Title: Pancreatic β-cell dysfunction in polycystic ovary syndrome: Role of hyperglycemia-induced nuclear factor-κB activation and systemic inflammation

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Funding: National Institutes of Health Grants HD048535 (F.G.), RO1 AG-12834 (J.P.K.), and NIH T32 DK007319 (S.K.M.).

Key Words: insulin secretion, glucose intolerance, androgens, insulin resistance, mononuclear cells

Authors Disclosure: No conflicts of interest to report

Running Head: Inflammation and β-cell dysfunction in PCOS

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Abstract Word Count: 247

Main Text Word Count: 3635 (not including acknowledgement, references, tables, and figures).

Tables: 3

Figures: 4
In polycystic ovary syndrome (PCOS), oxidative stress is implicated in the development of β-cell dysfunction. However, the role of mononuclear cell (MNC)-derived inflammation in this process is unclear. We determined the relationship between β-cell function and MNC-derived NFκB activation and tumor necrosis factor-α (TNF-α) secretion in response to a 2h 75g oral glucose tolerance test (OGTT) in normoglycemic women with PCOS (15 lean, 15 obese) and controls (16 lean, 14 obese). 1st and 2nd phase β-cell function was calculated as glucose-stimulated insulin secretion (GSIS; insulin/glucose area under the curve for 0-30min and 60-120min, respectively) x insulin sensitivity (Matsuda Index; ISOGTT). Plasma thiobarbituric acid (TBARS; oxidative stress), high-sensitivity C-reactive protein (hs-CRP), and nuclear factor κB (NFκB; inflammation) and TNF-α secretion from MNCs were also assessed. In obese women with PCOS, 1st and 2nd phase β-cell function was lower compared with lean and obese controls. Compared with lean controls, women with PCOS had greater change from baseline in NFκB activation and TNFα secretion, and higher plasma TBARS. β-cell function was inversely related to NFκB activation (1st and 2nd) and TNFα secretion (1st), and plasma TBARS and hs-CRP (1st and 2nd). 1st and 2nd phase β-cell function also remained independently linked to NFκB activation after adjustment for body fat percentage and TBARS. Therefore, we conclude that β-cell dysfunction is linked to hyperglycemia-induced MNC activation of NFκB activation and systemic inflammation. In PCOS, these data suggest that inflammation may play a role in impairing insulin secretion prior to developing overt hyperglycemia.

Key Words: obesity, hyperglycemia, inflammation, insulin sensitivity
INTRODUCTION

Up to 70% of women with polycystic ovary syndrome (PCOS) exhibit insulin resistance and are at risk for type 2 diabetes (T2D; 7, 10, 40). The conventional glucose-regulatory response to insulin resistance is a reciprocal rise in pancreatic β-cell insulin secretion that maintains normal blood glucose concentrations (29). While this compensatory hyperinsulinemia in PCOS has been reported and linked to elevated androgens (3, 38), defects in β-cell function are observed independent of body weight and the degree of insulin resistance (5, 6, 14, 27, 40). Consequently, it is not surprising that nearly 50% of women with PCOS develop prediabetes or T2D before the age of 40 (7, 10, 40). Further work is required to elucidate the mechanism involved in this attenuated β-cell function to better understand how interventions may prevent hyperglycemia in women with PCOS.

Hyperglycemia-induced reactive oxidative species (ROS) from mononuclear cells (MNC) is directly related to insulin resistance and circulating androgens in PCOS (20-23). The resultant oxidative stress leads to lipid peroxidation and DNA damage, and activation of nuclear factor-κB (NFκB), the cardinal signal of inflammation. Activated NFκB dissociates from inhibitory-κB (IκB) in the cytoplasm and undergoes nuclear translocation to promote the transcription of tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine known to impair insulin signaling and action (46). Although the link between insulin resistance and inflammation in the pathogenesis of T2D is well established, inflammation may also play an important role in disrupting pancreatic insulin secretion (13, 50).

Within the β-cell, there is a readily available pool of insulin released upon initial glucose ingestion (1st phase) that is followed by synthesis of new insulin to manage post-prandial glucose fluctuations (2nd phase) (30). Recent studies demonstrate that macrophages derived from circulating MNC infiltrate pancreatic islets in primates (39) and humans with T2D (15) and disrupt pancreatic insulin secretion. Indeed, TNFα from MNC-derived macrophages activate NFκB within the β-cell thereby...
inducing endoplasmic reticulum stress and subsequent β-cell apoptosis (50). We recently reported that MNC-derived reactive oxygen species (ROS) generation was directly associated with low 1st phase β-cell function in women with and without PCOS (34), and proposed that inflammation may trigger attenuated β-cell function in women with PCOS. However, to date, no study has specifically examined the interaction between hyperglycemia-induced MNC inflammation and decompensated 1st or 2nd phase pancreatic β-cell function in humans. Therefore, we tested the hypothesis that MNC-derived NFκB activation and TNFα secretion in response to oral glucose ingestion would be associated with β-cell dysfunction independent of body fat and oxidative stress.

