

Endothelial Function Varies According to Insulin Resistance Disease Type

Short Title: Insulin Resistance and Vascular Function

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Abstract

Objective: We examined the relationship between insulin resistance and vascular function in three insulin resistant states (type 2 diabetes mellitus, non-HIV lipodystrophic diabetes, and non-diabetic polycystic ovary syndrome (PCOS)) and in healthy controls.

Research Design and Methods: The population included 12 women with type 2 diabetes, 6 with lipodystrophic diabetes, 10 with polycystic ovary syndrome, and 19 healthy female subjects. Metabolic measures included insulin sensitivity by the homeostasis model assessment, lipids, free fatty acids, and adiponectin. High-resolution B mode ultrasound was used to determine endothelium-dependent and –independent vasodilation.

Results: Type 2 diabetic, lipodystrophic, and polycystic ovary syndrome subjects were insulin resistant compared with control subjects ($p = 0.001$). Flow mediated vasodilation (FMD) was reduced in diabetic subjects ($3.4 \pm 1.3\%$), compared with control ($7.3 \pm 1.1\%$) but not in lipodystrophic ($7.7 \pm 1.2\%$) or polycystic ovary syndrome subjects ($9.9 \pm .7\%$) ($p = 0.005$). Nitroglycerin-mediated vasodilation (NMD) was attenuated in both diabetic ($15.2 \pm 2.0\%$) and lipodystrophic subjects ($16.7 \pm 3.6\%$) compared to healthy control subjects ($24.6 \pm 2.4\%$) and polycystic ovary syndrome subjects ($23.2 \pm 1.8\%$) ($p = 0.019$). Neither insulin resistance, free fatty acids, adiponectin, nor C-reactive protein associated with vascular dysfunction.

Conclusions: Among these different types of patients with insulin resistance, we found abnormal endothelium-dependent vasodilation only in the patients with type 2 diabetes. We postulate that variations in the mechanism of insulin resistance may affect endothelial function differently than glucose homeostasis.

Insulin resistance, typically defined by vascular dysfunction across insulin resistance impairment of insulin-mediated actions on states.

glucose uptake, affects a wide range of tissues including adipose, skeletal muscle, and the vascular endothelium. Insulin, via a sequence of intracellular signals, activates endothelial nitric oxide synthase (eNOS) (1) and increases production of nitric oxide. Reductions in the bioavailability of nitric oxide (NO) are associated with atherosclerosis. Impaired insulin action, when assessed by fasting serum insulin level or the homeostasis model assessment (HOMA-IR) (2, 3), is associated with atherosclerosis and an increased risk of myocardial infarction. Insulin resistance is associated with endothelial dysfunction (4) and may serve as a link between insulin resistance and atherosclerosis.

Research Design and Methods

Subjects

Forty seven non-smoking women, including 12 with type 2 diabetes, 10 with polycystic ovary syndrome (PCOS), 6 with non-human immunodeficiency virus (HIV)-negative lipodystrophic diabetes, and 19 healthy controls were recruited through newspaper advertisement and from the Joslin Diabetes Center. All subjects underwent medical history, physical examination, and laboratory analysis including complete blood count, serum electrolytes, glucose, blood urea nitrogen, and creatinine, total cholesterol, and low density lipoprotein (LDL) cholesterol. Among the type 2 diabetic, PCOS, and healthy control subjects, those with hypertension, history of single entity and occurs as a consequence of a variety of mechanisms and disparate presentations, unified phenotypically by impaired insulin-mediated glucose uptake. Because the mechanisms of insulin resistance were excluded. Women with PCOS had 6 or vary in different conditions, its impact on other tissues remains unclear. Determining the effect of various insulin resistant states on endothelial function may provide insight in non-sex hormone binding globulin bound NO bioavailability in each disease and testosterone (unbound testosterone or uT) >15 ng/dl (0.5 nmol/L) levels greater than two prevalence of atherosclerosis in insulin resistant patients.

