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Comparative effects of acute hypoglycemia and hyperglycemia on pro-atherothrombotic biomarkers and endothelial function in non-diabetic humans

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ABSTRACT

Background: The comparative effects of acute moderate hyperglycemia and hypoglycemia on in vivo endothelial function together with pro-inflammatory and pro-atherothrombotic responses in healthy individuals have not been determined.

Methods: To investigate this question, 45 healthy subjects were compared during glucose clamp studies consisting of euinsulinemic hyperglycemia and hyperinsulinemic hyperglycemia (plasma glucose 11.1 mmol/L, both with pancreatic clamps) and hyperinsulinemic euglycemia and hyperinsulinemic hypoglycemia (plasma glucose 5.1 and 2.9 mmol/L, respectively). Two-dimensional Doppler ultrasound was used to determine brachial artery endothelial function.

Results: Insulin levels during euinsulinemia hyperglycemia were 194 ± 23 and (850 ± 49–988 ± 114) pmol/L during all hyperinsulinemic protocols. Responses of VCAM-1, ICAM-1, E-selectin, P-selectin, PAI-1, and IL-6 were increased ($p < 0.05$–0.0001) during euinsulinemic hyperglycemia or hypoglycemia as compared to hyperinsulinemic euglycemia or hyperinsulinemic hyperglycemia. PAI-1 was increased ($p < 0.04$) during hypoglycemia as compared to euinsulinemic hyperglycemia, and TNF-α responses were also increased during hypoglycemia as compared to hyperinsulinemic euglycemia or hyperinsulinemic hyperglycemia ($p < 0.05$). In vivo endothelial function was similarly blunted by acute moderate hyperglycemia or hypoglycemia.

Conclusion: In summary, acute moderate hypoglycemia and euinsulinemic hyperglycemia can result in similar endothelial dysfunction and pro-atherothrombotic responses. Fibrinolytic balance was reduced by a greater extent by hypoglycemia as compared to moderate hyperglycemia. Acutely, hyperinsulinemia can prevent the acute pro-atherothrombotic and pro-inflammatory effects of moderate hyperglycemia but not hypoglycemia.

1. Introduction

Recent data have suggested an association between severe hypoglycemia and increased serious cardiovascular morbidity and mortality (Desouza, Salazar, Cheong, Murgo, & Fonseca, 2003; Duckworth et al., 2009; Meigs et al., 2000; Ohkita, Takaoka, Shiota, Nojiri, & Matsumura, 2002). Moreover, several studies have demonstrated or reported that acute hyperglycemia (Aljada et al., 2004; Beckman, Creager, & Libby, 2002; Ceriello et al., 2013; Festa et al., 1999; Haubner et al., 2007) or hypoglycemia (Bedenis et al., 2014; Gogitidze Joy et al., 2010; Kvasnicka et al., 2000; Razavi Nematollahi et al., 2009; 52) can result in increased atherothrombotic mechanisms, reduced fibrinolytic balance, and impaired endothelial function in both non-diabetic and diabetic individuals (Beckman et al., 2002; Ceriello et al., 2002; Gogitidze Joy et al., 2010; Kvasnicka et al., 2000; Munshi et al., 2016).

Despite recent data reporting the acute effects of hyperglycemia and hypoglycemia on pro-atherothrombotic responses, only one study has compared their relative effects in humans (Ceriello et al., 2013). Ceriello et al., studying effects of GLP-1 in individuals with type 1 DM, suggested that both hyperglycemia and hypoglycemia could be considered equivalent pro-atherosclerotic risk factors (Ceriello et al., 2013). However, there are no data available directly comparing the acute pro-inflammatory and pro-atherothrombotic effects of hypoglycemia and hyperglycemia in non-diabetic individuals.

Therefore, in this present report, we have reanalyzed two of our recent articles (Kvasnicka et al., 2000; Rana et al., 2011) in order to compare the acute effects of moderate hyperglycemia (11.1 mmol/L) with and without hyperinsulinemia and moderate hypoglycemia (2.9 mmol/L) on pro-inflammatory, pro-atherothrombotic and pro-coagulant biomarker responses in healthy individuals. The pancreatic clamp technique and various glucose clamp methodologies

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(euglycemic, hyperglycemic and hypoglycemic) (Rana et al., 2011) were combined so that the acute independent effects of glycemia and hyperinsulinemia could be determined.

2. Methods

2.1. Subjects

45 adult volunteers (21 M/24 F, 38 ± 3 years, BMI 29 ± 2 kg/m^2 (23–36 kg/m^2), HbA1C 5.2 ± 0.2%) were studied. None of the participants smoked and received anticoagulants, clopidogrel, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or oral insulin sensitizers (metformin, thiazolidinediones). Each participant had normal blood count, plasma electrolytes, liver and renal function and no evidence of either impaired fasting glucose or overt diabetes mellitus. Study volunteers participated in four single-blind one-day protocols consisting of: 1) euinsulinemic/hyperglycemia (n = 15), 2) hyperinsulinemic euglycemia (n = 16), 3) hyperinsulinemic hyperglycemia (n = 14) and 4) hyperinsulinemic hypoglycemia (n = 16). Protocols 1 and 3 consisted of an initial pancreatic clamp (Rana et al., 2011) combined with a single-step 4-hour hyperglycemic (11.1 mmol/L) clamp with either euinsulinemia or hyperinsulinemia. Protocol 2 and 4 consisted of a one-step hyperinsulinemic euglycemic clamp (5 mmol/L) or hyperinsulinemic hypoglycemic clamp (2.9 mmol/L, both without pancreatic clamps). Data from this manuscript have been included in 2 separate reports (Kvasnicka et al., 2000; Rana et al., 2011) examining 1) the independent effects of insulin and hyperglycemia on pro-inflammatory responses (Rana et al., 2011) and 2) effects of antecedent hypoglycemia on pro-inflammatory and pro-atherothrombotic responses in healthy humans (Kvasnicka et al., 2000). All study participants gave written informed consent. Studies were approved by the Vanderbilt University and University of Maryland Human Subjects Institutional Review Board.

