Shimane J. Med. Sci., Vol.24 pp.29-35, 2007

Effects of Buckwheat Flour With Fiber, Izumo Soba, on Preventing Liver Steatosis in Mice

Masayuki YAMASAKI[1], Susumu MURATA[2], Mariko ISHIHARA[2], Takuya KATSUBE[3], Wang LI[1], Mamiko IWAMOTO[1], Rumi IKENISHI[1], Kuninori SHIWAKU[1]

[1]*Department of Environmental and Preventive Medicine,* [2]*Faculty of Medicine, Shimane University, Izumo 693-8501, Japan*

[3]*Shimane Institute for Industrial Technology, Matsue 690-0816, Japan*

(Received September 27, 2007; Accepted December 3, 2007)

Nutritional characteristics of buckwheat flour and fiber were studied for their effects on obesity and metabolic parameters in mice. Three types of diets with differing fiber content were used for the study. The Gozenko diet consisted of buckwheat flour with 3.3 wt% fiber. The Izumo Soba diet consisted of buckwheat flour with chaff and contained 6.0 wt% fiber. The third diet was the Cornstarch diet, consisting of cornstarch flour with 3.1 wt% fiber. After the mice were fed their respective diets with equal calorie for 30 days (16.6 kcal / day / mouse), the liver weight of the Izumo Soba diet group was significantly less than that of the Cornstarch or Gozenko diet groups. Additionally, the Cornstarch and the Gozenko diet groups had higher values for liver triglyceride contents than did the Izumo Soba diet group. Plasma γ-GTP and GPT for the Izumo Soba diet group were significantly lower than those for the Cornstarch diet groups, and GOT for Izumo Soba diet group was significantly lower than that for the Cornstarch or Gozenko diet groups. We concluded that the Izumo Soba diet, containing large amount of dietary fiber from the buckwheat chaff, has the potential for preventing liver steatosis.

Key words: Izumo Soba, dietary fiber, *de novo* lipogenesis, liver steatosis.

INTRODUCTION

Over the past decade, attention has focused on the high carbohydrate diets, due to its associate with dyslipidemia and overweight in humans (1-3). We have previously reported that high carbohydrate diets contributed to obesity and metabolic syndrome in Japanese (4-7). More particularly, we indicated that a decrease in dietary carbohydrate intake was a main contributor to weight loss in overweight Japanese (8-9). Dietary carbohydrates are major role-players in postprandial glycemic and insulinemic response. Postprandial hyperglycemia is one leading factor in increased *de novo* lipogenesis and triglyceride accumulation in the liver and fat tissues (10-12). The lowering of postprandial glycemic and insulinemic response is an essential objective in the prevention of those abnormalities (13).

There is no doubt that the quality and quantity of carbohydrates considerably affect their rate of digestion and absorption. Particularly, the quality of carbohydrate is represented as the Glycemic Index (GI) concept, a tool for ranking foods with respect to their postprandial glycemia-raising potential. The use of the GI concept is now widely accepted (14-15) and various foods have been tested (16-17). Low GI diets are those in which carbohydrates are digested and absorbed slowly, while high GI diets are those in which are rapidly digested and absorbed, with resulting differ glycemic response. Metabolic advantages of a low GI diet from a long-term perspective have been noted in several studies, particularly for those individuals with diabetes or hyperlipidemia (18). One nutritional variable frequently linked to low GI properties is fiber (19-21). A variety of fiber components, especially soluble fiber including pectin, Oatrim (oat fiber extract), guar gum, gum tragacanth, and methyl cellulose fiber, beneficially lower postprandial glycemic and insulinemic responses, when compared with meals without soluble fiber (22-23). Moreover, there

Correspondence: Masayuki Yamasaki, Department of Environmental and Preventive Medicine, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo 693-8501, Japan
Tel: +81-853-20-2167
Fax: +81-853-20-2165
E-mail: myamasak@med.shimane-u.ac.jp

are several indications that metabolites, formed during fermentation of fiber and other indigestible carbohydrates in the large intestine, contribute to the maintenance of colon health (24-25). Thus, for most healthy adults, foods with higher fiber likely have beneficial effects.

