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Article

Acetate wheat starch improving blood glucose response and bilan lipid on obesity dyslipidemia mice

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Resistant starch is particularly concerned with beneficial effects in regulating blood glucose concentration and lipid metabolism, reducing the risk of diabetes and cardiovascular diseases. This study aimed to validate the effects of wheat starch acetate containing 32.1% resistant starch on postprandial blood glucose response and lipid profile on obesity, dyslipidemia *Swiss* mice induced by a high-fat diet. The result showed that there was a restriction on postprandial hyperglycemia and remained stable for 2 hours after meal efficiently comparing with the control group fed natural wheat starch. Simultaneously, when maintaining the dose of 5g/kg once or twice a day for 8 weeks, wheat starch acetate to be able to reduce body weight and blood glucose, triglyceride, cholesterol levels compared to the control group ($p<0.05$).

Keywords: Acetate wheat starch. Glucose. Lipid. Cholesterol.

INTRODUCTION

The prevalence of obesity and overweight has increased dramatically in recent years. In particular, a high-carb diet is the main reason for obesity, overweight, hyperlipidemia, cardiovascular diseases, and diabetes. Currently, some resistant starches (RS), which are resistant to amylase enzyme activity in the small intestine to limit the glucose release and metabolizing to short-chain fatty acid (SCFAs) in the colon by the fermentation of intestinal microorganisms, have been used to replace natural starches in the daily diet to limiting the postprandial hyperglycemia while still providing enough energy for normal daily activities (Lockyer, Nugent, 2017). The prior studies have shown that RS consumption supports the treatment of lipid metabolism disorders by weight loss, postprandial glucose regulation, and improving triglyceride and cholesterol levels (Lee, Yoo, Lee, 2011).

In 2018, our research group synthesized acetate wheat starch (AWS) by the acetylation (Uyen, Nam, Dung, 2018). This type of RS_4 is formed by a modified chemical that shows strong resistance to amylase enzyme activity *in vitro*. This study aims is to clarify the effect of AWS in improving body weight, postprandial blood glucose response, and bilan profile on obese, dyslipidemia *Swiss* mice.

MATERIAL AND METHODS

Material

Natural wheat starch (NWS) and acetate wheat starch (AWS) were used for this research. NWS was purchased from Meizan Limited Company, Vietnam. AWS was prepared by the acetylation of NWS with ahydrid acetide. This kind of starch has a 2.4% acetyl index, containing 59.1% of digestible starch (DS) and 32.1% of resistant starch (Uyen, Nam, Dung, 2018).

The standard diet (60% glucid, 10% lipid, 30% protein, minerals, and vitamins) and the high-fat diet (30% glucid, 52% lipid, 18% protein, minerals, and vitamins)

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processed to the different tablets and stored at room temperature were used for this reseach.

Five-week-old male *Swiss* mice, weighing 20-24g purchased from the Institute of Vaccines and Medical Biological, Nha Trang, Viet Nam. All animal procedures were approved by the Ethics Council in Biomedical Research of University of Medicine and Pharmacy, Hue University and followed the Guidelines for Care and Use of Experimental Animals, 2011 of National Academy of Sciences, USA.

Model of obesity dyslipidemia mice by a high-fat diet

Mice were housed with access to food and water, maintained on a 12:12 hour light: dark cycle, the temperature was at 25-28^{o}C for a week prior and then divided into two groups including the control diet group (CD group) fed with a standard diet (60% glucid, 10% lipid, 30% protein, minerals, and vitamins) and high-fat diet group (HFD group) fed with a high-fat diet (30% glucid, 52% lipid, 18% protein, minerals, and vitamins). After 8 weeks, mice in the HFD group with bodyweight 30% higher than the CD group were collected blood from the tail vein with a heparin syringe. The plasma from the blood was collected by centrifugation at 4000 x g for 30 mins after settling the blood for 30 mins at 4^{o}C. The glucose, triglyceride, and cholesterol levels were measured by using automatic biochemical Cobas 6000 (Roche). Mice obtaining all biochemical levels higher than the CD group significantly reaching the threshold of obese and dyslipidemia condition were chosen for the experiment.