2.2. Experimental design

All individuals refrained from caffeine, exercise, and alcohol for 24 hours prior to the study. Participants were instructed to not use aspirin, NSAIDs or COX 2 inhibitors for three days prior to a study. Subjects were admitted to the General Clinical Research Center (GCRC) the night prior to the study at 5 pm. After an overnight 10 h fast, two intravenous cannulae were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand of the non-dominant arm. This hand was placed in a heated box (55–60 °C) during the study so that arterialized blood could be obtained (Abumrad, Rabin, Diamond, & Lacy, 1981). The other cannula was also placed in the non-dominant arm for infusions.

2.3. Pancreatic clamp and glucose clamp studies

At time −120 min, the somatostatin analogue, octreotide, was infused at 30 ng/kg/min to inhibit endogenous insulin, glucagon, and growth hormone secretion. Basal replacement amounts of human regular insulin (1.8 pmol/kg/min) (Lilly USA, Indianapolis, IN), human growth hormone (3 ng/kg/min) (Pharmacia & UpJohn, New York, NY) and a variable basal amount of glucagon (Boehringer Ingelheim, Ridgefield, CT) were infused during the pancreatic clamp to maintain euglycemia of approximately 5 mmol/L.

After stable euglycemia was established during the pancreatic clamp, four-hour single-step hyperglycemic clamps at differing insulinemia were performed (protocols 1 and 3). At time 0 min, the insulin infusion was either maintained at 1.8 pmol/kg/min (protocol 1) or increased to 9 pmol/kg/min (protocol 3) and continued until 240 min (Fig. 1). End of pancreatic clamp glucagon and growth hormone infusion rates were maintained unchanged during the 240-min glucose clamp procedures. Glucose targets of 5 mmol/L or 11.1 mmol/L were achieved using a modification of the glucose clamp technique (DeFronzo, Tobin, & Andres, 1979). Individuals participating in protocols 2 and 4 underwent a 2-h euglycemic or hypoglycemic clamp at an insulin infusion dose of 9 pmol/kg/min (Kvasnicka et al., 2000). Potassium chloride (20 mmol/L) was infused during hyperinsulinemic clamp studies to reduce insulin-induced hypokalemia. End of clamp blood samples were drawn at the same time of the day during all protocols.

2.4. Analytical methods

The collection and processing of blood samples have been described elsewhere (Cherrington, Lacy, & Chiasson, 1978). Plasma glucose concentrations were measured in triplicate every 5 min using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Insulin was measured as previously described (Wright et al., 2010) with an interassay CV of 9%. Catecholamines were determined by HPLC (Causon, Carruthers, & Rodnight, 1981) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. NEFA was measured using the WAKO kit adopted for use on a Packard Instrument (Meriden, CT). Glucagon was measured according to a modification of the method of Aguilar-Parada, et al. with an interassay coefficient of variation (CV) of 12% (Aguilar-Parada, Eisentraut, & Unger, 1969). Cortisol was assayed using the Clinical Assays Gamma Coat Radioimmunoassay (RIA) kit with an interassay CV of 6%. Growth hormone was determined by RIA (Hutton, Mikhailidis, Dormandy, & Ginsburg, 1979) with a CV of 8.6%.

All vascular adhesion molecules were assayed using LINCO Research Kits (St. Charles, MO) with an interassay CV of 8.5% (VCAM-1), CV of 9.7% (ICAM-1), CV of 13.4%, (E-selectin), CV of 9.02% (IL-6), and CV of 9.98% (TNF-α), respectively. PAI-1 and tissue plasminogen activator (TPA) were determined by TintElize® Platinum Kit with interassay CV of 3.3%. P-selectin was measured using the Meso Scale Discovery assay kit (Gaithersburg, MD) with a CV of 9.9%. All hormones, pro-inflammatory biomarkers and NEFA included in this report were measured using identical assay methodologies, equipment and personnel (Kvasnicka et al., 2000; Rana et al., 2011).

2.5. Endothelial function

Measurements of endothelial function were conducted at baseline and during the final 30 minutes of each glucose clamp as previously described (Kvasnicka et al., 2000; Rana et al., 2011). In brief, endothelial measurements of the dominant brachial artery were measured using 2D Doppler ultrasound during reactive hyperemia and exogenous nitroglycerin administration. Flow-mediated dilation was obtained by inflating the sphygmomanometric cuff around the proximal forearm. Brachial artery diameter measurements were taken at time points 30 seconds, 60 seconds, 90 seconds, 120 seconds and after cuff deflation. Then, after a 15–20-minute rest period, subjects received 0.4 mg sublingual nitroglycerin (as an exogenous nitric oxide donor). Additional scans were performed as above with vessel diameter measurements obtained at 1, 2, 3 and 4 minutes (Faulx, Wright, & Hoit, 2003). Endothelial function measurements were performed by JP and NJ (Kvasnicka et al., 2000; Rana et al., 2011). The coefficient of variation (CV) at baseline and end of clamp measurements was <1%.

2.6. Statistical analysis

Data are expressed as mean ± SE and were analyzed using standard, parametric, one- and two-way analysis of variance and with repeated measures where appropriate (Graph Pad Software, Inc., San Diego, CA). Tukey's post hoc analysis was used to delineate