Glucose homeostasis can be differentially modulated by varying individual components of a western diet[☆]

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Abstract

Chronic overconsumption of a Western diet has been identified as a major risk factor for diabetes, yet precisely how each individual component contributes to defects in glucose homeostasis independent of consumption of other macronutrients remains unclear. Eight-week-old male Sprague Dawley rats were randomized to feeding with one of six semi-pure diets: control, processed (high advanced glycation end products/AGE), high protein, high dextrose (glucose polymer), high in saturated fat (plant origin), or high in saturated fat (animal origin). After chronic feeding for 24 weeks, body composition was determined by bioelectrical impedance spectroscopy and glucose homeostasis was assessed. When compared to the control and high AGE diets, excess consumption of the diet high in saturated fat (animal source) increased body weight and adiposity, and decreased insulin sensitivity, as defined by HOMA IR, impaired skeletal muscle insulin signaling and insulin hypersecretion in the context of increased circulating glucagon-like peptide (GLP-1). Compared to the control diet, chronic consumption of the high AGE, protein or dextrose diet increased fasting plasma glucose, decreased fasting plasma insulin and insulin secretion. These diets also reduced circulating GLP-1 concentrations. These data suggest that individual components of a western diet have differential effects in modulating glucose homeostasis and adiposity. These data provide clear evidence of a link between over-consumption of a western diet and the development of diabetes.
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1. Introduction

Diabetes prevalence, now estimated as 171 million people worldwide, is expected to double within the next 20 years [1]. Diabetes is characterized by both hyperglycemia and a relative deficiency in insulin secretion, which precede the development of overt disease. In type 2 diabetes this is seen in the context of reduced insulin sensitivity, whereas in type 1 diabetes, autoimmune destruction of the pancreatic beta cells leads to absolute insulin deficiency. Reducing the global burden of diabetes is a high priority for the World Health Organization [1].

The global increase in diabetes has arisen in parallel with the increasing popularity of Western-style diets, so that it has been argued that dietary factors and diabetes are closely associated [2–5]. The adverse effects of the Western diet are most often attributed to its high energy density and poor nutrient profile with large amounts of saturated and *trans* fatty acids and poor quality carbohydrate. Yet other adverse features that derive from modern methods of food processing need to be considered. One of these is the generation of advanced glycation end products (AGEs) [6], formed through the non-enzymatic irreversible modification of free amino groups within proteins and amino acids by reducing sugars and reactive aldehydes, which can add functional properties such as increased shelf-life and taste of manufactured foods [8]. Once ingested, it is postulated that 10 to 30% of dietary AGEs are absorbed into the circulation [7,8], where they can form deleterious cross-linkages with many body tissues before excretion into the urine via the kidneys [8]. Some AGEs can also

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arise endogenously under physiologic conditions within tissues particularly in individuals with diabetes [9].

Recent studies in rodent models indicate that the restriction of dietary AGE intake not only improves insulin sensitivity, but can also extend lifespan [10,11]. Moreover, other rodent studies suggest an association between AGEs and type 1 diabetes [12–14]. Overall, there remains a need to distinguish the specific effects of dietary AGEs *per se* from events arising from other dietary excesses within the western diet, particularly in relation to adiposity, insulin sensitivity and pancreatic function. We are also yet to understand if chronic restriction of a single dietary component can prevent the development of abnormalities in insulin secretion and signaling. Within this study, undertaken in healthy rats, comparisons have therefore been made among groups chronically fed a highly processed diet (high in AGEs) with unprocessed diets high in either saturated fatty acids, dietary protein or refined carbohydrates.

