Asian Journal of Clinical Nutrition

ISSN 1992-1470

science alert

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OPEN ACCESS Asian Journal of Clinical Nutrition

ISSN 1992-1470
DOI: 10.3923/ajcn.2018.32.36

Research Article
Resistant Starch Modified Cassava Flour (MOCAF) Improves Insulin Resistance

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Abstract

Background and Objective: Type 2 diabetes mellitus is characterized by insulin resistance responsible for the occurrence of various complications. An approach to improve insulin resistance is through consumption of resistant starch type 3 (RS3). A substance that has potential to become RS3 is modified Cassava Flour (MOCAF) made from cassava tubers fermented with lactic acid bacteria. This study assessed the potential of MOCAF and its RS3 to improve insulin resistance in type 2 diabetes mellitus. **Materials and Methods:** Twenty four diabetes-induced rats were randomly selected and grouped into 4 groups (normal, MOCAF, RS3 and negative control). They were treated with different diets (standard, MOCAF and RS3) as much as 20 g each day for 4 weeks. In the end of study, blood were collected to measure fasting and post prandial blood glucose, plasma GLP-1, plasma insulin and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR).Stool sample were collected from colon for Short Chain Fatty Acid (SCFA) analysis. Data analysis of insulin was performed using one-way ANOVA test, other data analyzed using Kruskal-Wallis. **Results:** Fasting blood glucose decreased in MOCAF (446-105 mg dL^{-1}) and in RS3 group (494-97 mg dL^{-1}). Post prandial blood glucose decreased in MOCAF (485-136 mg dL^{-1}) and in RS3 group (526- 96 mg dL^{-1}). Significant higher GLP-1 production was found in normal, MOCAF and RS3 groups compare to negative control group ($p = 0.004$). RS3 consumption stimulated insulin production higher than MOCAF ($p = 0.018$). HOMA-IR calculation showed normal value in MOCAF and RS3 groups. The SCFA analysis showed that both MOCAF and its RS3 induce the production of valerate beside the three main fatty acids (propionate, acetate and butyrate). **Conclusion:** This study demonstrated that both MOCAF and its RS3 had the ability to improve insulin resistance in type 2 diabetes mellitus.

Key words: Type 2 diabetes mellitus, modified cassava flour, resistant starch type 3, short chain fatty acid, glucagon like peptide-1

Citation: Jauhar Firdaus, Erma Sulistyaningsih and Achmad Subagio, 2018. Resistant starch modified cassava flour (MOCAF) improves insulin resistance. Asian J. Clin. Nutr., 10: 32-36.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) occur because of the lack of insulin production and/or inability of the body to properly utilyze insulin (insulin resistance) and cause hyperglycemia. Almost 90% of DM cases are non insulin-dependent or type 2 diabetes mellitus which characterized by insulin resistance. Insulin resistance is responsible for the occurrence of various diabetic complication. Many studies has shown association between the occurrence of accelerated cardiovascular disease and insulin resistance[1]. Research showed that beside using hypoglycemic agents, one of approaches to control blood glucose and insulin production is through Glucagon-like peptide-1 (GLP-1) pathway. GLP-1 has mainy function such as stimulate insulin production and secretion, protect β-pancreatic cells and also reduce glucagon secretion so that it can control blood glucose and increase insulin sensitivity[2,3]. Unfortunately GLP-1 was degraded rapidly after its secretion inside the blood by dipeptidyl peptidase-IV (DPP-IV). In diabetic patient GLP-1 production was decreased. Many new drugs had the activity either by increase GLP-1 production, playing as GLP-1 analog or inhibiting the activity of dipeptidyl peptidase-IV (DPP-IV inhibitor). Recent studies showed that there are increased of GLP-1 secretion after consumption of resistant starch (RS)[4,5]. The effect is associated with RS function as a prebiotics in the gut. The RS will be fermented by gut microbiota to produce Short Chain Fatty Acid (SCFA) that induce "L" cell inside the gut wall to produce GLP-1[2]. Other study found that RS can also induce GLP-1 production independently from gut microbiota by altering bile acid signaling and adipose tissue immune modulation[6].

