A randomized control trial related to meal order of fruit, vegetable and high glycaemic carbohydrate in healthy adults and its effects on blood glucose levels and waist circumference

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ABSTRACT

Background: Fruit consumption before meals is known to decreases blood glucose level in patients with diabetes. The aim of this study was to investigate the effects of meal order between fruit, vegetable and high glycaemic carbohydrate on blood glucose levels and WC in healthy adults.

Methods: Thirty teachers of primary schools in Mantrijeron district Yogyakarta City, aged 30-40 years old, and had BMI ≥ 20, participated in this trial study and were randomly divided into five groups with 6 persons each: control group (C) given only 175 g steamed white rice (SWR), treatment (T1) group given a meal with order 50g banana (BN) -175 g SWR-75 g broccoli (BR), T2 group with SWR-BN - BR, T3 group with BN+BR-SWR7 and T4 group with SWR – BN+BR. Waist circumference and blood glucose levels were measured in the first and last days of intervention while daily intake was collected using the FFQ.

Results: The T3 group had lower mean FBG and 1h ppBG levels than other groups while the lowest 1h ppBG levels group were in the T1 group after 3 days intervention. In addition, the highest reduction of WC was found in men and women in the T1 group. Inversed fruit consumption was positively associated with increase of FBL levels (OR=10.77; 95% CI: 0.393 – 295.109; p= 0.159).

Conclusion: Fruit and vegetable consumptions before eating high glycaemic carbohydrate lower blood glucose levels while fruit consumption alone reduces WC in healthy adults.

INTRODUCTION

The global prevalence of degenerative diseases such as obesity and diabetes mellitus has increased among healthy adults in the last decade due to changes in their behaviors and lifestyles. In 2016, more than 1.9 billion adults around the world have overweight and 650 million among them with obesity. Sedentary lifestyle, fast food and low fiber consumptions are the most common risk factors of obesity. Central obesity is frequently found in adults with high body mass index (BMI) and have a higher risk to metabolic related disease compared to adults with normal BMI. The metabolic disorder is characterized by glucose intolerance, hypertension, dyslipidemia, insulin resistance, and increase of C-reactive protein levels. In addition, obesity is strongly associated with type 2 diabetes which accounts for 44% cases.

Body weight management is one of the important ways to reduce obesity in adults. Healthy dietary intake, which is combined with physical exercise, can effectively reduce body weight by lowering energy intake and increase its expenditure. However, some adult patients with obesity require behavioural training instead of controlling food intake and doing physical exercise in order to reduce their body weight (BW). Reduction of energy intake can be achieved through several ways such as changing meal portion, choosing less energy foods, modify food composition and eating pattern. For example, 19 obese adults who ate fat-restricted foods have greater reduction of their BW than their counterparts who ate carbohydrate-restricted foods for 6 days. Greater weight loss was also observed in 35 obese women who daily consumed foods with fat restriction with more vegetables and fruits, compared with 36 obese women who consumed foods with fat restriction daily for one year. Furthermore, a 2-day consumption of soup or salad before meal time decreased total energy intake and increased their satiety in young adults.

Glycaemic index (GI) is an important indicator for determination of carbohydrate effect on serum glucose concentration. Molecular structure, interaction with other macromolecules, food processing and
cooking and ripeness are the most common factors that influence the GI values of carbohydrates. In general, high GI foods are attributable to weight gain through increase of blood glucose levels and insulin secretion, which results in glucose oxidation and fat storage. Juanola-Falgarona and her colleagues reported that reduction of BW, blood glucose levels and insulin secretion was greater in 41 overweight and obese adults who received a diet containing moderate carbohydrate with low GI than 40 overweight and obese adults with moderate carbohydrate with high GI for 6 months. However, the effect of fruits or vegetables consumption before eating high glycaemic carbohydrates in adults with obesity has only been studied recently. Patients with type 2 diabetes mellitus (T2DM) who consumed 3 x 50g Ambon banana or equivalent fruits per day before having meals for 7 days have lower mean fasting blood glucose (FBG) and 1h postprandial blood glucose (1h ppBG) levels (60±98.67 and 53.33±105.51 mg/dL respectively). Another study reported that consumption of assorted vegetables before having breakfast for 3 days significantly decrease FBG levels to 7.75±32.25 mg/dL and 1h ppBG levels to 18.58±3.50 mg/dL in patients with T2DM. Therefore, the aim of this study was to investigate the effects of fruit and or vegetable consumptions before eating high glycaemic carbohydrate on blood glucose levels and WC in healthy adults.