Evaluation of AWS's ability to limit postprandial glucose response on obese dyslipidemia mice

To assess the regulation of postprandial glucose response of AWS, obese, dyslipidemia mice were randomly divided into 3 groups (N=6), fasting 16hr overnight and administered a single dose 5g/kg body weight of NWS in the NWS group, or a mixture AWS and NWS (1:1 w/w) in AWS/NWS group; or AWS in AWS group through the gastric tube. Glucose concentrations were determined immediately before and at 30, 60, 90, 120 minutes after diet by using Accu-Chek Performa (Roche Diagnostics).

Evaluation of AWS's ability to improve the body weight, glucose, triglyceride, and cholesterol levels on obese dyslipidemia mice

To evaluate the improvement of body weight, glucose, triglyceride, and cholesterol levels, these obese, dyslipidemia mice were divided into groups including the NWS1 group fed NWS at dose 5g/kg once a day; AWS1 group fed AWS at dose 5g/kg once a day; AWS2 group fed AWS at dose 5g/kg twice a day and a normal group (SF) fed standard diet for 8 weeks. During the trial period, all mice groups were permitted to eat standard diet and distilled water free daily. At the 4th and 8th weeks of the test, food was removed for 12hr before the experiment. Blood was collected from the tail vein with a heparin syringe. The plasma from the blood was collected by centrifugation at 4000 x g for 30 min after settling the blood for 30 min at 4^{o}C. The glucose, triglyceride, and cholesterol levels were measured by using automatic biochemical Cobas 6000 (Roche).

Numerical data were correlated with SPSS 20.0 statistic software (Chicago, IL, USA). Group differences were analyzed with the Kruskal-Wallis or Mann-Whitney test. Data are presented as mean ± SD. The significance level was set to $p<0.05$.

RESULTS AND DISCUSSION

The effect of high fat diet-induced obesity dyslipidemia *Swiss* mice

After 8 weeks, the HFD group was obese significantly with the mean body weight (51.4 g) 61.1% higher than the CD group (31.9 g) ($p<0.05$). All mice with bodyweight 30% higher than the CD group continuously chosen to collect the blood from the tail vein for measurement of glucose, triglyceride, and cholesterol concentrations.

TABLE I - The change in the biochemical index of mice groups after 8 weeks

Index (mean value)	CD group	HFD group	Increasing ratio (%)
Body weight (gram)	31.9 ± 2.6	51.4 ± 3.1	↑ 61.1*
Glucose (mmol/l)	6.1 ± 0.6	7.8 ± 0.7	↑ 27.9*
Triglyceride (mmol/l)	1.1 ± 0.2	1.9 ± 0.4	↑ 72.7*
Cholesterol (mmol/l)	1.7 ± 0.2	3.2 ± 0.8	↑ 88.2*

** $p<0.05$ compared CD group with HFD group*

The results in Table I show that there is an increase of all determined index including glucose, triglyceride, and cholesterol concentrations of these mice in the high-fat diet group (7.8, 1.9, and 3.2 mmol/l) 27.9%, 72.7%, and 88.2% higher compared to control group (6.1, 1.1 and 1.7 mmol/l), respectively (Table I).

In this model, after 8 weeks of being fed with a high-fat diet, the HFD group had 65.1% being obese and 36.1% being obese and dyslipidemia. This is explained that the consumption of excess in fat over the requirements of the body‘s energy for a long time increased fat accumulation in adipose tissue, causing obesity and leading to disorders of lipid metabolism.

The limit of postprandial glucose response on obese dyslipidemia mice of AWS

The results in Figure 1 show that there was an suddenly increase of postprandial glucose level in the NWS group which reached to the peak at 30 minutes (11.5 mmol/L), then rapidly decreased to 10.9; 10.3 and 8.7 mmol/L at 60, 90, 120 minutes, respectively. Meanwhile, in AWS/NWS and AWS groups, glucose levels were significantly lower than NWS group at each time ($p<0.05$) and it took a long time to reach the peak at 60 minutes in AWS/NWS group (9.2 mmol/L) and up to 90 minutes in AWS group (8.6 mmol/L). After that, both groups show the decrease of glucose levels to the initial level (8.1 mmol/L) at 120 minutes. This suggests that there is an improvement of postprandial glucose response in the mice groups fed a diet containing AWS compared with the NWS group. This is explained by AWS containing 32.1% of resistant starch, which resisted efficiently the hydrolysis of amylase enzyme leading to the limitation of glucose release and absorption in small intestine of obese dyslipidemia mice. The resistance of AWS to amylase activity is generated from the steric hindrence caused by the acetyl groups attached to the surface of starch molecule inhibiting the binding of enzyme amylase, which is the bulky protein macromolecule, on the receptor site of starch molecules supporting for the hydrolysis to release free glucose units (Uyen, Nam, Dung, 2018).