Of the foods screened for metabolic response and fiber content, little attention has been paid to buckwheat (*Fagopyrum esculentum* Moench). For the Japanese, the buckwheat noodle is represented in a main staple called soba, usually prepared in noodle-form. Buckwheat is similarly a staple for some rural populations in Nepal, Bhutan, and China. The Izumo area in Shimane prefecture, Japan, is famous for Izumo Soba, which is unique in that it contains buckwheat chaff, which is added when the buckwheat is ground into flour. In the other area in Japan, it no contains buckwheat chaff in soba flour, such as most famous Gozenko flour. Because the buckwheat chaff contains large amounts of fiber, Izumo Soba is naturally higher in fiber content than are the other types of soba.

Buckwheat flour noodles without the chaff eaten in Japan contain 75-90 wt% of carbohydrates, with a relatively low fiber content, thus being a potential contributor to postprandial hyperglycemia. Replacement of the lower fiber buckwheat flour with the higher fiber buckwheat flour with chaff added may contribute to the suppression of postprandial hyperglycemia, and prevention followed by *de novo* lipogenesis and triglyceride accumulation in the liver. We conducted an animal study to clarify the beneficial effects of the fiber-rich Izumo Soba flour, as compared to buckwheat flour without chaff.

MATERIALS AND METHODS

Animals and diets

Male ICR mice, aged 6 weeks and weighing 21-23 g, were purchased from Oriental Yeast Co., Ltd (Tokyo, Japan). All mice were allowed to acclimate two weeks before use in the present study. The mice were randomly divided into three groups, each group consisting of 9 mice. Three types of diets, Izumo Soba diet, Gozenko diet and Cornstarch diet, were prepared in pellet form and its compositions are shown in Table1. The mice were fed their respective diets for four weeks, all at the same calorie rate (16.6 kcal / day / mouse). All mice were housed on a 12-h light (07:00-19:00 h) / dark (19:00-07:00 h) cycle. Body weight was measured once a day. After four weeks fed, all mice were fasted for 16 hr, and then dissected under ether anesthesia. All procedures in the mice experiments were approved by the Animal Care and Use Committee of Shimane University School of Medicine, Japan.

Measurements

After dissection, liver, epididymal fat pad and soleus muscle weights were immediately measured. Blood samples were collected from the abdominal vein, and plasma was separated by centrifugation (1000 g × 10 min). Plasma glucose, triglycerides (TG), total cholesterol (T-Chol), HDL cholesterol (HDL-C) and nonesterified free fatty acids (FFA) were measured by Glucose C-II test, triglyceride E test, cholesterol E test, HDL cholesterol E test, and NEFA C test, respectively (Wako Pure Chemicals, Osaka, Japan). For the liver function test, plasma

Table 1. Composition of high carbohydrate diets

	Cornstarch diet	Gozenko diet	Izumo Soba diet
Calorie (kcal/100g)	359	348	348
Protein (% kcal)	21.7	19.8	19.3
Fat (% kcal)	7.2	7.8	8.4
Carbhydrate (% kcal)	71.1	72.3	72.2
Fiber (g/100g)	3.1	3.3	6.0
Cornstarch flour (g/100g)	63.4	27.0	14.0
Buckwheat flour (g/100g)	–	44.0*	70.0**

*: Gozenko flour was used as buckwheat flour.

**: Izumo Soba flour was used as buckwheat flour.

γ-glutamyltransferase (γ-GTP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were tested by γ-GTP C test, and Transaminase CII test, respectively (Wako Pure Chemicals, Osaka, Japan).

The liver lipid contents were extracted according to the method used by Carroll NV. *et al.* (1956) (26), and TG was measured by using the triglyceride E test (Wako Pure Chemicals, Osaka, Japan). The liver glycogen was extracted and measured according to Folch, J. *et al.* (1955) using anthrone reagent (27).

Statistical analyses

Statistical analyses of data were done with SPSS software version 13.0J (SPSS Inc., Tokyo, Japan). In this study, results are expressed as means ± S.D. Comparisons of the three diet groups of mice were performed by one-way analysis of variance (ANOVA) and Bonferroni correction for multiple comparisons, and *P*-value of less than 0.017 was used to assess significance.

RESULTS

Body weight changes

Fig. 1 shows changes in mean body weight gains for each diet group. After 10 days feeding, the mice fed with the Cornstarch diet (lowest fiber content,) and the Gozenko diet (second lowest fiber content,) gained body weight dramatically. These two groups had no significant difference body-weight curves and total body weight gain. The body-weight curve of the Izumo Soba diet group leveled off after 15 days feeding in contrast to those of the Cornstarch and Gozenko diet groups, which contrived to show rapid increases after the 10-days mark. Finally after 30days feeding, though there were no significant differences, the body-weight gain of the Izumo Soba diet group was lower than that of the Cornstarch or Gozenko diet groups (4.1±1.2 g *versus* 5.8±1.6 g, 6.4 ±1.9 g, *P* >0.017).

