ABSTRACT: This study was to evaluate the effects of lower-GI starch on the insulin signaling in STZ/nicotinamide-induced hyperglycemic rats. We divided 13 hyperglycemic rats and 6 normal rats randomly into two groups respectively and fed a diet containing 575 g/kg as either low-GI sweet potato starch (designed as “S”) or high-GI potato starch (designed as “P”). We labeled these four groups as HG-P, HG-S (hyperglycemic rats), N-P and N-S (normal rats). After consuming the diets for 4 weeks, the skeletal muscle was collected to measure the protein expression of the insulin receptor (IR), IR substrate (IRS)-1, and glucose transporter (GLUT)4. Two intraperitoneal glucose tolerance tests (IPGTTs) were also performed at 0th and 4th week to evaluate the postprandial glycemic response. The results showed that the area under the curve (AUC) for blood glucose at 4th week was significantly lower than that at 0th week in the HG-S group. The protein expression of IRS-1 and GLUT4 in the skeletal muscle was significantly upregulated in the HG-S group compared to the HG-P group. We concluded that starch with lower-GI improved the postprandial glycemic response of hyperglycemic rats and may associate with the upregulating the proteins involved in insulin signaling.

KEY WORDS: Glycemic index, Hyperglycemic rats, Insulin signaling, Starch

INTRODUCTION

Due to ever increasing prevalence of type 2 diabetes mellitus worldwide, we need to understand its different risk factors, including obesity, physical inactivity, and lifestyle factors (such as the dieting habit). Consumption foods with a higher glycemic index (GI), meaning producing higher postprandial blood glucose, tends to cause type 2 diabetes (Tuomilehto et al., 2001). Foods with higher GI values can cause higher postprandial blood sugar and insulin concentration (Brand-Miller and Holt, 2004). The results of epidemiologic studies suggest that eating food with a lower GI value can prevent diabetes (Salmeron et al., 1997; Schulze et al., 2004). Starchy foods are the main carbohydrate sources worldwide. For example, many Taiwanese consume sweet potato to substitute for rice. Adapting Western-style diets in recent years, many Taiwanese, especially young people, often consume potatoes in their diet. Choosing higher quality of carbohydrates in starchy foods is important to prevent and manage type 2 diabetes. Consuming diets rich in whole-grain cereals and starchy foods with a lower GI can protect against type 2 diabetes (Salmeron et al., 1997a; Schulze et al., 2004; Salmeron et al., 1997b). In FUNGENUT study, the investigators found that the insulinogenic index is improved after eating a rye-pasta diet (a lower-GI diet) but worsen after consuming an oat-wheat-potato diet (a higher-GI diet), and that modifying of rye- and pasta-based carbohydrates can enhance early insulin secretion in persons with metabolic syndrome (Kallio et al., 2007).

Eating starchy foods with different GI values can affect the postprandial blood sugar and insulin concentrations, resulting in developing type 2 diabetes. Impaired regulation of insulin signaling is a critical factor in the development of insulin resistance and type 2 diabetes. Insulin signaling frequently occurs in skeletal muscles and is a complicated cascade process. Insulin through the insulin signaling plays an important role in nutrient metabolism, including glycogen synthesis, glucose transport, lipid synthesis, and protein synthesis (Saltiel, 1996; Cheatham and Kahn, 1995). Briefly, insulin binds to
the insulin receptor (IR), to activate the IR and IR substrates (IRSs, with IRS-1 being the major substrate). Finally, this signal can induce the membrane translocation of glucose transporter (GLUT)4, and to increase the use of glucose and the synthesis of glycogen, lipids, and proteins.

In type 2 diabetic patients, the body becomes resistant to the effects of insulin presumably because of defects in insulin signaling (Lizcano and Alessi, 2002). We are still unclear whether the association between the foods with lower GI values and a reduced risk of hyperglycemia is mediated through an improvement in insulin signaling. Choosing a low GI diet has been already in practice in clinical dietetics. But very little mechanistic study in animal has been reported. Although there are a lot of studies reported that some Chinese medicine or herbs would upregulate the proteins involved in insulin signaling, the effects of starches with different GIs are needed. Therefore, in this study, we intended to evaluate the GI values of two kinds of starchy foods, sweet potato and potato, and aimed to assess the effects of starches with lower GI values on insulin signaling in skeletal muscle in rats with streptozotocin (STZ)/nicotinamide-induced hyperglycemia.

