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Effect of Low and High Glycaemic Index Diets on Developing the Risk of Metabolic Syndrome in Rats

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ABSTRACT. *Several observational studies have shown that the chronic consumption of high glycaemic index diet is associated with an increased risk of developing metabolic syndrome. This study was performed to identify the direct influences on the lipid profile and the adipose tissue deposition and the subsequent development of the risk of metabolic syndrome in rats by feeding diets of low glycaemic index (LGI) and high glycaemic index (HGI). Fifty rat weanlings (three weeks old) were equally divided into two groups and fed on either low glycaemic index diet based on high amylose, or isocaloric high glycaemic index diet for 12 weeks. Postprandial blood and tissue samples were collected at the end of the 12 weeks of feeding. The total white adipose tissue weights of the HGI fed rats (24.74 ± 0.53 g/rat) were significantly higher than the LGI fed rats (15.37 ± 0.36 g/rat). The HGI fed rats had higher postprandial leptin concentrations (1.86 ± 0.17 ng/ml) than LGI fed rats (1.34 ± 0.12 ng/ml). The postprandial insulin, and postprandial insulin glucose ratio were higher in the HGI fed rats (7.06 ± 0.90 ng/ml and 0.67 ± 0.01 ng/mlxmM) compared to the LGI fed rats (3.91 ± 0.4 ng/ml and 0.44 ± 0.01 ng/mlxmM). Triglycerides of the HGI fed rats showed higher values (1.56 ± 0.10 mM) than the LGI fed rats (1.07 ± 0.08 mM). The results indicated that LGI feeding was beneficial in preventing the conditions enhancing the cardio vascular disease whereas long-term feeding of HGI diet may increase the risk of developing metabolic syndrome in rats.*

INTRODUCTION

People with a combination of risk factors for cardio vascular disease (CVD) are reported to have "insulin resistant syndrome" or "metabolic syndrome" (Opara and Levine, 1997). The metabolic syndrome is characterized by risk factors, which include central obesity, dyslipidaemia, hypertension and insulin resistance or glucose intolerance. Cardiovascular disease is one of the major causes for deaths and disabilities of men and women all over the world (Srisawat *et al.*, 2003). CVD accounts for highest deaths each year than any other single cause, mostly by way of myocardial infarction (heart attack) and cerebro-vascular accidents (strokes).

Diabetes mellitus is characterized by alteration in the carbohydrate, fat and protein metabolism. These alterations are caused by a relative or absolute deficiency of insulin

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secretion and varying levels of insulin resistance (Eriksson *et al.*, 2002). It is evident that insulin resistance (IR) is usually overcome (normalised blood glucose levels) by increasing insulin secretion and maintaining high serum insulin level. Some individuals with IR then develop impaired glucose tolerance (IGF) followed by type 2 diabetes mellitus.

Obesity and increased blood lipids (low density lipoproteins and very low density lipoproteins and triglycerides), decreased high-density lipoprotein (HDL) and elevated total cholesterol/HDL ratio may place individuals at increased risk of CVD. Studies confirm that plasma cholesterol is lowered by consumption of polyunsaturated fatty acids (Edinton *et al.*, 1987).

The glycaemic index (GI) was proposed in 1981 as an alternative system for classifying carbohydrate-containing food (Le Leu *et al.*, 2003). GI describes the effect of a carbohydrate source on the level of blood glucose, is determined by much more than just its chemical composition. GI of a food depend on a variety of factors including type of carbohydrate (monomers or polymers), rate of ingestion, constituents (fat, protein and carbohydrate), method of cooking, hydrolysis, gastric empting rate, absorption and colonic effects. A range of factors associated with food consumption was shown to alter the rate of glucose absorption and subsequent glycemia. Starch is a polymer of the monosaccharide glucose. Starch granules are composed, in varying proportions, of a linear molecule amylose (200-300 glucose residues) and a branched molecule amylopectin (over 1000 glucose residues). Starch is digested by amylase and maltase, which hydrolyse the glucosidic bonds. The resulting glucose molecules are absorbed in the small intestine causing a rise in blood glucose concentration (Burkitt and Trowell, 1977). Foods containing more amylopectin which are digested easily, causing rapid blood glucose increase are categorised into high glycaemic index (HGI). By contrast amylose is resistant to digestion due to the unbranched linear nature of the molecule. These resistant starches are used in most of the low glycaemic index (LGI) diet preparations.