METHODS

Subjects: Sixty women, 30 with PCOS (14 lean, 16 obese) and 30 ovulatory controls (16 lean, 14 obese) 18-40 years of age volunteered for this cross-sectional study. Forty-seven of these subjects were involved in our previous work on PCOS and β-cell function of whom had complete data to assess inflammation (34), and half of the cohort was also involved in our prior work with insulin resistance (22). Subjects were non-smoking, weight stable (<2 kg weight loss in the previous 6 months), free of type 2 diabetes or cardiovascular disease, and not involved in habitual exercise for at least 6 months prior to the study. Subjects were excluded if they were taking supplements or medications known to influence glucose metabolism or immune responses. Lean and obesity were defined as having a BMI between 18-25 kg/m² or 30-40 kg/m², respectively. Women with PCOS were selected using the National Institute of Health criteria. As such, the presence of oligomenorrhea (i.e. intermenstrual intervals >35 days) and hyperandrogenemia (i.e. testosterone >60 ng/dl; androstenedione >3 ng/ml; or dehydroepiandrosterone-sulfate [DHEA-S] >300 µg/dl) was required after excluding nonclassic congenital adrenal hyperplasia, Cushing’s Syndrome, hyperprolactinemia and thyroid disease. All subjects with PCOS also exhibited polycystic ovaries on ultrasound. All
control subjects had regular menses lasting 25 to 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. Subjects received both verbal and written information about the study before signing informed consent documents approved by our Institutional Review Board.

Body Composition: Body weight was recorded on a digital platform scale with subjects wearing a hospital gown to the nearest 0.1 kg. Height was measured without shoes using a wall-mounted stadiometer to the nearest 1.0 cm. All subjects underwent dual-energy x-ray absorptiometry (DXA) to determine total body fat and truncal body fat (Hologic Inc., Waltham, MA). Truncal body fat was defined as the area between the diaphragm and the top of the greater trochanter.

Pancreatic β-cell Function: Subjects were provided weight-maintenance meals (resting metabolic rate x 1.2; ~50% CHO, 30% fat and 20% protein) and were instructed to refrain from strenuous activity during the 3 days before testing. An oral glucose tolerance test (OGTT) was performed in all women 5 to 8 days following the onset of menstruation. After an 8-12 hour overnight fast, a 75 gram OGTT was performed, and blood samples were obtained from an antecubital vein at 0, 30, 60, 90, and 120 minutes for the determination of plasma glucose and insulin. Insulin sensitivity derived from the OGTT (IS\textsubscript{OGTT}) was estimated using the Matsuda Index (36), and total area under the curve (AUC) during the OGTT was calculated using the trapezoidal method. 1\textsuperscript{st} and 2\textsuperscript{nd} phase glucose-stimulated insulin secretion (GSIS) was calculated by dividing plasma insulin by glucose AUC during the first 30 and last 60 minutes of the OGTT as previously described (35). Because the amount of insulin secreted to maintain normoglycemia is influenced by ambient level of insulin sensitivity, the product of GSIS and IS\textsubscript{OGTT} (i.e. disposition index) was calculated to characterize
pancreatic β-cell function.

Molecular Assays: Nuclear-bound NFκB was quantified by electrophoretic mobility shift assay (EMSA) as described before (19, 22). EMSA band specificity was verified by incubating the samples with specific antibodies against the p65 (H-286) and p50 (H-119) subunits of the NFκB complex (Santa Cruz Biotechnology, Santa Cruz, CA) to supershift the bands, and by competition with cold oligonucleotides. The protein content of IκB was quantified by Western blotting using a 1:1000 dilution of a monoclonal antibody against IκB (Transduction Laboratories) and actin (Santa Cruz Biotechnology, Santa Cruz, CA) (1). Densitometry after EMSA and Western blotting was performed on scanned films using Carestream Molecular Imaging software version 5.0.2.30 (Rochester, NY), and values for IκB were corrected for loading use those obtained from actin. A pooled control sample from all study subjects was also loaded on all Western blot gels to adjust for differences in exposure among gels.

MNC Isolation and Culture: MNCs were isolated via Histopaque-1077 density gradient centrifugation from blood samples obtained during the OGTT at 0 and 120 minutes (2 hours). As shown previously, these time points reflect baseline and peak or near-peak glucose-stimulated proinflammatory responses, respectively (2). The cells were washed and resuspended in RPMI (Sigma) and seeded in coated cell culture plates as previously described (31). The cells were incubated for 24 hours (humidified, 5% CO2, 37°C) and cell supernatants were collected (10,000 x g for 2 min) and stored at -80°C for subsequent analysis.