**MATERIALS & METHODS**

**Measurement of the GIs of sweet potato and potato:** We enrolled 13 healthy people with a mean age of 22.1 years in the study, to measure GI values of sweet potato (Tainun no. 57) and potato (Kennebec). Tainun no. 57 sweet potato and Kennebec potato are most popular in Taiwan. The study was approved by the Institutional Review Board of Taipei Medical University, and written informed consent was obtained from every subject. GI values were determined according to the standard GI testing protocol (Brouns et al., 2005); the reference food was 50 mL of a 50% glucose solution (25 g glucose). Briefly, on each test day, all subjects consumed a starchy food containing 25 g of carbohydrates with 100 mL water in 15 min. Sweet potato and potato were steamed for 20 minutes. One edible portion of starchy food that contained 25 g carbohydrates was calculated using the amount of carbohydrates listed for it in the Taiwanese Nutrient Database (Food and Drug Administration, Department of Health, Executive Yuan, 2012). Venous blood was sampled in a heparin-containing tube at 0 (initiation of ingestion), 30, 45, 60, 90, and 120 min after ingestion. Blood samples were centrifuged (1400 xg for 10 min at 4 °C) to obtain plasma. We measured blood sugar with a commercial kit (glucose oxidase and peroxidase, Randox Lab, Co. Antrim, United Kingdom). Antrim, United Kingdom).

To calculate GI values, the area under the curve (AUC) must be identified in advance. The AUC refers to the area included between the baseline and incremental blood glucose points when connected by straight lines. We identified the GI of a food as (Jenkins, 1987): the area under the glycemic curve of the test starchy food/ the area under the glycemic curve of glucose.

According to our results, the respective GI values of sweet potato and potato were 55 and 85, they belong to low and high GI category, respectively. In this study, we, therefore, used sweet potato as the source of lower-GI starch, and the potato as the source of higher-GI starch.

**Animals:** Male Sprague-Dawley rats (n = 19, aged 8 weeks) were obtained from BioLASCO Taiwan, (Taipei, Taiwan). Taipei Medical University approved the use of those laboratory animals. After a two-weeks adaptation, 13 hyperglycemic rats were induced by an intraperitoneal injection of streptozotocin (STZ) at 45 mg/kg body weight (BW) (Sigma, St. Louis, Missouri, USA) and by an injection of nicotinamide (150 mg/kg BW) 15 min later. After two days, this step was repeated using the modified Masiello method (Masiello et al., 1998; Chen and Cheng, 2006). Nicotinamide and STZ were freshly prepared in a 0.9% sodium chloride solution. A rat was considered to be hyperglycemia when its fasting plasma glucose concentration was greater than 180 mg/dL. 14 days after the last induction date. At this time point, we collected baseline blood samples from the tail vein of rats.

**TABLE 1. Composition of the diets used in this study.** 1In g/kg of the diet: corn oil 13, peanut oil 14, and lard 28.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>AMOUNT (G/KG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato starch or potato starch</td>
<td>575</td>
</tr>
<tr>
<td>Vegetable and animal fat 1</td>
<td>55</td>
</tr>
<tr>
<td>Casein</td>
<td>230</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>70</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose</td>
<td>60</td>
</tr>
</tbody>
</table>

**Diets:** We divided 6 normal rats and 13 hyperglycemic rats randomly into two groups respectively and fed a diet containing 575 g/kg (Lerer-Metzger et al., 1996) as either cooked 100% sweet potato starch (designed as “S”, from Abundant States Starch Manufacturing Factory, Chiayi County, Taiwan, produced from Tainun no. 57 sweet potato) or cooked 98% potato starch (designated as “P”, from Kuo Chi Trading Co., Taipei, Taiwan, produced from Kennebec potato) (Table 1). We labeled these 4 groups as HG-P, HG-S (hyperglycemic rats), N-P and N-S (normal rats). To verify the GI values of these two kinds of commercial starches, we measured the GI values according to the protocol mentioned previously. The results showed the GI of commercial sweet potato starch and potato starch is 52.2 and 91.0 and belongs to low and high GI category, respectively. This demonstrates that the 2 are suitable low and high GI starches for our study.

**Intraperitoneal glucose tolerance test (IPGTT):** Two IPGTTs were conducted 2 days prior the beginning and the
end of feeding study. The rats fasted overnight and the blood samples were taken from the tail vein (time 0). A glucose challenge was given intraperitoneally (2 g glucose/kg BW), other blood samples were taken after 15, 30, 60, 90, 120, 150, and 180 min. Plasma glucose concentrations were determined and the AUC for blood glucose was also calculated.

