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Failure of hyperglycemia and hyperinsulinemia to compensate for impaired metabolic response to an oral glucose load

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ABSTRACT

Objective: To evaluate whether the augmented insulin and glucose response to a glucose challenge is sufficient to compensate for defects in glucose utilization in obesity and type 2 diabetes, using a breath test measurement of integrated glucose metabolism.
Methods: Non-obese, obese normoglycemic and obese type 2 diabetic subjects were studied on 2 consecutive days. A 75 g oral glucose load spiked with ^{13}C-glucose was administered, measuring exhaled breath $^{13}CO_2$ as an integrated measure of glucose metabolism and oxidation. A hyperinsulinemic euglycemic clamp was performed, measuring whole body glucose disposal rate. Body composition was measured by DEXA. Multivariable analyses were performed to evaluate the determinants of the breath $^{13}CO_2$.
Results: Breath $^{13}CO_2$ was reduced in obese and type 2 diabetic subjects despite hyperglycemia and hyperinsulinemia. The primary determinants of breath response were lean mass, fat mass, fasting FFA concentrations, and OGTT glucose excursion. Multiple approaches to analysis showed that hyperglycemia and hyperinsulinemia were not sufficient to compensate for the defect in glucose metabolism in obesity and diabetes.
Conclusions: Augmented insulin and glucose responses during an OGTT are not sufficient to overcome the underlying defects in glucose metabolism in obesity and diabetes.

1. Introduction

Impaired carbohydrate oxidation is a well-recognized feature of obesity and diabetes. Investigations to date have implicated defects in multiple stages of glucose handling and metabolism, including the pancreatic response to ingestion (Del Prato & Marchetti, 2004), glucose absorption (Nielsen et al., 2000), glucose and insulin delivery to peripheral tissues (Baron & Clark, 1997; Hallsten et al., 2003), tissue insulin receptor signaling responses (Caro et al., 1987; Draznin, 2006; Petersen & Shulman, 2006), and transmembrane transport and intracellular glucose trapping by phosphorylation (Petersen & Shulman, 2006; Williams et al., 2003) following insulin stimulation (insulin-mediated glucose uptake) or driven by mass action (glucose-mediated glucose uptake) (Ahren & Pacini, 2002; Viviani & Pacini, 1999; Williams, Price, & Kelley, 2001). Most recently impaired mitochondrial function (Coletta & Mandarino, 2011; Goodpaster, 2013; Parish & Petersen, 2005) has been added to this list.

The relative importance of these phenomena in impaired net carbohydrate oxidation in obesity and type 2 diabetes remains unclear. One argument has been that net tissue glucose uptake, and oxidation are normalized by compensatory hyperinsulinemia and/or hyperglycemia, an idea that has found some experimental support (Egan & Stepniakowski, 1994; Natalucci, Boemi, Fumelli, Fumelli, & Burattini, 2002). However, other studies suggested that neither postprandial hyperinsulinemia nor postprandial hyperglycemia were sufficient to overcome the net metabolic defects in obesity and type 2 DM (Klauser, Prager, Schernthaner, & Olefsky, 1991; Mandarino, Consoli, Jain, & Kelley, 1996; Prager, Wallace, & Olefsky, 1987). Our group demonstrated using limb balance methods in humans that the net glucose delivery to skeletal muscle under steady-state conditions did not differ across insulin resistance groupings, suggesting that hyperinsulinemia was sufficient to compensate for this aspect of glucose handling (Mather, Laakso, Edelman, Hook, & Baron, 2000). In a recent paper, Galgani and Ravussin (Galgani & Ravussin, 2012) studied integrated whole-body glucose metabolism, measuring total carbohydrate oxidation by indirect calorimetry and production of labeled water following ingestion of labeled glucose as part of a traditional 75 gram glucose load. Nondiabetic subjects were studied, divided by clamp-derived insulin sensitivity into insulin resistant and insulin sensitive groups.

Conflict of interest: MJ and SS are employees of BioChemAnalysis Corp, which was the recipient of the NIH SBIR grant that supported these studies. Processes of data collection and data analysis were independent of this company. The remaining authors have no conflicts to declare.

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There was no difference in rates of net glucose oxidation following glucose ingestion between these groups. There was however a lower glucose oxidation rate in insulin resistant subjects when adjusted for their greater glucose exposure following glucose ingestion. These results suggest that the modest hyperglycemic response was compensatory in this nondiabetic group.

We have recently utilized exhaled labeled CO_2 following ingestion of labeled glucose as an index of integrated carbohydrate handling (Dillon et al., 2009; Hussain et al., 2014; Singal, Janghorbani, Schuette, Chisholm, & Mather, 2010). This method has been found to distinguish between lean, obese and obese/type 2 diabetic subjects, showing stepped decrements in areas under the curve for net glucose utilization (Dillon et al., 2009; Singal et al., 2010). Analogous to the Galgani method, by measuring a post-metabolism product of ingested glucose the breath method integrates all of the components of tissue and cellular glucose handling. This method is clearly dependent on the glucose excursion in response to ingestion, and in fact can serve as a surrogate measure of this phenomenon (Singal et al., 2010); we have also recently shown that it is inversely correlated to fasting indices of insulin resistance and to clamp-derived measures of insulin resistance (Hussain et al., 2014). In contrast to effects observed using the deuterium labeling method of Galgani, our method demonstrates early and continuing divergence of tracer appearance between subject groups (arguing that this is not simply an effect of delayed glucose metabolism) (Dillon et al., 2009). For current purposes the breath method offers a new opportunity to evaluate the question whether hyperinsulinemia and/or hyperglycemia following an oral challenge is sufficient to normalize tissue glucose metabolism.