One type of RS that has been widely used is RS type 3 (RS3) that can be produced by using retrogradation process. The process occurs through reassociation (realignment) of hydrogen bonding between short chain amylose formed after the heating process[7]. Different sources of raw materials and different methods of producing RS will result in different composition of RS and further causing different microbiota colony growth[8]. One of product that has potention to become RS3 is MOCAF, a flour made from cassava tubers fermented with lactic acid bacteria so that it changes its physical, chemical and microbiological aspects. Because its properties is similar to wheat flour and has no gluten, MOCAF is now widely used as the substitution of wheat flour. Meanwhile, Asbar *et al.*[9] studied the use of 3 cycle of autoclaving-cooling in MOCAF as raw material which resulted the increased of RS3 content from 0.79-8.73%. Another method to increase RS3 content has been reported by Zahruniya[10] using autoclaving-cooling combined with debranching enzyme pullulanase which later adopted as standard method for increasing RS3 content in this study. The fact showed that DM was associated with the metabolism disorder that is why nutritional therapy played important role in diabetic management[11]. Based on the author knowledge, there was no previous study on MOCAF and its RS3 in association with type 2 diabetic management. The aim of this study was to find out the potention of MOCAF and its RS3 as a nutritional therapy in type 2 diabetes mellitus by increasing insulin sensitivity through GLP-1 production.

MATERIALS AND METHODS

Experimental animals and research protocols: The study was conducted in Department of Physiology, Faculty of Medicine University of Jember from April-June 2017. The study used 24 male rats (Rattus norvegicus) aged 2 months with body weight ranged from 200-250 g. Rats were treated based on the Helsinki convention. An ethical approval was obtained from the Ethical Committee of the Faculty of Medicine, University of Jember (No. 1.137/H25.1.11/KE/2017). Rats were placed in cages individually, in a room that has proper ventilation and the temperature is between 18-26°C, humidity was between 30-70%. The lighting was regulated light and dark for 12 h. The cages were cleaned every day. After one week of acclimatization rats were randomly selected and grouped into 4 groups, (1) Normal rat feed with standard diet (3% protein, 50% fat, 24-26% fiber) and given tap water for drink, (2) Diabetic rat feed with MOCAF and tap water for drink, (3) Diabetic rat feed with RS3 and tap water for drink and (4) Diabetic rat as negative control feed with standard diet and tap water for drink. All food was given as much as 20 g each day *ad libitum* simultaneously for 4 weeks. Diabetic induction was conducted as previously describe by Srinivasan method by injecting normal rat intraperitoneally with low dose of streptozotocin (35 mg kg^{-1} b.wt.). After one week of induction, rats were fasted for 6 h then blood samples were collected form the tail vein to measure fasting blood glucose and then oral glucose tolerance test (OGTT) were performed. Diabetic condition was achieved when after OGTT procedure blood glucose reach >300 mg dL^{-1} [12].

Measurement of post prandial blood glucose, fasting blood glucose, plasma GLP-1, plasma insulin and HOMA-IR: One day before the end of the study, post prandial blood glucose was measured using portable glucometer (Easy Touch GCU) from tail vein of rats. In the next day rats were fasted for 6 h then blood samples were collected from the tail vein of rats for

fasting blood glucose measurement and from the heart for plasma GLP-1 using a standard Rat GLP-1(Glucagon Like Peptide 1) ELISA kit and plasma insulin using a standard Rat INS (Insulin) ELISA kit (Elabscience). Insulin resistance was calculated using HOMA-IR formula:

$$\text{Fasting glucose (mg dL}^{-1}\text{)} \times \text{Fasting insulin (mI U L}^{-1}\text{)}/405$$

Optimal cutt-off point was 1.775 for non diabetic and 4 for diabetic individual (Conversion of insulin units: 1 ng is equal to 0.1190 IU or 119.04 mIU)[13].