Insulin resistance, however, is not a single entity and occurs as a consequence of a variety of mechanisms and disparate presentations, unified phenotypically by impaired insulin-mediated glucose uptake. Because the mechanisms of insulin resistance were excluded. Women with PCOS had 6 or vary in different conditions, its impact on other tissues remains unclear. Determining the effect of various insulin resistant states on endothelial function may provide insight in non-sex hormone binding globulin bound NO bioavailability in each disease and testosterone (unbound testosterone or uT) >15 ng/dl (0.5 nmol/L) levels greater than two prevalence of atherosclerosis in insulin resistant patients.

Accordingly, we sought to investigate the role of insulin resistance on endothelial function in three distinct populations of insulin resistant women (polycystic ovary syndrome (PCOS), type 2 diabetes and lipodystrophic diabetes) compared with healthy subjects. We also measured a marker of atherosclerosis or risk factors (except for inflammation (C-reactive protein (CRP)) smoking) for atherosclerosis. The and two adipokines (adiponectin and free fatty acids) to determine whether these familial partial lipodystrophy (FPLD), two factors, known to be abnormal in insulin resistance, may serve as mechanism of partial lipodystrophy - mandibuloacral

dysplasia variety, and one with acquired diastole. After baseline image acquisition, a lipodystrophy after dermatomyositis. One forearm sphygmomanometric cuff was subject was blind, had renal insufficiency, and inflated to suprasystolic pressure (200 mm peripheral arterial disease and died 3 months Hg) for five minutes. Upon cuff release, after the study; one subject had renal reactive hyperemia causes flow to increase insufficiency and died 1 year after the study; through the brachial artery subserving the and one subject had coronary artery disease. forearm. Flow-induced, endothelium-Two lipodystrophic subjects were taking ACE dependent vasodilation of the brachial artery inhibitors, and they withheld these was determined by acquiring images at one medications for 24 hours prior to vascular minute after cuff deflation. Flow-mediated testing. Diabetes medications were held on vasodilation at this time point is largely the day of study. Other potential subjects endothelium-dependent and nitric oxide taking an angiotensin converting enzyme mediated, and can be inhibited by (ACE) inhibitor, angiotensin receptor blocker, administration of the nitric oxide synthase or statin were excluded. All participants antagonist, N^G-monomethyl-L-arginine (5). provided written, informed consent. The Ten minutes after cuff release, the brachial protocol was approved by the Human artery was imaged again to re-establish basal Research Committees of the Joslin Diabetes conditions. Then, to determine endothelium-Center and the Brigham and Women's independent vasodilation, subjects received Hospital. 0.4 mg of nitroglycerin, sublingually. The

Vascular Reactivity Studies

All subjects were studied in the later. Brachial artery blood flow velocity was morning in the post-absorptive state, fasting determined via time-velocity integral after the previous midnight. Cyclooxygenase measurement. Nitroglycerin was not inhibitors, alcohol, and caffeine were administered if the systolic blood pressure prohibited for 24 hours prior to the study. was below 110 mm Hg or the subject refused Subjects were studied in a quiet, temperature- nitroglycerin, usually to avoid a severe controlled, dimly-lit room, after resting supine headache during the second and third visits.

