

Epicardial Adipose Tissue as a Source of Nuclear Factor- κ B and c-Jun N-Terminal Kinase Mediated Inflammation in Patients with Coronary Artery Disease

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Context: Visceral adipose tissue (AT) is known to confer a significantly higher risk of type 2 diabetes and cardiovascular disease. Epicardial AT has been shown to be related to cardiovascular disease and myocardial function through unidentified mechanisms. Epicardial AT expresses an inflammatory profile of proteins; however, the mechanisms responsible are yet to be elucidated.

Objectives: The objectives of the study were to: 1) examine key mediators of the nuclear factor- κ B (NF κ B) and c-Jun N-terminal kinase (JNK) pathways in paired epicardial and gluteofemoral (thigh) AT from coronary artery disease (CAD) and control patients and 2) investigate circulating endotoxin levels in CAD and control subjects.

Design: Serums and AT biopsies (epicardial and thigh) were obtained from CAD (n = 16) and non-CAD (n = 18) patients. Inflammation was assessed in tissue and serum samples through West-ern blot, real-time PCR, ELISAs, and activity studies.

Results: Western blotting showed epicardial AT had significantly higher NF κ B, inhibitory- κ B kinase (IKK)- γ , IKK β , and JNK-1 and -2 compared with thigh AT. Epicardial mRNA data showed strong correlations between CD-68 and toll-like receptor-2, toll-like receptor-4, and TNF- α . Circulating endotoxin was elevated in patients with CAD compared with matched controls [CAD: 6.80 ± 0.28 endotoxin unit(EU)/ml vs. controls: 5.52 ± 0.57 EU/ml; $P < 0.05$].

Conclusion: Epicardial AT from patients with CAD shows increased NF κ B, IKK β , and JNK expression compared with both CAD thigh AT and non-CAD epicardial AT, suggesting a depot-specific as well as a disease-linked response to inflammation. These studies implicate both NF κ B and JNK pathways in the inflammatory profile of epicardial AT and highlight the role of the macrophage in the inflammation within this tissue. (*J Clin Endocrinol Metab* 94: 261–267, 2009)

Adipose tissue (AT) can be considered to integrate both metabolic and immune functionality (1), and it has been established that abdominal, as opposed to gluteofemoral, AT is

critical to both metabolic and inflammatory dysfunction. This difference in risk may be attributable to the depot-specific characteristics of expression and secretion of proinflammatory adi-

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Abbreviations: AT, Adipose tissue; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; CT, threshold cycle; CV, coefficient of variation; CVD, cardiovascular disease; EU, endotoxin unit; IKK, inhibitory- κ B kinase; JNK, c-Jun N-terminal kinase; LAL, limulus amoebocyte lysate; LPS, lipopolysaccharide; NF κ B, nuclear factor- κ B; sCD-14, soluble CD-14; T2DM, type 2 diabetes mellitus; TLR, toll-like receptor.

pocytokines (2, 3). These depot-specific effects appear to include the relatively understudied epicardial AT depot. This site has also been linked to the pathogenesis of cardiovascular disease (CVD) because clinical studies have noted a strong correlation between epicardial AT fat mass, central abdominal fat, and the associated risk of metabolic syndrome and type 2 diabetes mellitus (T2DM) (4–6).

In understanding the biology of epicardial AT, studies by Mazurek *et al.* (7) compared the expression of pathogenic factors between epicardial AT and sc fat derived from the thigh of coronary artery disease (CAD) patients undergoing coronary artery bypass grafting (CABG). These studies highlighted the potential importance of the inflammatory response of epicardial tissue, which may therefore represent a negative influence on cardiovascular function; this finding is affirmed by our previous studies, which highlighted a similar adipocytokine profile in epicardial and omental AT, the latter of which is closely linked to CVD (7, 8).

In addressing the mechanisms of inflammation, our previous studies demonstrated that human isolated abdominal sc adipocytes possess many of the key components of the nuclear factor- κ B (NF κ B) inflammatory pathway (9, 10). This is supported further by both human (11) and murine studies (12, 13).

Innate immunity represents one of the potential pathways for proinflammatory cytokine release, and subsequent increase in CVD risk, with increasing adiposity (14). Innate immunity can be activated via the toll-like receptors (TLRs), which recognize antigens such as lipopolysaccharide (LPS), viral CpG-rich DNA, and zymosan, allowing a rapid reaction to infection (15). Activation of TLRs leads to translocation of NF κ B into the nucleus to initiate the transcription of IL-6, IL-1, and TNF- α (16) and the release of IL-6, TNF- α , and resistin, as noted in human adipocyte studies (9, 10). These findings indicate increased activation of the innate immune pathway in response to LPS (endotoxin) *in vitro*; however, this inflammatory response may also occur *in vivo*, through stimulation of the TLRs by gut-derived LPS. Such *in vivo* observations have previously been reported in CVD and T2DM subjects as well as subjects with impaired glucose tolerance. In which soluble CD-14 (sCD-14), a monocytic marker for LPS activity, is up-regulated (1, 9, 17).

