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Glucose and lipopolysaccharide regulate proatherogenic cytokine release from mononuclear cells in polycystic ovary syndrome

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ABSTRACT

Women with polycystic ovary syndrome (PCOS) have chronic low-grade inflammation, which can increase the risk of atherogenesis. We examined the effect of glucose ingestion and lipopolysaccharide (LPS) on markers of proatherogenic inflammation in the mononuclear cells (MNC) and plasma of women with PCOS. Sixteen women with PCOS (8 lean, 8 obese) and 15 weight-matched controls (8 lean, 7 obese) underwent a 3-h oral glucose tolerance test (OGTT). Interleukin-6 (IL-6) and interleukin-1β (IL-1β) release from MNC cultured in the presence of LPS and plasma IL-6, C-reactive protein (CRP), and soluble vascular adhesion molecule-1 (sVCAM-1) were measured from blood samples drawn while fasting and 2 h after glucose ingestion. Truncal fat was measured by dual-energy absorptiometry (DEXA). Lean women with PCOS and obese controls failed to suppress LPS-stimulated IL-6 and IL-1β release from MNC after glucose ingestion. In contrast, obese women with PCOS suppressed these MNC-derived cytokines under the same conditions. In response to glucose ingestion, plasma IL-6 and sVCAM-1 increased and CRP suppression was attenuated in both PCOS groups and obese controls compared with lean controls. Fasting plasma IL-6 and CRP correlated positively with percentage of truncal fat. The absolute change in plasma IL-6 correlated positively with testosterone. We conclude that glucose ingestion promotes proatherogenic inflammation in PCOS with a systemic response that is independent of obesity. Based on the suppressed MNC-derived cytokine responses suggestive of LPS tolerance, chronic low-grade inflammation may be more profound in obese women with PCOS. Excess abdominal adiposity and hyperandrogenism may contribute to atherogenesis in PCOS.

1. Introduction

In polycystic ovary syndrome (PCOS), glucose ingestion triggers an inflammatory response that is independent of obesity (González et al., 2005, 2006b, 2012a). Indeed, peripheral blood mononuclear cells (MNC) of lean women with PCOS exhibit increased nuclear factor κB (NFκB) activation following an oral glucose challenge (González et al., 2006b, 2012a). NFκB regulates transcription of a variety of proatherogenic inflammatory mediators that include interleukin-6 (IL-6), interleukin-1β (IL-1β), and soluble vascular cell adhesion molecule-1 (sVCAM-1). IL-6 and IL-1β are endocrine cytokines that stimulate synthesis of C-reactive protein (CRP) in the liver (Barnes and Karin, 1997; Moshage et al., 1988). Whereas sVCAM-1 causes attachment of MNC to the vascular endothelium, CRP promotes the uptake of lipids into MNC-derived foamy macrophages within atherosclerotic plaques (Carlos et al., 1991; Zwaka

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et al., 2001). Positive feedback by IL-6 and IL-1β perpetuates the preceding molecular events (Romano et al., 1997; Wang et al., 1995). Thus, IL-6, IL-1β, CRP, and sVCAM-1 work in concert in the development of atherosclerosis.

Lipopolysaccharide (LPS) is a bacterial component capable of activating NFκB in MNC upon binding to toll-like receptor 4 (TLR4), a pathogen pattern recognition receptor implicated in the development of atherosclerosis (Covert et al., 2005; Xu et al., 2001). LPS originating from gut-related bacteria can access the circulation in the presence of nutrients that increase intestinal permeability (Deopurkar et al., 2010; Neyrinck et al., 2008). High circulating LPS activity is associated with components of the metabolic syndrome (Lassenius et al., 2011). In our previous studies on glucose-stimulated inflammation and insulin resistance in PCOS, we evaluated TNFα, the proinflammatory cytokine that is a known mediator of insulin resistance. TNFα release from MNC obtained while fasting is increased in PCOS after direct *in vitro* exposure to glucose alone (González et al., 2006a) or LPS alone (unpublished results), indicating that MNC in the disorder are already pre-activated. Furthermore, the TNFα response from MNC is altered in PCOS when hyperglycemia is followed in tandem by *in vitro* LPS exposure. This alteration is characterized in lean women with PCOS by a failure to elicit the normal suppression of TNFα release from MNC that occurs in an uninflamed population, and in obese women with PCOS by paradoxical TNFα suppression akin to a phenomenon known as LPS tolerance (González et al., 2005; Ziegler-Heitbrock, 2001). To our knowledge, the hyperglycemic and LPS-related responses of MNC-derived proatherogenic cytokines such as IL-6 and IL-1β have never been explored in women with PCOS.