Some epidemiological studies find that foods rich in dietary fibre and resistant starch intake are protective against cancer, cardiovascular disease, diabetes and obesity. It was also found that LGI feeding has a beneficial effect by preventing metabolic disorders (Kabir, *et al.*, 2002 , Sievenpiper *et al.*, 2002, Le Leu *et al.*, 2003). Consumption of a high-dose of resistant starch enhanced postprandial glucose tolerance potentially due to the increased rate of colonic fermentation (Robertson *et al.*, 2003). Diets rich in insoluble-fibre are linked to a reduced risk of both diabetes and cardiovascular disease; however, the mechanism of action remains unclear. The carbohydrates that escape digestion (*e.g.* resistant starch) in the small intestine are fermented in the gut, producing short-chain fatty acids (SCFA), which lower colonic pH and alter blood lipids (Slavin, 2003).

This study examined the effects of long-term consumption of LGI or HGI diet in the risk of developing of metabolic syndrome in rats.

MATERIALS AND METHODS

Fifty male Wistar rats (three weeks old weanlings) were randomly divided into two groups. They were assigned to two feeding groups of either LGI or HGI diet (Table 1). For

the first two weeks they received 20g/rat/day, then 40g/rat/day diet for next 10 weeks (155 KJ/rat/day in 1st two weeks then 310 KJ/rat/day).

Table 1. Composition of experiment diets[1]

Ingredient	HGI	LGI
	g/kg	g/kg
Casein	200	200
Methionine	2	2
Starch[2]		
High Amylose	-	514
Glucose Polymer (Polycose)	514	-
Canola oil ml/kg	25	25
Sunflower oil ml/kg	25	25
Wheat Bran	50	50
Minerals[3]	67	67
Vitamins[4]	13	13
Sucrose	85	85

[1] All diets were prepared weekly and stored in dry place (room temperature).
[2] High-amylose was 60% amylose & 40% amylopectin. Polycose was 100% glucose polymer.
[3,4] Vitamins and minerals were standard rat premix formula purchased from International Animal Health Products, NSW, Australia.

Each group was fed an isocalorically macronutrient-controlled diet. The only difference between the two diets was the type of starch used to provide the carbohydrate, HGI (100% glucose polymer) and LGI (60% amylose 40% amylopectin) (Pawlak *et al.*, 2001). All diets were prepared weekly and stored in a dry place.

After 12 weeks of feeding experimental diets (15 week old) rats were fasted overnight (12 hours). They were given their usual diet for two hours in the morning and anaesthetized with sodium pentobarbital (15 mg/kg body weight) (Saade *et al.*, 1996). Before they were killed but under anaesthesia, post-prandial plasma and serum blood samples were collected. Separated adipose tissues from the body were freeze-clamped and stored at – 80 °C. Blood samples were analysed for total cholesterol, triglycerides and HDL cholesterol using CHOL reagent and with Precipath, Precinorm quality control kits (Roche Diagnostics, Mannheim, Germany) in Roche Hitachi 912 autoanalyzer. Plasma insulin and leptin were measured by radioimmunoassay using rat leptin and insulin antibodies and standards (Linco Research Inc., USA). Plasma glucose concentrations were measured by reading absorbance spectrophotometrically at 492 nm. Adipose tissue free fatty acids were extracted using gas chromatograph. Data were analysed using SPSS statistical software. All values were expressed as the mean ± SEM unless otherwise stated. Significance was assumed if $P < 0.05$ after applying Student's t test. Ethical clearance for this study was obtained, from the Sydney University Animal Ethics Committee (L02/8-2003/3/33792).

RESULTS

Table 2 illustrates the biochemical and tissue measurements of the rats after 12 weeks of experimental diet feeding.