Biochemical Analysis: Serum glucose was determined during the OGTT using a glucose oxidase assay (YSI 2300 STAT Plus, Yellow Springs, OH). All remaining blood was centrifuged at 1,000 rpm for 10 minutes at 4°C, and stored at -80°C until analysis. Plasma insulin was measured by
radioimmunoassay (Millipore, Billerica, MA). Plasma high-sensitivity C-reactive protein (hs-CRP) was determined by a high-sensitivity enzyme-linked immunosorbent assay (ELISA; Alpha Diagnostics International, San Antonio, TX). Serum luteinizing hormone (LH), testosterone, androstenedione, and DHEA-S concentrations were measured by radioimmunoassay (Diagnostics Products Corporation, Los Angeles, CA). Plasma thiobarbituric acid-reactive substances (TBARS; an index of oxidative stress related to lipid peroxidation) was measured by fluorescence (OXItex; ZeptoMetric Corp. Buffalo, NY) (23). MNC-derived TNFα was determined via high-sensitivity ELISA (R & D systems, Minneapolis, MN). All samples from each subject were measured in duplicate in the same assay to minimize variance.

Statistical Analysis: Data were analyzed using the statistical program R (Leopard build 64-bit, The R Foundation, Vienna, Austria 2013). Skewed data were log transformed for statistical analysis to meet normality requirements. Because prior work by our group suggests that obesity reduces insulin sensitivity in PCOS (2, 20-23), data from this study were compared across groups using analysis of variance (ANOVA). In the event of statistical significance, pairwise comparisons with Bonferroni adjustments were used to identify the source of significance. The absolute change between pre- and 2-hours post-glucose challenge was used to characterize release of TNFα from MNCs. The percent change between alterations in NFκB and IκB were determined for each subject to account for inter-subject variability. Pearson’s product moment correlation was used to determine associations. Linear regression analysis was used to adjust for body fat and oxidative stress to confirm the relationship between β-cell function and inflammation. Data are expressed as mean ± standard error of mean, and significance was accepted as p≤0.05.
RESULTS

Age, Body Composition, and Serum Androgen Levels: Groups were similar in age, but body weight and total body fat were significantly greater (p<0.05) in obese subjects compared with those who were lean (Table 1). Compared with weight-similar controls, women with PCOS exhibited significantly higher (p<0.05) serum levels of LH, testosterone, androstenedione and DHEA-S.

Glucose Regulation: OGGT glucose and insulin curves are shown in Fig. 1A and 1B and clinical values are shown in Table 1. Fasting and 2-hour glucose levels were comparable across groups, although glucose at 60 and 90 min were statistically higher in obese PCOS women compared to lean and obese controls (p<0.05). Compared with lean controls, women with PCOS and obese controls had higher fasting and 2-hour insulin levels with a tendency towards higher 2nd phase insulin AUC (p=0.11) and 2-hour insulin levels (p=0.07) in obese subjects regardless of PCOS status. 1st phase GSIS was higher in obese women with PCOS (p<0.05) and obese controls (p=0.06) compared with lean controls, but was comparable in lean women with PCOS and lean controls. 2nd phase GSIS was higher (p<0.01) and 2SOGTT was lower (p<0.05) in women with PCOS compared with weight-similar controls (Table 1). 1st and 2nd phase β-cell function (Fig. 2A and 2B) were significantly lower (p<0.05) in obese women with PCOS and modestly lower (p=0.08, 1st phase only) in lean women with PCOS compared with weight-similar controls.

Inflammation and Oxidative Stress in MNC and Plasma: Basal and 2 hour inflammatory levels of NFκB, IkB, and TNFα are shown in Table 3. Basal and 2 hour values of NFκB and IkB were similar across groups. TNFα values were higher in lean controls compared with other groups. In response to glucose ingestion, the change from baseline (%) in activated NFκB was significantly greater (p<0.01) in women with PCOS compared with weight-similar controls (Fig. 3A). The change from baseline
(%) in IκB protein content was significantly lower (p<0.05) and the absolute change in TNFα secretion was significantly higher (p<0.05) in both PCOS groups and obese controls compared with lean controls (Fig. 3B and 3C). Fasting plasma TBARS was significantly higher (p<0.05) in lean and obese women with PCOS and obese controls compared with lean controls (Table 1). In contrast, fasting plasma hs-CRP was higher (p<0.05) in both obese groups compared with either lean group.

