c-Jun NH$_2$-Terminal Kinase Activity in Subcutaneous Adipose Tissue but Not Nuclear Factor-κB Activity in Peripheral Blood Mononuclear Cells Is an Independent Determinant of Insulin Resistance in Healthy Individuals

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OBJECTIVE—Chronic low-grade activation of the immune system (CLAIS) predicts type 2 diabetes via a decrease in insulin sensitivity. Our study investigated potential relationships between nuclear factor-κB (NF-κB) and c-Jun NH$_2$-terminal kinase (JNK) pathways—two pathways proposed as the link between CLAIS and insulin resistance.

RESEARCH DESIGN AND METHODS—Adiposity (dual-energy X-ray absorptiometry), waist-to-hip ratio (WHR), and insulin sensitivity (M, hyperinsulinenic-euglycemic clamp) were measured in 22 healthy nondiabetic volunteers (aged 29 ± 11 years, body fat 28 ± 11%). NF-κB activity (DNA-binding assay) and JNK1/2 activity (phosphorylated JNK) were assessed in biopsies of the vastus lateralis muscle and subcutaneous adipose tissue and in peripheral blood mononuclear cell (PBMC) lysates.

RESULTS—NF-κB activities in PBMCs and muscle were positively associated with WHR after adjustment for age, sex, and percent body fat (both P < 0.05). NF-κB activity in PBMCs was inversely associated with M after adjustment for age, sex, percent body fat, and WHR (P = 0.02) and explained 16% of the variance of M. There were no significant relationships between NF-κB activity and M in muscle or adipose tissue (both NS). Adipose-derived JNK1/2 activity was not associated with obesity (all P > 0.1), although it was inversely related to M (r = −0.54, P < 0.05) and explained 29% of its variance. When both NF-κB and JNK1/2 were examined statistically, only JNK1/2 activity in adipose tissue was a significant determinant of insulin resistance (P = 0.02).

CONCLUSIONS—JNK1/2 activity in adipose tissue but not NF-κB activity in PBMCs is an independent determinant of insulin resistance in healthy individuals. Diabetes 58:1259–1265, 2009
is abnormally elevated in obesity, and an absence of JNK1 results in decreased adiposity, improved insulin sensitivity, and enhanced insulin receptor signaling in two different mouse models of obesity (9). Therefore, JNK1 is now considered an important player in the modulation of insulin action and a critical component of the pathogenesis of obesity, insulin resistance, and type 2 diabetes (9).

There is a paucity of data exploring the hypothesis that either or both of these pathways play a role in CLAIS-induced insulin resistance in humans. In particular, no study investigated these pathways in healthy individuals before they develop diabetes, which is essential with respect to etiology of insulin resistance. An association between NF-κB activation and glucose metabolism has been suggested in patients with type 2 diabetes, in whom there was a 60% reduction in the protein levels of IKKβ and a parallel elevation of NF-κB activity in skeletal muscle biopsies, which correlated with insulin resistance (16). In another study in insulin-resistant women with polycystic ovary syndrome (a condition also associated with CLAIS) NF-κB expression correlated with 2-h glucose levels (17). Furthermore, increased NF-κB activity in mononuclear cells has been shown in patients with type 2 diabetes (18,19) as well as in obese individuals (20). However, none of these studies investigated NF-κB activity in relation to direct assessment of insulin sensitivity, as measured by the hyperinsulinemic-euglycemic clamp. With regard to JNK1 activity, we have also previously shown that obese insulin-resistant humans (insulin sensitivity assessed by homeostasis model assessment [HOMA]) have increased JNK phosphorylation in skeletal muscle (21). Only one other study demonstrated that JNK1 activity in skeletal muscle was increased in obesity and type 2 diabetes and was an independent determinant of insulin resistance as measured by euglycemic clamp (22), but in that study adipose tissue and PBMCs were not examined.

Thus far, no studies have investigated the relative contribution of NF-κB and JNK pathways to development of insulin resistance in humans. Because it is likely that the relationship between CLAIS and insulin resistance can be initiated and perpetuated systemically by cross talk among insulin-sensitive tissues, i.e., adipose tissue, skeletal muscle, liver, and circulating immune cells, we sought to investigate these pathways in relation to insulin resistance in skeletal muscle, adipose tissue, and PBMCs. Therefore, we hypothesized that sustained activation of NF-κB and/or JNK1 in PBMCs and/or insulin-sensitive tissues such as skeletal muscle and subcutaneous adipose tissue contributes to development of insulin resistance in nondiabetic and otherwise healthy individuals.

