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Research article

Metabolic, stress, and inflammatory biomarker responses to glucose administration in Fischer-344 rats: intraperitoneal vs. oral delivery

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ABSTRACT

Introduction: Metabolic effects of anthropogenic chemicals are a focus of environmental health research due to the significant public health implications. Conventional glucose tolerance tests (GTTs) do not generally examine multiple metabolic, inflammatory, and endocrine factors; however, responses to exogenous glucose can provide insight into mode-of-action and disease processes, and warrant consideration in developing models for toxicological assessment.

Methods: GTTs were conducted on male Fischer-344 rats to 1) assess the feasibility of measuring multiple analytes in small sample volumes; 2) monitor analyte response; and 3) determine whether route of glucose delivery (oral, OGTT vs. intraperitoneal, IPGTT, 2 g/kg) modified responses. Plasma samples (0, 30, 60, 90, 120 min post-glucose administration) were analyzed for triglycerides; hormones involved in glucose regulation (insulin, glucagon, glucagon-like peptide (GLP)-1)), energy homeostasis (ghrelin, leptin), and stress response (corticosterone); cytokines (TNF, IL-6); and markers of endothelial dysfunction (VEGF, PAI-1).

Results: Glucose peaked at 30 min during the IPGTT but not the OGTT ($p < 0.001$), a trend paralleled by insulin, while triglycerides decreased following the IPGTT (transient) and the OGTT (sustained). GLP-1 was transiently decreased while ghrelin and leptin levels increased progressively during the IPGTT alone. Corticosterone was increased during both the IPGTT (sustained) and OGTT (transient). TNF and VEGF were unchanged, while PAI-1 and IL-6 were not detected. Increasing the oral glucose dose to 3 g/kg did not significantly alter profiles.

Discussion: Results confirm the feasibility of measuring multiple analytes during a GTT, and indicate that administration of glucose can impact metabolic and endocrine profiles in a route-dependent manner.

1. Introduction

Metabolic impacts of environmental chemicals are an important current focus of environmental health research due to the significant public health implications, particularly in light of global trends towards increased obesity and type 2 diabetes (Chen, Magliano, & Zimmet, 2011; Ng et al., 2014). In addition to obesity and diabetes, chemicals in the environment have been linked with measures of disrupted glucose homeostasis, including impaired glucose tolerance, metabolic syndrome, and insulin resistance (Heindel et al., 2016; Russ & Howard, 2016). While experimental studies have demonstrated that environmental chemicals can produce such effects, there remains a need to develop appropriate models to investigate mechanisms underlying these associations and identify chemicals that may be contributing to metabolic dysfunction (Lind et al., 2016; Neel & Sargis, 2011).

Metabolic homeostasis is tightly linked to innate immune and stress responses, such that dysregulation of one will tend to produce changes in the others that collectively contribute to disease pathogenesis (Duncan et al., 2003; Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014; Vegiopoulos & Herzig, 2007; Wellen & Hotamisligil, 2005). Substantiating this notion, pharmacological intervention targeting postprandial hyperglycemia is associated with improvements in inflammation, endothelial dysfunction, and oxidative stress pathways (Giugliano, Ceriello, & Esposito, 2008). Existing pathological states may also modify endocrine and inflammatory responses to glucose. For example, individuals with impaired glucose tolerance exhibit a cytokine response to glucose of greater magnitude and duration than that seen in individuals with normal glucose tolerance (Esposito et al., 2002). Higher triglyceride levels following glucose administration in individuals with signs of insulin resistance has been proposed as a potential early indicator of metabolic dysfunction (Vossen, Todter, Altenburg, Beisiegel, & Scheja, 2011). Similarly, plasma leptin was found to

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increase in response to glucose administration only in obese individuals (Corica et al., 2001). Given such relationships, approaches that enable simultaneous assessment of metabolic, inflammatory, and endocrine factors following administration of glucose may provide valuable insight into processes impacted by various risk factors, including environmental chemicals, relevant to disease pathogenesis.

The glucose tolerance test (GTT) is the most common method used to assess glucose homeostasis in humans and in experimental animals. After fasting, a single dose of glucose is administered and blood glucose levels are measured at regular intervals over a period of time to assess clearance from circulation. Measurement of insulin in blood samples collected at the time of glucose measurement can provide additional information on potential underlying causes for differences in glucose tolerance. In GTTs conducted on mice and rats, the limited sample volumes that can be collected restrict the number of assays that can be conducted (Bowe et al., 2014), and other factors implicated in glucose metabolism and metabolic dysfunction are generally not measured. The development of highly sensitive immunoassays and multiplexing capabilities opens the possibility of assessing multiple analytes despite the sample volume limitations. In the present study we assessed the feasibility of measuring a panel of metabolic, inflammatory, and endocrine markers during a GTT using the Fischer rat model commonly used in toxicological studies. Furthermore, given known impacts of route of glucose administration on metabolic endpoints (e.g. through the incretin effect), we compared oral (OGTT) vs. intraperitoneal (IPGTT) administration of glucose on these endpoints.