MATERIALS AND METHODS

Study design
This research study was conducted in Mantrijeron district, Yogyakarta City. Teachers from 5 public primary schools aged 30-40 years, had BMI ≥ 20 and were physically healthy were participated in the Randomized Control Trial (RCT) study with pre- and post-tests design group control design. They participated in this study after reading and signing the informed consent. The research subjects were excluded from this study if they were athletes, smoked, and consuming alcohol. This research protocol has been approved by the ethics committee of Dr. Moewardi General Hospital Surakarta/Faculty of Medicine, Universitas Sebelas Maret with the letter number 1.188/XII/HREC/2017.

Dietary intervention
Before the treatment was commenced in the breakfast time, 30 selected subjects were randomly divided into 5 groups: 6 persons in the control group (C) given 175 g of steamed white rice, 6 in the treatment group (T1) given 50 g banana 5 min before eating 175 g steamed white rice and then eating 75 g broccoli, 6 in the T2 group given 175 g steamed white rice 5 min before eating 50 g banana plus 75 g broccoli, 6 in the T3 group given 50 g banana plus 75 g broccoli 5 min before eating 175 gr steamed white rice and 6 in the T4 group given 175 g steamed white rice and after 5 min given 50 g banana and 75 g broccoli. The research experiment was performed in 3 consecutive days. All research subjects could drink water freely and eat lunch and dinner meals as usual.

Anthropometric and biochemical measurements
In the day 1 and 3, anthropometric data (body weight, height, and waist circumference) were measured using SMIC digital personal scale (China), GEA Medical SH-2A (China), and standard tape, respectively, while blood pressure was measured using the Mercury Sphygmomanometer (GEA China). Eight hours fasting and 1h post meal blood glucose (FBG) levels were measured using a Semi-Automatic Chemistry Analyzer BM-sp7000S (China). Data of food consumption were collected using the Food Frequency Questionaire (FFQ) method in the end of the treatment. The daily requirement of food intake of all research subjects was calculated using the Mifflin formula.

Statistical analysis
All collected data were analyzed using Statistical Product and Service Solutions (SPSS) for Windows series 20 and presented as mean±SD. Categorical data were statistically analyzed using the chi square test and numeric data with the Independent Paired T and Anova tests, followed by Tukey Post-Hoc. To identify the effects of consumption of fruits and or vegetables before eating high GI rice on blood glucose levels, multiple logistic regression test was performed before and after adjustment for sex, BMI, WC and intake of energy, carbohydrate and fiber with the significant value was set up at <0.05.

RESULTS
Table 1 show the baseline characteristics of all research subjects. Mean age ranged from 34 to 37 years old, in which the T2 group had the oldest mean age (37.83±14) while T4 group had the youngest mean age. The different mean in age was not statistically significant (p=0.359). The gender distribution was unequal among C and T groups (p=0.031) with more women than men in the C, T1 and T3 groups. In contrast with subject age, the highest mean BW was found in the T4 group (77.88±14.06 kg) while C and T2 groups had similar mean BW. T1 and T3 groups had the lowest mean BW (around 58 kg). The difference of mean BW was statistically significance (p=0.030). According to BMI, C and T2-4 groups
Table 1  Baseline characteristics of 30 healthy adults who participated in this study