Statistical analysis: Data processing was performed using IBM SPSS statistics software (version 19.0.0 for Windows, SPSS. Inc., Chicago, IL). Data were previously analyzed for normality with Shapiro-wilk test and for homogeneity of variances with levene statistic. Statistical analysis of insulin was performed using one-way ANOVA test. Then, *post-hoc* analysis was performed. Statistical analysis of fasting blood glucose, post prandial blood glucose and GLP-1 was perform using Kruskal-wallis because they donot have either normal distribution nor homogeneous data. The data then further analyzed using Mann-whitney to find the differences with probability level 5%.

RESULTS

Blood glucose profile was analyzed before induction of streptozotocin, after induction and after dietary treatment. After induced with streptozotocin, blood glucose profile in MOCAF, RS3 and negative control groups were increased compare to blood glucose profile before induction. The fasting blood glucose in MOCAF groups was increased from 102-446 mg dL^{-1}. The fasting blood glucose in RS3 groups was increased from 97-494 mg dL^{-1} and in negative control groups the fasting blood glucose was increased from 108-209 mg dL^{-1}. The postprandial blood glucose in MOCAF groups was increased from 105-485 mg dL^{-1}. The postprandial blood glucose in RS3 groups was increased from 97-526 mg dL^{-1} and in negative control groups the postprandial blood glucose was increased from 101-311 mg dL^{-1}. After being treated, the blood glucose profile were decreased in MOCAF and RS3 groups. The fasting blood glucose after treatment was 105 mg dL^{-1} in MOCAF groups and 97 mg dL^{-1} in RS3 groups. The postprandial blood glucose after treatment was 136 mg dL^{-1} in MOCAF groups and 96 mg dL^{-1} in RS3 groups. The blood glucose profile in negative control groups was still high which is 338 mg dL^{-1} of fasting blood glucose and 362 mg dL^{-1} of postprandial blood glucose. The blood glucose profile in normal groups was in normal value from the beginning until the end of this study which is 104, 94 and 96 mg dL^{-1} of fasting blood glucose and 101, 110, 98 mg dL^{-1} of postprandial blood glucose (Fig. 1).

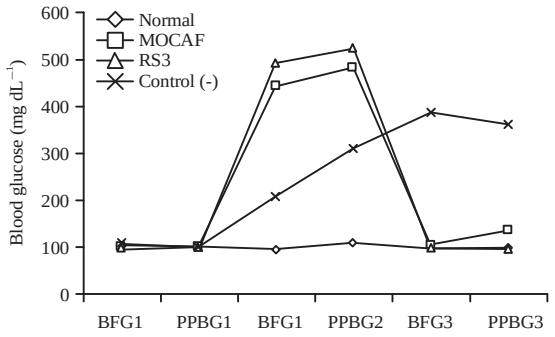

Fig. 1: Blood glucose analysis, BFG1: Blood fasting glucose pretreatment, PPBG1: Post prandial blood glucose pre treatment, BFG2: Blood fasting glucose post STZ induction, PPBG2: Post prandial blood glucose post STZ induction, BFG3: Blood fasting glucose post treatment and PPBG3: Post prandial blood glucose post treatment

Analysis using Kruskal-Wallis of plasma GLP-1 test showed that plasma GLP-1 levels in normal, MOCAF and RS3 groups were not significantly different each other. The plasma GLP-1 levels in negative control group was 0.082+0.00005 pg dL^{-1}, significantly lower than normal (0.083+0.00023 pg dL^{-1}), MOCAF (0.083+0.00015 pg dL^{-1}) and RS3 (0.083+0.00044 pg dL^{-1}) with $p = 0.004$.

The plasma insulin level analysis showed high production and secretion of insulin in all groups except in MOCAF groups. Although the plasma insulin level in MOCAF group was significantly lower than RS3 group ($p = 0.018$), the calculation of insulin resistance index (HOMA-IR) in MOCAF group showed normal value (Table 1).

The SCFA analysis revealed that all the given diets has prebiotic effect. In the standard diatary group (normal and negative control) there was high concentration of acetate, propionate and butyrate but low concentration of valerate. Different results were found in MOCAF and its RS3 groups, they showed medium concentration of acetate, propionate and valerate but low concentration of butyrate.