for a minimum of 5 minutes. High-resolution

Laboratory Analyses

B-mode ultrasonography of the brachial artery Total cholesterol, triglycerides, HDL was performed using a Toshiba 270 SSA cholesterol, LDL cholesterol and blood (Toshiba America Medical Systems, Inc., glucose levels were measured by standard Tustin, CA) ultrasound machine and 7.5 MHz laboratory techniques. hs-CRP levels were linear array probe. The brachial artery was measured using the Beckman LX-20 imaged longitudinally just proximal to the (Beckman Coulter, Brea, CA). This assay has antecubital fossa. Transducer position was been validated against the Dade Behring adjusted to obtain optimal images of the near hsCRP method and has an inter and intra- and far wall of the intima. Images were assay CV of < 8%. Adiponectin levels were simultaneously recorded on super VHS video measured using a sandwich ELISA (Linco tape. The video output and Research, St. Charles, MO) and had an inter-electrocardiographic signal of the ultrasound and intra-assay CV of <9%. Insulin levels machine were connected to a computer were measured using a two-site immunoassay equipped with a Data Translation frame- (Linco Research, St. Charles, MO) with an grabber videocard, (Dataviz, Trumbull, CT). inter and intra-assay CV of <11%. Free fatty The 'R' wave on the electrocardiogram acids in serum were measured using reagents served as a trigger to acquire frames at end- from Wako diagnostics. Free fatty acids were

measured based the acylation of coenzyme density lipoprotein (LDL) cholesterol levels, A(CoA) by the fatty acids in the presence of blood pressure, and body mass index did not added acyl-CoA synthetase (Wako differ significantly among the groups. High Chemicals, Richmond, VA). The acyl-CoA density lipoprotein (HDL) cholesterol levels produced is oxidized by added acyl-CoA were lower and triglycerides levels were oxidase (ACOD) generating hydrogen higher in each insulin resistant group peroxide that is measured compared with healthy subjects. Insulin spectrophotometrically. The inter- and intra-resistance, as measured by HOMA-IR, was assay CVs for this assay are between 3-7%. greater in lipodystrophy (27.6 ± 16.4), PCOS The homeostasis model assessment (HOMA- (6.6 ± 1.9), and type 2 diabetic subjects (6.1 ± 1.4) compared to control subjects (2.3 ± 0.4) IR) of insulin resistance was calculated as fasting glucose x fasting insulin/22.5 (6). ($p = 0.001$, by ANOVA). Similarly, insulin

Statistical Methods

Descriptive measures are reported as means \pm standard deviation (SD). control subjects. The insulin level and HOMA-IR in lipodystrophic subjects were \pm standard error (SE). Demographic data, more than twice that of any other group. Flow-mediated vasodilation was arterial diameter, reactive hyperemia, and reduced in type 2 diabetic subjects ($3.4 \pm 1.3\%$) compared with healthy control subjects ($7.3 \pm 1.1\%$), but was unexpectedly preserved in subjects with lipodystrophy ($7.7 \pm 1.2\%$) and with PCOS ($9.9 \pm 0.7\%$) ($p = 0.005$ by ANOVA, Figure 1). In post-hoc testing, flow-mediated vasodilation was significantly reduced in subjects with type 2 diabetes mellitus when compared to healthy subjects ($p = 0.02$). This was not accounted for by differences in baseline arterial diameter. Baseline arterial diameter was similar in each group: 3.11 ± 0.12 mm in healthy control subjects, 3.27 ± 0.14 mm in type 2 diabetic subjects, 3.20 ± 0.15 mm in PCOS, and 3.31 ± 0.29 mm in lipodystrophic subjects, ($p > 0.2$). Nitroglycerin-mediated vasodilation was reduced in both type 2 diabetic ($15.2 \pm 2.0\%$) and lipodystrophic subjects ($16.7 \pm 3.6\%$) compared with healthy control subjects ($24.6 \pm 2.4\%$) and PCOS subjects ($20.7 \pm 2.8\%$) ($p = 0.02$, by ANOVA, Figure 2). In post-hoc testing, nitroglycerin-mediated vasodilation was reduced in subjects with type 2 diabetes mellitus ($p = 0.02$) significantly and trended towards attenuation in lipodystrophic subjects ($p = 0.09$) when compared to healthy subjects. Six subjects did