We examined the effect of glucose ingestion on IL-6 and IL-1β release from MNC exposed *in vitro* to LPS, as well as the effect of glucose ingestion alone on circulating IL-6, CRP, and sVCAM-1 in women with PCOS. We hypothesized that in response to glucose ingestion, LPS-stimulated IL-6 and IL-1β release from MNC and plasma IL-6, CRP, and sVCAM-1 are altered in women with PCOS compared with weight-matched controls and that these markers of atherogenesis are related to abdominal adiposity, insulin sensitivity, and circulating androgens.

2. Methods

2.1. Subjects

Sixteen women with PCOS (8 lean and 8 obese) 20–34 years of age and 15 weight-matched control subjects (8 lean and 7 obese) 20–39 years of age volunteered for study participation. Some subjects in the current study had been involved in our previous work on PCOS and insulin resistance (González et al., 2006b). The women with PCOS were selected using NIH criteria because this particular PCOS phenotype is most associated with metabolic dysfunction (Wild et al., 2010). All control subjects had regular menses lasting 25–35 days and a luteal range serum progesterone level consistent with ovulation (>5 ng/ml). All control subjects exhibited normal circulating androgen levels and did not have any skin manifestations of androgen excess or polycystic ovaries on ultrasound.

Diabetes and inflammatory illnesses were excluded in all subjects. None of them smoked tobacco or used medications that could have an impact on carbohydrate metabolism or immune function for a minimum of 6 weeks before beginning the study. None of the subjects exercised regularly during the 6 months before study participation. Written informed consent was obtained in all subjects according to Institutional Review Board guidelines for the protection of human subjects.

2.2. Study design

All subjects ingested a 75-g glucose beverage after an overnight fast of ~12 h. Blood samples were drawn at 0, 30, 60, 90, and 120 min for glucose and insulin determination. Additional plasma was isolated from the fasting and 120-min (2-h) blood samples and stored at −80 °C until assayed for IL-6, CRP, and sVCAM-1. The early glucose response was determined by calculating the area under the curve during the first 30 min of the OGTT (AUC_{0-30} glucose) using the trapezoidal method. Insulin sensitivity was derived from the OGTT (IS_{OGTT}) using the following formula: 10,000 divided by the square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin) (Matsuda and DeFronzo, 1999). Dual-energy absorptiometry (DEXA) was used to determine the percentage of total body fat and the percentage of truncal fat (Hologic Inc., Waltham, MA, USA), as previously described (González et al., 2005).

Isolation and culture of MNC were performed as previously described (González et al., 2005). MNC were incubated for 24 h with 1 ng/ml of LPS endotoxin from *Escherichia coli* 0127:B8 (Sigma–Aldrich Co. LLC, St. Louis, MO, USA). Culture supernatants were collected and stored at −80 °C until assayed for IL-6 and IL-1β.

Plasma glucose, insulin, IL-6, and CRP concentrations along with those of IL-6 and IL-1β in MNC culture supernatants were measured as previously described (González et al., 2005, 2006a). Serum luteinizing hormone (LH), testosterone, androstenedione, and dehydroepiandrosterone-sulfate (DHEA-S) were also measured as previously described (González et al., 2005, 2007). Plasma sVCAM-1 was measured by ELISA (R&D Systems, Minneapolis, MN, USA). All samples were measured in duplicate in the same assay. The interassay and intra-assay coefficients of variation for all assays were 7% and 12% respectively.

2.3. Statistics

Data were analyzed using StatView (SAS Institute, Cary, NC, USA). All values were initially examined graphically for departure from normality, and the natural logarithm transformation was applied as needed. Descriptive data and change from baseline of variables were compared between groups using ANOVA for multiple group comparisons. The source of significance by ANOVA was identified using Tukey's *post hoc* test. Paired Student's *t*-tests were performed for within-group comparisons. Treatment effects of glucose ingestion were determined by calculating the absolute change in proatherogenic inflammation markers