Table 2. Biochemical and tissue measurements of LGI and HGI diet fed rats of 15 weeks of age

Variable	Measurements (Mean ± SE) LGI	HGI
Body Weight (g/rat)	390 ± 10	398 ± 12
Total WAT (g/rat)	15.4 ± 0.4	24.7 ± 0.5 *
Plasma Post-Prandial Insulin (ng/ml)	3.91± 0.4	7.06 ± 0.90*
Insulin-Glucose Ratio (ng/mlxMm)	0.44 ± 0.01	0.67 ± 0.01*
Plasma Post-Prandial Leptin (ng/ml)	1.34 ± 0.12	1.86 ± 0.17*
Total Plasma Cholesterol (mM)	1.93 ± 0.11	2.24 ± 0.19
Plasma Triglycerides (mM)	1.07 ± 0.08	1.56 ± 0.10*
Plasma HDL (mM)	1.05 ± 0.04	1.04 ± 0.06

White adipose tissue (WAT), $n = 25$ for each group, * $P<0.05$

There was no significant difference in the body weights between the two diet groups. But total white adipose tissue (WAT) weights of HGI fed rats were significantly higher than that of LGI fed rats. Subcutaneous WAT, epididymal WAT, peritoneal WAT and post peritoneal WAT of the HGI fed rats showed significant increase compared to the LGI fed rats (Figure1)

These HGI fed rats also showed elevated postprandial plasma leptin, and triglycerides concentrations. The increase of total cholesterol and HDL in HGI fed rats was not statistically significant (Table 2) ($P>0.05$). There was higher postprandial plasma insulin secretion and higher postprandial insulin glucose ratio showed in HGI diet group than LGI diet fed rats ($P<0.05$) (Table 2).

There were significant differences in epididymal white adipose tissue fatty acid profiles between the two diet groups of rats. Saturated fatty acid percentage found in HGI rats was about 28% while in LGI it was 25%. Mono-unsaturated fatty acids percentage found in HGI rats was about 49% and in LGI it was about 43%. Poly-unsaturated fatty acids percentage found in LGI diet fed rats was about 10% higher than the HGI diet fed rats (Figure 2). Presence of Arachidonic acid (C20: 4) was characteristically significant for LGI diet fed rats only. This poly-unsaturated fatty acid percentage was non detectable in HGI rat adipose tissue.

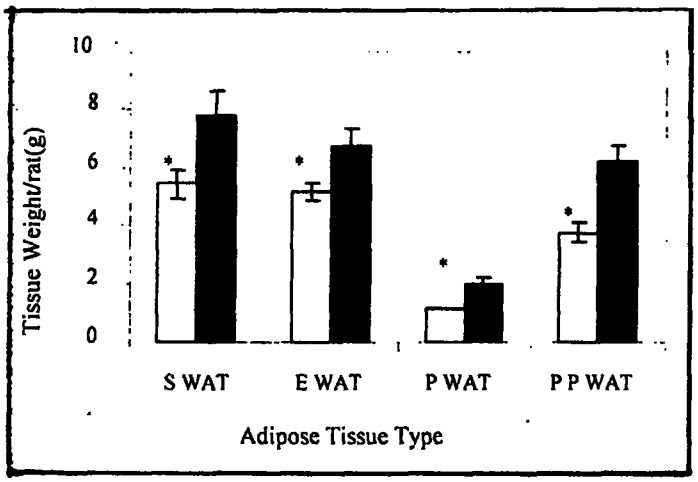

Fig. 1. Epididymal adipose tissue weights of rats after feeding

LGI (□) or HGI (■) for a period of 12 weeks. Subcutaneous White Adipose Tissue (S WAT), Epididymal White Adipose Tissue (E WAT), Peritoneal White Adipose Tissue (P WAT), Post Peritoneal White Adipose Tissue (P P WAT) n= 25 for both groups. * P>0.05

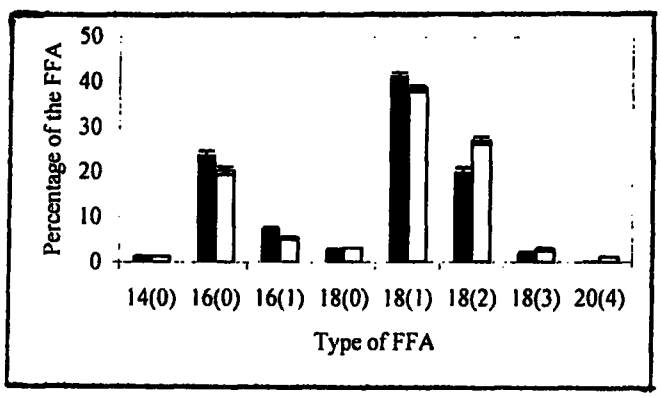

Fig. 2. Epididymal adipose tissue Free Fatty Acids profile of rats of 12 weeks feeding of diet consisting LGI (□) or HGI (■) for period of 12 weeks

Free Fatty Acid Concentrations were expressed as percentages. 14(0)- Myristic acid, 16(0)- Palmitic acid, 16(1)- Palmioleic acid, 18(0)-Stearic acid, 18(1)-Oleic acid, 18(2)-Linoleic acid, 18(3)-Linoleneic acid, 20(4)- Arachidonic acid. n= 25 for both groups.