Correlations: 1st and 2nd phase β-cell function was negatively correlated with glucose-stimulated NFκB activation (Fig. 4A and 4B) and 1st phase β-cell function was positively correlated with IκB protein content (Table 2). 1st and 2nd phase β-cell function remained independently linked to NFκB activation after adjustment for body fat percentage (Estimate: -0.001, t-value: -1.95, p=0.05) and oxidative stress (Estimate: -0.001, t-value: -2.7, p=0.008). The relationship between NFκB activation and 1st phase β-cell function was also independent of the ratio of trunk fat to total fat (Estimate: -0.001, t-value: -1.95, p=0.05), and there was only a trend for impact on this relationship when trunk fat percentage was used in the model (Estimate: -0.001, t-value: -1.90, p=0.06). Furthermore, the relationship between NFκB activation and 2nd phase β-cell function was independent of the ratio of trunk fat to total fat and trunk fat percentage (Estimate: -0.001, t-value: -2.69, p=0.009).

Glucose-stimulated NFκB activation was positively correlated with insulin sensitivity (r=-0.43, p=0.006), MNC-derived TNFα secretion (r=0.46, p<0.001), plasma hs-CRP (r=0.39, p<0.001) and plasma TBARS (r=0.35, p<0.006). Serum levels of testosterone and androstenedione were positively correlated with NFκB activation and TNFα secretion, and negatively correlated with IκB protein content (Table 2). Serum androstenedione was also positively correlated with plasma TBARS and hs-CRP, and serum DHEA-S was positively correlated with plasma TBARS.
DISCUSSION

These data show for the first time that MNC-derived NFκB activation in response to glucose ingestion is inversely related to in vivo measures of 1st and 2nd phase β-cell function. Suppression of post-prandial inflammation appears to be a normal physiologic response to glucose ingestion as this is associated with adequate matching of GSIS to the level of insulin sensitivity. This is reflected by decreases in NFκB activation and TNFα secretion and increases in IκB protein content from MNC in normoglycemic lean reproductive-age women. This is in contrast to lean women with PCOS where glucose ingestion increases NFκB activation and TNFα secretion and decreases IκB protein content from MNC compared with lean controls. These findings are consistent with our previous reports in women with PCOS (19, 23) and older adults (32), and in impaired glucose tolerance (16) and type 1 diabetes (26). This observation is important because it illustrates that in PCOS, feeding alone is capable of triggering inflammation in the absence of obesity (17, 48).

Elevated β-cell mass is a typical consequence of insulin resistance that allows for increased insulin secretion to maintain normoglycemia (42). In fact, insulin resistant obese controls in our study cohort exhibit greater 1st and 2nd phase GSIS compared with lean controls. In contrast, lean women with PCOS with a comparable degree of insulin resistance to obese controls do not exhibit greater 1st phase GSIS. This latter finding suggests that in PCOS, early β-cell decompensation can occur prior to the development of obesity, and that MNC-derived inflammation may reduce the readily available pool of insulin to respond to glucose ingestion (41, 44). Interestingly, 2nd phase GSIS reflecting the synthesis of new insulin to manage fluctuations in post-prandial glucose is increased in lean PCOS women compared with lean controls and comparable to that of obese controls. Despite differences between 1st and 2nd phase GSIS, these observations should be viewed with caution given that the amount of insulin that is secreted to maintain normoglycemia is influenced by the prevailing insulin
sensitivity. Consequently, β-cell function is best characterized by the product of GSIS and ISOGTT. In this context, only 1st phase β-cell dysfunction is evident after correcting for insulin resistance in lean women with PCOS. Thus, our data suggest the possibility that the stark differences in 1st vs. 2nd phase β-cell function in lean women with PCOS are linked to inflammation-induced deficits in β-cell mass and/or function (37, 49).

