected using a purposive sampling. Subjects who were pregnant, diagnosed with degenerative diseases, routinely taking drugs using any means of weight loss program (e.g pills or liposuction) were excluded in this study. The minimal number of subjects was calculated in this study. With the β (power) value of 10%, and α (significancy) value 0.05, we calculated the minimum of subjects in this study based on prefered changes on total triglyceride level was 16 subjects. A total of 37 subjects were involved in this study but only 28 subjects were finished the whole intervention. Subjects were asked to maintain their usual diet while receiving the intervention. This study has been approved by the ethical committee of Faculty of Medicine, Universitas Gadjah Mada. The subjects were given the informed consent regarding the study design, what is the purpose of the study and what will be measured.

The lesser yam was given as cookies for snacking purpose. Subjects were asked to finish their daily intake 87 gram of cookies a day, which contains 37 gram lesser yam flour. An optimation to the cookies were done before the study was started by mixing the flour with wheat flour (80% of lesser yam and 20% of wheat flour). The purpose of this combination was to improve the acceptability of the cookies. The amount of cookies eaten by each subjects was recorded. This intervention was done in 6 consecutive weeks.

Measurements include fasting blood glucose, lipid profile, GLP-1 and anthropmetric indices were done twice, before and after the intervention. After the subjects signed informed consent, an interview was done to collect the characteristic data of our subjects. Following the interview, anthropometric measurements were done; those include height, weight, waist circumference, hip circumference, and % body fat. Afterwards, subjects were asked to fast 8 hours before the blood collection at the following day.

Overweight and obesity status were determined based on body mass index (BMI) (overweight >25 kg/m^2 and obese >27 kg/m^2). BMI was calculated by dividing body weight (in Kg) with squared height (in meter). Height was measured using microtoise while body weight was measured using digital scale. Waist and hip circumference were measured using non elastic tape. Body fat was measured using body impedance analyser.

The blood collection was done in an EDTA tube. Subjects were initially asked to fast 8 hours before the blood collection. After the blood was collected, plasma were separated and it then distributed into several aliquots for different analysis. Glucose and lipid profile includes total cholesterol, LDL, high density lipoprotein (HDL) and triglyceride were analysed using Diasys Kit. Plasma GLP-1 concentration were measured using enzyme immunoassay (Sigma Aldrich).

All dataset were subject to Saphiro-Wilk normality test, the normally distributed and not normally distributed will be analysed accordingly to type of dataset. Paired t-test was used to examine the changes on anthropometric measures, metabolic profile and GLP-1 concentration. A spearman test was used to examine the correlation between changes in GLP-1 and glucose level as well as lipid profile after the intervention. The significance was based on p value less than 0.05.

Results

A total of 37 overweight and obese adults were enrolled in this study but only 28 subjects were finished the 6 weeks intervention. From those 28 subjects not all finished the snacks, therefore we chose only 20 subjects with the highest cookies intake to be further analysed. And because 4 subjects had a very low GLP-1 concentration, only 16 subjects were reported in this study. We reported that the significantly high number of drop out was because the bitter after taste of this cookies. Additionally, some subjects experienced gastrointestinal discomfort after consuming the product. After the intervention we recorded that from 16 subjects average daily intake of this cookies was 35 gram a day.

Anthropometric indices and metabolic profile were analysed (Table 1). Although there was a slight reduction in waist circumference after the intervention, in general this lesser yam based cookies intervention for 6 weeks did not change body weight and body composition. We also found no changes in fasting blood glucose after the intervention. The significant change of lipid profile was seen in total cholesterol level and LDL cholesterol level only while triglyceride and HDL cholesterol were remained unchanged.

Further analysis was done to understand the mechanism on how cholesterol level was affected by the intervention. We proposed that GLP-1 is responsible for this lipid profile amelioration. After the intervention there were significant changes in fasting GLP-1 concentration (p=0.06) (Table 1). A correlation analysis was done to examine the role of fasting GLP-1 concentration on lipid profile and glucose level. As showed in Table 2, GLP- is not correlated with fasting blood glucose (p=0.29). Interestingly, GLP-1 was inversely correlated with total cholesterol and LDL cholesterol level (p=0.001 and p=0.002 respectively). A change in GLP-1 concentration was not related to changes in anthropometric indices such as waist circumference or % body fat. Additionally, we also found no correlation between changes in GLP-1 concentration on initial body weight, waist circumference or % body fat.

