Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes

S. J. Creely,1,2 P. G. McTernan,1 C. M. Kusminski,1 ff. M. Fisher,1 N. F. Da Silva,1 M. Khanolkar,3 M. Evans,3 A. L. Harte,1 and S. Kumar1,2

1Diabetes and Metabolism Research Laboratories, Clinical Sciences Research Institute, Warwick Medical School; 2University Hospital of Coventry and Warwickshire, Coventry, England; and 3Heart Research Institute, Cardiff University, Wales, United Kingdom

Submitted 23 June 2006; accepted in final form 5 November 2006


Obesity is known to represent one of the single most important risk factors for the increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease. In addition, an increase in central (visceral) adiposity confers higher metabolic risk. This increased metabolic risk is associated with subclinical inflammation, with several studies demonstrating increased levels of proinflammatory adipokines, such as IL-6 and TNF-α (32, 33), in patients with obesity and T2DM. Activation of proinflammatory adipokines in adipose tissue (AT) is coordinated through NF-κB, a key transcription factor in the inflammatory cascade (2, 10, 11, 18, 21, 22, 33, 35, 37, 38). Adipocytes also secrete adiponectin (29, 30, 36, 41, 42), which has been shown to possess anti-inflammatory properties through its action on NF-κB and is inversely correlated with obesity and diabetes (29, 30, 36, 41, 42). Evidence for the role of NF-κB in AT has been shown in studies overexpressing the NF-κB activator IKKβ in mice, which resulted in increased inflammatory cytokine production and the onset of diabetes (7). In contrast, hepatocyte IKKβ knockout (KO) mice demonstrated a decrease in circulating proinflammatory cytokines (3). This indicates that IKKβ KO mice do not develop hepatic insulin resistance and glucose intolerance compared with their high-fat diet-fed counterparts. Further studies also illustrate that an inflammatory reaction, induced by the bacterial endotoxin lipopolysaccharide (LPS), is markedly attenuated in the IKKβ KO mice (3).

The interactions among obesity, hyperinsulinemia, and endotoxin have clear implications for the immune system. Studies examining immune competence of Kupffer cells in the liver
have highlighted that these cells exhibit decreased functionality during hyperinsulinemia (14). This may cause a possible reduction in clearance of LPS [absorbed from the gastrointestinal (GI) tract] in hyperinsulinemic subjects. Impairment of Kupffer cell function can also lead to increased systemic insulin secretion in response to glucose stimulation (13). In addition, endotoxemia may aggravate hyperinsulinemia, as studies have shown a direct link between increasing systemic LPS and the secretion of insulin (12). This may, therefore, form a positive “feed forward” mechanism leading to insulin resistance, the oversecretion of insulin, and subsequent inflammation.

Our hypothesis is that chronic activation of the innate immune system in AT, a feature of obesity-related hyperinsulinemia, is a consequence of low-grade endotoxemia caused by the effect of insulin on immune competence and/or intestinal motility and/or intestinal permeability. Therefore, this present study will 1) investigate the protein expression of key components of the innate immune pathway in human AT as well as the differential regulation in obesity and T2DM; 2) demonstrate the in vitro capacity of LPS to stimulate the secretion of proinflammatory cytokines through activation of the innate immune pathway in human AT; 3) assess the associations among LPS, insulin, proinflammatory cytokine release, and the serum receptors for LPS clearance, sCD14, in the defined cohorts; and 4) examine the potential proinflammatory effects of an insulin sensitizer in reducing circulating insulin and LPS in T2DM subjects.

