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ORIGINAL CONTRIBUTION

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Adaptation of Sprague Dawley rats to long-term feeding of high fat or high fructose diets[1]

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Summary *Background* Present animal models used to emulate type 2 diabetes may not accurately reflect the metabolic changes that occur in humans. *Aim of the study* The purpose of this research was to evaluate diets reported to induce insulin resistance and impaired glucose metabolism in rats as a potentially useful model for studying type 2 diabetes. *Methods* Three groups of male Sprague Dawley rats (n=7) were fed either a control diet, based on AIN recommendations (53 % cornstarch, 10 % sucrose and 7 % soybean oil), a high fat diet (25 % soybean oil, 35 % cornstarch) or a high fructose diet (53 % fructose, 10 % sucrose) for a 3 month period. Glucose tolerance tests were carried out in week 3 and week 9 of the experiment. At the termination of the experiment, serum insulin, glucose, cholesterol and triacylglycerols were measured. Glucose incorporation into glycogen and glycogen synthase activity were measured in soleus muscles. *Results* Similar weight gain was observed for all three groups of rats. Glucose tolerance curves and fasting glucose levels were not significantly different at any time point in the experiment. Insulin levels were unchanged for the controls (171±21 pM), high fructose (164±16 pM) and high fat (181±30 pM) diets. Fasting serum triacylglycerols and cholesterol levels were not significantly elevated by dietary treatment. In soleus muscles, rats on all three diets had a significant increase in glycogen synthesis in response to insulin, but synthesis was similar in all three groups. Glycogen synthase activity was also not significantly affected by long-term dietary intervention. *Conclusions* In this study, healthy Sprague Dawley rats fed high fat or high fructose diets for 3 months adapted to the nutritional intervention without developing classical signs of insulin resistance and impaired glucose tolerance.

Key words Glucose intolerance – insulin resistance – fat – fructose – rats

Introduction

The ability for foods and food constituents to prevent or delay the onset of several diseases has led to an increased interest in evaluating the effectiveness of dietary components in disease prevention. Rigorous scientific evaluation of the therapeutic value is not available for the vast majority of nutraceutical products. Only a few have been tested in long-term prospective human trials [1, 2]. The high cost of such studies, and the inability to control confounding factors, accentuate the need for finding appropriate animal models to assess the efficacy of the numerous compounds considered to have therapeutic properties. This study was designed to evaluate rat models of diet-induced insulin resistance in order to establish a reproducible model that closely resembles the pre-diabetic state in humans.

Numerous studies report that feeding a high fat diet to

rats leads to insulin resistance and impaired glucose metabolism [3–7]. It has also been shown that high fructose diets fed to rats causes insulin resistance, hypertriacylglycerolemia and hypertension [8, 9]. These two models were chosen for evaluation as potential nutritional animal models to be used in future experiments to evaluate the efficacy of various nutraceuticals in preventing or delaying type 2 diabetes.

One of the enzymes which is considered to play an important regulatory role in glucose metabolism is glycogen synthase (GS); its activity level changes in conditions such as glucose intolerance as well as in animals with insulin resistance. Studies have demonstrated that patients with type 2 diabetes have low GS activity in skeletal muscle as well as decreased GS response to insulin [10–12]. This mechanism has been suggested as the primary reason for the decreased stored glycogen found in diabetes type 2. Similar findings were observed in animals exposed to high fat diets, insulin resistance developed as well as decreased response of skeletal muscle GS to injected insulin [13, 14]. Although it is thought that a high fructose diet causes insulin resistance, little is known regarding its affects on glycogen metabolism. The purpose of this study was to evaluate two nutritional models, a high fat diet and a high fructose diet, fed to rats, and assess effects on glucose metabolism and glycogen synthesis.

Materials and methods

Animals

Male Sprague Dawley rats (Harlan, Israel) were housed in individual suspended stainless-steel cages in a controlled environment (22–24 °C and 12 h light–12 h dark) with food and water freely available. Food consumption was recorded and animals were weighed weekly. The animals were cared for under the guidelines set forth by the Animal Care Committee of the Hebrew University, Jerusalem, Israel.

Experimental design

Twenty-one rats, age 6 weeks, were divided into 3 groups such that the average weight was similar in each group. Animals were fed either a control diet (10 % sucrose, 53 % starch, 7 % soybean oil), high fat (10 % sucrose, 35 % starch, 25 % soybean oil) or high fructose diet (10 % sucrose, 53 % fructose, 7 % soybean oil) for a 3 month period. A glucose tolerance test was performed in week three and week nine of the experiment. At the termination of the experiment, fasting serum glucose, insulin, triacylglycerols and cholesterol levels were measured. Glucose incorporation into glycogen was assessed in the soleus muscle in the presence and absence of insulin. Glycogen synthase activity (EC 2.4.1.11) was determined in soleus muscles that were incubated with glucose, or glucose and insulin. Heart glycogen content was also measured.