Results

Baseline characteristics are presented in Table 1. Type 2 diabetic subjects were older than lipodystrophic diabetic, PCOS, and healthy control subjects. Hemoglobin A1c was $7.8 \pm 1.9\%$ in the subjects with type 2 diabetes and $8.8 \pm 2.3\%$ in the subjects with lipodystrophy. Of the 12 subjects with type 2 diabetes, glucose lowering was achieved in 7 with a sulfonylurea alone, in 2 with insulin alone, and in 3 with metformin and a sulfonylurea. All lipodystrophic subjects were taking high dose insulin and 2 were taking metformin. Total cholesterol and low

not receive nitroglycerin. One PCOS and 3 was preserved in subjects with lipodystrophic healthy control subjects had systolic blood diabetes and with PCOS. Our results do not pressure below our cutoff level of 110 support a direct relationship between insulin mm/Hg, while 2 subjects with type 2 diabetes resistance and endothelial function across a refused.

Free fatty acids (FFA) were surprising, for worsening insulin resistance is significantly higher in each insulin resistant broadly associated with increases in group compared with healthy subjects ($p =$ atherosclerosis across these same disease 0.004, by ANOVA) (Table 1). Adiponectin states, from least in PCOS to most in levels were significantly lower in the type 2 lipodystrophic diabetes (7-9). Phenotypic diabetic and PCOS groups compared with variations in insulin-affected tissues suggest healthy control ($p = 0.04$, by ANOVA) and that the mechanism of insulin resistance, lipodystrophic subjects. CRP was not instead of the severity of either insulin significantly different among the four groups resistance or glucose disturbance, may be ($p > 0.2$, by ANOVA).

Insulin resistance, whether measured of these disease states on vascular function. by HOMA-IR or fasting insulin level, In type 2 diabetes mellitus, serine correlated inversely with HDL and phosphorylation of the insulin receptor adiponectin levels and directly with substrate attenuates normal activation of triglycerides levels and BMI (Table 2). Free phosphatidylinositol 3-kinase and AKT fatty acid levels correlated indirectly with attenuating endothelial nitric oxide synthase HDL ($r = -0.31$, $p = 0.04$) and directly with activity (10). The molecular basis for defects triglycerides levels ($r = 0.39$, $p = 0.008$). in insulin signaling differs in lipodystrophy Neither markers of insulin sensitivity, BMI, and PCOS compared with type 2 diabetes. CRP, nor adipokines correlated with For example, lamin or peroxisome endothelium-dependent vasodilation or with proliferator-activated receptor-gamma endothelium-independent vasodilation. mutations in some forms of lipodystrophy but Glucose levels correlated inversely with not common type 2 diabetes or PCOS suggest endothelium-independent vasodilation ($r = -$ a different origin for the resistance to insulin 0.46, $p = .009$) but not endothelium- action (11). Differing responses to leptin dependent vasodilation. administration (12), PPAR- γ therapy (13), and variations in insulin-mediated FFA and ketone body suppression (14, 15) further

Conclusions

In this investigation, we evaluated indicate that lipodystrophy causes insulin vascular function in subjects with different resistance differently than type 2 diabetes. types of insulin resistance including those Previous investigations by Dunaif and with type 2 diabetes mellitus, lipodystrophic colleagues have demonstrated increased diabetes, and polycystic ovary syndrome, and insulin receptor serine phosphorylation and compared those patients with healthy control decreased IRS-1 tyrosine phosphorylation subjects. Insulin-resistance, as determined by (16, 17) in obese women with PCOS. Several HOMA-IR, was more profound in each studies, including ours, demonstrate preserved insulin resistant group compared with control endothelial function in women with PCOS subjects. Despite the presence of insulin who are either non-obese or without morbid resistance, endothelium-dependent obesity (18, 19), but this remains vasodilation was reduced only in subjects controversial (20-22). The presence of with type 2 diabetes. Endothelial function obesity may contribute importantly to the

