Insulin Suppresses Endotoxin-Induced Oxidative, Nitrosative, and Inflammatory Stress in Humans

PARESH DANDONA, MD, PHD
HUSAM GHANIM, PHD
ARINDAM BANDYOPADHYAY, MD
KELLY KORZENIEWSKI, BSC
CHANG LING SIA, BSC
SANDEEP DHINDSA, MD
AJAY CHAUDHURI, MD

OBJECTIVE — To investigate whether insulin reduces the magnitude of oxidative, nitrosative, and inflammatory stress and tissue damage responses induced by endotoxin (lipopolysaccharide [LPS]).

RESEARCH DESIGN AND METHODS — Nine normal subjects were injected intravenously with 2 ng/kg LPS prepared from *Escherichia coli*. Ten others were infused with insulin (2 units/h) for 6 h in addition to the LPS injection along with 100 ml/h of 5% dextrose to maintain normoglycemia.

RESULTS — LPS injection induced a rapid increase in plasma concentrations of nitric oxide metabolites, nitrite and nitrate (NOM), and thiobarbituric acid–reacting substances (TBARS), an increase in reactive oxygen species (ROS) generation by polymorphonuclear leukocytes (PMNLs), and marked increases in plasma free fatty acids, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibition factor (MIF), C-reactive protein, resistin, visfatin, lipopolysaccharide binding protein (LBP), high mobility group-B1 (HMG-B1), and myoglobin concentrations. The coinfusion of insulin led to a total elimination of the increase in NOM, free fatty acids, and TBARS and a significant reduction in ROS generation by PMNLs and plasma MIF, visfatin, and myoglobin concentrations. Insulin did not affect TNF-α, MCP-1, IL-6, LBP, resistin, and HMG-B1 increases induced by the LPS.

CONCLUSIONS — Insulin reduces significantly several key mediators of oxidative, nitrosative, and inflammatory stress and tissue damage induced by LPS. These effects of insulin require further investigation for its potential use as anti-inflammatory therapy for endotoxemia.

Diabetes Care **33:2416–2423, 2010**

Endotoxin (lipopolysaccharide [LPS]) induces inflammation by binding to its specific receptor, Toll-like receptor-4 (TLR-4) (1,2). This leads to the nuclear translocation and activation of nuclear factor κB (NFκB), the major proinflammatory transcription factor, and an increase in the transcription of proinflammatory genes with a corresponding increase in the concentration of the products of these genes in plasma. Endotoxemia in association with clinical Gram-negative septicemia syndrome in humans leads to a high incidence of morbidity and mortality, especially in patients in intensive care units (ICUs).

Insulin has been shown to suppress inflammatory changes in vitro and in vivo. It suppresses intranuclear NFκB and Egr-1 binding and the expression of several proinflammatory mediators including intercellular adhesion molecule-1 and monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-9, C-reactive protein (CRP), and serum amyloid A (3,4). In addition, it suppresses the expression of several Toll-like receptors (TLRs) (5). It also suppresses reactive oxygen species (ROS) generation and $p47^{phox}$ expression.

Resistin and visfatin, two peptides originally discovered as adipokines (6,7), have now been shown to be products of proinflammatory myeloid cells (8,9) and to induce proinflammatory cytokines, chemokines, and ROS. Both have been implicated in atherogenesis (10).

LPS induces an increase in the expression of inducible nitric oxide synthase and NO release (11) from macrophages. LPS is also known to stimulate the generation of ROS including superoxide.

There are recent data showing that LPS injection in experimental animals leads to a reduction in myocardial function. Insulin administration in LPS-injected animals reduces LPS-induced damage (12).

On the basis of the above, we hypothesized that *1*) the injection of LPS in normal human subjects will induce an increase in ROS generation, lipid peroxidation, nitrosative stress as reflected in plasma concentrations of NO metabolites, nitrite and nitrate, plasma free fatty acid concentration, plasma myoglobin concentrations. novel adipokines, resistin and visfatin, and lipopolysaccharide binding protein (LBP) in parallel with proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and macrophage migration inhibitory factor (MIF), and high mobility group box-1 (HMG-B1) protein, which acts as a proinflammatory cytokine when released into the circulation and 2) insulin will suppress the LPS-induced increases in ROS generation, lipid peroxidation, plasma concentrations of nitrite and nitrate (NOM), free fatty acids (FFAs), resistin, visfatin, and markers of tissue damage including myoglobin, HMG-B1, TNF-α, and IL-6.