These results proved that AWS can regulate the blood glucose to change very well, to avoid immediately rising and falling to prevent excessive hyperglycemia. The final result is to maintain postprandial glucose response and satiety (Shimotoyodome *et al*., 2010).

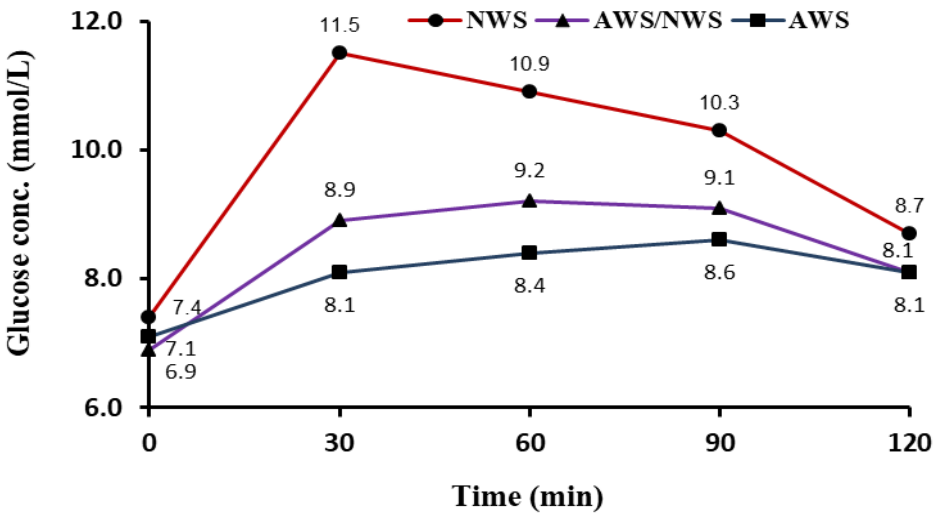

FIGURE 1 - Postprandial glucose concentrations of three experimental mice groups.

Comparing the area under the glucose concentration curve ($AUC_{glu.}$) of the mice groups showed that $AUC_{glu.}$ of the AWS group is only 79.9% ($p < 0.05$) compared to the NWS group and 94.2% compared to the AWS/NWS plot. Based on the glycemic index of food (GI = glycemic index), it shows that the GI of NWS is 75 and AWS is 62.2. This suggests that AWS is a medium GI feed (Dung, Thuy, 2012; Brand-Miller *et al*., 2003).

The improvement of body weight, glucose, triglyceride, and cholesterol levels of AWS

The change in body weight after 8 weeks is shown in Figure 2. While the bodyweight of the SF group increased to 14.5% and maintained in the NWS1 group, whereas in the AWS1 and AWS2 groups tended to decrease by 8.2 and 19.6%, respectively ($p<0.05$). Moreover, the AWS2 group was able to lose weight in the 4th week of the trial (17.6%) compared to the NWS1 group ($p<0.05$).