| Groups | C (n=6) | T1 (n=6) | T2 (n=6) | T3 (n=6) | T4 (n=6) | p     |
|--------|---------|----------|----------|----------|----------|-------|
| Age (years) | 36.67±3.50 | 35.33±3.67 | 37.83±2.14 | 35.00±2.61 | 34.50±3.39 | 0.359 |
| Sex; n (%) | | | | | | |
| -Men | 2 (16.67%) | 2 (16.67%) | 4 (33.33%) | 1 (8.33%) | 3 (25.00%) | 0.031' |
| -Women | 4 (22.22%) | 4 (22.22%) | 2 (11.11%) | 5 (27.77%) | 3 (16.67%) | 0.030' |
| BW (kg) | 67.27±6.66 | 58.30±5.44 | 67.88±17.23 | 58.58±6.35 | 77.88±14.06 | 0.030 |
| Height (cm) | 157.67±5.28 | 158.33±3.67 | 160.67±6.31 | 152.33±3.27 | 164.50±9.91 | 0.083 |
| BMI (kg/m2) | 27.08±2.71 | 23.19±1.90 | 26.03±4.89 | 25.21±3.36 | 29.36±4.35 | 0.078 |
| Systolic blood pressure (mmHg) | 116.67±8.16 | 76.50±10.61 | 92.00±14.72 | 85.00±0.00* | 88.67±22.03 | 0.830 |
| Diastolic blood pressure (mmHg) | 76.67±8.16 | 75.00±13.78 | 81.67±7.53 | 76.67±10.33 | 75.33±15.06 | 0.699 |
| FBG level (mg/dl) | 90.50±6.53 | 84.00±7.10 | 90.00±4.43 | 89.67±6.92 | 81.50±8.89 | 0.159 |
| Energy Intake (kcal) | 1287.25±10.82 | 1158.80±45.54 | 1509.78±245.73 | 1062.10±0.00* | 1571.17±183.00 | 0.274 |
| Carbohydrate Intake (g) | 206.05±19.87 | 181.70±1.41 (68%) | 197.43±28.17 | 170.70±0.00* (66.9%) | 191.70±25.27 | 0.591 |
| Protein Intake (g) | 45.00±7.64 | 34.20±1.27 (54.5%) | 51.75±11.63 (113.3%) | 27.50±0.00* (64.2%) | 57.40±10.31 | 0.121 |
| Fat Intake (g) | 25.90±4.38 | 31.80±4.95 (63.2%) | 59.48±12.33 (118%) | 30.30±0.00* (63.8%) | 65.83±24.23 | 0.098 |
| Fiber Intake (g) | 11.90±5.37 | 7.10±0.99 (21.27%) | 9.58±1.59 (23.84%) | 5.90±0.00* (19.45%) | 9.77±2.14 (25.66%) | 0.369 |

C= Control; T= Treatment; Analyzed using one way Anova with p<0.05; % food intake was calculated from collected data consumption divided by daily individual requirement in the same age and gender with the Mifflin formula
### Table 2  Mean differences of FBG and 1h ppBG levels in C and T groups in day 1 and day 3 treatments

| Blood Glucose Level (mg/dL) | C        | T1       | T2       | T3       | T4       | p        |
|----------------------------|----------|----------|----------|----------|----------|----------|
| Day 1                      |          |          |          |          |          |          |
| FBG level                  | 90.50±6.53 | 84.00±7.10 | 90.00±4.43 | 89.67±6.92 | 81.50±8.89 | 0.159    |
| 1h ppBG level              | 121.33±8.29 | 74.67±9.61 | 112.33±5.24 | 86.50±16.15 | 107.83±15.37 | <0.001*  |
| Day 3                      |          |          |          |          |          |          |
| FBG level                  | 86.33±6.77 | 81.50±12.82 | 79.67±8.21 | 77.00±9.21 | 72.33±7.55 | 0.131    |
| 1h ppBG level              | 106.17±15.17 | 71.83±9.97 | 95.00±5.25 | 77.33±12.58 | 93.83±19.84 | 0.048*   |