Obese women with PCOS exhibit the greatest degree of insulin resistance and compromised β-cell function, which may confer the greatest risk for developing T2D compared with obese controls or lean women with PCOS. Because obesity is associated with elevated β-cell mass (42), it is not surprising that obese women with PCOS have exaggerated 1st and 2nd phase GSIS compared with lean women with PCOS. It is evident after correction of GSIS for insulin resistance that 1st and 2nd phase β-cell function is significantly lower compared with both lean groups and obese controls. This decrease in β-cell function may very well reflect the initial sign of disruption in the balance between inflammation-induced β-cell injury and compensatory β-cell function (9, 28). TBARS, a common index of lipid peroxidation, was similar in both obese groups and lean women with PCOS, suggesting that differences in insulin resistance among groups are independent of oxidative stress alone. Instead, the combination of excess total body fat and/or trunk fat and PCOS may promote greater inflammation to account for the profound decrease in insulin sensitivity and β-cell function in obese women with PCOS. Glucose-stimulated NFκB activation from MNC is greater in obese women with PCOS compared with lean women with PCOS. Furthermore, measures of adiposity including abdominal adiposity in our study cohort are inversely associated with insulin sensitivity and directly associated with MNC-derived NFκB activation and TNFα secretion as well as plasma TBARS and hs-CRP. Abdominal adiposity has been linked to elevations in circulating mediators of inflammation such as hs-CRP that have been shown to impair β-cell function, although our study cannot rule out a
role of adipokines (e.g. adiponectin or leptin) or elevated free fatty acids in this process (9, 28). Nevertheless, our data indicate that the regulation of early and late phase blood glucose control in response to glucose ingestion appears to be synergistically hampered by inflammation unique to PCOS in conjunction with the inflammatory load of excess body fat. Our findings are of clinical relevance because they highlight the unique pancreatic endocrine phenotypes in lean and obese women with PCOS that may differentially elevate risk of developing fasting vs. post-prandial glucose intolerance (30).

Hyperandrogenism in PCOS may contribute to β-cell dysfunction. It remains controversial whether androgens are capable of imparting a direct effect on the β-cell. In rodents, androgen receptors have been identified in pancreatic β-cells, and prenatal androgen exposure decreases β-cell sensitivity to GSIS (2, 3, 18, 25, 27, 43, 45). In humans, however, induction of hyperandrogenism in healthy individuals has no effect on β-cell function or viability (11). The ability of hyperandrogenism to contribute to pancreatic dysfunction in PCOS is most likely through the induction of oxidative stress and inflammation from MNC that may ultimately promote β-cell failure in susceptible individuals (33). Circulating androgens are directly associated with glucose-stimulated NFκB activation and p65 protein content along with plasma TBARS and hs-CRP, and negatively associated with IκB protein content. This corroborates similar results from our other studies (2, 20-23). Furthermore, we have recently reported that hyperandrogenism is capable of activating MNC to increase MNC sensitivity to glucose ingestion in a receptor-dependent fashion (19, 20, 24). Thus, it appears that in PCOS, hyperandrogenism serves as an additional indirect mechanism to accentuate the deleterious effects of oxidative stress and inflammation on β-cell function.
Our study has certain limitations that may affect our interpretation. We recognize that associations do not equate to causality and that further studies are needed to elucidate the role of MNC-derived inflammation on β-cell function in PCOS. In addition, we acknowledge that our study cannot completely rule out the specific role of visceral fat as opposed to subcutaneous fat on pancreatic function since we did not use MRI or CT scans to determine abdominal fat. We also recognize that subtle differences in plasma glucose during 60 and 90 min of the OGTT could cause some degree of impairment in pancreatic insulin secretion between PCOS and control subjects. It is likely that elevated blood glucose in obese women with PCOS is an effect of excess body fat that promotes insulin resistance and β-cell dysfunction. In contrast, it is unlikely that hyperglycemia per se is the primary factor explaining our results since lean women with PCOS exhibit β-cell dysfunction in the face of glucose levels comparable to lean and obese controls. Moreover, the distinct differences in metabolic and inflammation parameters we report in women with PCOS compared with weight-similar controls lend support to the contention that in PCOS, a distinct β-cell dysfunction is present prior to developing overt impaired glucose intolerance or T2D. The reason for increased basal TNFα secretion from MNC of lean healthy controls is unclear. These data should be interpreted with caution as opposed to inferring that healthy controls have greater inflammation than PCOS or obese counterparts. We have not observed increased basal TNFα secretion from MNC in lean controls compared with other groups in our previous work (19), and there is no clinical correlate with TNFα secretion from MNC. In fact, hs-CRP, a well-established biomarker of inflammation, was low in healthy controls, suggesting that these individuals are not characterized by clinical inflammation. Most importantly, TNFα secretion from MNC decreased in response to glucose ingestion, suggesting that suppression of MNC-derived inflammation goes hand-in hand with adequate pancreatic function. We also acknowledge that using plasma C-peptide as a measure of pre-hepatic insulin secretion may provide more direct measures of β-cell function. Nevertheless, we have previously shown that
hepatic insulin extraction does not affect insulin-derived calculations of β-cell function from the OGTT before or after lifestyle modification (35). Use of the euglycemic and hyperglycemic clamp or frequently sampled intravenous glucose tolerance test may provide a more accurate assessment of insulin resistance and GSIS, respectively compared with OGTT-derived measures (8). However, these intravenous techniques exclude the gastrointestinal tract which limits the physiologic understanding of in vivo β-cell function. Nevertheless, the IS\textsubscript{OGTT} has been shown to correlate strongly with euglycemic clamp derived measures of insulin sensitivity (36), and our IS\textsubscript{OGTT} results for lean and obese women with PCOS compared with weight-similar controls mimic those from seminal articles that used the euglycemic clamp (12). Thus, utilizing the OGTT may provide more “real-world” advantage to discerning links between MNC- and systemic markers of inflammation and in vivo β-cell function.