Organ weight

Table 2 shows summarized organ weight at 30 days after ingestion. The liver weights for the Cornstarch diet and Gozenko diet groups were significantly heavier than for the Izumo Soba diet group (2.57±0.28 g, 2.50±0.43 g *versus* 1.93±0.53 g, *P* =0.012 and 0.015, respectively). Although there were no significant differences between them, the fat pads weight of the Izumo Soba diet group was slightly less than that for either the Cornstarch or Gozenko diet

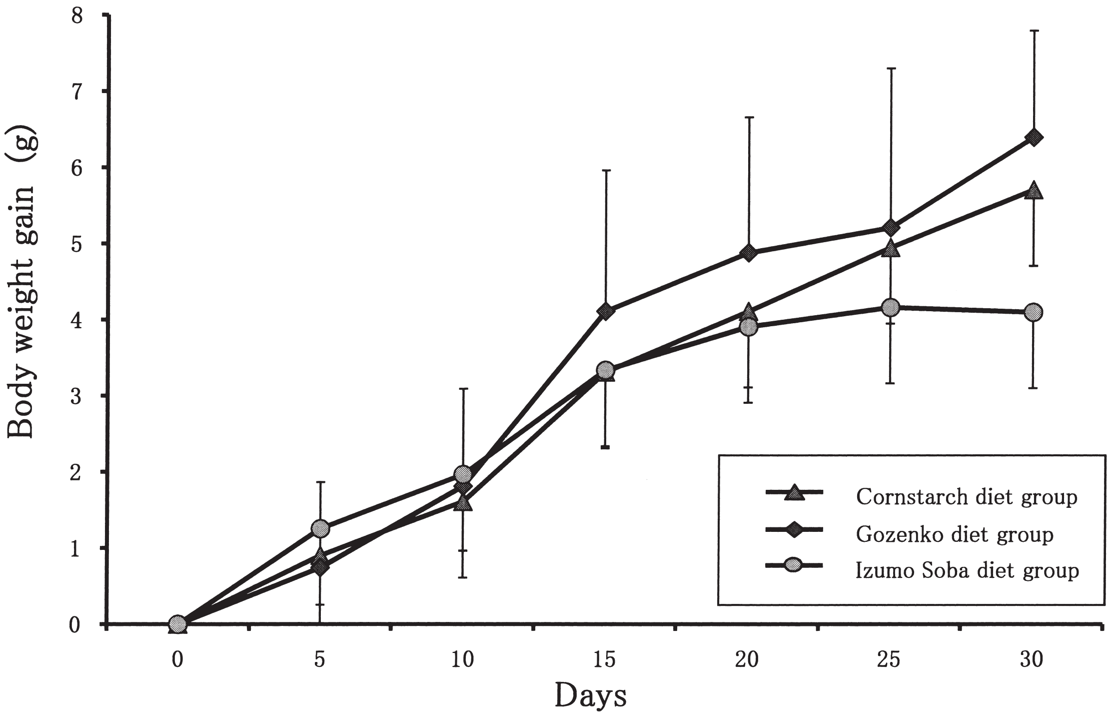

Fig. 1. Changes in mean body weight gain. Closed triangles, squares and circles indicate the Cornstarch, Gozenko and Izumo soba diet groups, respectively. Vertical bars indicate S.D.

Table 2. Summary of measurment variables

	Cornstarch diet group	Gozenko diet group	Izumo Soba diet group
Body weight gain (g) (after day 30)	5.8 ±1.6	6.4 ±1.9	4.1 ±1.2
Organ weight (g)			
Liver	2.57 ±0.28	2.50 ±0.36	1.93 ±0.53 *
Epididymal fat pad	0.71 ±0.21	0.85 ±0.23	0.66 ±0.37
Soleus muscle	0.40 ±0.03	0.42 ±0.07	0.43 ±0.04
Liver glycogen content			
Absolute (mg)	110.9 ±32.4	73.7 ±23.9	65.4 ±32.7
Relative (mg/g organ wt.)	43.1 ±12.4	31.4 ±11.1	33.6 ±14.7
Liver TG content			
Absolute (mg)	24.8 ±14.0	24.7 ±14.7	15.6 ±8.7
Relative (mg/g organ wt.)	9.9 ±6.0	10.1±4.9	8.2 ±4.8
Plasma			
Glucose (mg/dl)	140 ±22	139 ±42	129 ±28
TG (mg/dl)	82 ±21	94 ±32	90 ±37
TC (mg/dl)	115 ±17	143 ±32	126 ±17
HDL-C (mg/dl)	80 ±17	90 ±20	81 ±13
FFA (mEq/dl)	9.2 ±1.4	9.6 ±2.2	8.4 ±1.5
γ -GTP (IU/l)	4.0 ±0.6	3.4 ±0.3	2.9 ±0.5 **
GOT (IU/l)	115 ±19	105 ±13	81 ±10 *
GTP (IU/l)	25.0 ±2.5	22.1 ±2.2	19.2 ±3.3 **