Δ Blood Glucose Level

| FBG level                  | -4.17    | -2.50    | -10.33   | -12.67   | -9.17    | 0.296    |
| 1h ppBG level              | -15.17   | -2.83    | -17.33   | -9.17    | -14.00   | 0.491    |

Analyzed using multiple Anova with p<0.005

### Table 3  Mean differences of WC in all groups with fruit consumption only or fruit and vegetable consumption before eating carbohydrate in day 1 and day 3

| WC (cm) | C        | T1       | T2       | T3       | T4       | p        |
|---------|----------|----------|----------|----------|----------|----------|
| Day 1   |          |          |          |          |          |          |
| Men     | 91.00±1.41 | 76.50±10.61 | 92.00±14.72 | 85.00±0.00 | 88.67±22.03 | 0.830    |
| Women   | 85.50±6.03 | 76.75±5.06 | 77.00±7.07 | 75.00±6.12 | 90.00±7.55 | 0.957    |
| Day 3   |          |          |          |          |          |          |
| Men     | 91.00±0.00 | 75.50±10.61 | 92.50±15.42 | 86.00±0.00 | 89.67±21.52 | 0.702    |
| Women   | 84.25±6.18 | 75.75±4.43 | 76.00±5.66 | 75.00±6.08 | 89.00±8.08 | 0.780    |

Δ WC

| Men     | 0.00     | -1.00    | 0.50     | 1.00     | 0.33     | 0.080    |
| Women   | -1.25    | -1.00    | -1.00    | 0.00     | -0.33    | 0.033*   |

Analyzed using multiple Anova with p<0.05; a indicated only one man in the T3 group

### Table 4  Multiple logistic regression of fruit consumption only or fruit and vegetable consumption before eating carbohydrate on FBG levels in healthy adults

| OR (95% CI)  | P-value | Nagelkerke R Square (%) |
|--------------|---------|-------------------------|
| Model 1      |         |                        |
| Fruit and vegetable consumption after carbohydrate | 1.40 (0.195 – 10.032) | 0.738 |
| Fruit consumption after carbohydrate               | 2.80 (0.361 – 21.727)  | 0.325 |
| Model 2      |         |                        |
| Fruit and vegetable consumption after carbohydrate | 2.69 (0.134 – 53.734)  | 0.518 |
| Fruit consumption after carbohydrate               | 10.77 (0.393 – 295.109) | 0.159 |
Our findings showed that men in T1 and T3 groups had inadequate intake of energy while women had adequate intake of energy in all groups. Carbohydrate intake was lower than 80% in all men of C and T groups whereas only women in T2 group had 55% carbohydrate intake. In contrast to carbohydrate intake, all women and men in C, T2, and T4 groups seemed to have excessive protein intake while inadequate intake of protein was found in the two remaining groups. This pattern of protein intake was similar to the pattern of lipid intake in women but not in men. Men in T2 and T4 groups had excessive lipid intake while the other groups were inadequate. Surprisingly, insufficient fiber intake was observed in both sexes.

Fasting and 1 hour postprandial blood glucose (1h ppBG) levels were examined to evaluate the effect of fruit and vegetables consumption before eating carbohydrate. In general, the consumption of fruit only or fruit and vegetables before eating carbohydrate significantly reduced FBG and 1h ppBG levels (Figure 1 and Table 2). In the day 1, mean FBG levels were similar in all groups except in T1 and T4 groups (84.00±7.10 and 81.50±8.89 mg/dL respectively). Reduction of mean FBG levels was observed in all groups after 3 days treatment but only significant in T2 and T3 groups. The mean of 1h ppBG level was increased in C, T2 and T4 groups but decreased in T1 and T3 groups, compared with mean FBG levels in the day 1 of treatment. After 3 days of treatment, the mean differences of 1h ppBG levels were the same with the first day of treatment except the T3 group which had similar mean 1h ppBG and FBG levels. Significantly lower mean of 1h ppBG levels was found in T1 and T3 groups compared with C group (p<0.001). In addition, the mean of 1h ppBG levels declined in