In conclusion, lean women with PCOS exhibit MNC-derived inflammation and lower 1\textsuperscript{st}, but not 2\textsuperscript{nd} phase β-cell function, suggesting that inflammation may affect distant pathways regulating insulin secretion physiology prior to the development of obesity. In contrast, obese women with PCOS exhibit greater insulin resistance than lean subjects along with 1\textsuperscript{st} and 2\textsuperscript{nd} phase β-cell dysfunction, indicating that the combination of PCOS and obesity induces greater decompensation of pancreatic responses to insulin resistance. Most importantly, β-cell function is directly related to MNC-derived NFκB activation in response to glucose ingestion along with plasma TBARS and hs-CRP, and inversely related to IκB protein content, which implicates inflammation in the impairment of β-cell function in PCOS (4, 44, 47). Our findings highlight the need for further investigation to determine the mechanism by which inflammation interacts with the pancreatic β-cell to increase diabetes risk in PCOS.
ACKNOWLEDGEMENTS

S.K.M developed the study hypothesis for this report, and J.P.K and F.G were responsible for the study design. C.L.S and F.G. performed the laboratory measurements. S.K.M and F.G. wrote the manuscript. F.G. is the guarantor of this work. All authors assisted with study organization, data analysis, and manuscript editing. The authors report no conflict of interest.

This paper was presented at the 74th Annual Meeting of the American Diabetes Association, San Francisco, California, June 13-17th 2014.

This research was supported by National Institutes of Health Grants HD048535 (F.G.), RO1 AG-12834 (J.P.K.), and NIH T32 DK007319 (S.K.M.).

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REFERENCES


TABLE LEGENDS

Table 1. Body Composition, androgen levels, glucose homeostasis, hs-CRP and oxidative stress. Data are expressed as mean ± standard error of mean. P-value represents analysis of variance. BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone-sulfate. AUC, area under the curve, ISOGTT, insulin sensitivity derived from the Matsuda index; 1st and 2nd phase GSIS, glucose-stimulated insulin secretion was calculated as insulin/glucose area under the curve 0-30 min and 60-120 min of the oral glucose tolerance test, respectively. #Data log-transformed for statistical analysis. †Significantly different compared with lean controls (p<0.05). ‡Significantly different compared with obese controls (p<0.05). §Significantly different compared with lean women with PCOS (p<0.05).

Table 2. Pearson’s correlations between insulin, body composition and serum androgen levels and markers of inflammation from MNC and plasma for the combined groups. ISOGTT, insulin sensitivity derived from the Matsuda index; 1st and 2nd phase GSIS, glucose-stimulated insulin secretion calculated as insulin/glucose area under the curve during 0-30min and 60-120min, respectively; BMI, body mass index; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone-sulfate; β-cell function, disposition index (ISOGTT x GSIS); hs-CRP, high sensitivity-C-reactive protein; TBARS, thiobarbituric acid-reactive substances. Fasting insulin, 2-hour insulin, GSIS, hs-CRP, LH, DHEA-S, and androstenedione were log-transformed for statistical analysis. Statistically significant correlations are represented by *p<0.05, †p<0.01, and ‡p<0.001.

Table 3. Baseline and 2-hour levels of NFκB, IκB protein content, and TNFα secretion. Data are expressed as mean ± standard error of mean. *Significantly different compared with all other groups (p<0.05).
Table 1. Body Composition, androgen levels, glucose homeostasis, hs-CRP and oxidative stress.