MATERIALS AND METHODS

Subjects. Whole abdominal subcutaneous (AbdSc) AT was obtained from lean nondiabetic (ND) subjects (BMI 23.6 ± 1.5 kg/m², age 45.7 ± 7.5 yr, n = 23 female) and obese ND subjects (BMI 32.5 ± 4.0 kg/m², age 46.8 ± 11.0 yr, n = 16, female:male 4:1) during elective abdominal surgery. T2DM subjects provided AbdSc AT biopsy (age 61 ± 10.2 yr, BMI: 28.2 ± 3.6 kg/m², male n = 5). Subjects providing fat samples were not on endocrine therapy, (e.g., steroids, hormone replacement therapy, thyroxine) or receiving any antihypertensive therapy and were not diabetic, unless otherwise stated. All studies were performed with the approval of the local ethics committee (Cardiff/Coventry and Warwickshire/South Birmingham), and patients provided informed consent.

Isolation and cell culture of mature adipocytes. AbdSc AT was digested in collagenase (2 mg/ml; Worthington Biochemical) to isolate the AbdSc adipocytes, as previously described (25). Following isolation of these cells, compacted aliquots of mature adipocytes (1 ml, 500,000 adipocytes) were cultured in phenol red-free DMEM (96°C) with 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris. These samples were subsequently flash-frozen in liquid nitrogen, thawed, and centrifuged (800 g, 4°C). The resulting infranatant was extracted from under the lipid layer and stored immediately at −80°C. Viability of adipocytes was assessed using the trypan blue exclusion dye method as previously documented (25) (Sigma UK).

Extraction of adipocyte RNA and quantitative RT-PCR. RNA was extracted from human whole AbdSc AT using the RNeasy Lipid Tissue kit (Qiagen UK) according to the manufacturer’s instructions. Extraction was followed by a DNase digestion step to remove any contaminating genomic DNA. RNA was quantitated using the Nanodrop ND-1000 spectrophotometer (LabTech UK) and 1 μg of RNA from each sample was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen UK) and random hexamers according to the manufacturer’s instructions. RT-PCR was performed in a reaction mix containing TaqMan Universal PCR Master Mix (AmpErase UNG), 100–200 nmol TaqMan probe, 900 nmol primers, and 25 ng of cDNA. All reactions were multiplexed with the housekeeping gene 18S, provided as a preoptimized control probe (Applied UK) enabling data to be expressed as Δ cycle threshold (ΔCT) values (where ΔCT = C T 18S − C T gene of interest). Measurements were carried out on at least three occasions for each sample. Reactions were as follows: 50°C for 2 min and 95°C for 10 min and then 44 cycles of 95°C for 15 s and 60°C for 1 min.

Primer and probe sequences for TLR-2 and TLR-4 expression were assessed using a predefined assay-on-demand kit (Applied UK). The expression of each gene was compared between depots using the ΔΔCT method (1). All statistics were performed at the ΔCT stage to exclude potential bias due to averaging of data transformed through the equation 2−ΔΔCT. Statistical analysis was undertaken using an unpaired Student’s t-test (SPSS 12, SPSS).

Protein determination and Western blot analysis. Homogenized AbdSc AT and isolated AbdSc adipocytes were resuspended in 4% RIPA buffer, as previously described (26). Protein concentrations were determined using the Bio-Rad DC (Detergent Compatible) protein assay kit (5). Western blot analysis was performed and relative expression standardized using densitometry quantification software (GeneTools; Geneflow, Fradley, UK). In brief, 10–40 μg of protein were loaded onto a 10% polyacrylamide gel (Geneflow). A human monoclonal TLR-2 primary antibody (97 kDa, TLR-2; HyCult Biotechnology, Uden, The Netherlands) and a human polyclonal anti-TLR 4 (97 kDa; Santa Cruz Biotechnology, Santa Cruz, CA) were used to assess the presence of the TLRs in AbdSc AT and AbdSc adipocytes. TLR-2 and TLR-4 were developed using mouse and rabbit horseradish-peroxidase (HRP) secondary antibodies, respectively (2 mg/ml; The Binding Site, Birmingham, UK). A polyclonal myeloid differentiation marker 88 (MyD88) antibody (32 kDa, 1:250; TCS Cellworks UK) and a polyclonal TNF receptor-associated factor 6 (TRAF6, 54 kDa) antibody (1:500, TCS, Cellworks UK) were used to assess MyD88 and TRAF6 protein expression in AbdSc AT and control and LPS-treated adipocytes. MyD88 and TRAF6 were both developed using an anti-rabbit HRP secondary antibody (The Binding Site). Protein expression in AbdSc AT for NF-κB (65 kDa, 1:250, TCS Cellworks) was assessed, as described above, using mouse monoclonal antibodies. Equal protein loading for these samples was confirmed by examining α-tubulin expression by means of Western blotting, as previously described (25). A chemiluminescent detection system ECL/ECL+ (GE Healthcare, Little Chalfont, UK) enabled visualization after exposure to X-ray film. Autoradiographs were quantified by densitometry using Synoptics Group Gene tools Bio Imaging system software (Syngene, Cambridge, UK) according to the manufacturer’s guidelines.