As such, the interaction between fat mass, inflammatory response, and endotoxin has clear implications for the immune system. In addition, endotoxemia may aggravate hyperinsulinemia/insulin resistance because studies have shown a direct link between increasing systemic LPS and the secretion of insulin as well as association studies between LPS and insulin (9, 18). This may therefore form a positive feed-forward mechanism leading to insulin resistance, the oversecretion of insulin, and subsequent inflammation.

To shed light on the function of epicardial fat in CAD and non-CAD patients and the signaling pathways that may lead to an inflammatory response, we sought here to: 1) investigate systemic LPS levels in preoperative CABG patients compared with age and body mass index (BMI)-matched controls, 2) examine the protein expression of key inflammatory signaling molecules such as NF κ B and c-Jun N-terminal kinase (JNK) in epicardial AT compared with paired sc thigh AT, and 3) investigate the gene

expression of TLR-2 and -4 and determine any correlations with markers of inflammation, such as TNF- α , and markers of macrophages.

Subjects and Methods

Tissue collection

Paired human epicardial and thigh AT biopsies were taken from a subgroup of the CAD patients undergoing CABG (age 63.9 ± 9.2 yr; BMI 27.1 ± 3.5 kg/m², $n = 16$). Epicardial biopsies were taken approximately 1 h after anesthetic. Thigh AT biopsies were taken when the section of saphenous vein was obtained, 30–45 min after anesthesia. For non-CAD patients nonpaired epicardial and thigh biopsies were taken from different patients. Epicardial biopsies were taken approximately 1 h after anesthetic from patients with no history of CVD disease who were undergoing valve replacement. Subcutaneous thigh tissue was taken from non-CAD patients undergoing elective surgery. All tissues were flash frozen immediately. All studies were performed with the approval of the local ethics committee with informed consent being obtained from all subjects before enrollment. Patients were on a range of medications as outlined in Table 1. Diabetic patients were excluded from this study.

Serum collection

Fasted blood samples ($n = 72$) were taken before surgery, and serum levels were analyzed for a panel of proteins [C-reactive protein (CRP), CD-14, adiponectin, and insulin] and compared with non-CAD and nondiabetic control subjects, which were age, gender, and BMI matched with no previous history of CVD (Table 2).

Protein determination and Western blot analysis

Human AT was homogenized and resuspended in radioimmunoprecipitation assay buffer, as previously detailed (19). Protein concentrations were determined using the detergent-compatible protein assay kit (Bio-Rad Laboratories, Hercules, CA) (20). Western blot analysis was performed using a method previously described (21). In brief, 10–30 μ g of protein were loaded on to a denaturing polyacrylamide gel (10–12%; GeneFlow, Leicestershire, UK). Protein expression of NF κ B (1:250; TCS Cellworks, Buckingham, UK), inhibitory- κ B kinase (IKK)- β (1:500; TCS Cellworks), and IKK α (1:500; Abcam, Cambridge, UK) was assessed using mouse monoclonal antibodies. A polyclonal, phosphospecific, anti-JNK-1 and -2 antibody was also used (1:1750; BioSource International, Camarillo, CA). Equal protein loading was confirmed by examining α -tubulin protein expression (1:5000; The Binding Site, Birmingham, UK). No statistical difference was observed in α -tubulin expression for all samples analyzed. For CAD patients ($n = 4$), paired sc and epicardial AT was used. Epicardial from a further four non-CAD patients were used as controls. A chemiluminescent detection system, ECL/ECL⁺ (Amersham Pharmacia Biotech, Little Chalfont, UK), enabled visualization of bands, whereas intensity was determined using densitometry (Genesnap; Syngene, Cambridge, UK).

Assessment of NF κ B activity

NF κ B activity was assessed with Trans-AM NF κ B p65 transcription factor assay kit (Active Motif, Rixensart, Belgium; detection limit < 40 ng of whole cell extract). In brief, a 96-well plate coated with an oligonucleotide for the NF κ B consensus binding site was incubated with 1.5 μ g of AT extract for 1 h. The NF κ B complex bound to the oligonucleotide is detected by binding of the NF κ B p65 antibody. A secondary antibody conjugated to horseradish peroxidase was added. Activity was determined by luminescence (22).