**RESEARCH DESIGN AND METHODS**

Twenty-two volunteers (10 women) aged between 18 and 50 years underwent a rigorous protocol using standard measures of obesity and insulin resistance. Specifically, participants were nondiabetic (normal glucose tolerance or impaired glucose tolerance) according to an oral glucose tolerance test (OGTT) (World Health Organization 1990 criteria), nonsmokers at the time of the study, and healthy according to a detailed physical examination and routine blood analyses. No participants had clinical or laboratory signs of acute or chronic infection or took any medication or used illicit drugs at the time of the study. The protocol was approved by the Alfred Hospital Ethics Committee and complied with the Declaration of Helsinki 2004. All subjects provided written informed consent before participation. All participants were characterized for plasma inflammation markers, body composition, glucose tolerance, insulin action, and NF-κB and JNK1 activity in PBMCs and muscle and adipose tissue (Table 1).

All subjects underwent medical screening, which included history, physical examination, and basic laboratory tests (including fasting plasma lipid levels, liver function tests, urea, creatinine, and electrolyte levels, urinary illicit drug screening, an anthropometric assessment, an OGTT, and a hyperinsulinemic-euglycemic glucose clamp. Before metabolic testing, participants were asked to abstain from strenuous exercise and caffeine for 3 days. All of the metabolic testing was performed after a 12-h overnight fast.

**Anthropometric measurements.** Body composition was estimated by total-body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) with calculations of percent body fat, fat mass, and fat-free mass as described previously (11). Waist and hip circumferences were measured and waist-to-hip ratio (WHR) was calculated as an index of body fat distribution.

**Metabolic studies.** A 2-h 75-g OGTT was performed after a 12-h overnight fast, and glucose tolerance status was determined by World Health Organization 1999 criteria. Plasma glucose concentrations were determined by the glucose oxidase method (EML 105; Radiometer, Copenhagen, Denmark). Insulin action was assessed at physiological insulin concentrations during a hyperinsulinemic-euglycemic clamp. In brief, after an overnight fast, a primed continuous intravenous insulin infusion (9 mU/kg) was administered for 120 min at a constant rate of 40 mU/m² body surface area per minute (M). Plasma glucose was measured every 5 min during the clamp, and the variable infusion rate of glucose was adjusted to maintain blood glucose at a constant value of 4.5–6.5 mmol/L. This infusion achieved steady-state plasma insulin concentrations. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 40 min of the insulin infusions. Blood samples for analysis of inflammation markers were drawn before the start of the glucose clamp.

**Muscle and adipose tissue biopsies.** Biopsies of subcutaneous adipose and vastus lateralis muscle were performed on a separate day using standard aseptic technique and local anesthesia. In brief, before a percutaneous muscle biopsy of the vastus lateralis muscle, a scalpel blade was used to make a 7-mm skin incision and to cut the fascia. A side-cutting muscle biopsy needle was passed through the incision to obtain ~100 mg of muscle tissue. Adipose tissue was obtained by needle biopsy from the abdominal area, 1–2 cm superior to McBurney’s point. After making an ~5-mm skin incision, the needle biopsy was performed using a 50-ml plastic syringe attached to a 13-gauge aspiration needle. The muscle and adipose tissues were immediately placed in liquid nitrogen and then stored at −80°C.

**Blood sample analysis.** Blood samples were drawn using standard phlebotomy techniques into sodium citrate Vacutainers for measurement of inflammatory markers. The tubes were centrifuged immediately (1,500g, 15 min, 4°C), and the plasma was stored at −80°C until analyses were performed. Monocyte chemoattractant protein-1 (MCP-1) was measured using a Beadlyte kit (Upstate Cell Signaling Solutions, Charlotte, VA). Multiplexed assays were run according to the manufacturer’s instructions on a Luminex 100 Bioplex machine using Luminex Pro software version 1.7 (Bio-Rad, Atherton, CA). Plasma high-sensitivity C-reactive protein (hsCRP) was measured by an immunoturbidimetric assay. Hepatic enzymes, alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT), were measured by NAH (without P-5-P) and L-γ-glutamyl-γ-carboxy-4-nitroanilide substrates, respectively. hsCRP and hepatic enzymes (ALT and GGT) were measured by Alfred Hospital Pathology Services using the Architect system (Abbott Diagnostics, Abbott Park, IL).