Values are mean ± SD.
* indicates a significant difference between Izumo Soba diet and the other two diet groups ($P < 0.017$).
** indicates a significant difference between Izumo Soba diet and Cornstarch diet group ($P < 0.017$).

groups (0.66 ±0.37 g *versus* 0.71 ±0.21 g, 0.85 ±0.23 g, $P > 0.017$). There were no significant differences in soleus muscle weight among all diets groups.

Biochemical analyses of fasting plasma

The results of biochemical analyses of fasting plasma were shown in Table 2. There were no significant differences in fasting plasma glucose and lipids among all diet groups, but the values of fasting plasma glucose and of plasma FFA for the Izumo Soba diet group were slightly lower than those of Cornstarch or Gozenko diet groups. On the liver function test, the values of γ-GTP and GTP for the Izumo Soba diet group were significantly lower than those for Cornstarch diet group ($P = 0.007$ and > 0.001, respectively). And the value of GOT for the Izumo Soba diet group was significantly lower than that for Cornstarch or Gozenko diet groups ($P < 0.001$ and $= 0.003$, respectively).

Liver glycogen and triglyceride contents

Liver glycogen content for the Izumo Soba diet group was 40% lower than that of Cornstarch diet group (65.4 ±32.7 mg *versus* 110.9 ±32.4 mg, $P > 0.017$). Although the liver weight of the Gozenko diet group was higher and close to that of the Cornstarch diet group, liver glycogen was lower and comparatively close to the Izumo Soba diet group. The absolute liver triglyceride contents of the Izumo Soba diet group were 40% lower than that of Cornstarch and Gozenko diet groups, though there were no significant differences between them (15.6 ±8.7 mg *versus* 24.8 ±14.0 mg, 24.7 ±14.7 mg).

DISCUSSION

The highlight of this study was that the Izumo Soba diet, containing large amounts of dietary fiber from buckwheat chaff, suppressed increasing of liver TG contents and liver-derived blood γ-GTP, GOT and GTP, despite all diets used in this study were rated as high carbohydrate diets. And more, though there were slight effects, suppressed body-weight gain and improved blood glucose and lipids metabolism. The other two diets, the Gozenko and Cornstarch diet, had high carbohydrate and low fiber content, seemingly contributing to liver steatosis and slightly body weight gain in the mice, in contrast to the Izumo Soba diet did not, even though all had the same calories and similar carbohydrate content. These results suggest the unique properties of the Izumo Soba diet. We have previously indicated that high carbohydrate diets were one of main contributing factors to metabolic syndrome and obesity in Japanese (4-7). Metabolic syndrome and obesity are frequently accompanied by the liver steatosis. The liver steatosis is believed to be one of causes for abnormal lipid metabolism on metabolic syndrome and obesity. Thus, dietary fiber intake by individuals with healthy or metabolic syndrome might possibly help to prevent or improve metabolic syndrome and obesity by suppressing liver steatosis.

After 30 days feeding, fasting plasma glucose in the Izumo Soba diet group remained lower compared with the other two diet groups. Previously, we demonstrated that a low GI diet used with amylose, a major resistant starch and one of the undigestible components of carbohydrates as well as dietary fiber, strongly suppressed acute postprandial glycemic and insulinemic responses (28). The cumulative effects of repeated suppression of acute postprandial blood glucose by regular intake of a low GI diet likely lowers fasting plasma glucose levels. Pawlak *et al*. (2004) also reported on the long-term effects of different GI diets on normal rats; in that study, the body fat mass increases for the low GI group were significantly lower than those for the high GI group after 17 weeks of feeding (29). Oral glucose tolerance tests were performed at the 5th and 14th weeks, with the area under the curves (AUCs) for blood glucose and insulin of the low GI group being significantly lower. Lerer-Metzer *et al*., (1996) also reported that the low GI diet produced favorable effects in controlling postprandial glycemic and insulinemic responses (30). In these two studies, the high GI diet groups had a much greater proportion of distinctly abnormal islets than did the low GI groups, that showed severely disorganized architecture and extensive fibrosis, and both studies concluded that the proportion of fibrotic islets in each individual strongly correlated to AUC of glycemic response for both high and low GI groups.