RNA extraction and quantitative RT-PCR

RNA was extracted from samples using RNeasy lipid tissue kit (QIAGEN, Crawley, UK) according to the manufacturer's instructions.

TABLE 1. CAD patient data

	CAD subjects (serum study, n = 72)	CAD subjects (tissue study, n = 16)	Control subjects (serum study, n = 60)	Control subjects (tissue study, n = 18)
Age (yr)	63.8 ± 8.4	66.7 ± 6.7	61.8 ± 8.0	72.8 ± 9.9
BMI (kg/m ²)	27.8 ± 3.5	28.4 ± 3.6	27.9 ± 3.3	27.5 ± 4.9
Male, %	84	93	83	62
Medications (% receiving treatment)				
Statin	93	94	12	50
Aspirin	93	100	20	72
β-Blocker	82	88	20	61
ACEI	36	75	10	16
Ca ²⁺ channel blockers	51	13	18	28
Nicorandil	30	31	0	

Anthropometric data and medication details of study participants. BMI and age are given as mean ± sd; the remaining values are given as percentages rounded to the nearest whole number. ACEI, Angiotensin-converting enzyme inhibitor.

Extraction was followed by a DNase digestion step to remove any contaminating genomic DNA. RNA was quantified using the Nanodrop ND-1000 spectrophotometer (Labtech, International, East Sussex, UK), and 1 μg of RNA from each sample was reverse transcribed using Superscript III reverse transcriptase (Invitrogen, Paisley, UK) and random hexamers, according to the manufacturer's instructions. Real-time PCR was performed in a reaction mix containing TaqMan universal PCR master mix (AmpErase UNG, Applied Biosystems, Foster City, CA), 100–200 nM TaqMan probe, 900 nM primers, and 25 ng cDNA for all PCRs. All reactions were multiplexed with the housekeeping gene 18S, provided as a preoptimized control probe (Applied Biosystems), enabling data to be expressed as δ -threshold cycle (CT) values (where Δ CT = CT of 18S subtracted from CT of gene of interest). Measurements were carried out on at least three occasions for each sample. Reactions were as follows: 50 C for 2 min, 95 C for 10 min; then 40 cycles of 95 C for 15 sec and 60 C for 1 min. Adiponectin and CD-45 mRNA were assayed using probes of previously described sequences (8). All other gene expression was assessed using predesigned gene expression assay-on-demand kits (Applera).

Serum sample analysis

Serum samples were analyzed for the determination of insulin (Linco Research, St. Charles, MO), CRP (Life Diagnostics Inc., West Chester, PA), sCD-14 (R&D Systems, Abingdon, UK), adiponectin (Linco Research). Insulin, CRP, sCD-14, and adiponectin levels were analyzed by a solid-phase ELISA [insulin: coefficient of variation (CV) intraassay 5.96%, interassay 10.3 ± 0.9%; CRP: intraassay CV 4.4%, interassay CV 3.3%; sCD-14: intraassay 5.4%, interassay 6.3%; adiponectin: in-

traassay CV 3.4%, interassay CV 5.7%]. For these studies, anthropometric data were collected and are detailed in Table 2. Fasting glucose was analyzed using a glucose oxidase method (YSL 200 STAT plus; Yellow Springs Instruments, Yellow Springs, OH).

Measurement of circulating endotoxin levels

Serum endotoxin was assayed using a chromogenic limulus amoebocyte lysate (LAL) test, which is a quantitative test for Gram-negative bacterial endotoxin (Cambrex, Walkersville, MD). 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 *p*-nitroaniline from the colorless substrate Ac-Ile-Glu-Ala-Arg-pNA. The *p*-nitroaniline released was measured photometrically at 405–410 nm after termination of the reaction. The correlation between the absorbance and the endotoxin concentration is linear in the 0.1–1.0 EU/ml range (intraassay CV 3.9 ± 0.46%, interassay CV 9.6 ± 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. Our previous studies assessed specificity of the assay as well as cross-reactivity (9). In brief, to assess recovery of endotoxin within the assay, known concentrations of endotoxin (*Escherichia coli* origin) (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 was any interference from plasma contents. Lyophilized endotoxin (*E. coli* origin) was used to generate a standard 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 a combination of both. No interference was observed for any of the tested concentrations of lipid and/or glucose.