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<td>Insulin AUC0-30 (μU/ml-min) †</td>
<td>743±78</td>
<td>1673±331</td>
<td>1334±296</td>
<td>208±365</td>
<td>†</td>
</tr>
<tr>
<td>Glucose AUC60-120 (mg/dl-min)</td>
<td>6904±395</td>
<td>6760±401</td>
<td>7292±424</td>
<td>8319±342</td>
<td>0.84</td>
</tr>
<tr>
<td>Insulin AUC60-120 (μU/ml-min) †</td>
<td>1659±170</td>
<td>4015±618 †</td>
<td>4152±526 †</td>
<td>8478±1478 †‡§</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ISOGTT</td>
<td>9.8 ± 0.4</td>
<td>4.8 ± 0.6 †</td>
<td>4.9 ± 0.4 †</td>
<td>2.8 ± 0.4 †‡§</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1st Phase GSIS0-30 †</td>
<td>0.22 ± 0.02</td>
<td>0.51 ± 0.10</td>
<td>0.39 ± 0.09</td>
<td>0.60 ± 0.10 †</td>
<td>0.009</td>
</tr>
<tr>
<td>2nd Phase GSIS60-120 †</td>
<td>0.24 ± 0.02</td>
<td>0.62 ± 0.09 †</td>
<td>0.60 ± 0.08 †</td>
<td>1.01 ± 0.16 †‡§</td>
<td>0.004</td>
</tr>
<tr>
<td>hs-CRP (mg/l) †</td>
<td>1.1 ± 0.6</td>
<td>4.5 ± 0.8 †</td>
<td>1.3 ± 0.3</td>
<td>6.4 ± 1.0 †‡§</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>0.6 ± 0.1</td>
<td>1.2 ± 0.1 †</td>
<td>1.2 ± 0.2 †</td>
<td>1.3 ± 0.2 †</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of mean. P-value represents analysis of variance. BMI, body mass index; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone-sulfate. AUC, area under the curve, ISOGTT, insulin sensitivity derived from the Matsuda index; 1st and 2nd phase GSIS,
glucose-stimulated insulin secretion was calculated as insulin/glucose area under the curve 0-30 min and 60-120 min of the oral glucose tolerance test, respectively. #Data log-transformed for statistical analysis. †Significantly different compared with lean controls (p<0.05). ‡Significantly different compared with obese controls (p<0.05). §Significantly different compared with lean women with PCOS (p<0.05).
Table 2. Pearson’s correlations between insulin, body composition and serum androgen levels and markers of inflammation from MNC and plasma for the combined groups.

<table>
<thead>
<tr>
<th>Correlation coefficients (r)</th>
<th>NFkB Activation from MNC (change from baseline, %)</th>
<th>IkB Protein from MNC (change from baseline, %)</th>
<th>TNFα Secretion from MNC (change from baseline, Δ)</th>
<th>Plasma TBARS</th>
<th>Plasma hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin Metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.47 †</td>
<td>-0.36 †</td>
<td>0.36 †</td>
<td>0.61 †</td>
<td></td>
</tr>
<tr>
<td>2-hour Insulin</td>
<td>0.21</td>
<td>-0.31 †</td>
<td>0.19</td>
<td>0.12</td>
<td>0.46 †</td>
</tr>
<tr>
<td>ISOGTT</td>
<td>-0.43 †</td>
<td>0.37 †</td>
<td>-0.37 †</td>
<td>-0.62 †</td>
<td>0.87 †</td>
</tr>
<tr>
<td>1st Phase GSIS</td>
<td>-0.08</td>
<td>-0.08</td>
<td>0.09</td>
<td>0.36 †</td>
<td>-0.06</td>
</tr>
<tr>
<td>2nd Phase GSIS</td>
<td>0.12</td>
<td>-0.27 *</td>
<td>0.23</td>
<td>0.17</td>
<td>0.39 †</td>
</tr>
<tr>
<td>1st Phase β-cell Function</td>
<td>-0.37 †</td>
<td>0.31 †</td>
<td>-0.27 *</td>
<td>-0.41 †</td>
<td>-0.26 *</td>
</tr>
<tr>
<td>2nd Phase β-cell Function</td>
<td>-0.37 †</td>
<td>0.09</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.30 *</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.33 †</td>
<td>0.21</td>
<td>0.35 †</td>
<td>0.46 †</td>
<td>0.69 †</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>0.31*</td>
<td>0.01</td>
<td>-0.40 †</td>
<td>0.17</td>
<td>0.62 †</td>
</tr>
<tr>
<td>Truncal fat (%)</td>
<td>0.35 †</td>
<td>-0.02</td>
<td>0.21</td>
<td>0.21</td>
<td>0.68 †</td>
</tr>
<tr>
<td><strong>Androgens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>0.23</td>
<td>-0.34 †</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.33 †</td>
<td>-0.25 *</td>
<td>0.28 *</td>
<td>0.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.38 †</td>
<td>-0.44 †</td>
<td>0.27 *</td>
<td>0.28 *</td>
<td>0.28 *</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>0.17</td>
<td>0.23</td>
<td>0.07</td>
<td>0.32 †</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*ISOGTT*, insulin sensitivity derived from the Matsuda index; 1st and 2nd phase GSIS, glucose-stimulated insulin secretion calculated as insulin/glucose area under the curve during 0-30 min and 60-120 min, respectively; BMI, body mass index; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone-sulfate; β-cell function, disposition index (ISOGTT x GSIS); hs-CRP, high sensitivity-C-reactive protein; TBARS, thiobarbituric acid-reactive substances. Fasting insulin, 2-hour insulin, GSIS, hs-CRP, LH, DHEA-S, and androstenedione were log-transformed for statistical analysis. Statistically significant correlations are represented by *p<0.05, †p<0.01, and ‡p<0.001.
Table 3. Baseline and 2-hour levels of NFκB, IκB protein content, and TNFα secretion.