**NF-κB activity measurements.** PBMCs were isolated from whole blood using Ficol-Paque Plus density centrifugation (Amersham Biosciences, Upp.

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**TABLE 1**

|                | All   | Women | Men  |
|----------------|-------|-------|------|
| n              | 22    | 10    | 12   |
| Age (years)    | 29±1  | 31±1  | 27±1 |
| Weight (kg)    | 75±1  | 71±1  | 79±9 |
| BMI (kg/m²)    | 25±4  | 24±5  | 26±4 |
| Waist circumference (cm) | 84±10 | 82±11 | 85±10 |
| WHR            | 0.85±0.08 | 0.82±0.07 | 0.88±0.08 |
| Body fat (%)   | 28±11 | 36±7  | 22±9*|
| Fasting glucose (mmol/l) | 4.5±0.5 | 4.3±0.4 | 4.7±0.5 |
| 2-h glucose (mmol/l) | 4.6±1.3 | 4.7±1.6 | 4.6±1.1 |
| M (mg kg⁻¹ min⁻¹) | 10.3±4.4 | 10.7±5.3 | 9.9±3.8 |
| White blood cells (10⁹/l) | 5.8±1.0 | 5.8±1.1 | 5.9±1.0 |
| hsCRP (mg/l)   | 1.1±1.1 | 1.4±1.2 | 0.9±0.9 |
| MCP-1 (ng/ml)  | 15.3±17.5 | 10.7±11.7 | 19.1±20.0 |
| ALT (units/l)  | 17±5  | 13±4  | 20±10|
| GGT (units/l)  | 21±14 | 16±5  | 26±18|

Data are means ± SD. *P < 0.05 male vs. female subjects.

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*Note: All Women Men

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fluorescence for each cycle was quantitatively analyzed by an ABI Prism 7700 sequence detection system (PE Biosystems, Foster City, CA). Samples were then rotated for 30 min, end-over-end, at 4°C, before being centrifuged (14,000 × g, 4°C), to remove residual FBS and DMSO. The supernatant was discarded, and the pellets were resuspended in 150 μl Tris-HCl, pH 8.0, 150 mmol/l NaCl, 0.02% NaN₃, 0.1% SDS, 100 mmol/l Tris, pH 8.0, 150 mmol/l NaCl, 1 mmol/l EDTA, and 0.1 mmol/l sodium pyrophosphate, 250 μmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotilin, and 10 μg/ml leupeptin) with a hand-held homogenizer (Polytron- Aggregate; Kinematica, Switzerland). Supernatant was removed, and the pelleted was resuspended in 150 μl buffer C (20 mmol/l HEPES, pH 7.9, 100 mmol/l KCl, 0.1 mmol/l EDTA, and 0.1 mmol/l EGTA), incubated on ice for 20 min, and then centrifuged (10,000 × g, 30 min, room temperature). The resulting supernatant was used in the NF-κB DNA-binding assay.

PBMC samples were washed in excess PBS and centrifuged (400 × g, 5 min, 4°C), to remove residual FBS and DMSO. The supernatant was discarded, and the pellets were resuspended in 100 μl triple detergent buffer (50 mmol/l Tris-HCl, pH 8.0, 150 mmol/l NaCl, 0.02% NaN₃, 0.1% SDS, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotilin, 1% Nonidet P-40, and 0.5% sodium deoxycholate, in Milli-Q H₂O). These were then sonicated for 1 min and centrifuged (400g, 3 min, 4°C). The resulting supernatant was used in the NF-κB assay. The protein concentration of all protein isolates was determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL), performed according to the manufacturer’s instructions.