2. Methods and Materials

2.1. Rodents

All animal experiments were performed in accordance with guidelines from the Alfred Medical Research and Education Precinct Animal Ethics Committee. Healthy male 8-week-old Sprague Dawley rats, weighing 250 to 300 g, were randomized into groups ($n=10$/group) and given one of the following diets: a control (C) diet (unbaked AIN93G [15]); a baked diet high in AGEs (AGE diet) (AIN93G processed at 160°C for 1 h); a high protein (Pr) diet with 48% of total energy (%E) as protein; a high glucose (Glu) diet (with 636 g dextrose/kg); a high saturated fat diet of plant origin (Pla Fat) (40%E from hydrogenated coconut oil) or a high saturated fat diet of animal origin (Ani Fat) (40%E from clarified butter, ghee) and followed for a period of 24 weeks.

All diets used were AIN-93G with one modification only. AIN-93G is a semi-pure formulation manufactured by Specialty Feeds (Western Australia, Australia). Unlike the high AGE diet, the control, high protein, high dextrose, and high fat diets were not processed (i.e., were kept raw) and all diets were not dehydrated nor formed into solid pellets. The thiamine content of the control and high AGE diets was determined by Lactobacillus fermentum (Difco, Detroit, MI, USA) performed by PathWest Laboratory Medicine, Western Australia, Australia. The AGE content of the rodent chow was determined by an in-house ELISA to carboxymethyllysine (CML) previously described [16]. Prior to analysis, food samples were homogenized and dissolved in phosphate buffered saline and the supernatants used in the ELISA.

Rats were housed in groups of three per cage with a 12 h light/dark cycle and ad libitum access to food and water. At week 0, 12 and 23 weeks after feeding, rats were placed individually in metabolic cages (Tecniplast, VA, Italy) for 24 hours to collect urine samples and to accurately measure water and food intake. At week 24 of the study, rats were anaesthetized with pentobarbitone sodium (50 mg/kg body weight) and perfused via the abdominal aorta with 0.1 mol/L phosphate-buffered saline for 1–2 min to remove circulating blood. The liver, gastrocnemius skeletal muscle, visceral fat pads (all adipose tissue within the abdominal cavity with the exception of those depots specifically associated with reproductive organs) and pancreas were removed, snap frozen in liquid nitrogen and stored at −80°C. Glycated hemoglobin was determined by HPLC as previously described [17].

2.2. Bioelectrical impedance spectroscopy (BIS)

At 23 weeks and after feeding, bioelectrical impedance spectroscopy was performed in rats ($n=6$/group) anaesthetized with 2.5% isoflurane in 1.75 L/min of oxygen delivered via nose cone. The animals were kept idle during BIS and placed supine. Subcutaneously placed stainless steel needles (gauge 25) were used as electrodes at the midline. The 2 source electrodes (S1 and S2) and 2 detecting electrodes (D1 and D2) were placed as follows: S1, back of the head; D1, upper margin of the clavicula; D2, upper margin of the pelvic bone; S2, at the base of the tail. Both sets of electrodes were coupled to an Ipedimed SF BIS 256 (ImpSFB7, Impedimed, Brisbane, Australia) and frequencies from 4 to 1000 kHz recorded as three readings over 20 seconds. The average of these three reading was used to provide estimates of fat mass, total body water and fat free mass as previously described [18,19].

2.3. Intravenous glucose tolerance testing (IVGTT)

After 24 weeks of feeding, intravenous glucose tolerance testing was performed [20]. In brief, rats ($n=6$/group) were anaesthetized and the left carotid artery cannulated. After equilibration and a bolus glucose injection of 1 g/kg, 0.5 ml blood samples were taken at 2, 5, 10, 15, 30 and 45 min for the measurement of plasma glucose (glucose oxidase method using an autoanalyser, Beckman Coulter LX20PRO) and plasma insulin by radioimmunoassay (Rat Sensitive RIA, Linco Research, MO, USA). Whole blood was reconstituted in saline and returned to the rats after plasma was

extracted. Area under the curve (AUC) was calculated by the trapezoidal rule (GraphPad Prism, GraphPad Software, San Diego, CA, USA).