Biochemical variables

Glucose was determined by a glucose oxidase method using a glucose analyzer (Beckman Bun 2).

Insulin was assayed in serum samples using a standard radioimmunoassay kit (DPC, Los Angeles, Ca.).

Cholesterol and triacylglycerol levels were determined using enzymatic assays available in commercial kits (Raichem, San Diego, Ca.).

Glucose tolerance tests were carried out using whole blood from rat tail tips and a hand held glucometer (Elite, Bayer Diagnostics, Germany). Fasting glucose levels were measured at time zero (0 min) and animals were then intubated with 300 mg glucose/100 g body weight. Additional blood samples were taken at 30, 60 and 120 min following the glucose load.

Glucose incorporation into glycogen was determined in soleus muscles using the method described by Katz & Westerblad [15] with slight modifications. A preincubation of 30 min in 2 mM pyruvate in Krebs-Henseleit buffer was followed by 60 min incubation in medium containing glucose (5 mM) with or without insulin (120 pM) and [^{14}C-U]glucose (18.5 kBq/mL). Samples were kept under a constant stream of 95 % O_2: 5 % CO_2. Glycogen was isolated from muscle samples using the method of Rigden et al. [16], and radioactivity was measured.

Glycogen synthase activity in the soleus muscles was determined following incubation with or without insulin as described above for glucose incorporation into glycogen. The only variation being that no radioactive glucose was added to the incubation medium. Following incubation and freezing, samples were homogenized to determine (GS) activity using the methods of Thomas *et al.* [17] and Madar [18]. GS activity was expressed as nmole UDP glucose · mg protein^{-1} · min^{-1}. Results were also reported as GS_I/GS_{Total}.

Glycogen content of heart muscle was assayed using a modification of the method described by Rigden et al. [16]. Heart muscle tissue was boiled in 7.7 mM KOH, precipitated in 3 volumes ethanol + Na_2SO_4 and centrifuged. The precipitate was washed and then suspended in water and incubated with amyloglucosidase, glucose oxidase, peroxidase and 0-dianisidine dihydrochloride; glucose content was determined spectrophotometrically at 450 nm. Recovery of pure glycogen using this method was ~90 %.

Statistical analysis

Data are expressed as mean ± SE. Differences between means were determined by analysis of variance (ANOVA),

and two-way ANOVA was used in multivariable analyses. Differences were considered significant at probability levels of $p \leq 0.05$ using the Fisher's protected least significant difference method.

Results

Rats fed the high fat diet had a significantly lower daily food intake than control or fructose-fed animals, although weight gain in all three groups was similar throughout the experiment (Table 1). Fig. 1 shows the results of glucose tolerance tests carried out in weeks 3 and 9 of the experiment. Figure 1 A, at 3 weeks, shows no differences in fasting glucose levels among treatments. At 30 min, the animals fed the high fructose diet, and to a lesser extent the animals fed the high fat diet, showed an increase in blood glucose levels above control animals, but the trend was not significant. A second glucose tolerance test carried out in week 9 of the experiment (Fig. 1 B) showed similar curves among the experimental groups. There was no indication that the high fat or high fructose diets led to impaired glucose tolerance. Fasting glucose levels were similar in all 3 groups. In contrast to the earlier test at 3 weeks, at 30 min, control rats had the highest glucose levels.

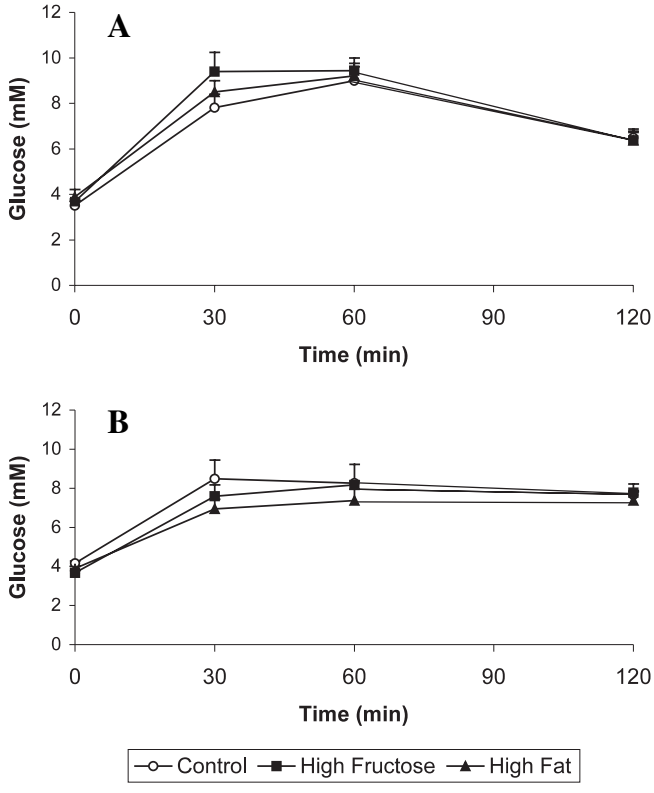

Fig. 1 *A* Glucose tolerance curves following an overnight fast in rats after three weeks on control, high fructose or high fat diets. *B* Glucose tolerance curves in the same rats after nine weeks on control, high fructose or high fat diets. Results are expressed as mean ± SE.