Conditioned medium cytokine assessment. Conditioned media from untreated (control) AbdSc adipocytes and LPS-treated AbdSc adipocytes were assayed for IL-6 and TNF-α (Quantiglo ELISA; RandD Systems, Abingdon, UK) by use of a solid-phase enzyme-linked immunosorbant assay (IL-6 intra-assay CV 3.1%, interassay CV 2.7%; TNF-α intra-assay CV 6.7%, interassay CV 11.0%).
Assessment of circulating levels of inflammation markers in subjects. Fasting serum samples were obtained from 25 ND subjects who had no significant medical illness and 25 sex-, BMI-, and age-matched T2DM patients without known diabetic complications or inflammatory conditions. These patients had a history of T2DM for at least 1 yr. From this diabetic cohort, 11 of the patients were diet controlled, 10 treated with oral hypoglycemics, and 4 treated with insulin and oral hypoglycemics. All patients remained on therapy during sampling. Ethical approval was obtained and informed consent gained from subjects for all studies undertaken. Samples were analyzed for assessment of cytokines, insulin, and glucose levels, as further described below.

**T2DM patients treated with rosiglitazone.** Fasting baseline and 10-wk-posttreatment sera were obtained from a cohort of newly diagnosed, previously untreated T2DM patients treated with the insulin sensitizer rosiglitazone (RSG; see Table 2). These samples were utilized to examine the effect of reducing circulating insulin levels on endotoxin concentrations. Samples were analyzed for assessment of cytokines, insulin, and glucose levels, as further described below.

Measurement of circulating cytokines and hormones. Serum samples were analyzed for the determination of insulin (Linco Research, St. Charles, MO), leptin (Linco Research), IL-6 and TNF-α (Bender MedSystems, Vienna, Austria), and sCD14 (RandD Systems, Abingdon, UK) protein concentrations. Insulin, leptin, sCD14, TNF-α, and IL-6 levels were analyzed by a solid-phase enzyme-linked immunosorbent assay (Insulin CV intra-assay 5.96%, interassay 10.3 ± 0.9%; leptin CV intra-assay 2.6%, interassay 3.8 ± 3.4%; sCD14 intra-assay 5.4%, interassay 6.3%; TNF-α CV intra-assay 6.0%, interassay 9.3%; IL-6 CV intra-assay 6.2%, interassay 7.0%). For these studies, anthropometric data were collected and are detailed in Tables 1 and 2. Fasting glucose was analyzed using a glucose oxidase method (YSL 200 200 STAT plus). Insulin resistance [homeostasis model assessment (HOMA-IR)] was derived using the HOMA equation (24).

**Measurement of circulating endotoxin levels.** Serum endotoxin was assayed using a chromogenic limulus amebocyte lysate (LAL) test, which is a quantitative test for gram-negative bacterial endotoxin (Cambrex). Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the LAL. The initial rate of activation is directly determined by the concentration of endotoxin. The activated enzyme catalyzes the splitting of n-nitroaniline (pNA) from the colorless substrate Ac-Ile-Glu-Ala-Arg-pNA. The pNA released was measured photometrically at 405–410 nm following termination of the reaction. The correlation between the absorbance and the endotoxin concentration is linear in the 0.1–1.0 EU/ml range (intra-assay CV 0.75%). For the purposes of this study, all samples were run in duplicate within the same plate; therefore, no interassay variability was observed in this study.