DISCUSSION

Type 2 diabetes mellitus is a complex disease that associated with life style and diet. Many studies suggested the addition of RS3 in daily food may play roles in diabetes route of treatment associated with or without gut microbiota[6,14]. Different source of RS3 will give different functional value. One

Table 1: Analysis of GLP-1, insulin, HOMA-IR and short chain fatty acid

Groups	GLP-1 (pg dL^{-1})	Insulin (ng mL^{-1})	HOMA-IR	Short chain fatty acid (mM)			
				Acetate	Propionate	Butyrate	Valerate
Normal	0.083	47	1.30	35.16	13.15	6.48	0.31
MOCAF	0.083	21	0.65	19.83	5.17	0.32	2.35
RS3	0.083	41	1.15	16.18	5.51	0.57	2.52
Negative control	0.082	50	5.53	25.28	11.48	4.00	0.42

substance that suitable as raw material to produce RS3 is MOCAF, a modified flour made from cassava, because it contains higher amylose which is required to be a source of RS. Based on the author knowledge, there was no previous study on MOCAF and its RS3 in association with type 2 diabetic management. In this study it demonstrated that MOCAF and its RS3 were potential to use as dietary regiment for type 2 diabetic patient.

In this study, type 2 diabetic rat model fed with MOCA For its RS3 have shown to have an improvement in their condition. Both fasting blood glucose and post prandial blood glucose were within normal value. Both MOCAF and its RS have shown the same stimulating effect on production and secretion of GLP-1. They had an equal plasma GLP-1 to the normal group which mean that both had the similar ability to induce the production and secretion of plasma GLP-1. The plasma insulin level in RS3 group was significantly higher than MOCAF group, where the MOCAF group showed the lowest level. This is likely that MOCAF consumption does not stimulate insulin secretion although it can decrease blood glucose. In contrast with RS3 group, which showed a normal HOMA-IR and high level of plasma insulin, indicating an effect of insulin production and further decreasing blood glucose. The negative control group demonstrated high plasma insulin concentration and high HOMA-IR index which mean that the pancreas can produce high insulin but the body can not properly use it to take the blood glucose up into peripheral tissue such as adipose and skeletal muscle, which resemble insulin resistance, a condition of type 2 diabetes mellitus.

The ability of RS3 to prevent insulin resistance and to increase insulin sensitivity have been shown in many studies but its mechanism of action are not fully understand yet. One possibility is that resistant starch plays a role as prebiotic inside colon. RS3 granules make specific attachment pattern on upper intestine so that it can be attached by probiotic bacteria such as *Lactobacillus* sp. The RS then fermented to produce short chain fatty acids (SCFA). These products have role on increasing production and secretion of GLP-1 and Peptide YY (PYY) endogenous in the intestinal wall. The increasing of GLP-1 will induce β-pancreatic cell proliferation, increase insulin secretion and control glucagon, while the increase of PYY will reduce apetite[15-17]. SCFA such as valeric acid and propionic acid play a role as GPR41 agonist, they increase insulin-stimulated glucose uptake in 3T3-L1 adipocytes and basal glucose uptake on myotube[18]. Other mechanism that might responsible for RS3 to prevent insulin resistance are not associated with gut microbiota, possibly by altering bile acid signaling as well as adipose tissue immune modulation[6,19]. This study also provided the data about the ability of MOCAF to improve insulin sensitivity but the exact mechanism whether it has the same path as RS3 or other mechanism needed further studies.

CONCLUSION

Present study suggested that the administration of both MOCAF and resistant starch type 3 MOCAF can improve insulin sensitivity in type 2 diabetes mellitus. Further studies are needed to determine the molecular mechanisms and the optimum dose to provide recommendations for it uses as nutritional therapy in type 2 diabetes mellitus in human.

SIGNIFICANCE STATEMENTS

This study discovers the possible of MOCAF and its RS3 that can beneficial for diabetic-induced rats. This study will help the researcher to uncover the critical area of nutritional therapy for type 2 diabetes mellitus. Thus, a new finding on these nutrients may be use to improve insulin resistance in type 2 diabetes mellitus and prevent further complication of disease.

ACKNOWLEDGMENT

This research receives no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors thank to Faculty of Medicine, University of Jember for providing research facilities.

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