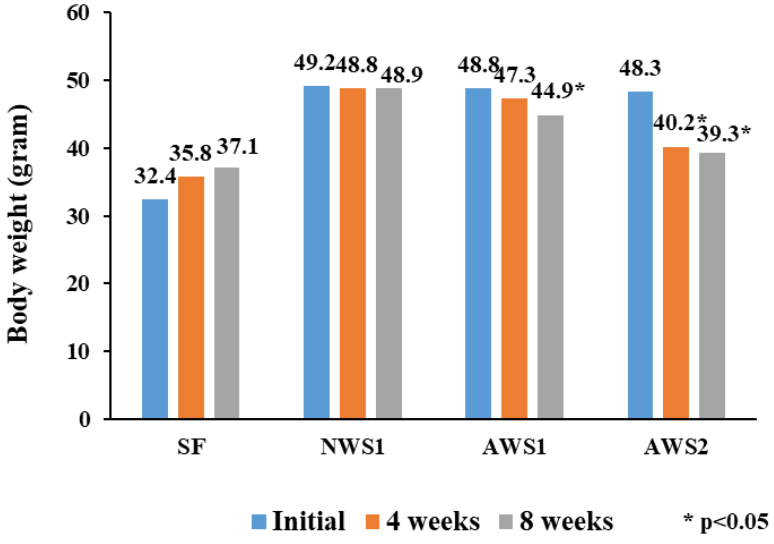

FIGURE 2 - Bodyweights of four experimental mice groups after 8 weeks.

The change in glucose level after 8-week testing is shown in Figure 3. AWS was able to reduce glucose concentration to 20.8 and 24.2% in the AWS1 and AWS2 groups compared to the NWS1 group, respectively ($p < 0.05$), and reached the initial level equivalent to the SF group ($p > 0.05$). However, there was no significant difference between the AWS1 and AWS2 groups. At the 4th week, glucose concentration decreased (4.2%) in both AWS1 and AWS2 groups but there was no significant difference compared to the NWS1 group.

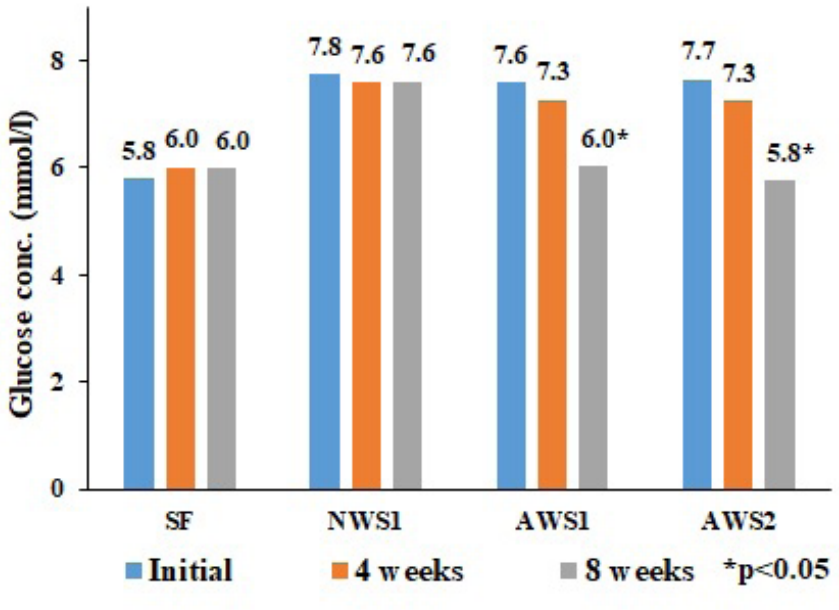

FIGURE 3 - Glucose concentrations of four experimental mice groups after 8 weeks.

A significant decrease in body weight and blood glucose level on AWS groups compared to the NWS group was the basis to evaluate the improvement in triglyceride and cholesterol index. In the 8th week, only triglyceride levels in AWS1 and AWS2 groups were significantly lower to 36.3 and 32.5% compared with the NWS1 group, respectively ($p < 0.05$). In particular, it has reached a normal physiological value and equivalent to the SF group ($p > 0.05$) (Figure 4).