resistance did not correlate with FFA levels, adipokines, and inflammation did not. Also, triglycerides were directly associated with vascular smooth muscle and in HDL was inversely associated with function. Glucose levels correlated inversely with insulin resistance. Similarly, across a population of healthy control, type 2 diabetic subjects without microalbuminuria, and type 2 diabetic subjects with albuminuria, free fatty acid levels did not associate with endothelium-independent vasodilation (35). Moreover, diabetes (44, 45). Despite the attenuation in Ballotshafer and colleagues demonstrated no endothelium-independent vasodilation in the association between ambient free fatty acid levels and flow-mediated vasodilation in first degree relatives of patients with type 2 diabetes (36). Thus, abnormalities in these suggest that endothelial vasodilator adipocyte factors in insulin resistance do not independently mediate vascular dysfunction across a range of insulin resistance states.

Inflammation

Inflammation has been demonstrated to associate with endothelial dysfunction in healthy subjects and patients with coronary artery disease (37, 38). Moreover, when inflammation is induced by vaccination, endothelial function is depressed and the increase in inflammatory cytokines prevents endothelial dysfunction (39, 40). Despite this relationship between inflammation and endothelial function in non-diabetic populations, several studies have demonstrated that CRP does not correlate with vascular function in diabetic subjects (41-43). Similarly, in our cohort, CRP levels did not vary significantly across our resistant populations and did not correlate with endothelial function. CRP, as a marker of inflammation, however, may not represent all inflammatory markers.

Vascular smooth muscle dysfunction

The response to nitroglycerin was attenuated in subjects with type 2 lipodystrophic diabetes mellitus. We have previously reported an impaired response to nitroglycerin in subjects with type 2 diabetes mellitus (44), but this is the first report of a similar finding in subjects with lipodystrophic diabetes. Markers of insulin resistance, atherosclerosis

Limitations

In this investigation of vascular function in insulin resistance, HOMA-IR was used to approximate insulin resistance. This measure, in large part, reflects the relationship between insulin production and hepatic glucose output and is reported to correlate well with dynamic measures of insulin resistance, such as a euglycemic clamp or the minimal model (46). However, HOMA-IR may underestimate the severity of insulin resistance in subjects with long-standing type 2 diabetes because of an inability to make insulin. It is unlikely that insulin resistance in the type 2 diabetic subjects would approach the same severity in subjects with lipodystrophy. Lipodystrophic subjects were the most insulin resistant and had preserved endothelial function unlinking a direct relationship between the two parameters.

In studying these populations, the number of subjects with lipodystrophy was small compared to the other groups because of their rarity in the population. Moreover, two of the subjects were taking ACE inhibitors which may improve endothelial function while 3 had microvascular disease or diabetes which should worsen it.

Although these study conditions were bioavailability of nitric oxide and risk of imperfect, the similarity of vascular function atherosclerosis.

within subjects with lipodystrophy and between them and healthy control subjects suggests our observations are valid and not likely to be altered if a greater number of lipodystrophic patients were included.

Age varied significantly among the groups. Several investigations have noted that older subjects have attenuated endothelial function compared with younger controls (47-50). Despite this difference, in this study, endothelial function remained significantly different among the groups after controlling for age.

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Conclusion

How, then, to explain our findings? Our measure of insulin resistance is strongly correlated with impairment of insulin-mediated skeletal-muscle glucose uptake. Although there is a link between the skeletal muscle defect and vascular defect in obesity/diabetes, different vascular insulin signaling disturbances are likely operational in the other insulin resistance states. Insulin levels in the lipodystrophic diabetic subjects were markedly higher than every other group, possibly stimulating eNOS enough to overcome the disturbances in vascular smooth muscle function demonstrated in our cohort.