Table 1 Weight gain, blood parameters and heart glycogen content in rats fed a control, high fructose or high fat diet for 3 months

	Control	High Fructose	High Fat
Initial weight (g)	100±2	99±2	100±3
Final weight (g)	396±12	372±11	371±17
Glucose (mmol/L)	8.05±0.22	8.12±0.39	7.27±0.33
Insulin (pmol/L)	171±21	164±16	181±30
Cholesterol (mmol/L)	2.32±0.14	2.21±0.12	2.10±0.15
Triacylglycerols (mmol/L)	0.81±0.11	1.03±0.18	0.80±0.06
Heart glycogen (mg/g tissue)	2.57±0.37[ab]	3.06±0.49[a]	1.98±0.32[b]

Data is mean ± SE of 7 rats per group

[ab] numbers in same row with different superscripts are significantly different ($P<0.05$)

Other parameters measured show little evidence of impaired glucose tolerance or insulin resistance. At the termination of the experiment, after 12 weeks on the specified diets, fasting glucose levels were similar for all 3 groups (Table 2). In this chronic study, no differences in fasting insulin levels were observed. Levels were 171±21, 164±16 and 181±30 pmol/L for control, high fructose and the high fat fed animals, respectively. Total cholesterol levels were also unchanged following dietary treatment (Table 2). Serum triacylglycerols levels tended to be higher in the fructose fed animals, but significant differences were not reached.

Table 2 Total glucose incorporation into glycogen (nmole glucose·g tissue^{-1} · min^{-1}), total glycogen synthesis activity (nmole UDP glucose·mg protein^{-1} · min^{-1}) and glycogen synthase fractional activity (GS_I/GS_{Total}) in rat soleus muscles in the presence or absence of insulin. Rats were fed control, high fructose or high fat diets for 12 weeks. Results are expressed as mean ± SE

	Control		High Fructose		High Fat	
	–insulin	+insulin	–insulin	+insulin	–insulin	+insulin
Glycogen synthesis	3.75±0.42	8.97±0.92	4.10±0.63	11.80±0.87	4.25±0.57	11.09±1.0
Total GS activity	1.88±0.07	1.96±0.07	2.08±0.15	1.78±0.16	1.62±0.13	1.98±0.13
GS_I/GS_{Total}	0.15±0.04	0.17±0.04	0.17±0.04	0.23±0.04	0.22±0.05	0.24±0.04

Total glycogen levels in heart muscle were determined (Table 2). Although a significant difference was observed between the high fat and the high fructose diets, neither diet was different from the control.

Glycogen synthesis was determined using two different methodologies: glucose incorporation into glycogen and GS activity. To assess the basal rate of glucose incorporation into glycogen, whole soleus muscles were incubated without added insulin (Table 2). No difference in basal glycogen synthesis between the three groups was measured. The contralateral soleus muscle of each rat was used to assess glycogen synthesis in the presence of insulin. All three groups had similar glucose incorporation into glycogen in the presence of insulin. Overall, a 1.5- to 1.7-fold increase in glucose incorporation to glycogen was observed.

Glycogen synthase activity was measured in muscle homogenates previously incubated with or without added insulin and results are shown in Table 2. No significant inhibition of total enzyme activity was observed in rats fed high fat or high fructose diets and GS fractional activity (GS_I/GS_{Total}) was also similar. In muscles incubated with insulin, a small non-significant increase in GS fractional activity was observed for all three treatments. Overall, no differences were measured in GS enzyme activity following the dietary intervention.

Discussion

This study examined the effectiveness of a diet-induced model of insulin resistance in rats in an attempt to establish a reliable nutritional model for evaluating the efficacy of various nutraceuticals in preventing or delaying disease onset. In contrast to expected outcomes based on previous studies reported in the literature, none of the common signs of insulin resistance or glucose intolerance were observed in rats fed high fat or high fructose diets.

Elevated fasting glucose levels, a common sign of glucose intolerance, were not observed at any of the time points measured throughout the 12 week experiment. This result was not unexpected as numerous studies using high fat or high fructose diets report glucose intolerance, with normal fasting glucose levels [5, 8, 20, 21]. Han et al. [3] fed Wistar rats 50% fat diets for 32 weeks and observed increased weight gain after 10 weeks and elevated glucose levels from 16 weeks onward. Fasting insulin levels were significantly increased (1- to 2-fold) from 4 weeks on the high fat diet. In contrast to the findings of Han et al. [3], significant differences in animal weight, fasting insulin levels and glucose levels were not found in this study. The inability of high fat or high fructose diets to modify fasting insulin levels has also been reported by others [5, 8, 21].