| Table 4 | Continue |
|---------|----------|
| OR (95% CI) | P-value | Nagelkerke R Square (%) |
| Sex | | |
| Female (ref) | 1.497 | 0.745 |
| Male | (0.131 – 17.053) | |
| BMI (kg/m²) | | |
| < 24 (ref) | 5.412 | 0.435 |
| > 24 | (0.078 – 375.521) | |
| WC | 1.335 | 0.882 |
| (0.030 – 59.888) | |
| Energy intake | 0.628 | 0.679 |
| (0.069 – 5.712) | |
| Carbohydrate intake | 0.376 | 0.542 |
| (0.016 – 8.733) | |
| Fiber Intake | 2.525 | 0.621 |
| (0.064 – 99.652) | |

Model 1: crude model without adjustment and Model 2: adjusted for sex, BMI, WC, and intake of energy, carbohydrate, and fiber

**Figure 1** Fasting and 1 hour postprandial blood glucose levels in all groups with fruit consumption only or fruit and vegetable consumption before eating carbohydrate in day 1 and day 3. (a) represented control group and (b-e) represented treatment groups. Data were presented as mean±SD and * indicated a significant difference between FBG and 1 h ppBG day 1 or day 3 while ** indicated a significant difference of FBG/1 h ppBG between day 1 and day 3 with p<0.05
all groups after 3 day treatment and the difference was statistically significant (T1 and T3 vs C). The highest decline of FBG and 1h ppBG levels after the third day treatment was found in T3 and T2 groups (-12.67 and -17.33 mg/dL respectively).

To find out the effect of consumption of fruits and or vegetables before eating carbohydrate on fat storage in the abdomen, we measured WC in the first and third day treatments. Table 3 indicated that consumption fruit only before eating carbohydrate reduced WC in both men and women. In the day 1, higher mean WC (compared to cut-off point) was found in men of C and T2 groups and in women of C and T4 groups. After 3 days treatment, mean WC increased in men subjects in T2 (0.5 cm), T3 (1 cm) and T4 (0.33 cm) groups whereas decreased mean WC was observed in both sexes of the T1 (-1 cm) and in women of the C group (1.25 cm). The mean differences of WC reduction in women was statistically significant (p=0.033).

For further investigation of fruit of vegetable effects on FBG levels, a statistical analysis with the multiple logistic regressions was performed with adjustment for some confounding factors. Table 4 showed that fruit and or vegetable intake after eating high GI carbohydrate increased FBG levels in healthy adults. Healthy adults consumed fruit after eating high GI carbohydrate had 10.77 times risk of higher FBG levels than that of consumed fruit before eating high GI carbohydrate but it was not statistically significant (95% CI: 0.393 – 295.109; p= 0.159). In comparison with consumption of fruit and vegetable before eating GI carbohydrate, 2.69 times risk of increased FBG levels was observed in healthy adults who consumed fruit and vegetable after eating high GI carbohydrate (95% CI: 0.134 – 53.734; p= 0.518). But again, the finding was not statistically significant.

**DISCUSSION**

We have documented meal order between fruit and vegetable in the high glycaemic breakfast on blood glucose levels and central fat accumulation in healthy adults. Consumption of 50g Ambon banana before having the breakfast is able to reduce blood glucose levels and WC in healthy adults with normal weight while only reduction of blood glucose levels occur in healthy adults with overweight who consumed 50g Ambon banana and 75g broccoli before having the breakfast. Furthermore, fruit and or vegetable consumptions after having the breakfast increase at least 2.69 times risk of high FBG levels on healthy adults. Overall these findings suggest that fruit and vegetable consumptions before meal time may reduce blood glucose levels and rise BW loss in healthy adults with overweight or obesity.