Discussion

To our knowledge, this is the first study investigating the effect of lesser yam based cookies on metabolic profile in human. This study did not able to prove the effect of lesser yam based cookies on fasting glucose. However adding lesser yam based cookies on an *ad libitum* diet was able to reduce LDL and total cholesterol level in overweight/obese adults. Interestingly, we reported that the role of prebiotic on reduction of LDL and total cholesterol level was due to changes in GLP-1 production.

Prebiotic has long been investigated to improve glucose homeostasis in animal trial (11–14) but the results in human are still conflicting (9,15,16). A study done by Luo et al. (15) showed that 4 weeks treatment with 20 gram short-chain fructan was able to reduce basal hepatic production but not insulin stimulated glucose. Additionally, in patients with hypercholesterolemia, administration of short-chain fructan was able to reduce postprandial insulin response (16). It was proposed that GLP-1 production is one of the important mechanisms on how prebiotic improve glucose homeostasis. Administration with inulin type fructan was able to improve endogenous GLP-1 production in animal model (8). In human, administration of 16 gram for 2 weeks was able to increase GLP-1 production and reduce postprandial glucose response (9). Unfortunately, there was no change in GLP-1 and glucose level after the intervention. In addition to that we found no correlation between fasting GLP-1 and glucose concentration.

It is proposed that the effect of inulin on lipid profile and GLP-1 was due to its fermentation properties that produces of short chain fattty acids (SCFAs). In contrary to previous studies which used extracted inulin, in this study we used lesser yam as the natural source of inulin. A study done by Winarti et al (17) showed that lesser yam has a supperior prebiotic effect compared to the inulin extract especially in the production of SCFAs. Several studies reported that intraluminal injection of SCFAs increased GLP-1 secretion (18,19). In human lumen, SCFAs targetted free fatty acid (FFA) receptors (FFA2 and FFA3) which are located in L-cells. And because L-cells produce GLP-1 as well as other gut derived peptides such as peptide YY (PYY), it was suggested that the relationship between SCFAa and GLP-1 was through activation of FFA2 and FFA3 (20).

GLP-1 was proven has a potential effect to ameliorate lipid profile. A meta-analysis study showed that GLP-1 receptor agonist was able to reduce LDL level in type 2 diabetic patients (7). Although there is no clear explanation on how GLP-1 ameliorates lipid profile, it has been suggested that GLP-1 receptor agonist did prevent dyslipidemia through alteration of energy utilization, reduction on hepatic lipid synthesis and interaction with parasympathetic signalling pathway. In this study, we showed that fasting GLP-1 concentration was negatively correlated with changes LDL and cholesterol level. This suggested that increasing production of GLP-1 in overweight and obese individuals was protective towards dyslipidemia and cardiovascular diseases (21). The relationship between fasting GLP-1 concentration and lipid metabolism has also been suggested by Wadden *et al* (22). In contrary to our study, they investigated the effect of overfeeding on GLP-1 production and metabolic profile in overweight and normal weight adults. In the study, they found that changes in GLP-1 were positively correlated with changes in HDL cholesterol but not with LDL and total cholesterol. Aside from the role of inlulin content and its fermentation properties, to date there were no information regarding other bioactive components of lesser yam that could affect cholesterol level.

There were some limitations in this study. The after taste and the variability of the product was the major challenge we have to increase the optimum inulin intake in our subjects. We assumed that the lack of changes in glucose and HDL level was due to low compliances. And because not all of the subjects finished their cookies, only subjects with the higher intake then further analysed and this limited the number of sample used in this study. There was only limited number of subjects who finished this study thus we cannot generalized this results into the population. The result also cannot be generated into the general population because in our study because in average the subjects only consume 35 gram out of 87 gram a day. We also did not provide control group therefore no comparison to control group can be made.