The TransAM NF-κB DNA-binding activity assay (Active Motif, Carlsbad, CA) was used to detect and quantify NF-κB transcription factor activity, specifically of the p65 subunit. Nuclear extracts obtained from muscle (1 μg protein/well) and adipose tissue (5 μg protein/well) biopsies and PBMC (30 μg protein/well) samples were analyzed for their binding capacity to an NF-κB consensus sequence in labeled DNA in a 96-well plate format. The assays were performed according to the manufacturer’s instructions, and absorbance was measured on a Victor3 Multilabel plate reader (PerkinElmer, Wellesley, MA). Results are expressed as nanograms of p65 activity per microgram of protein.

**JNK1/2 activity measurements.** Phosphorylation of JNK ([(Thr^{183}/Tyr^{185}]/ total JNK) was used as a surrogate for JNK1/2 activity as described previously (21). PBMC, subcutaneous adipose tissue, and skeletal muscle samples were homogenized, and protein concentrations were determined. Total protein (40 μg) was separated by SDS-PAGE and blotted onto a polyvinylidene fluoride membrane. Blots were performed for phosphorylated JNK (1:1,000) and total JNK (1:1,000) using antibodies from Cell Signaling Technologies. Bands were visualized using a Bio-Rad anti-rabbit IgG horseradish peroxidase (1:4,000) conjugated antibody and ECL solution (Sigma) and developed on film. Bands were quantified using Quantity One Imaging software. JNK1/2 activity in muscle and adipose tissue was available for only 18 individuals because insufficient tissue was available for analyses.

**Real-time analysis (RT-PCR).** Gene expression of the p65 subunit of NF-κB and IκBα in PBMCs was assessed by real-time quantitative RT-PCR, performed using the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7700 sequence detection system; PE Biosystems, Foster City, CA) as described previously (23). Briefly, 4 μg total RNA was isolated from PBMCs with TRIzol according to the manufacturer’s instructions (Gibco BRL, Grand Island, NY). The resulting RNA was used to synthesize cDNA with a Superscript First Strand synthesis system (Gibco BRL). To control for variation in the amount of DNA available for PCR in the different samples, gene expression was normalized simultaneously (by multiplexing) in relation to the expression of the housekeeping gene 18S rRNA (18S rRNA TaqMan Control Reagent kit) as an endogenous control. Primers and TaqMan probes for the genes described above were constructed with the help of Primer Express (PerkinElmer) (for sequences, see Table 2) and fluorescence for each cycle was quantitatively analyzed by an ABI Prism 7700 sequence detection system. Results are expressed as arbitrary units, related to minor groove binding (MBG) probe sample fluorescence, corrected to endogenous 18S expression.

**Statistical analysis.** Statistical analyses were performed using SAS Jump statistics software (SAS Institute, Cary, NC). Results are given as means ± SD (unless indicated otherwise). The values for inflammatory markers and M and I were logarchronically transformed before analysis to approximate normal distributions.

The relationships between inflammatory markers, NF-κB, JNK1/2, and anthropometric and metabolic variables were examined by calculating Pearson correlation coefficients, and P values were corrected for multiple comparisons (Benjamini-Hochberg procedure). Multiple linear regression models and partial correlations were used to examine the relationships after adjustment for covariates. Stepwise regression was used to determine a relative contribution of each of the variables to the model. Differences between men and women were assessed by unpaired Student’s t test. Statistical significance was assumed when P < 0.05.

Our correlation tests are sufficiently sensitive at α = 0.05 with a power of 1 − β = 0.80 for two-sided correlations at r = 0.53 and for one-sided correlations at r = 0.48 (n = 22 degrees of freedom = 20). Because inflammation markers are known to be positively associated with obesity and negatively with insulin sensitivity, the one-sided calculation is most relevant to the current analyses. Post hoc statistical power calculations for the multiple regression models of our main findings, which all had R² > 0.6, give power estimates greater than 0.90 for up to five independent variables in the model at α = 0.05.

**RESULTS**

The anthropometric and metabolic characteristics of the whole study population are summarized in Table 1. All subjects were deemed healthy according to their physical examination, were normotensive with normal lipid profile, full blood count, plasma CRP levels, liver function tests (Table 1), and renal function (estimated glomerular filtration rate < 60 ml/min per m²), and therefore subclinical inflammation was ruled out. Women had higher percent body fat and HDL cholesterol levels, but there were no other sex differences seen among any of the other anthropometric and metabolic variables.