Statistical analysis

Determination of correlations in gene expression analysis was performed using Pearson's correlation coefficient, whereas due to the non-normal distribution of serum LPS, a Spearman's rank correlation coefficient was used to determine any correlations involving these data (SPSS 14.0 for Windows; SPSS UK Ltd., Surrey, UK). Significance in protein expression data and activity was determined by paired and unpaired *t* test as appropriate and in serum analysis by unpaired student *t* test (SPSS 14.0 for Windows; SPSS UK Ltd., Surrey, UK).

TABLE 2. Serum biochemical profile

	CAD (mean ± sd; n = 72)	Control (mean ± sd; n = 60)	Significance
Fasting glucose (mmol/liter)	5.6 ± 0.1	5.4 ± 0.07	NS
Fasting insulin (IU/ml)	9.9 ± 0.69	8.3 ± 0.68	NS
CRP (μg/ml)	7.1 ± 1.0	2.6 ± 0.2	<i>P</i> < 0.05
Adiponectin (μg/ml)	7.05 ± 0.3	9.5 ± 0.6	<i>P</i> < 0.01
sCD-14 (μg/ml)	1.26 ± 0.03	1.18 ± 0.03	<i>P</i> < 0.05
LPS (EU/ml)	6.8 ± 0.28	5.5 ± 0.57	<i>P</i> < 0.05
Cholesterol (mmol/liter)	4.0 ± 0.69	5.59 ± 0.99	<i>P</i> < 0.01

Serum profiles for samples used in the serum study. Values are presented as mean ± SEM. *P* values were determined by unpaired *t* test; NS, No significant difference between groups. 1 EU = 0.56 ng LPS.

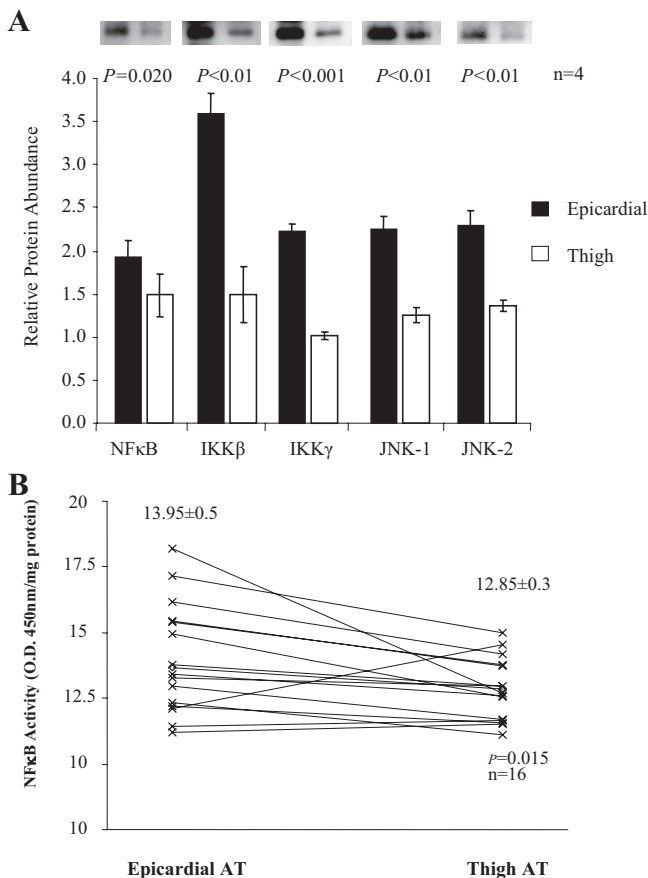


FIG. 1. A, Western blot analysis of components of the NFκB and JNK pathways observed in epicardial and sc thigh AT taken from CAD subjects with representative bands shown above. Densitometry was carried out and data are presented as a comparative analysis between matched pairs; P values are detailed above. B, Differences in NFκB activation in paired epicardial and thigh AT depots as assessed by trans-AM NFκB p65 transcription factor assay (n = 16). Activity was determined by luminescence and is expressed per milligram of protein. Means and ses are given for each graph.

Results

Abundance of NFκB, IKKβ, and IKKγ in epicardial and thigh AT taken from CAD subjects

Protein content for NFκB, IKKβ, and IKKγ in paired, epicardial and thigh AT samples showed higher protein expression for all proteins in epicardial AT when compared with sc thigh AT. The higher levels are statistically significant for all analyzed proteins when assessed by paired student t test (Fig. 1A).

Assessment of phosphorylated JNK-1 and JNK-2 in paired epicardial and thigh AT taken from CAD subjects

Protein expression of JNK-1 and -2 isoforms was assessed using a phospho-specific antibody that indicated that there was a significant increase in protein abundance of JNK 1/2 in the epicardial AT when compared with the thigh AT from the same subject (P < 0.01; Fig. 1A).