It is believed that a main cause of developing liver steatosis is increased *de novo* lipogenesis and TG accumulation in the liver, which, in turn, are proceeded by postprandial hyperglycemia and hyperinsulinemia. Hudgins (2000) and Parks (2002) indicated that a feature of *de novo* lipogenesis was the occurrence of abnormal lipid circulation between the liver and the adipose tissue, mainly through of FFA (10-12). Our results indicate that the Izumo Soba diet had an advantageous effect on the limiting of the liver *de novo* lipogenesis and TG accumulation, with the Izumo Soba diet-fed mice exhibiting lower the liver weight and liver TG content (both absolute and relative values), though all three diets used in this study contained large amounts of carbohydrates materials (about 73% kcal) and all mice fed same calorie rate (16.6 kcal / day). Further, the plasma FFA in the mice fed the Izumo Soba diet remained low after 30 days ingestion. In addition to these facts, the liver-derived blood γ-GTP, GOT and GTP for the Izumo Soba diet group, which enzymes were major parameter of liver function, kept lower than that for the Gozenko or Cornstarch diet groups. We concluded that the Izumo Soba diet has the effects of limiting the liver *de novo* lipogenesis and TG accumulation, and thus the potential for a significant impact on the prevention of liver steatosis.

Finally, we surmise that reducing the postprandial glycemic response is of great importance in maintaining good general health in individuals, as well as in the treatment of metabolic syndrome. While further acute and chronic studies with GI foods, focusing on liver *de novo* lipogenesis, are needed to examine this presumption more closely, we anticipate that future findings will support our conclusion that the high-fiber diet has the potential for prevention of liver steatosis.

CONCLUSION

This study found that the Izumo Soba diet, which contains large amount of dietary fiber from buckwheat chaff, helped to suppress increasing liver TG, and had a potential to limit liver *de novo* lipogenesis and for prevention of liver steatosis.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (to M. Yamasaki and to K. Shiwaku), and grants for promotion of scientific research from Shimane University School of Medicine (to S. Murata).

REFERENCES

1) Coulston AG, Liu GC and Reaven GM (1983) Plasma glucose, insulin and lipids responses to high-carbohydrate low-fat diets in normal human. *Metabolism* 32: 52-56.

2) Parks EJ and Hellerstein MK (2000) Carbohydrate-induced hypertriacylglcerolemia: histrical perspective and review of biological mechanism. *Am J Clin Nutr* 71: 412-433.

3) Reven GM (1995) Pathophysiology of insulin resistance in human disease. *Physiol Rev* 75: 473-486.

4) Shiwaku K, Anuurad E, Enkhmaa B, Nogi A, Kitajima K, Shimono K, Yamane Y and Oyunsuren T (2004) Overweight Japanese with body mass indexes of 23.0-24.9 have higher risks for obesity-associated disorders: a comparison of Japanese and Mongolians. *Int J Obes Relat Metab Disord* 28: 152-158.

5) Shiwaku K, Hashimoto M, Kitajima K, Nogi A, Anuurad E, Enkhmaa B, Kim JM, Kim IS, Lee SK, Oyunsuren T, Shido O and Yamane Y (2004) Triglyceride levels are ethnic- specifically associated with an index of stearoyl-CoA desaturase activity and n-3 PUFA levels in Asians. *J Lipid Res* 45: 914-922.

6) Shiwaku K, Hashimoto M, Nogi A, Kitajima K and Yamasaki M (2004) Traditional Japanese dietary basics: a solution for modern health issues? *Lancet* 363: 1737-1738.

7) Shiwaku K, Anuurad E, Enkhmaa B, Kitajima K and Yamane Y (2004) Appropriate BMI for Asian populations. *Lancet* 363: 1077.