**Relationship between NF-κB and JNK1/2 activity.** NF-κB activity in PBMCs was positively associated with NF-κB activity in the skeletal muscle (r = 0.68, P = 0.0004) but not in adipose tissue (r = −0.17, P = 0.4). JNK1/2 activity in PBMCs was not related to JNK1/2 activity in skeletal muscle (r = −0.14, P = 0.6) or subcutaneous adipose tissue (r = −0.34, P = 0.2). In addition, no relationship between JNK1/2 activity and NF-κB activity was identified in any of the tissues studied (all P > 0.2).

**Relationship of NF-κB and JNK1/2 to inflammation markers.** White blood cell count (r = 0.39, P < 0.05) and plasma levels of MCP-1 (r = 0.63, P < 0.05) were each associated with NF-κB activity in PBMCs but not with NF-κB activity in the skeletal muscle or subcutaneous adipose tissue (Table 3). Plasma hsCRP (Table 3) was not related to NF-κB activity in any of the tissues investigated and did not correlate with insulin sensitivity (r = 0.20, P = 0.4). JNK1/2 activity was not associated with any of these markers of inflammation (P > 0.1).

**Relationship of NF-κB and JNK1/2 to obesity.** NF-κB activity in either PBMCs or skeletal muscle was not associated with an overall measure of obesity, percent body fat (Table 3, Fig. 1). NF-κB activity in subcutaneous adipose tissue also was not related to any of the anthropometric variables. Furthermore, the gene expression of IκBα and NF-κB (p65) in PBMC did not correlate with any other sex differences seen among any of the other anthropometric and metabolic variables.
of the anthropometric or metabolic parameters or NF-κB activity in PBMCs (data not shown). However, we found that NF-κB activity in both PBMCs and skeletal muscle was positively associated with WHR (r = 0.48, P = 0.02; r = 0.68, P = 0.003) (Table 3, Fig. 1) before and after adjustment for age and sex (both P < 0.05). Tissue JNK1/2 activity was not related to any measures of obesity (both P > 0.1) (Table 3).

**Relationship of NF-κB and JNK1/2 to insulin sensitivity.** NF-κB activity in PBMCs was inversely associated with insulin sensitivity before (r = −0.46, P = 0.03) (Table 3) and after adjustment for age, sex, percent body fat, and WHR (P = 0.02). In stepwise regression, elevated NF-κB activity in PBMCs explained 16% of the variance in the model after adjustment for the variables stated above. NF-κB activity in skeletal muscle and adipose tissue were not associated with insulin sensitivity (both P > 0.1).

JNK1/2 activity in the adipose tissue was related to insulin sensitivity (r = −0.54, P < 0.05) (Fig. 2) before and after adjustment for age, sex, percent body fat, and WHR (P = 0.04). In stepwise regression with age, sex, percent body fat, and WHR, JNK1/2 activity in adipose tissue explained the 29% variance in insulin sensitivity (P = 0.02).

### TABLE 3
Pearson correlation coefficients between NF-κB and JNK1/2 activity in PBMCs and muscle and adipose tissue with anthropometric and metabolic variables

|            | NF-κB                | JNK1/2              |
|------------|----------------------|---------------------|
|            | PBMCs | Muscle | Adipose tissue | PBMCs | Muscle | Adipose tissue |
| Age        | −0.16 | −0.11  | 0.35           | −0.009 | −0.09  | 0.01           |
| % body fat | 0.20  | −0.09  | 0.21           | −0.14  | −0.12  | 0.07           |
| Waist      | 0.17  | 0.29   | 0.08           | 0.06   | −0.29  | 0.32           |
| WHR        | 0.48* | 0.68*  | 0.03           | 0.14   | −0.40  | 0.04           |
| M          | −0.46* | −0.29  | −0.15          | −0.08  | 0.23   | −0.54*          |
| WBC        | 0.39  | 0.20   | 0.18           | 0.13   | −0.20  | 0.04           |
| hsCRP      | 0.11  | 0.13   | −0.03          | −0.41  | 0.39   | −0.002          |
| MCP-1      | 0.63* | 0.36   | 0.21           | 0.02   | 0.03   | 0.21           |

*P < 0.05. WBC, white blood cell count.