NFκB activity data in paired epicardial and thigh AT taken from CAD subjects

Assessment of NFκB activity determined that activity was significantly higher in epicardial AT compared with thigh AT

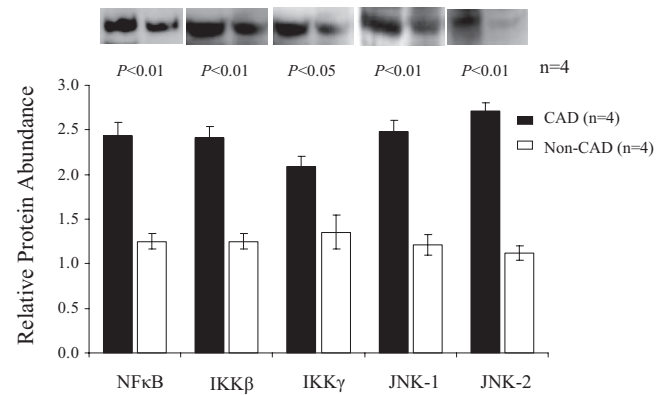


FIG. 2. Western blot analysis comparing components of the NFκB and JNK pathways between CAD (n = 4) and non-CAD (n = 4) subjects for epicardial adipose tissue. Representative bands are shown above the histograms along with t test. P values were derived from comparison of patient parameter-matched pairs by densitometric analysis of the Western blot signals.

from the same subject (Epicardial AT: NFκB 13.95 ± 0.05 OD/mg protein (mean ± SEM) vs. Thigh NFκB 12.85 ± 0.03 OD/mg protein; P = 0.015, Fig. 1B).

Comparative protein expression of NFκB, IKKβ, and IKKγ between CAD and non-CAD subjects for epicardial AT

Assessment of protein content for NFκB, IKKβ, and IKKγ in epicardial AT samples taken from CAD and non-CAD patients highlighted a higher protein expression for all proteins in epicardial AT from CAD subjects when compared with epicardial AT taken from non-CAD subjects (Fig. 2). The higher levels are statistically significant for all proteins examined when assessed by Student’s t test (Fig. 2).

mRNA expression of TLRs, TNF-α, and CD-68 in epicardial and thigh AT taken from CAD subjects

TNFα and adiponectin mRNA levels were compared between epicardial and thigh AT from CAD patients. TNFα mRNA levels were similar between depots (ΔCt 19.72 and 19.76 for epicardial and thigh depots, respectively). Adiponectin was determined to be expressed in epicardial fat at 58% of the level in thigh fat, but this trend did not attain statistical significance (P < 0.09).

To ascertain the importance of the macrophage cell type in the inflammatory process within the AT sites, we used CD-68 and CD-45 as markers for macrophage presence. Assessment of CD-68 mRNA expression highlighted a strong positive correlation with both TLR-2 and -4 in the epicardial AT (TLR-2: r = 0.94, P < 0.001; TLR-4: r = 0.95, P < 0.001, Fig. 3A). These positive correlations were also observed in thigh tissue, although the correlations were less pronounced (TLR-2: r = 0.75, P < 0.001; TLR-4: r = 0.65, P < 0.01; Fig. 3B). The correlation between gene expression of CD-68 and the proinflammatory TNF-α was also examined. The mRNA expression of TNF-α was positively correlated with CD-68 mRNA expression in both epicardial (TNF-α: r = 0.81, P < 0.001 Fig. 3C) and thigh tissue (TNF-α: r = 0.59, P < 0.05; Fig. 3D), again with the stronger correlation noted in the epicardial AT.