### Table 1. Clinical characteristics for ND and T2DM subjects

<table>
<thead>
<tr>
<th></th>
<th>T2DM (n = 25)</th>
<th>ND (n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>31.8 ± 4.5</td>
<td>29.5 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>20.5</td>
<td>20.5</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>52.2 ± 11.7</td>
<td>48.1 ± 19.2</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>8.6 ± 2.5</td>
<td>5.6 ± 0.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, inv log IU/ml (geometric mean)</td>
<td>12.0 (1.8)</td>
<td>8.9 (1.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Endotoxin, inv log EU/ml (geometric mean)</td>
<td>5.5 (1.6)</td>
<td>3.1 (1.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>16.8 ± 7.0</td>
<td>6.61 ± 4.3</td>
<td>0.00865</td>
</tr>
<tr>
<td>sCD14, million IU/ml</td>
<td>2.81 ± 1.2</td>
<td>1.39 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>4.95 ± 2.7</td>
<td>4.24 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>24.2 ± 21.1</td>
<td>28.2 ± 22.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD or geometric means. Glucose, insulin, and endotoxin data for BMI-, age-, and sex-matched control [nondiabetic (ND)] and type 2 diabetes (T2DM) serum samples.

### Table 2. Clinical characteristics before and following RSG treatment

<table>
<thead>
<tr>
<th></th>
<th>Pre-RSG (n = 10)</th>
<th>Post-RSG (n = 10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>34.7 ± 7.3</td>
<td>36.2 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4:3</td>
<td>N/A</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>55.7 ± 0.2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>9.6 ± 3.2</td>
<td>7.1 ± 1.6</td>
<td>0.0313</td>
</tr>
<tr>
<td>Insulin, IU/ml (geometric mean)</td>
<td>14.27 (1.6)</td>
<td>7.0 (2.1)</td>
<td>0.0395</td>
</tr>
<tr>
<td>Endotoxin, EU/ml (geometric mean)</td>
<td>6.94 (1.65)</td>
<td>4.5 (1.96)</td>
<td>0.0139</td>
</tr>
</tbody>
</table>

Values are means ± SD or geometric means. Glucose, insulin, and endotoxin data for treatment-naive T2DM patients before and after rosiglitazone (RSG) treatment, respectively.

To assess recovery of endotoxin within the assay, known concentrations of recombinant endotoxin (0.25 and 1.00 EU/ml) were added to diluted, pooled plasma to determine whether the expected concentration correlated closely with the actual observed value and whether there were any variations due to reaction with plasma contents.Lyophilized endotoxin (E. coli origin) was used to generate a standard curve with the chromogenic LAL test kit from Cambrex and produced a corresponding curve in accordance with the manufacturer’s instructions. In plasma, the recovery of spiked endotoxin was 82.0 ± 3.3% efficient. Plate-to-plate variability within the same experiment was 7.4 ± 0.9%. These findings were similar to those observed from assessment by Cambrex. Experiments were also performed to assess cross-reactivity between glucose, mixed triglycerides (Sigma Aldrich, Gillingham, UK), and LPS in the LAL test kit. Saline (endotoxin level <0.1 EU/ml, Sigma Aldrich) was spiked with 5 and 20 mM glucose, 1 and 5 mM lipid, and glucose in combination with lipid. These were then analyzed with the LAL test kit. For all the different concentrations of glucose, lipid, or glucose combined with lipid, there was no interference within the assay.