![FIG. 1. NF-κB p65 activity in PBMCs and central obesity, insulin sensitivity, and JNK1 activity in subcutaneous adipose tissue and insulin sensitivity. A: NF-κB p65 activity in PBMCs and WHR. B: NF-κB p65 activity in PBMCs and total body fat. C: NF-κB p65 activity in PBMCs and M. D: JNK1/2 activity and insulin sensitivity (M).](image-url)
When both JNK1/2 in adipose tissue and NF-κB activity in PBMCs were entered in the same model, only JNK1/2 activity in adipose tissue remained a significant determinant of insulin sensitivity, explaining 29% \((P = 0.02)\), whereas percent body fat contributed 21% \((P = 0.02)\) and sex 10% \((P = 0.07)\). When forced into this model, NF-κB activity in PBMCs explained only 4% of the variance in insulin sensitivity \((P = 0.1)\). JNK1/2 activity in PBMCs and muscle was not associated with any measures of obesity or glucose metabolism \((all \ P > 0.2)\). Exclusion of the two subjects with impaired glucose metabolism (one with impaired glucose tolerance and one with impaired fasting glucose) did not significantly alter the correlations presented.

### DISCUSSION

In the present study, we showed for the first time that, among healthy, nondiabetic individuals, both NF-κB activity in PBMCs and JNK1/2 activity in subcutaneous adipose tissue were important determinants of insulin resistance and explained 16 and 29%, respectively, of its variance after adjustment for appropriate covariates. Moreover, NF-κB activity in both PBMCs and skeletal muscle was also associated with central obesity.

The cross-sectional relationships between NF-κB activity in PBMCs and adiposity were consistent with previous findings in obese patients in whom NF-κB activity in PBMCs was elevated \((20,24)\) and correlated with BMI \((20,25)\). We added to the current evidence by showing this relationship across a wide range of adiposity. We also provided more precise measures of overall adiposity, specifically percent body fat by dual-energy X-ray absorptiometry as opposed to BMI, but, interestingly, our study showed that NF-κB activity in PBMCs was specifically associated with central obesity but not overall adiposity \((measured \ as \ total \ percent \ body \ fat \ or \ BMI)\), perhaps because we have studied participants with a wide range of adiposity as opposed to only morbidly obese patients. In the above-mentioned studies \((20,24)\), the relationship between central adiposity and NF-κB activity was not investigated. It is well established that central adiposity is a stronger predictor than overall adiposity of insulin resistance and progression to type 2 diabetes than overall adiposity and is likely to be responsible for the proinflammatory phenotype seen in these conditions \((26–31)\). Thus, our additional findings showed for the first time a relationship between NF-κB activity in skeletal muscle and central obesity, suggesting that activation of the NF-κB/IκB pathway in muscle may contribute to a local inflammatory environment due to lipid accumulation and contributing to insulin resistance.

In accordance with two other studies \((20,24)\), we also found a relationship between increased NF-κB activity in PBMCs and insulin resistance. In contrast to these studies, which used a calculated HOMA as a proxy for insulin resistance, we examined insulin resistance directly using the standard hyperinsulinemic-euglycemic clamp. Moreover, we showed that the relationship between NF-κB activity in PBMCs and insulin resistance is independent of the degree of obesity and explained 16% of variance in insulin resistance after adjustment for age, sex, percent body fat, and WHR. Although previous studies have shown that NF-κB activation in skeletal muscle is related to insulin resistance in obese humans \((20,24)\), this was not seen in our study in nondiabetic healthy individuals. Our data are consistent with rodent studies in which overexpression of the p65 subunit of NF-κB in skeletal muscle had no effect on insulin resistance as measured by glucose clamp \((32)\). In our study, we also found no relationship between activation of NF-κB in adipose tissue and any of the anthropometric or metabolic parameters. Animal models of type 2 diabetes have demonstrated an association between insulin resistance and elevated levels of the activator of NF-κB, IκkB, at the gene level, in perigonadal but not in mesenteric adipose tissue \((33)\). Gene levels of the inhibitor of NF-κB (IkBα) have also been associated with central adiposity in obese elderly women \((34)\). Both of these studies \((33,34)\), however, examined only gene expression as opposed to activity. The absence of an association between NF-κB activity in adipose tissue and anthropometric or metabolic parameters in the present study could also be due to differences in the populations examined. Both of the previously reported studies described the relationship in obese and insulin-resistant animals/populations \((33,34)\), whereas our study was conducted in a healthy population.