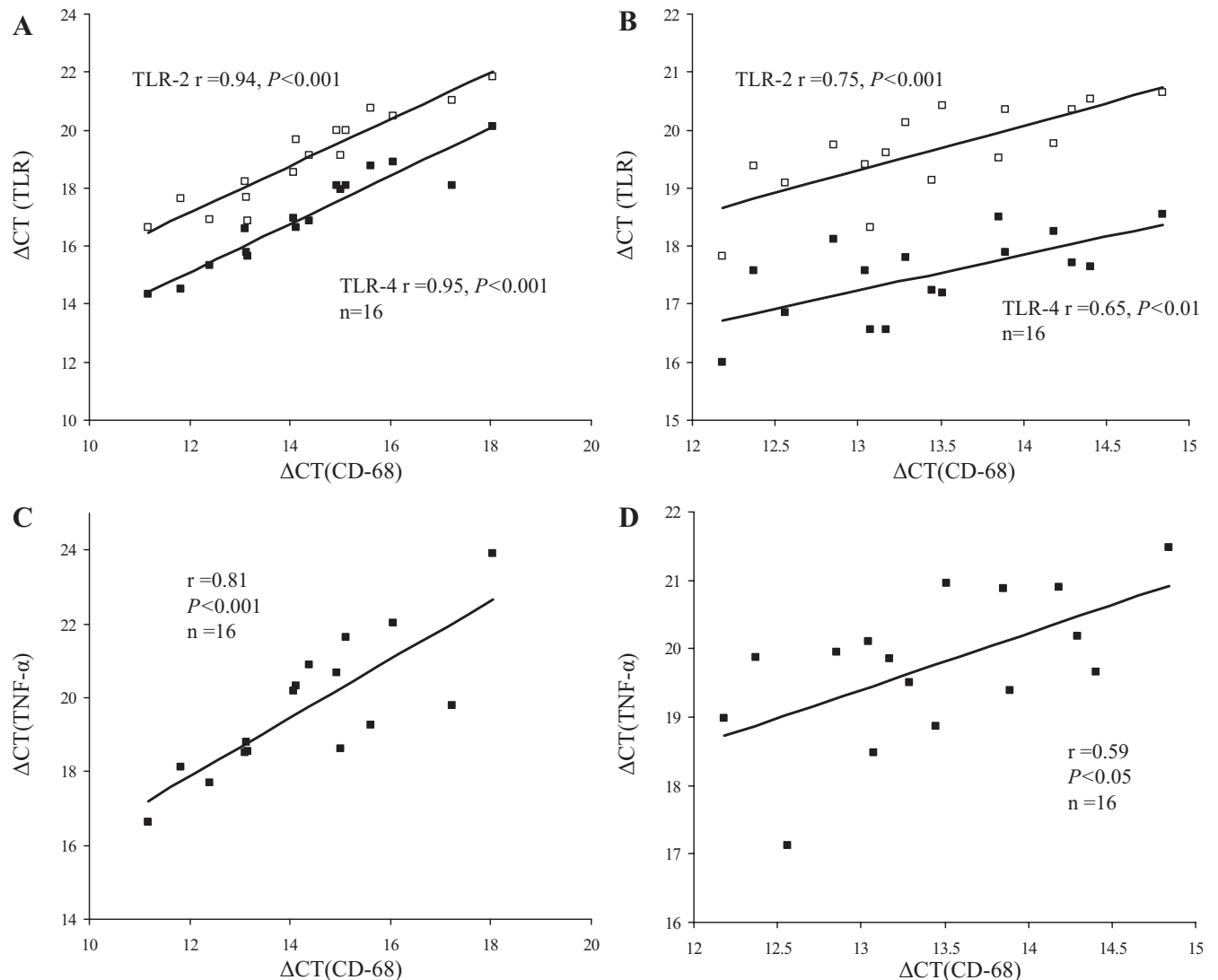


FIG. 3. Correlations between the Δ CTs of CD-68 and TLR-2 (open box) and TLR-4 (filled box) in epicardial (A) or thigh (B) AT and correlations between the Δ CTs of CD-68 and TNF- α in epicardial AT (C) and thigh AT (D) taken from CAD patients. Correlation coefficients and associated P values are shown on each plot.

Similar findings were observed when assessing the relationship between expression of TNF- α and the TLRs, with strong positive correlations noted between TNF- α expression and that of both of the analyzed TLRs in epicardial AT (TNF- α vs. TLR-2 mRNA $r = 0.81$, $P < 0.001$, vs. TLR-4 $r = 0.82$, $P < 0.001$). Weaker correlations were observed in the thigh AT samples between TNF- α mRNA expression and TLR-2 ($r = 0.58$, $P < 0.05$) and between TNF- α and TLR-4 ($r = 0.64$, $P < 0.01$). Similar results were observed when using CD-45 as a marker for macrophage presence (data not shown).

mRNA expression of TLRs, TNF- α , and CD-68 in epicardial and thigh AT taken from non-CAD Subjects

Possible correlations between the TLRs or TNF- α and CD-68 were also examined in non-CAD samples. Weak correlations for TLR-2 and TLR-4 against CD-68 in non-CAD epicardial AT ($r = 0.288$, $P = 0.247$ and $r = 0.480$, $P < 0.05$ for TLR-2 and -4, respectively) and non-CAD thigh AT ($r = 0.826$, $P < 0.01$ and $r = 0.698$, $P < 0.01$ for TLR-2 and -4, respectively) were observed (Fig. 4, A and B). Similarly, TNF- α

was weakly correlated to CD-68 in epicardial ($r = 0.636$, $P < 0.01$, Fig. 4C) and thigh ($r = 0.423$, $P = 0.091$, Fig. 4D) AT.