**Statistical analysis.** All variables were initially analyzed using a bivariate Pearson correlation to address any associations between clinical parameters. Serum levels of endotoxin were not normally distributed; therefore, analyses were performed on log-transformed data. Linear logged regression was used to assess the relationship between measurements of endotoxin and insulin, with residual plots applied. Protein expression data between control and treatment regimen were compared using an unpaired t-test. The threshold for significance was P < 0.05. Data in the text and figures are presented as means ± SD unless otherwise stated. Analyses were carried out using the SPSS v. 12 (SPSS, Chicago, IL) software package.

### RESULTS

**mRNA and protein expression of key innate immune pathway receptors in human isolated AbdSc adipocytes.** mRNA analysis demonstrated the presence of TLR-2 (ΔC_T 16.1 ± 0.52) and TLR-4 (ΔC_T 18.4 ± 0.29) in adipocytes across a range of BMIs (BMI 29.3 ± 3.2 kg/m², age 47 ± 10.0 yr, n = 10). Similarly, we also demonstrated TLR-2 and TLR-4 protein expression in AbdSc adipocytes. Treatment of AbdSc adipocytes with LPS increased the protein expression of TLR-2 twofold compared with control (P < 0.05; Fig. 1), whereas there was no change observed in TLR-4 protein expression with LPS treatment (P = NS; Fig. 1).

IL-6 and TNF-α secretion in response to LPS in cultured AbdSc adipocytes. Secretion of IL-6 and TNF-α was increased in the cultured adipocytes treated with 10 ng/ml LPS (IL-6 P < 0.01; TNF-α P < 0.05; Fig. 2).
Effect of NF-κB blockade in cultured AbdSc adipocytes. NF-κB inhibitor reduced the levels of IL-6 in AbdSc adipocytes compared with untreated adipocytes (control) (Control 2.7 ± 0.5 vs. NF-κB inhibitor 2.1 ± 0.4 ng/ml, P < 0.01; n = 6), whereas there was no significant change in TNF-α levels (Control 2.3 ± 0.9 vs. NF-κB inhibitor 2.0 ± 0.7 ng/ml, P = NS; n = 6).

Ex vivo expression of TLRs, MyD88, TRAF6, and NF-κB in AbdSc adipose tissue from T2DM subjects and matched ND controls. mRNA studies determined TLR-2 and TLR-4 mRNA expression in human AbdSc AT from the different cohorts. Furthermore, mRNA expression data demonstrated that both TLR-2 and TLR-4 were significantly higher in obese subjects compared with lean subjects (TLR-2 obese ΔC_T 18.19 ± 0.17, lean ΔC_T 18.84 ± 0.21, P < 0.05; TLR-4 obese ΔC_T 15.48 ± 0.16, lean ΔC_T 16.09 ± 0.17, P < 0.05; Fig. 3). The mRNA findings were confirmed by protein expression data, as TLR-2 was significantly higher in obese AbdSc AT when compared with AbdSc AT taken from lean subjects (P < 0.01, n = 5; Fig. 4). Additionally, TLR-2 was also higher in diabetic AbdSc AT compared with ND matched controls (P = 0.013, n = 5; Fig. 5). Although TLR-4 showed no change in AT expression in the obese cohort [lean TLR-4 5,993 ± 863 (SE) OD units, obese TLR-4 4,384 ± 1,098 OD units, P = NS] or diabetic cohort (ND TLR-4 4,805 ± 263 OD units, T2DM TLR-4 4,355 ± 269 OD units, P = NS). Analysis of MyD88, TRAF6, and NF-κB by Western blot confirmed that protein expression was increased within AbdSc AT taken from T2DM subjects compared with ND AbdSc AT controls (MyD88: T2DM > ND, P < 0.05; TRAF6: T2DM > ND, P < 0.01; NF-κB: T2DM > ND, P < 0.01; Fig. 6).