8) Anuurad E, Shiwaku K, Enkhmaa B, Nogi A, Kitajima K, Yamasaki M and Yamane Y (2004) Ethnic differences in the formation of small LDL particles in Asians: a comparison of Koreans, Japanese and Mongolians. *Eur J Clin Invest* 34: 738-746.

9) Nogi A, Shiwaku K, Kitajima K, Yamasaki M, Anuurad E, Enkhmaa B, Yoneyama T, Hashimoto M, Kihara I, Yagura C, Iyama Y, Mihara S and Yamane Y (2004) Weight loss and improvement of metabolic syndrome by interventinal program based on health education. *Journal of the Japanese Association of Rural Medicine* (*JJRM*) 53: 649-659 (in Japanese, Eng Abstr).

10) Hudgins LC (2000) Effect of high-carbohydrate feeding on triglyceride and saturated fatty acid synthesis. *Proc Soc Exp Biol Med* 225: 178-183.

11) Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD and Hirsch J (2000) Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J Lipid Res* 41: 595-604.

12) Parks EJ (2002) Dietary carbohydrate's effects on lipogenesis and the relationship of lipogenesis to blood insulin and glucose concentrations. *Br J Nutr* 87 Suppl 2: S247-253.

13) Frost G, Wilding J and Beecham J (1994) Dietary advice based on the glycaemic index improves dietary profile and metabolic control in type 2 diabetic patients. *Diabet Med* 11: 397-401.

14) Wolever TM, Jenkins DJ, Jenkins AL and Josse RG (1991) The glycemic index: methodology and clinical implications. *Am J Clin Nutr* 54: 846-854.

15) Jenkins DJ, Wolever TM, Collier GR, Ocana A, Rao AV, Buckley G, Lam Y, Mayer A and Thompson LU (1987) Metabolic effects of a low-glycemic index diet. *Am J Clin Nutr* 46: 968-975.

16) Ludwig DS (2000) Dietary glycemic index and obesity. *J Nutr* 130: S280-283.

17) Macdonald IA (1999) Carbohydrate as a nutrient in adults: range of acceptable intakes. *Eur J Clin Nutr* 53: S101-106.

18) Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL and Goff DV (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34: 362-366.

19) Ritz P, Krempf M and Cloarec D (1991) Comparative continuous indirect calorimetry study of two carbohydrates with different glycemic indices. *Am J Clin Nutr* 54: 855-859.

20) Dahlquist A and Semenza G (1985) Disaccharidases of small-intestinal mucosa. *J Pediatr Gastroenterol Nutr* 4: 857-865.

21) Heijnen ML, van Amelsvoort JM and Weststrate JA (1995) Interaction between physical structure

and amylose:amylopectin ratio of foods on postprandial glucose and insulin responses in healthy subjects. *Eur J Clin Nutr* 49: 446-457.

22) Jenkins DJ, Jenkins AL, Wolever TM, Vuksan V, Rao AV, Thompson LU and Josse RG (1994) Low glycemic index: lente carbohydrates and physiological effects of altered food frequency. *Am J Clin Nutr* 59: S706-709.

23) Wursch P and Pi-Sunyer FX (1997) The role of viscous soluble fiber in metabolic control of diabetes. *Diabetes Care* 20: 1774-1780.

24) Bingham SA (1990) Mechanisms and experimental and epidemiological evidence relating dietary fiber (non starch polysaccharides) and starch to protection against bowel cancer. *Proc Nutr Soc* 49: 153-171.

25) Thorburn A. Muir J and Proietto J (1993) Carbohydrate fermentation decreases hepatic glucose output in healthy subjects. *Metabolism* 42: 780-785.

26) Carroll NV, Longley RW and Roe JH (1956) The determination of glycogen in liver and muscle by use of anthrone reagent. *J Biol Chem* 221: 583-593.

27) Folch J, Lees M and Sloane Stanly GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497-509.

28) Yamasaki M, Watanabe M, Yoneyama T, Nogi A, Wang L and Shiwaku K (2007) Influence of rice with different amylose contents on postprandial glycemic response. *Journal of Rural Medicine* (*JRM*) 2: 51-58.

29) Pawlak DB, Kushner JA and Ludwig DS (2004) Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *Lancet* 364: 778-785.

30) 30. Lerer-Metzger M, Rizkalla SW, Luo J, Champ M, Kabir M, Bruzzo F, Bornet F and Slama G (1996) Effects of long-term low-glycaemic index starchy food on plasma glucose and lipid concentrations and adipose tissue cellularity in normal and diabetic rats. *Br J Nutr* 75: 723-732.