Circulating analyte levels

There were no significant differences between the serum levels of fasting glucose or insulin between the CAD and non-CAD groups (Table 2). As expected, the serum levels of CRP were significantly higher in the CAD group when compared with the non-CAD group, and adiponectin levels were significantly lower (Table 2). Additionally, sCD-14 was significantly increased in CAD patients compared with the matched case controls (Table 2).

Circulating endotoxin levels

Endotoxin levels were significantly higher in the BMI- and age-matched CAD group than the BMI- and age-matched non-CAD subjects ($P < 0.05$; Table 2). Furthermore, fasting insulin significantly correlated with serum endotoxin levels in the whole population ($\rho = 0.19$, $P < 0.05$). No correlation was observed in any of the subjects between glucose and endotoxin. However, endotoxin concentrations significantly correlated with total triglyceride levels

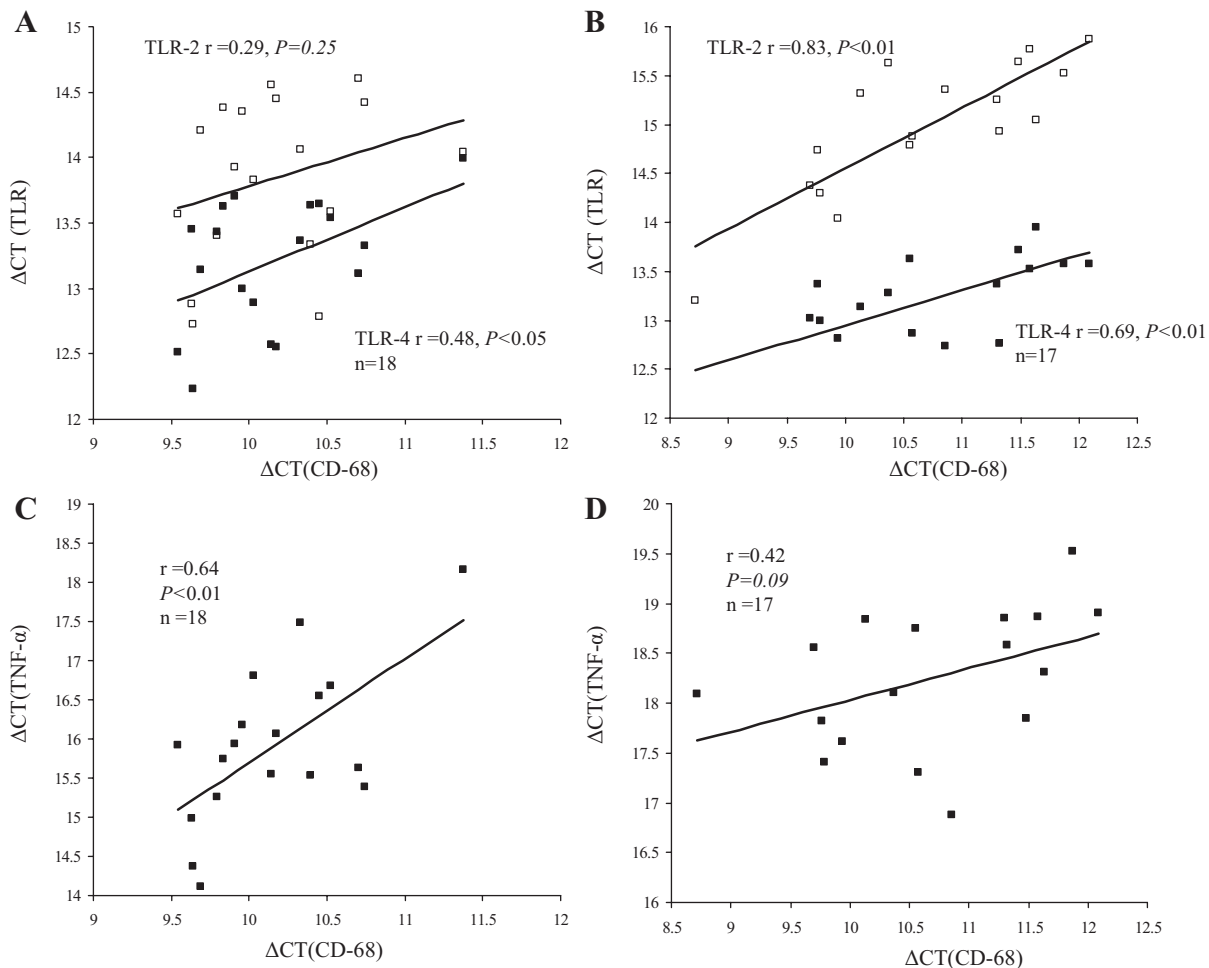


FIG. 4. Correlations between the Δ CTs of CD-68 and TLR-2 (open box) and TLR-4 (filled box) in epicardial (A) or thigh (B) AT and correlations between the Δ CTs of CD-68 and TNF- α in epicardial AT (C) and thigh AT (D) taken from non-CAD patients. Correlation coefficients and associated *P* values are shown on each plot.

($\rho = 0.26, P = 0.003$), although subgroup analysis determined significant correlations between endotoxin levels and triglycerides in CAD subjects ($\rho = 0.645, P < 0.001$) but not non-CAD subjects ($\rho = -0.148, P = 0.259$).

Discussion

The findings from these studies indicate that epicardial fat from CABG patients represents a site of increased inflammatory response in comparison with both paired sc thigh tissue and epicardial AT from non-CAD subjects. Due to the lack of fascia between the adipocyte and myocardial layers (8), this inflammation may have implications for cardiac function. Adipokines secreted by epicardial adipocytes could readily interact with the adjacent cardiomyocytes, which may influence myocardial function, as shown in previous rodent studies (23–25). Additionally these studies determined that mRNA expression of TLR-2, TLR-4, and TNF- α correlated closely with the levels of CD-68 mRNA in both AT depots taken from CAD subjects, implicating the macrophage as the protagonist in this increased inflammatory activation. Furthermore, our data indicated that increased systemic LPS levels in CAD *vs.* non-CAD subjects may represent an initiator of such inflammation.

These studies indicate that in an inflammatory state, as represented by CAD, the differences between the AT depots remain, thus demonstrating the intrinsic differences in the depots' response to the disease state. In this study the sc thigh tissue expressed consistently lower levels of the inflammatory mediators than epicardial AT, suggesting that it remains relatively benign in comparison with abdominal and, specifically, epicardial AT. The high levels of both NF κ B and JNK pathway components in the epicardial depot and the increased NF κ B activation, in comparison with the paired sc tissue, indicated that it may well be the activation of these pathways that is responsible for driving the inflammatory profile of gene expression that has been observed previously (7, 8).