Thus, the effects of insulin resistance on vascular function vary according to origin of impaired insulin signaling. Lipodystrophic and PCOS patients have normal endothelial function, indicative of preserved endothelial insulin signaling, despite impaired glucose handling. Abnormal endothelium-dependent vasodilation in subjects with type 2 diabetes mellitus suggests that attenuated endothelial activation of PI-3 kinase and Akt importantly affects endothelial function in this insulin resistance disorder. Mechanisms of insulin resistance that are associated with an increased risk of atherosclerosis require better characterization to explain the variations in

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Table 1: Baseline Characteristics

| | Lipodystrophy | PCOS | Type 2 Diabetes | Control | p |
|--------------------------------|---------------|-------------|-----------------|-----------|--------|
| N | 6 | 10 | 12 | 19 | |
| Age (years) | 47±13 | 31±6 | 56±14 * | 41±11 | <0.001 |
| Mean Arterial Pressure (mm Hg) | 96±8 | 94±9 | 108±13 | 98±12 | >0.2 |
| BMI (kg/m ²) | 27±3 | 30±5 | 31±7 | 26±7 | >0.2 |
| Total Cholesterol (mg/dL) | 200±47 | 192±31 | 178±37 | 172±13 | >0.2 |
| LDL Cholesterol (mg/dL) | 101±31 | 120±26 | 99±40 | 98±23 | >0.2 |
| HDL Cholesterol (mg/dL) | 34±8 † | 37±9 † | 49±14 | 61±12 | <0.001 |
| Triglycerides (mg/dL) | 311±165 † | 171±72 * | 153±95 | 70±39 | <0.001 |
| Insulin (uU/ml) | 58±85 * | 28±22 | 14±12 | 10±8 | 0.016 |
| Glucose (mg/dL) | 191±113 † | 88±9 | 156±68 † | 88±18 | <0.001 |
| HOMA-IR | 27.6±16.4 † | 6.6±1.9 * | 6.1±1.4 * | 2.3±0.4 | 0.001 |
| Adiponectin (ug/mL) | 16.1±13.5 | 8.3±4.2 | 9.0±3.8 | 13.4±5.8 | 0.041 |
| Free Fatty Acids (mEq/L) | 0.15±0.06 | 0.19±0.05 * | 0.20±0.07 † | 0.11±0.05 | 0.002 |
| C Reactive Protein (mg/L) | 2.25±1.61 | 1.49±1.12 | 3.74±4.09 | 1.54±2.26 | >0.2 |

Abbreviations: BMI: body mass index ; LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA-IR: homeostatic model assessment – insulin resistance. † p < 0.01 * p < 0.05 compared to control subjects

Table 2: Insulin Resistance Correlations

| | HOMA Correlation Coefficient | Spearman's Rho | Insulin Correlation Coefficient | Spearman's Rho |
|--------------------------|---|-----------------------|--|-----------------------|
| Age | 0.06 | p > 0.2 | 0.06 | p > 0.2 |
| BMI | 0.49 | p = 0.005 | 0.46 | p = 0.006 |
| Total Cholesterol | 0.2 | p > 0.2 | 0.23 | p > 0.2 |
| LDL Cholesterol | 0.23 | p > 0.2 | 0.28 | p > 0.2 |
| HDL Cholesterol | -0.55 | p = 0.001 | -0.53 | p = 0.001 |
| Triglycerides | 0.46 | p = 0.001 | 0.36 | p = 0.014 |
| FMD | -0.18 | p > 0.2 | -0.05 | p > 0.2 |
| NMD | -0.06 | p > 0.2 | 0.18 | p > 0.2 |
| Adiponectin | -0.53 | p = 0.001 | -0.47 | p = 0.001 |
| Free Fatty Acids | 0.17 | p > 0.2 | 0.01 | p > 0.2 |
| CRP | 0.26 | P = 0.13 | 0.11 | p > 0.2 |

Abbreviations: BMI: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; FMD: flow-mediated vasodilation; NMD: nitroglycerin-mediated vasodilation

Figure Legend

Figure: Vascular Function. (A) The mean percent increase in brachial artery size 1 minute after cuff release compared with baseline is illustrated. (B) The mean percent increase in brachial artery size 3 minutes after sublingual nitroglycerin administration compared with baseline is illustrated.



