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

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 [14C-U]glucose (18.5 kBq/mL). Samples were kept under a constant stream of 95% O2: 5% CO2. 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/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 + Na2SO4 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),