Interestingly, NF-κB activity in PBMCs was associated with circulating MCP-1 levels but not with CRP. MCP-1 is one of the many downstream cytokine targets of the key proinflammatory transcription factor, NF-κB. MCP-1 is known to recruit white blood cells to the site of inflammation and therefore may be considered an important driver and perpetuator of NF-κB activation and inflammation \((35)\). NF-κB activity in PBMCs and adipose tissue have been shown previously to correlate with the gene \((20,34)\) and protein \((20)\) expression of interleukin-6, macrophage inhibitory factor, and hsCRP in obese individuals. In our study, however, plasma levels of CRP were not associated with either NF-κB activity or insulin sensitivity probably because of the low plasma levels of CRP \((all \ values \ within \ normal \ range)\) in our healthy nondiabetic population. Therefore, NF-κB may be a more sensitive early marker of inflammation than CRP in healthy individuals. We have made the novel observation that NF-κB activity in PBMCs is an important determinant of both central adiposity and insulin resistance. Interestingly, Arkan et al. \((13)\) demonstrated that mice lacking IKK in PBMCs, which makes them unable to activate the NF-κB/IκB pathway, are protected from systemic insulin resistance. Our data demonstrated this relationship for the first time in humans.

To date, JNK1/2 activity has only been associated with obesity in animal models of obesity and diabetes \((9)\). In this study, we showed that JNK1/2 activity in PBMCs, skeletal muscle, and subcutaneous adipose tissue was not associated with any measure of obesity in humans.
In the current study we also showed that JNK1/2 activity in subcutaneous adipose tissue was associated with insulin resistance, independently of age, sex, percent body fat, and WHR and explained 29% of the variance in insulin resistance after adjustment for these covariates. In this study, we have made the novel observation that when both NF-κB activity in PBMCs and JNK1/2 activity in subcutaneous adipose tissue are included in this model, only JNK1/2 activity was a significant determinant of insulin resistance, suggesting not only that these pathways are codependent but also that JNK1/2 activity is an independent and therefore potentially more important determinant of insulin resistance in nondiabetic, otherwise healthy individuals. JNK activity in skeletal muscle has been shown to be associated with insulin resistance in obese and diabetic subjects (22). We have also shown previously that obese insulin-resistant humans (insulin sensitivity assessed by HOMA) have increased JNK1/2 phosphorylation in skeletal muscle (21). In our present study, however, we did not see a relationship between JNK1/2 activity in skeletal muscle and insulin resistance, most likely because of our different study population, i.e., healthy nondiabetic individuals versus obese, type 2 diabetic subjects. Adipose tissue JNK activity was not examined in either of these previous studies (21,22), so we cannot compare our results in this regard. It could be hypothesized that different inflammatory pathways may be important in the initiation of insulin resistance as opposed to when diabetes has already developed. In our study, tissue JNK1/2 activity was not associated with circulating inflammatory makers, suggesting that tissue-specific inflammation and activation of JNK are enough to cause insulin resistance.

Our study has several limitations. First, because of the cross-sectional nature of the study, we cannot delineate the cause and effect relationships between JNK and NF-κB activity and insulin resistance. Second, the sample size is small, and, hence, the results should be interpreted with caution. However, the strength of the correlations and their robustness in various adjusted models attests to the validity of our findings. Third, standard imaging methods such as computed tomography or magnetic resonance imaging would have provided a better assessment of central adiposity than waist circumference.

In conclusion, we have demonstrated that elevated NF-κB activity in PBMCs and JNK1/2 activity in subcutaneous adipose tissue are both important determinants of insulin resistance in a healthy nondiabetic population. Moreover, we show that JNK1/2 activity in adipose tissue but not NF-κB activity in PBMC is an independent determinant of insulin resistance in this population. Further investigation is warranted to determine the mechanisms by which changes in NF-κB and JNK1/2 activity contribute to the development of type 2 diabetes in humans.

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