<table>
<thead>
<tr>
<th></th>
<th>Lean Controls</th>
<th>Obese Controls</th>
<th>Lean PCOS</th>
<th>Obese PCOS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFκB Activation</td>
<td>0 Hour</td>
<td>111±17</td>
<td>107±18</td>
<td>134±18</td>
<td>110±23</td>
</tr>
<tr>
<td>(Densitometry)</td>
<td>2 Hours</td>
<td>104±16</td>
<td>128±26</td>
<td>158±22</td>
<td>131±24</td>
</tr>
<tr>
<td>IκB Protein Content</td>
<td>0 Hour</td>
<td>139±20</td>
<td>176±13</td>
<td>156±22</td>
<td>161±15</td>
</tr>
<tr>
<td>(Densitometry)</td>
<td>2 Hours</td>
<td>138±19</td>
<td>156±12</td>
<td>120±16</td>
<td>112±11</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>0 Hour</td>
<td>23.6±6.9*</td>
<td>8.6±2.6</td>
<td>2.6±0.3</td>
<td>5.0±1.1</td>
</tr>
<tr>
<td></td>
<td>2 Hours</td>
<td>17.5±6.0</td>
<td>11.9±3.4</td>
<td>3.1±0.7</td>
<td>9.9±3.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of mean. *Significantly different compared with all other groups (p<0.05).
FIGURE LEGENDS

**Figure 1.** Oral glucose tolerance test plasma glucose (A) and insulin (B) curves. Data are expressed as mean ± standard error of mean. †Significantly different compared with lean controls (p<0.05). ‡Significantly different compared with obese controls (p<0.05). ††Significantly different compared with lean and obese controls (p<0.05).

**Figure 2.** Effects of obesity and polycystic ovary syndrome on 1st phase (A) and 2nd phase (B) β-cell function. β-cell function was calculated as 1st or 2nd phase GSIS x ISOGTT. GSIS was calculated as area under the curve for insulin divided by glucose during 0-30 min and 60-120 min of the oral glucose tolerance test. ISOGTT, insulin sensitivity derived from the Matsuda index. Data are expressed as mean ± standard error of mean. *Obese controls compared with obese women with PCOS (p<0.05).

**Figure 3.** Effects of obesity and polycystic ovary syndrome (PCOS) on MNC-derived inflammation. Change from baseline (%) in NFκB activation (A) and IκB protein content (B) based on a densitometric quantitative analysis between fasting and 2-hour post-glucose ingestion samples. Representative EMSA bands of intranuclear NFκB and Western blots of IκB and actin protein are depicted above the histograms showing the change in content pre- and post-glucose ingestion. Samples from all four study groups were run on the same gel. Absolute change from baseline (Δ, pg/ml) in TNFα secretion (C) measured in culture supernatants between fasting and 2-hour post-glucose ingestion samples. Data are expressed as mean ± standard error of mean. *Lean controls vs. obese controls, lean women with PCOS and obese women with PCOS (p<0.05). †Lean women with PCOS compared with obese women with PCOS (p<0.05).

**Figure 4.** Correlations between the change from baseline (%) in MNC-derived NFκB activation and 1st phase (A) and 2nd phase (B) β-cell function for the combined groups.
**FIGURE 1**

A. Plasma Glucose (mg/dl) over time (min) for Lean Control, Obese Control, Lean PCOS, and Obese PCOS.

B. Plasma Insulin (μU/ml) over time (min) for Lean Control, Obese Control, Lean PCOS, and Obese PCOS.
FIGURE 2

A

1st Phase β-Cell Function

* p < 0.05

B

2nd Phase β-Cell Function

* p < 0.05

Lean Controls  Obese Controls  Lean PCOS  Obese PCOS
FIGURE 3

A
Pre (0 hour) and Post (2 Hours) Glucose Ingestion

Activated NFkB

Change in NFkB Activation (%)

* p < 0.04
† p < 0.05

B

Change in IκB Protein (%)

* p < 0.05

C

Change in TNFα Secretion (Δ)

* p < 0.05
FIGURE 4

A

1st Phase β-Cell Function

Change in NFκB Activation (%)

r = -0.34
p < 0.01

B

2nd Phase β-Cell Function

Change in NFκB Activation (%)

r = -0.36
p < 0.01

○ Lean Controls

● Lean PCOS

△ Obese Controls

▲ Obese PCOS