Furthermore, we addressed the influence of macrophages in this inflammatory process via gene expression studies, and these identified that TLR-2 and TLR-4 expression is closely linked to the presence of activated macrophages, with particularly strong correlations noted in epicardial AT. In conjunction with other studies (7), our previous findings (8) suggest that the epicardial depot exhibits a particularly high level of macrophage infiltration, thus supporting the concept that the macrophage plays a crucial role in the inflammatory response in epicardial AT.

The serum studies further confirmed previous observations (8) that the CAD patients were in a state of low-grade inflam-

mation with significantly higher levels of CRP and lower levels of adiponectin. In addition, the CAD patients were observed to have significantly higher levels of both sCD-14 and LPS when compared with healthy, matched controls. Recent studies have shown serum LPS levels to be elevated in diabetic subjects (9), suggesting that LPS may be involved in the chronic inflammation observed in T2DM. Creely and colleagues (9, 10) demonstrated an up-regulation of inflammatory cytokines in AT after LPS stimulation, which appears to be mediated via NF κ B activity. On this basis, the raised LPS levels observed in the CAD patient cohort may drive an inflammatory phenotype in the epicardial as well as other AT depots. Further studies in mice have linked hyperendotoxemia with an increase in gut permeability resulting from obesity (26). Whereas the patient and control cohorts in this study were BMI matched, the possibility of a link between the raised levels of LPS in the CAD cohort and the chronic inflammation associated with CVD remains an intriguing one. Together these results suggest that the epicardial AT may represent a further site for therapeutic action in CAD. This is reinforced due to the current findings highlighting increased expression of both JNK and NF κ B, with a clear association between macrophage and inflammation in epicardial fat. Therefore, it seems apparent that the use of antiinflammatory therapeutics, which have been shown to down-regulate these inflammatory pathways such as salicylates and statins (27), may exert additional beneficial effects on the local metabolism of AT around and within the heart to further reduce detrimental effects on cardiac function in patients with CAD. However, further studies are required to explore this line of research.

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