After consuming the diets for 4 weeks, the rats were starved overnight, then anesthetized them with Rompun and Zoletil. Blood was collected in no-anticoagulant tubes and EDTA-containing tubes. Serum and plasma were prepared and stored at -20 °C for further insulin and lipid measurements. In addition, another part of blood was collected in heparin-containing tubes and plasma was prepared and stored at -20 °C for further glucose measurements. The skeletal muscle (the gastrocnemius) was immediately removed, weighed, and frozen in liquid nitrogen. Tissues were then stored at -80 °C for later protein expression studies.

**Measurement of glucose, insulin, and lipid concentrations:** The plasma glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and non-esterified fatty acid (NEFA) concentrations were determined spectrophotometrically using a glucose kit, triglycerides kit, cholesterol kit, LDL-C kit, and NEFA kit, respectively (Randox). The plasma insulin level was measured by a Rat Insulin Enzyme-linked Immunosorbent Assay (ELISA) kit (Mercodia, Uppsala, Sweden).

**Western blotting:** Skeletal muscles were homogenized in Pro-Prep™ protein extraction solution (iNtRON Biotechnology, Gyeonggi-do, Korea) with a polytron (Brinkmann Instruments, Westbury, New York, USA). Protein concentrations in each sample were quantified by a commercial assay kit (Bio-Rad DC Protein Assay kit, Bio-Rad Laboratories, Hercules, California, USA) using bovine serum albumin (BSA) as a standard. Equal amount of proteins (30 μg) were denatured and separated by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). After separation, the proteins were electroblotted onto a polyvinylidene difluoride transfer membrane (Amersham Biosciences, Little Chalfont, Bucks, UK). These blots were then incubated with a rabbit anti-rat IR, rabbit anti-rat IRS-1 (Millipore, Billerica, Massachusetts, USA), and rabbit anti-rat GLUT4 (Chemicon, Billerica, California, USA). Finally, the blot was treated with goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase (HRP) (Santa Cruz Biotechnology, Santa Cruz, California, USA), and specific bindings of antibodies were detected using an enhanced chemiluminescence Western blot detection kit (Thermo Scientific, Rockford, Illinois, USA). The bands were quantified using an Image-Pro Plus 4.5 software analysis.

**RESULTS**

Table 2 and table 3 show the characteristics and the blood parameters levels of all rats after 4 weeks of consuming the experimental diets. There were no any significantly differences of body weight, feeding efficiency or HOMA values between four groups. The data showed that the fasting blood glucose and LDL/HDL in hyperglycemic rats were significantly higher than those in normal rats. The levels of blood insulin and HDL in hyperglycemic rats were significantly lower than those in normal rats.

### Table 2. Characteristics of rats after 4 weeks of consuming experimental diets. Data were presented as the mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>N-P (N=3)</th>
<th>N-S (N=3)</th>
<th>HG-P (N=7)</th>
<th>HG-S (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>346.3 ± 5.5</td>
<td>342.7 ± 7.0</td>
<td>335.6 ± 7.5</td>
<td>340.2 ± 8.1</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>496.0 ± 27.2</td>
<td>485.0 ± 4.4</td>
<td>463.1 ± 23.6</td>
<td>422.2 ± 17.3</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>149.7 ± 32.5</td>
<td>142.3 ± 8.4</td>
<td>127.3 ± 20.8</td>
<td>82.0 ± 22.2</td>
</tr>
<tr>
<td>Feeding efficiency (%)</td>
<td>21.4 ± 4.6</td>
<td>20.3 ± 1.2</td>
<td>18.2 ± 3.0</td>
<td>11.7 ± 3.2</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>14.0 ± 2.8</td>
<td>13.4 ± 0.6</td>
<td>11.5 ± 0.6</td>
<td>11.4 ± 2.1</td>
</tr>
</tbody>
</table>

### Table 3. Blood parameters of rats after 4 weeks of consuming experimental diets. Data were presented as the mean ± SD; Means with a different superscript differ significantly according to the two-way ANOVA with Fisher’s test.