Expression of MyD88, TRAF6, and NF-κB in AbdSc adipocytes treated with LPS in vitro. Western Blot analysis of MyD88, TRAF6, and NF-κB in isolated cultured AbdSc adipocytes treated with LPS showed a significant increase in the expression of NF-κB [Control 15,262 ± 37 (SE) OD units, LPS-treated 14,449 ± 131 OD units, P = 0.006, n = 4] and TRAF6 [Control 15,345.42 ± 73 (SE) OD units, LPS-treated 14,290 ± 229 OD units, P = 0.01, n = 4], but there was no significant difference in MyD88 [Control 5,980 ± 435 (SE) OD units, LPS-treated 6,134 ± 313 OD units, P = NS, n = 4].

Circulating endotoxin levels in ND and T2DM subjects. Endotoxin levels were significantly higher in the BMI-, sex-, and age-matched T2DM group than in the ND subjects (P = 0.0031; Table 1). Endotoxin was also found to be significantly lower in those subjects who were treated with oral hypoglycemics or insulin and oral hypoglycemics than those treated with diet alone [diet alone (geometric mean) 7.2 (1.6) EU/ml; insulin/oral hypoglycemics (geometric mean) 4.6 (1.4) EU/ml; P = 0.02]. The treated population, however, still had significantly higher endotoxin than the ND controls [insulin/oral hypoglycemics T2DM (geometric mean) 4.6 (1.4) EU/ml; ND (geometric mean) 3.09 (1.7) EU/ml; P = 0.005]. Furthermore, fasting insulin significantly correlated with serum endotoxin levels in the whole ND population (r = 0.679, P < 0.001; Fig. 7). This correlation remained when controlled for sex, age, and BMI (r = 0.731, P < 0.001). No correlation was observed in any of the subjects between glucose and endotoxin.
correlation between HOMA-IR and endotoxin in controls \( (r = 0.692; P < 0.001) \), which could be accounted for by the strong correlation with insulin.

**Effect of the insulin sensitizer RSG on circulating endotoxin levels in treatment-naive T2DM patients.** Patients treated with RSG exhibited a significant reduction in fasting insulin levels and endotoxin levels (endotoxin \( P = 0.0139 \); insulin \( P = 0.0395 \); Table 2). There was also a positive correlation between change in insulin level and change in endotoxin level, which was also highly significant \( (r = 0.673, P = 0.016) \); i.e., the greater the change in insulin, the greater the change in endotoxin.

**DISCUSSION**

Our present findings suggest that human AT represents an important site of innate immune activation, specifically that LPS-treated human isolated AbdSc adipocytes can significantly increase the secretion of the potentially diabetogenic proinflammatory cytokines. Our studies have further highlighted that there is increased expression of key components of the innate immune cascade in human AT in states of obesity and T2DM, in particular TLR-2, MyD88, and TRAF6 as well as NF-κB. Furthermore, in vitro blockade of NF-κB in AbdSc adipocytes reduced IL-6 secretion. Therefore, an increase in AT mass may induce the expression and further dysregulate the functional role of the TLRs, and the downstream transcription factor NF-κB, potentially linking the innate immune system, LPS, human obesity, and T2DM (23). One source of such subclinical inflammation may arise through commensal bacteria derived from the gut, as our in vivo findings highlight that circulating levels of LPS in T2DM subjects are increased. Therefore, these data highlight that LPS may be a mediator of inflammation in AT and present another potential mechanism for inflammation in obesity and T2DM.