2.4. Intra-peritoneal insulin tolerance testing (ipITT)

ipITT was performed after 23 weeks of feeding. After a fasting blood sample was collected, a 0.5 U/kg insulin bolus (Humalog, Insulin Lispro, Eli Lilly, USA) was injected intra-peritoneally into rats and blood samples were taken at 15, 30, 60 and 120 min post-bolus. Plasma glucose was measured as described above.

2.5. Homeostatic model assessment of insulin resistance (HOMA-IR)

HOMA-IR was calculated to determine the relative insulin sensitivity [21] using the formula (insulin (μU/ml)×glucose (mmol/L)) divided by 22.5.

2.6. pAKT/AKT immunoblotting

Western immuno-blotting was used to determine the ratio of phosphorylated Akt (pAkt) to total Akt as a marker of insulin signaling in both liver and skeletal muscle. Thirty μg of homogenized protein (liver or gastrocnemius skeletal muscle) was reduced with 2% β-mercaptoethanol and proteins were separated by polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Gladesville, Australia). Separated protein bands were transferred onto a Hybond-P PVDF membrane (Millipore, Maryland, USA) using a semi-dry blotting apparatus (Bio-Rad Laboratories, Gladesville, Australia). After transfer, membranes were blocked with 5% skim milk powder diluted in a 1 M Tris-buffered saline solution with 0.05% Tween-20 (TBS-T) for 1 h. After blocking, membranes were washed in 1 M TBS-T solution for 10 min before incubating overnight with either Akt or pAkt primary antibodies (rabbit anti-rat S473, Cell Signaling Technologies, MA, USA, Akt antibody at a dilution of 1/10,000 and pAkt antibody 1/5000). Akt and pAkt membranes were washed six times in 1M TBS-T solution before incubating for 1 h at room temperature with an anti rabbit, HRP-labeled polymer secondary antibody (Dako, California, USA). Membranes were probed with Chemiluminescent Peroxidase Substrate-3 (Sigma-Aldrich, St. Louis, USA) for 3 min. Light emission was captured on CL-XPosure film (Thermo Scientific, Rockford, IL, USA). The density of each band was quantitated using Adobe Photoshop. Results were expressed as a ratio of pAkt to Akt.

2.7. Plasma GLP-1 and glucagon

Plasma GLP-1 and glucagon were determined using ELISA kits from Wako (Osaka, Japan) in plasma samples taken after a 6 hour fast. All blood samples were obtained at the same time of the day.

2.8. Statistical analysis

All statistical computations were performed using GraphPad Prism version 4.0a for Mac OS X (GraphPad Software, San Diego, California, USA). Values for experimental groups are given as mean, with bars showing the SEM, unless otherwise stated. One-way ANOVA with Tukey's post-test analysis, was used to determine statistical significance. Where appropriate, two-tailed t tests were performed. A probability of $P<0.05$ was considered to be statistically significant.

3. Results

To determine the effects of excess consumption of macronutrients and heat treated foodstuffs on glucose homeostasis and pancreatic function, healthy Sprague Dawley rats were fed one of the following diets for 24 weeks: a control (unbaked; C) diet, a high AGE diet (AGE), a high protein (Pr) diet, a high dextrose (Glu) diet, or a high fat diet in saturated fat from either a plant (hydrogenated coconut oil; Pla Fat) or animal fat (clarified butter; Ani Fat) source. The nutrient and energy content of each diet are presented in Table 1. All diets were isoenergetic but differed in specific macronutrients.

The heat labile vitamin thiamine (B1) was measured in the control and high AGE diets to determine any effect of heat damage. The thiamine content of the control diet was 2.61 μg/g compared to 3.51 μg/g of thiamine in the high AGE (baked) diet.

The high AGE diet had a five fold increase in AGE content above all other diets (High AGE — 101.9 versus control — 20.9 nmol/mol lysine/100 mg), as determined by an ELISA specific to the AGE CML [16] with the exception of the high dextrose diet which was 3 fold higher in AGE content than the control diet. CML was chosen as a surrogate marker of AGEs because it is present in tissues and serum from humans and