We concluded that lesser yam based cookies was an interesting alternative for prevention of dyslipidemia in overweight and obese individuals. As shown in this study, plasma LDL and total cholesterol in overweight/obese adults was decreased after consuming lesser yam based cookies. And reduction of this cholesterol was negatively associated with GLP-1. Further study is needed to evaluate the effect of lesser yam based cookies on dyslipidemia in broader and bigger population. It will be interesting to study the effect of lesser yam among patients with type 2 diabetes and dyslipidemia. This food item is potential to become an alternative for nutritional intervention for patients with cardiovascular diseases and type 2 diabetes.

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Research involving human particioants and/or animal

All procedures performed in studies involving human were accordance with the ethical standards of the institution at which the studies were conducted. In this study, the ethical approval was obtained from Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine Universitas Gadjah Mada – Dr. Sardjito General Hospital

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Table 1.

Anthropometric measures, body composition and fasting serum parameters before and after the treatment. Subjects received lesser yam cookies every day for 6 weeks. No significant changes were seen in body composition including body weight, body mass index, % body fat or waist circumference. However, Total cholesterol and LDL cholesterol were significantly reduced after the treatment. All the data are presented in mean ± standard deviation.

Characteristics	Pre treatment (n=16)	Post treatment (n=16)	*p*
Age (years)	22.8 ± 6.1	22.8 ± 6.1	
Height (cm)	163.4 ± 10.2	163.4 ± 10.2	
Weight (kg)	81.5 ± 14.5	81.3 ± 14.6	0.73[a]
Body Mass Index (kg/m^2)	30.5 ± 4.9	30.4 ± 4.7	0.66[a]
Waist Circumference (cm)	92.7 ± 11.3	91.2 ± 11.6	0.06[a]
Hip Circumference (cm)	103.0 ± 8.3	104.4 ± 8.3	0.57[b]
Body fat (%)	31.9 ± 5.3	31.8 ± 5.5	0.93[a]
Glucose (mg/dL)	71.5 ± 15.9	79.0 ± 21.4	0.95[a]
Triglyceride (mg/dL)	116.4 ± 58.3	106.8 ± 61.4	0.58[b]
Cholesterol (mg/dL)	199.1 ± 48.9	178.3 ± 37.1	0.03*[a]
LDL (mg/dL)	149.9 ± 53.3	120.2 ± 39.1	0.03*[a]
HDL (mg/dL)	37.4 ± 8.5	35.16 ± 14.7	0.36[b]
GLP-1 (pg/dL)	62.2 ± 8.9	58.8 ± 5.2	0.06[b]

Analysis were done using independent t-test for normally distributed data[a] and Mann withney test for not normally distrubuted data[b]. LDL = low density lipoprotein; HDL = high density lipoprotein; GLP-1 = glucagon like peptide -1. *Statistically significant $p<0.05$.

Table 2.

Correlation between GLP-1 concentration and anthropometric and metabolic profiles. Fasting GLP-1 level was negatively correlated with total cholesterol dan LDL cholesterol level.

	r	95% CI	*p*
Body Mass Index	-0.02[b]	-0.52 to 0.49	0.94
Waist Circumference	0.13[a]	-0.40 to 0.60	0.62
Hip Circumference	-0.24[a]	-0.67 to 0.30	0.36
Percent Body Fat	-0.27[a]	-0.69 to 0.27	0.30
Glucose	-0.28[a]	-0.69 to 0.26	0.29
Triglyceride	0.27[a]	-0.27 to 0.68	0.30
Cholesterol	-0.73[a]	-0.90 to -0.36	0.001*
LDL cholesterol	-0.71[a]	-0.89 to -0.32	0.002*
HDL cholesterol	0.21[a]	-0.33 to 0.65	0.43
Initial body weight	0.28[b]	-0.26 to 0.69	0.29
Initial waist circumference	0.30[a]	-0.25 to 0.70	0.26
Initial percent body fat	0.04[a]	-0.47 to 0.54	0.87

Analysis were done using pearson correlation test for normally distributed data [a] and spearman correlation test for not normally distrubuted data [b]. LDL = low density lipoprotein; HDL = high density lipoprotein; GLP-1 = glucagon like peptide -1. *Statistically significant $p<0.05$.