<table>
<thead>
<tr>
<th></th>
<th>N-P (n=3)</th>
<th>N-S (n=3)</th>
<th>HG-P (n=7)</th>
<th>HG-S (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC (ante cibum) sugar (mg/dL)</td>
<td>140.7 ± 18.5 a</td>
<td>153.0 ± 8.9 a</td>
<td>227.9 ± 7.4 b</td>
<td>221.7 ± 13.7 b</td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>3.7 ± 0.5 b</td>
<td>3.5 ± 0.4 b</td>
<td>1.2 ± 0.2 a</td>
<td>1.2 ± 0.6 a</td>
</tr>
<tr>
<td>HOMA values</td>
<td>13.5 ± 6.1</td>
<td>12.5 ± 2.2</td>
<td>16.5 ± 3.3</td>
<td>16.8 ± 8.5</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>53.3 ± 3.8</td>
<td>60.7 ± 6.5</td>
<td>57.3 ± 7.2</td>
<td>55.3 ± 6.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>59.7 ± 6.8</td>
<td>56.3 ± 4.9</td>
<td>58.0 ± 11.9</td>
<td>51.5 ± 3.1</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>27.6 ± 2.5 b</td>
<td>29.1 ± 1.5 b</td>
<td>12.4 ± 2.5 a</td>
<td>14.5 ± 3.1 a</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>12.7 ± 2.0</td>
<td>12.2 ± 1.5</td>
<td>11.8 ± 1.9</td>
<td>11.3 ± 1.6</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.5 ± 0.07 a</td>
<td>0.4 ± 0.06 a</td>
<td>1.0 ± 0.3 b</td>
<td>0.8 ± 0.3 b</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.7 ± 0.004</td>
<td>0.7 ± 0.05</td>
<td>0.6 ± 0.07</td>
<td>0.6 ± 0.05</td>
</tr>
</tbody>
</table>

Table 4 listed the AUC for blood glucose in IPGTT of all rats. The AUC for blood glucose in hyperglycemic rats was...
significantly higher than that in normal rats. This result means that the glucose intolerance occurred in hyperglycemic rats. Figures 1 shows comparisons of AUC for blood glucose between 0th and 4th week for hyperglycemic rats. In the HG-P group, the AUCs for blood glucose at 0th and 4th week were 20304.6 ± 3782.1 and 20186.8 ± 2940.9, respectively (p>0.05). In the HG-S group, the AUCs for blood glucose at 0th and 4th week were 20270.0 ± 1433.8 and 17955.0 ± 1301.2, respectively (p=0.012412). The AUC for blood glucose at 4th week in the HG-S group was obviously lower than that at 0th week. This result showed that lower-GI sweet potato starch feeding for 4 weeks could improve the postprandial glycemic response in hyperglycemic rats.

Figures 2-4 illustrate the protein expressions of the IR, IRS-1, and GLUT4 in skeletal muscle from the Western blot analysis. Different GI starches intervention had no significant effect on the protein expression of IR in the skeletal muscle. In hyperglycemic rats, results showed that the lower-GI sweet potato starch feeding for 4 weeks significantly enhanced the protein expression of IRS-1 and GLUT4 compared to higher-
GI potato starch-fed group. This result demonstrates that the low-GI sweet potato starch feeding for 4 weeks upregulated the proteins involved in insulin signaling in the hyperglycemic rats.

DISCUSSION

Several beneficial effects of low-GI diets have been shown in non-diabetic persons, such as reduced risks of diabetes and coronary heart disease, and enhanced blood glucose regulation (Riccardi et al., 2008). A meta-analysis study mentioned that a low-GI diet can lower serum HbA1c levels by 0.4% and fructosamine levels by 0.2 mmol/L in patients with diabetes compared to the high-GI or other diets (Thomas and Elliott, 2010). A previous study also mentioned that in type 2 diabetes, insulin sensitivity is affected by the GI value of the diet, significantly increases in the low-GI group compared to the high-GI group (Rizkalla et al., 2004). Thus, the diet with a lower GI value is an effective method for improving glycemic control in diabetes, and low-GI diets should be incorporated into the lifestyle of diabetic patients.

The quality of carbohydrates in starchy foods is important to prevent and manage type 2 diabetes. Lin et al. evaluated the GI values and insulimetic indices of five kinds of Chinese starchy foods, including adlay, brown rice, mung bean noodles, taro, and yam (Lin et al., 2010). They found that brown rice has the highest GI value (of 82) compared to the other starchy foods, and brown rice also produces the highest glycemic and insulinemic responses. Mung bean noodles have the lowest GI value (of 28) and produce the lowest glycemic and insulinemic responses. In Bornet et al.’s study (1987), six starch-rich foods were tested in 18 NIDDM patients and compared using an oral glucose-tolerance test. The investigators found that bread and potato have the higher GI values and produce higher incremental plasma glucose responses. In examining changes in the blood glucose AUC over time with an oral glucose-tolerance test, Pawlak et al (2004) found that the AUC for blood glucose has significantly been increased in the high-GI group compared to the low-GI group at week 5, and that the difference persists for the duration of the experiment. Taken together, we summarize that diet with a lower-GI can stabilize postprandial glucose and produces a lower glycemic response. In our study, we found that the AUCs for blood glucose at 4th week in the sweet potato starch-fed group were significant lower than those at 0th week. This result showed that compared to the potato starch-fed group, lower-GI sweet potato starch improved the postprandial glycemic response in hyperglycemic rats. This result is similar to those we summarized previously.