DISCUSSION

After 12 weeks of feeding LGI diet had significant effect on rat metabolism. The HGI diet fed rats showed evidence of the development of early signs of metabolic disorders. HGI fed rats had higher postprandial insulin secretion, adipose tissue weight, triglyceride

and leptin concentration than LGI diet fed rats. The increase of total plasma cholesterol levels was not statistically significant. HGI fed rats are more prone to develop conditions such as insulin resistance, dyslipidaemia and other associated disorders of metabolic syndrome or "Syndrome X". The LGI diet resulted in lower postprandial plasma insulin and glucose profiles, which were associated with a decrease in total fat mass. This decrease in fat mass was accompanied by a decrease in leptin. Leptin levels reflect body fat content and act as an acute sensor of energy balance by signalling to the hypothalamus. It has been suggested that leptin itself may play an important role in developing of insulin resistance by increasing adipose tissue mass (Walder *et al.*, 1998). In this study HGI diet fed rats had greater visceral adiposity (epididymal, peritoneal and post peritoneal white adipose tissue) compared to LGI diet fed rats. Such increased visceral fat stores tend to flood portal circulation with free fatty acids exposing body tissues to excessive free fatty acid levels. The exposure of higher free fatty acid concentrations may increase the probability of developing insulin resistance in the body tissues (McCarty, 2001). These results help the speculation of higher insulin sensitivity of individuals who have lesser adipose tissues (LGI fed rat group). Since these adipose tissues have better insulin sensitivity, it increases the plasma glucose clearance by speedup glucose uptake.

This study suggests that intake of rapidly digested and absorbed carbohydrates with a high glycaemic index are associated with an increased risk of metabolic syndrome. In animals and in short-term human studies, a high intake of carbohydrates with a HGI produced greater insulin resistance than did the intake of LGI carbohydrates. In large prospective epidemiological studies, the higher glycaemic index diet has been associated with a greater risk of type-2 diabetes in both men and women. Resistance to the anti-lipolytic action of insulin could potentially lead to resistance to insulin-stimulated glucose uptake into muscle. By consuming HGI diet for longer period these rats become more prone to reduce the insulin sensitivity of the fat tissues, subsequently develop glucose intolerance and metabolic disorders in later stages of the life.

There is emerging evidence that excess adipose tissue, acting as an endocrine organ may act at distant sites as key modulators of risk for a number of chronic diseases such as insulin resistance, atherosclerosis, hypertension and dyslipidaemia. LGI fed rat adipose tissue consists of higher proportion of long chain polyunsaturated fatty acids (Linoleic acid) than that of HGI fed rats. A lower percentage of arachidonic acid (AA) can be seen in LGI fed rat adipose tissue but not in HGI rats. The AA is the precursor of prostaglandins (eicosanoids), leukotrines and lipoxines, which may important roles in inflammation, and regulation of immunity (Calder, 2001). Inflammation has been identified as an important predictor of cardiovascular risk and as one of the strongest mechanisms in CVD. This anti-inflammatory role of AA may have a protective action against the initiation of CVD.

Dietary starch that escapes digestion in the small intestine is quantitatively important as a substrate for fermentation. Subjects fed on stale maize porridge had significantly more production of SCFA together with a significant drop in stool pH (Ahmed *et al.*, 2000). These SCFA considered to be contributing substantially to the normal physiological functions, which may reduces serum and hepatic cholesterol. In the study the serum propionate concentration (type of SCFA) in the rats fed on resistant starch diets was significantly higher than that of other groups. Resistant starch may be fermented to produce propionate, which reduces serum cholesterol in substantial amount and triglycerides (Cheng and Lai, 2000).

CONCLUSIONS

The findings in this study provide scientific justification to the concept that HGI feeding for longer periods may increase the risk of developing metabolic syndrome. It also indicates that replacing HGI with lower glycaemic index diets may enhance better insulin sensitivity and ultimately reduces risk of developing metabolic syndrome.

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