The innate immune system in humans has probably arisen in evolution as an acute-phase response to localized inflammation. It may have been a means of first-line defense from bacterial and fungal organisms that breach the barrier of the skin and the GI mucosa, potentially causing deleterious systemic effects (28). However, this reaction could have become disadvantageous in those with increasing adiposity. The protective inflammatory effects are therefore exaggerated by the sheer volume of AT (and exacerbated by hyperinsulinemia) that accompanies obesity. As such, upregulation of TLR-2 and the inflammatory cascade components, including NF-κB, in T2DM secondary to LPS could have detrimental downstream effects on vascular biology (16, 34). Our current studies have shown that induction of the TLRs in AbdSc adipocytes increased proinflammatory cytokines. In the presence of the NF-κB inhibitor, by use of the same cellular system, the adipocytokine profile was altered, thus highlighting a role for NF-κB in proinflammatory cytokine release from AbdSc adipocytes. In addition to this, protein analysis demonstrated an increase in expression levels of components of innate immunity in AT taken from both obese and T2DM subjects. Our present in vivo findings may contribute to the current understanding of the role of obesity-related hyperinsulinemia/
insulin resistance and low-grade inflammation. These data demonstrate a highly significant correlation between LPS and insulin in healthy ND subjects. As hyperinsulinemia/insulin resistance may affect immune competence (14, 39) and subsequently LPS clearance, this could explain the raised LPS levels in T2DM and the observed correlation between LPS and insulin. Such a correlation, however, was not observed in the T2DM cohort. This may be explained, in part, by the closely BMI-matched subjects, as well as by the influence of antidiabetic medication, which may change circulating insulin levels. Indeed, the relationship between insulin and LPS levels was explored further in the RSG-treated patients, in whom the change in LPS correlated with the change in insulin. Glitazones have previously been shown to have immunomodulatory properties (8) and reduce inflammation in both in vitro and in vivo models. Also, the fall in insulin levels in our subjects may help to improve Kupffer cell immune function and LPS clearance. The reduction in the inflammatory response to antidiabetic medication may, therefore, partly relate to the change in LPS levels. Since there have been no reported effects between thiazolidinedione (TZD) use and gut flora, gut absorption, or Kupffer cell function, we concluded that LPS levels are probably influenced directly by a change in insulin concentration. Hence, the proinflammatory effects of LPS may be modulated by the insulin-sensitizing effects of TZDs. This may point to a mechanism for the anti-inflammatory role of TZDs, in addition to direct effects mediated via PPARγ. Other mechanistic considerations to be made are the role of the TZDs in HDL metabolism. Several studies have demonstrated that TZD use may increase the bioavailability of HDL (9). This may have a consequence of “mopping up” LPS, as HDL has been shown to have a positive effect on LPS clearance (31), and the effect of LPS on cytokine production seems to be ameliorated in the presence of elevated HDL (17).

The raised LPS in T2DM may arise as a function of either decreased GI transit time or increased GI absorption, secondary to insulin action. Studies have shown that bacterial overgrowth and delayed GI transit time occur in T2DM (15). Additionally, hyperinsulinemia, as well as hyperglycemia, affects jejunal motility even in normal ND subjects (6). Further evidence linking the GI tract and LPS is the observation that bacterial overgrowth is directly associated with serum LPS in cirrhotic subjects (4). This would therefore have obvious implications for the absorption of LPS from the GI tract in patients who are hyperinsulinemic. The second hypothesis is supported by previous observations that insulin directly stimulates nutrient absorption independently of hyperglycemic conditions (40).

In conclusion, our findings demonstrate that circulating serum LPS is higher in T2DM patients than in lean healthy subjects. Second, LPS can activate the innate immune pathway in isolated AbdSc adipocytes to stimulate secretion of proinflammatory cytokines. These observations, taken to-
together with the finding of increased expression of innate immune system pathway factors in AT taken from obese and T2DM patients, suggest a role for LPS in initiating the subclinical inflammation seen in these states. Hence, diabetic subjects appear to be more exposed to the risk of inflammation through endotoxin. We also noted an association between insulin and endotoxin, important in the hyperinsulinemic/insulin-resistant obese phenotype. Whether this is due to suppression of immune clearance of LPS by the liver or hyperinsulinemia/insulin resistance causing increased absorption or bacterial translocation/overgrowth requires further investigation. Collectively, the present study suggests a potential role for gut flora in the pathogenesis of obesity-related T2DM and the innate immune response.

ACKNOWLEDGMENTS

We thank the operative surgeons, in particular Mr. Paul Levick and staff, for the provision of human adipose tissue; Jacqueline Farmer for her continuing support in these studies; Diabetes UK; and The Wellcome Trust.

REFERENCES