In our study, women with overweight or obesity are associated with excessive intake of protein and fat but lack of fiber whereas only a few men with overweight or obesity have such condition. This study is in line with a research study conducted in women that central obesity were linked to high intake of energy, especially protein and fat consumptions and low physical activity. Several studies also have reported that fiber intake, breakfast habits, physical activity, and genetic factors were closely related to obesity. The low fiber intake in our research subjects was resulted from low variation of fruit and vegetable consumptions in their dietary intake. Most of them only consumed seasonal fruits such as mango, rambutan, or zalacca 2-3 times/week and vegetables 6-8 times/week. Another possibility was that they tend to have a daily habit to eat fruits and vegetables after carbohydrate consumption so that fibers derived from fruits or vegetables are chemically mixed with carbohydrates in the stomach, resulting in reduction of fiber absorption and bacterial fermentation in the intestines. Furthermore, we could not speculate that physical activity has no link to obesity in our research subjects because the study did not examine their physical activity.

Interestingly, the effect of fruit and vegetable consumptions before having the breakfast is higher than that of fruit consumption alone against blood glucose levels. Based on our knowledge, few studies have analyzed the effect of fruit and vegetable consumptions before eating high glycaemic meals. The decrease in blood glucose levels in this study may be related to the high content of flavonoids in Ambon banana and broccoli. Flavonoids play an important role in the prevention of obesity and T2DM by protecting pancreatic β cells from free radicals, increasing insulin sensitivity, and functioning as α-amylase inhibitors. These compounds also inhibit the expression of Glucose Transporters 2 on the mucous layer of the small intestine, which leads to reduced absorption of glucose and fructose. Beside these effects, fruit and vegetable consumptions also increase short chain fatty acid (SCFA) levels which can provide 10% of reserved energy, increase free fatty acids and increase glucose uptake in muscle and adipose cells. It is surprising that reduction of blood glucose levels appears not only in the treatment groups but also in the control group. This effect was likely related to adherence of research subjects who performed fasting 8-10 hours prior to blood sampling in the morning and their dietary habit to have more dense dinner than breakfast and lunch.
In addition to lowering blood glucose levels, fruit consumption before having the breakfast was also reduced fat accumulation in the abdominal wall. The high flavonoids in the banana ambon may inhibit fat absorption, lower lipid peroxidase and triglyceride levels, increase free fat oxidation and prevent LDL oxidation. Moreover, water-soluble fiber from banana is known to slow down the gastric emptying time, reduces lipid digestion rate and absorption as well as inhibits bile acid resorption and metabolism.

Although our results of this preliminary study showed that fruit and vegetable consumptions before eating high glycaemic breakfast reduced blood glucose levels and WC, there are some limitations to generalize these results to general adult population. At first, the changed meal order was only conducted at the breakfast time so that it did not represent daily diets and daily blood glucose levels. We also only used a moderate glycaemic fruit in this study and the effect would differ from using low and high glycaemic fruits. From this study, other parameters of carbohydrate and lipid metabolisms such as insulin, GLP-1 and SCFA levels were not measured. Thus, it is still unknown whether or not changes in meal order can improve insulin sensitivity, SCFA levels and lipid profile in blood circulation.

CONCLUSION

Fruit consumption before eating high glycaemic carbohydrate reduced blood glucose levels and waist circumference in healthy adults whilst fruit and vegetable consumptions before eating high glycaemic carbohydrate reduces blood glucose levels in healthy adults with obesity. Changes in meal order of fruit and vegetable can be used as a diet method to enhance weight loss in adults with obesity. Further investigations with different glycaemic fruits and vegetables which given in all meal time, are required to provide more comprehensive data.

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CONFLICT OF INTEREST

The author declares no conflicts of interest regarding the publication of this article.

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