2. Subjects, materials and methods

We performed secondary analyses of data from a recently published study (Hussain et al., 2014). Full details of the methodology and participant characteristics are previously published (Hussain et al., 2014); for convenience we provide a brief summary here. Participants were men and women, 18–65 years of age. Participants were categorized as non-obese (BMI <30 kg/m^2), or obese (BMI ≥ 30 kg/m^2). Diabetes mellitus was determined by prior physician diagnosis or defined at screening using a 2-hour 75-g oral glucose tolerance test (OGTT) under fasting conditions, applying the American Diabetes Association criteria of fasting glucose level >126 mg/dL or 2-h glucose level >200 mg/dL). Participants with diabetes could be treated with any combination of diet, exercise, insulin, or antidiabetes medications except thiazolidinediones or metformin. Volunteers were excluded if they had used thiazolidinediones within 6 months or metformin within 4 weeks. Stable antihypertensive or antihyperlipidemic medications were allowed. Volunteers were also excluded if not weight-stable for at least 6 months, if they had type 1 diabetes or rare variant forms of diabetes, were pregnant, had a concurrent acute or chronic medical illness likely to affect systemic fuel metabolism, used psychotropic medication including antidepressants, or had pulmonary disease or a history of current or past smoking.

Volunteers who qualified after this screening were scheduled for clinical research center admission on 2 consecutive days to undergo study measurements. Volunteers provided written informed consent for screening and main study participation. This project was overseen and approved by the Indiana University Institutional Review Board.

2.1. Measurements

The methodology has been previously published (Hussain et al., 2014); a brief summary is provided. Body composition was measured by dual energy x-ray absorptiometry at the time of screening evaluation. OGTT and clamp studies were done in the morning following an overnight fast; participants consumed their usual diet except where provided by the study while admitted to the clinical research center. Participants with diabetes continued to take their diabetes medications, except morning treatments were withheld on each of the 2 measurement days until after the completion of the studies.

Anthropomorphic and blood pressure measures were performed the morning of the first testing procedure. Beginning at 7:30 a.m., baseline blood samples were drawn through an indwelling intravenous catheter, followed by a standard 75-g OGTT containing an added 150 mg of [$^{13}C_6$] glucose (catalog number CLM-1396; Cambridge Isotope Laboratories, Andover, MA). Breath samples were obtained at half-hourly intervals during the following 3 h. Blood samples were obtained concurrent with these breath samples. Upon completion of the OGTT, subjects were fed lunch and dinner ad libitum. Fasting from 8 p.m. was again implemented in anticipation of the second measurement day.

A 4-hour hyperinsulinemic euglycemic clamp procedure was performed on the second day using established procedures in our laboratory (Mather et al., 2000), based on the original method of DeFronzo (DeFronzo, Tobin, & Andres, 1979). An insulin infusion rate of 120 mU/m^2/min was used to ensure full suppression of endogenous glucose production in all subject populations (Ferrannini & Mari, 1998; O'Shaughnessy et al., 1995).

2.2. Analytical procedures

Breath [^{13}C] glucose enrichment was measured using isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH). Screening laboratory measurements were performed by the Indiana University Health clinical laboratory using standard methodologies. Glucose measurements were performed at the bedside using a glucose oxidase method (YSI 2500 STAT glucose analyzer; Yellow Springs Instruments, Yellow Springs OH). Serum free fatty acids were measured using a colorimetric assay (Roche Diagnostics, Indianapolis, IN). Serum insulin levels were measured by radioimmunoassay (Millipore-Linco, St. Charles MO).

2.3. End points

Fasting and 2-hour glucose values and the area under the curve for glucose excursion above baseline to 180 min (glucose AUC180, calculated using the trapezoidal rule) were derived from OGTT glucose data. Similarly, breath measures of enrichment of exhaled breath for $^{13}CO_2$ were used to calculate breath AUC. In prior work we determined that the breath AUC180 value is the preferred single endpoint measure for this breath testing method (Dillon et al., 2009; Hussain et al., 2014; Singal et al., 2010). This value was used as the dependent variable for the current set of analyses.

The clamp glucose infusion rate-based calculation of glucose disposal rate (GDR) was adjusted for shifts in and out of the glucose space over each 20-min interval, with the average of values from the fourth hour of the clamp procedure taken as the GDR for each individual (Hussain et al., 2014). This measure was then expressed per Kg fat-free mass (GDRffm) for the current set of analyses. The oral disposition index (oDI) was calculated using the OGTT-derived insulinogenic index (insulin30-insulin0/glucose30-glucose0) multiplied by the clamp-derived GDRffm. Free fatty acids (FFA) were measured in blood samples from the OGTT and the glucose clamp procedure. From these measures we calculated the Adipose Insulin Resistance Index (Adipose-IRi $= FFA_0$ min OGTT $\times$ Fasting plasma insulin) (Lomonaco et al., 2012), and estimated adipose insulin sensitivity as the ability of insulin to suppress lipolysis (ΔFFA_{120}) $=$ free fatty acid $_{0min}$ $-$ free fatty acid $_{120min}$ using clamp data.

2.4. Statistical analysis

Due to technical or scheduling problems we were not able to obtain DEXA measures on all participants. For the purposes of the