Numerous studies have shown that hyperglycemia results from a disorder of the insulin signaling pathway (Ryder et al., 2001; Samad et al., 2000; Desrois et al., 2004). A previous study mentioned that insulin resistance from animals fed a high-fat diet was reflected in impaired activation of insulin signaling (Ouwens et al., 2005). Defects in insulin signaling are also reported in ob/ob mice and Zucker fatty rats (Kessler et al., 2001). In this study, we used the protein expression of IR, IRS-1 and GLUT4 as indicators of insulin signaling. A study have focused on IRS-1 as a major target for IRS kinase and it is probably essential for all of insulin’s biological responses, including fatty acid and glycogen metabolism and the regulation of glucose metabolism (Draznin, 2006).

Glucose uptake by skeletal muscles primarily occur through GLUT4, which GLUT4 is a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis (Tengholm and Meyer, 2002). Huang et al mentioned that either acute of long-term changes in the abundance of GLUT4 in the muscle cells could provoke systemic changes in glucose disposal in vivo (Huang and Czech, 2007). In this study, our results showed that the lower-GI sweet potato starch feeding for 4 weeks enhanced the proteins expression of IRS-1 and GLUT4 in the skeletal muscle of hyperglycemic rats. Our IPGTT results also suggest that a positive effect of lower-GI sweet potato starch on postprandial glycemic response of hyperglycemic rats. We supposed this positive effect on postprandial glycemic response probably associated with the enhanced expression of IRS-1 and GLUT4 and then upregulated the proteins involved in insulin signaling in the skeletal muscle.

In this study, we found that STZ/nicotinamide-induced hyperglycemia was associated with insulin deficiency. Masiello et al. (1998) demonstrated that STZ, plus nicotinamide, intravenous glucose tolerance tests revealed clear abnormalities in glucose tolerance and insulin responsiveness. Investigators of Korea has reported that male Sprague-Dawley rats showed rapid chemical destruction of the pancreatic β cells when they were given a single high-dose injection of STZ (80 mg/kg, IP) and affects the secretion of insulin (Kim et al., 2006). In Chen’s study (2011), they injected the rats with 50 mg/kg BW of STZ. They found the levels of free fatty acids, C-reactive protein (CRP) and TNF-α increased in these rats and they suggested that elevated circulating fatty acids and chronic inflammation causes insulin resistance via inhibiting the signaling downstream of insulin receptor. In the present study, we found that the levels of insulin show no significant differences between HG-S and HG-P groups after 4 weeks. This result suggests the sweet potato starch feeding for 4 weeks did not enhance the secretion of insulin effectively in STZ/nicotinamide-induced hyperglycemic rats. We supposed the sweet potato starch feeding improves glucose tolerance may via the upregulation of proteins involved in insulin signaling rather than increase the secretion of insulin. Choosing a low-GI diet has been already in practice in clinical dieticians. However, there are very few mechanistic study in animal has been reported. This study provides an evidence for the potential beneficial effects of lower-GI diet in diabetic patients.

This study has some limitations. First, owing to the limited number of our rats, in this study we evaluated only three factors in insulin signaling in response to the lower-GI starch. To note, some other factors related to insulin signaling, such as phosphatidylinositol-3 kinase, protein kinase Akt, and insulin...
receptor substrate 2, are all important. At the molecular level, these factors may alter the efficiency of glucose use (Huang and Czech, 2007). Second, the phosphorylated state after insulin stimulation, such as phosphorylated IRS-1 or phosphorylated IR, could also be monitored in the further, in addition, measure the protein expression of GLUT4 only located in the cell surface instead of the whole cell could observe the GLUT4 translocation (Bryant et al., 2002). Thus, a wide scale of analysis of these factors is required in the future to improve insight into the effects of lower-GI starch on regulating insulin signaling.

In conclusion, our study results suggest that lower-GI sweet potato starch improved the postprandial glycemic response in hyperglycemic rats, and may associate with the upregulation of the proteins involved in insulin signaling in the skeletal muscle. A wide scale of analysis is still required in the future study to evaluate the effects of lower-GI starch on regulating insulin signaling.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST DISCLOSURE

There is no conflict of interest.

REFERENCES