Abnormal glucose tolerance curves following a glucose load is one of the earliest diagnostic tests to determine glucose intolerance. Using this tool, no evidence of significant impairment in glucose uptake or utilization was observed in this experiment.

Glycogen synthesis was evaluated in soleus muscles by two methodologies and differences among experimental groups were not found. It had been hypothesized that if rats became insulin resistant, with impaired glucose transport, glycogen synthesis would be inhibited, and a blunted response to insulin stimulation would be observed. The results of glucose incorporation into glycogen showed no differences in basal rate of glycogen synthesis in muscle samples incubated without added insulin. When insulin was added, all three groups responded with a 1.5- to 1.7-fold increase in glucose incorporation into glycogen. Rats on the high fat or high fructose diets showed no evidence of insulin resistance. When glycogen synthase activity was directly measured in muscle homogenates, overall, GS activity was similar in all three groups. No evidence of impaired or decreased enzyme activity was observed. When muscles were exposed to insulin *in vitro*, the control animals and animals fed the high fat diet exhibited a small increase in GS_I, but differences were not significant. For total GS activity, no significant differences were measured.

Although glucose incorporation into glycogen was increased 1.5-fold in response to insulin, direct measurement of GS activity in muscles pre incubated with insulin did not reflect this level of stimulation in either control or experimental animals. Differences in methodologies may explain these inconsistencies. Overall, glycogen synthesis did not appear to be significantly modified in rats fed the high fructose or high fat diet in this chronic feeding model. Heart glycogen stores also did not reflect significant dietary induced changes in glycogen metabolism when compared to control animals.

Several explanations may account for the inability of high fat or high fructose diets to induce insulin resistance and glucose intolerance in this experiment. It appears that individual rat strains fed a diabetogenic diet have different propensities to develop features analogus to type 2 diabetes mellitus. Although work has been done in Sprague Dawley rats, this is an outbred strain, and genetic variability is great among colonies throughout the world. It is possible that the rats used here were resistant to diet-induced glucose impairment and represent a non-responsive colony of rats. Gayles et al. [22], working in Wistar rats fed high fat diets, report a wide range of susceptibility to diet-induced obesity with many animals exhibiting resistance.

Glucose uptake in muscle tissue was not determined in this study, although it has been reported that high fat diets decrease insulin stimulated glucose uptake by 35–45% in mice [23]. It is possible that regulation at the cell membrane level may occur, which is not perceptible using the glucose tolerance test. The time of exposure to the diet may also be important. In previous studies carried out in our laboratory, using Sprague Dawley rats from the same colony, signs of insulin resistance were observed in rats weaned directly onto experimental diets. Han et al. [3] also used

newly weaned rats in their experiments as did Zierath et al. [23]. In addition, the length of the experiment may be critical. It is possible that if the experiment had been terminated earlier, results would be different than those found in chronic feeding for 12 weeks reported here. It was thought that chronic feeding would exacerbate the pre-diabetic syndrome, thus 12 weeks would be preferable over a shorter experimental period. However, these results indicate that healthy animals were able to adapt to the diets without developing metabolic disorders. In a recent study by Pagliassotti et al. [24], rats were fed high-fat diets starting at 5 weeks of age up to 58 weeks of age. Although impaired glucose uptake was found to be diet and age dependent, diet alone had no significant effect on GS activity. Higgins et al. [25] found no impairment of whole body glucose disposal in rats fed high-fat diets for 32 weeks, although tissue specific glucose metabolism changed dramatically.

Diet composition may also play a critical role in inducing metabolic changes. Fat content of the diets used here may not have been "extreme" enough to induce insulin resistance. Many laboratories have used greater than 25 % fat in the diet (w/w), commonly of the highly saturated varieties [3, 20, 21]. Many of the studies sited in the literature only see definitive signs of insulin resistance when using the insulin clamp technique [5, 8, 21]. In general, sub-clinical glucose intolerance may be difficult to detect, and only with the aid of an insulin clamp can small changes in glucose metabolism be detected. Although this may be the case, the use of the insulin clamp technique is not a practical solution for studying larger numbers of animals.

In conclusion, Sprague Dawley rats fed a high fat or a high fructose diet for 3 months may not be a suitable model for investigating insulin resistance and impaired glucose tolerance. The findings reported here illustrate the problems of developing models for the investigation of human diseases. There remains a distinct need for establishing appropriate, reproducible animal models for nutritional research that closely resemble metabolic imbalances in humans.

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