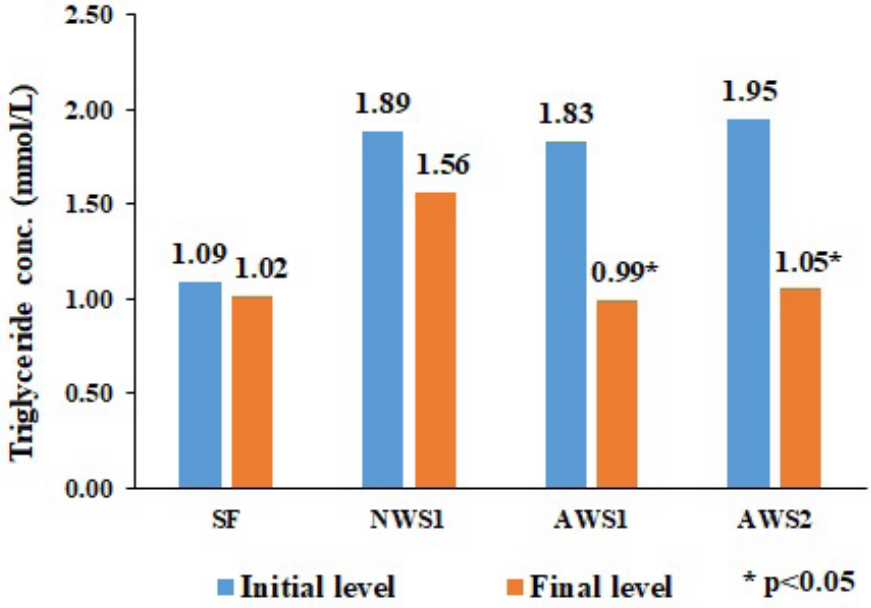

FIGURE 4 - Triglyceride concentrations of four experimental mice groups after 8 weeks.

Also, cholesterol concentrations in AWS1 and AWS2 groups were lower 24.2 and 27.2% than the NWS1 group and decreased to 32 and 33% compared to the original, respectively ($p < 0.05$) (Figure 5).

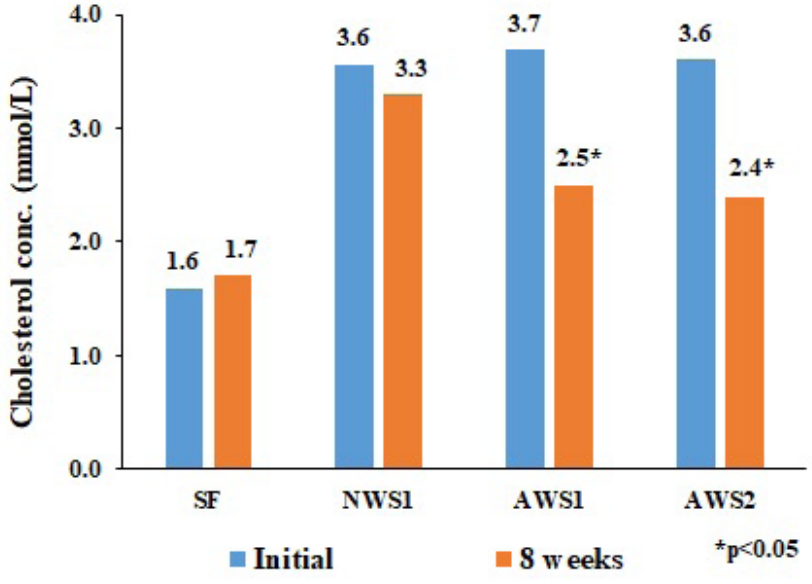

FIGURE 5 - Cholesterol concentrations of four experimental mice groups after 8 weeks.

These results were explained that resistant starch is not digested in the small intestine causing a decrease of blood glucose level in a long-term RS consumption which leads to the lack of body energy. It must mobilize energy by increasing lipid oxidation from adipose tissue to provide enough energy for the body's activities, which resulted in the decrease in body weight, triglyceride and finally increase the insulin sensitivity (Lerer-Metzger *et al*., 1996).

Besides, RS has more swelling properties than natural starches, so it bulges in the stomach and inhibits the reabsorption of bile acids and steroid compounds of the bile acid cycle in the intestine, resulting in reducing of cholesterol level (Han *et al*., 2004). Besides, propionic acid, a product of anaerobic fermentation in the colon, also contribute to the inhibition of cholesterol synthesis in the liver and reduces the level of cholesterol in the blood (Vanhoof, Schrijver, 1997, Chen, Anderson, Jennings, 1984).

Furthermore, the fermentation of RS by anaerobic bacteria in the colon to produce short-chain fatty acids which stimulate the secretion of GLP-1 and PYY hormones to reduce appetite (Keenan *et al*., 2006).

This research shows that AWS can control the postprandial glucose response in obese and dyslipidemia mice more effectively than NWS, helping to regulate blood sugar levels during the 120 minutes after a meal. Also, it showed the advance of AWS in the effects of weight loss, reducing blood glucose, and lipid profile on obese, dyslipidemia mice.

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