2. Methods

2.1. Animals

Specific pathogen-free male Fischer-344 rats (229 ± 15 g) were obtained from Charles River (St. Constant, Québec, Canada) and allowed to acclimatise to their surroundings for 1 wk. Animals were housed in individual plexiglass cages on wood-chip bedding under HEPA-filtered air and held to a 12 h dark/light cycle. Food and water were provided ad libitum. All experimental protocols were reviewed and approved by the Animal Care Committee of Health Canada.

2.2. Glucose tolerance tests and blood collection

Animals were fasted for 6 h prior to undergoing an OGTT or IPGTT. Rats were randomly assigned to groups according to glucose dose and route of administration: 2 g/kg body weight OGTT (n = 10), 3 g/kg body weight OGTT (n = 7), and 2 g/kg body weight IPGTT (n = 7), in line with glucose doses typically used in rodent studies (Bowe et al., 2014; Muniyappa, Lee, Chen, & Quon, 2008). Blood glucose was measured using a OneTouch®Verio™IQ glucometer (LifeScan Canada Ltd., Burnaby, British Columbia, Canada). A small blood sample (< 200 µl) was collected from the tail vein with a heparinized needle to obtain an intial (time 0) reading, and transferred to a BD microtainer with plasma separator (BD, Mississauga, Ontario, Canada, cat# B365985). Rats were subsequently administered glucose (50% dextrose, Vétoquinol N.-A.Inc., Lavaltrie, Québec, Canada) by gavage (OGTT) or intraperitoneal injection (IPGTT, 20% dextrose in sterile water). Glucose measurement and blood sampling was repeated at 30, 60, 90, and 120 min. Following centrifugation of blood samples, plasma was removed and aliquoted for subsequent analyses (Table 1) and frozen at − 80 °C.

2.3. Indices of insulin resistance, sensitivity and β-cell function

Surrogate indices of insulin resistance and β-cell function were calculated (Matthews et al., 1985) according to the following equations:

HOMA-IR = $G_0 \times I_0 / 22.5$ and HOMA %β = $(20 \times I_0) / (G_0–3.5)$.

where G_0 and I_0 are fasting glucose (mmol/L) and insulin (µIU/mL) values, respectively.

Table 1
Plasma volumes required for assays.

Assay	Volume (µL) required for duplicate assay reactions	Dilution (− fold)	Order of aliquoting
Insulin	10	–	1
Corticosterone	5	500	2
Triglycerides	20	–	3
Multiplex metabolic and cytokine factors	25	4*	4

* Dilutions were increased to up to 18-fold where required due to insufficient remaining plasma.

The Quantitative Insulin Sensitivity Check Index (QUICKI) (Katz et al., 2000) was calculated according to the equation:

QUICKI = $1 / (\log G_0 + \log I_0)$, where G_0 and I_0 are fasting glucose (mg/dL) and insulin (µIU/mL) values, respectively.

2.4. Plasma analyses

Triglycerides were analyzed using a colorimetric assay kit (Cayman Chemical Company, Ann Arbor, Michigan USA). Insulin was analyzed using an ultra-sensitive rat enzyme-linked immunosorbent assay (ELISA) kit (Crystal Chem Inc., Downers Grove, IL, USA). Corticosterone was analyzed using the DetectX ELISA kit (Arbor Assays, Ann Arbor, Michigan, USA). Cytokine and metabolic hormone levels were analyzed by multiplexing 5-plex diabetes (glucagon, glucagon-like peptide (GLP)-1, ghrelin, leptin, plasminogen activator inhibitor (PAI)-1) and 3-plex cytokine (tumour necrosis factor (TNF), interleukin (IL)-6, vascular endothelial growth factor (VEGF)) panels (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada). For multiplex analyses, plasma was diluted up to 18-fold as needed to compensate for insufficient volume. Samples run at such dilutions were excluded if results fell within background levels. All analyses were conducted in duplicate.

2.5. Statistical analyses

Two-way repeated measures ANOVA was conducted to determine the impact of the glucose administration regime (*Route*; 2 g/kg OGTT, 3 g/kg OGTT, 2 g/kg IPGTT) and time (*Time*; 0, 30, 60, 90, 120) on endpoints. A one-way ANOVA was conducted for comparison of area under the curve (AUC) across groups. Post-hoc Holm-Sidak pairwise comparisons were conducted as directed by ANOVA main effects or interactions; for significant *Route* effects, all three regimes were compared to each other, while for significant *Time* effects, pairwise comparisons were performed relative to time 0. Data were transformed to meet normality and equal variance assumptions as warranted. Statistical analyses were conducted using SigmaPlot 12.5 Systat Software, Inc., San Jose, California, USA.

3. Results

3.1. Baseline levels of glucose and insulin

As expected, average baseline glucose and insulin levels following a 6 h fast were similar in animals randomly assigned to different groups (2 g/kg body weight OGTT, 3 g/kg body weight OGTT, 2 g/kg body weight IPGTT; Supplemental Table 1). HOMA-IR, HOMAβ, and QUICKI indices confirmed that there were no significant differences across groups.

3.2. Glucose and insulin during GTT

Blood glucose was increased following the IPGTT but not the OGTT