RESEARCH DESIGN AND METHODS

— Nineteen normal-weight (BMI 20–25 kg/m^2) healthy male subjects aged between 20 and 33 years (mean age 26 ± 3 years) were recruited for this study. After an overnight fast, nine subjects were injected intravenously with 2 ng/kg of LPS prepared from *Escherichia*

From the Division of Endocrinology, Diabetes and Metabolism, State University of New York at Buffalo and Kaleida Health, Buffalo, New York.
Corresponding author: Paresh Dandona, pdandona@kaleidahealth.org.
Received 13 May 2010 and accepted 31 July 2010. Published ahead of print at http://care.diabetesjournals.org on 10 August 2010. DOI: 10.2337/dc10-0929.

coli along with saline at 100 ml/h. The other 10 were infused with insulin (2 units/h) 1 h before the LPS injection along with 100 ml/h of 5% dextrose coinfused with insulin to maintain normoglycemia. Insulin/dextrose or saline infusions were continued for 6 h after the LPS injection while subjects were in the fasting state to avoid the potential proinflammatory effect of a meal (13). They were then provided with a 900-calorie meal at 6:00 P.M. after which they ate nothing till the next morning. Subjects were monitored for vital signs (temperature, pulse, blood pressure, headaches, body aches, and chills) for 24 h after the LPS injection. Blood samples were collected 1 h before the LPS injection and at 0, 1, 2, 4, 6, and 24 h after the injection. The protocol was approved by the internal review board of the State University of New York at Buffalo, and written consent was obtained from all subjects.

Polymorphonuclear leukocytes isolation and ROS generation

Blood samples were collected and polymorphonuclear leukocytes (PMNLs) were isolated and ROS generation was measured as described previously (14). The intra-assay coefficient of variation (CV) for ROS generation is 8%.

Measurement of glucose, insulin, FFAs, NOM, and thiobarbituric acid–reacting substance concentrations and homeostasis model assessment of insulin resistance calculation

Plasma concentrations of glucose were measured by a 2300 STAT Plus glucose analyzer (YSI, Yellow Springs, OH). Insulin concentrations were measured from plasma samples using an ELISA kit (Diagnostics Systems Laboratories, Webster, TX). FFA concentrations were measured using the Half-Micro calorimetric kit from Roche Diagnostic (Indianapolis, IN). NOM (NO_2/NO_3) were assayed by the Griess reaction (R&D Systems, Minneapolis, MN), and thiobarbituric acid–reacting substances (TBARS) were assayed by spectrofluorometry with a kit from Zeptometrix (Buffalo, NY). The CVs for these assays ranged from 2 to 7 and 4 to 11% for intra- and interassay variations, respectively. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: (fasting insulin [microunits per milliliter) × fasting glucose [millimoles per liter])/22.5.

Measurements of plasma cytokines, CRP, visfatin, resistin, myoglobin, HMG-B1, and LBP concentrations

Commercially available ELISAs were used to measure concentrations of circulating cytokine concentrations (R&D Systems), CRP (American Diagnostica, Stamford, CT), visfatin (Phoenix Pharmaceuticals, Belmont, CA), myoglobin (Life Diagnostics, West Chester, PA), HMG-B1 (IBL Transatlantic), and LBP (Cell Sciences, Canton, MA). The CVs for these assays ranged from 3 to 8 and 6 to 11% for inter- and intra-assay variations, respectively.

Statistical analysis

Statistical analysis was conducted using SigmaStat software (SPSS, Chicago, IL). All data are means ± SEM. Statistical analysis from baselines was preformed using Holm-Sidak one-way repeated-measures analysis of variance (RMANOVA). Dunnett's two-factor RMANOVA method was used for multiple comparisons between different groups. Paired *t* tests were used to compare changes from baseline at 24 h.

RESULTS

Clinical features

LPS injection induced an increase in temperature from 97.7 ± 0.5°F to a peak of 100.9 ± 0.9°F at 4 h, systolic blood pressure increased from 113 ± 11 mmHg to a peak of 129 ± 13 mm Hg at 2 h, and pulse rate increased from 63 ± 7/min to a peak of 97 ± 9/min at 6 h and was not affected by insulin (Table 1). Headaches, chills, and body ache scores increased after LPS injection with peaks at 1 to 2 h with insulin infusion reducing body ache score significantly (Table 1) without affecting headaches and chills.

Blood leukocyte counts

After LPS, total leukocyte count increased from a baseline of 4,300 ± 900 at −1 h to a peak of 11,800 ± 1,200 cells/mm^3 at 6 h and was still elevated (7,000 ± 1,100 cells/mm^3) at 24 h, mainly attributable to polymorphonuclear leukocytosis (Table 1). Monocytes and lymphocytes fell rapidly from 6 ± 0.4 to 0.5 ± 0.1% and from 39 ± 5 to 3 ± 0.8%, respectively. Insulin infusion did not alter this pattern. The marked reduction in monocytes and lymphocytes prevented us from examining ROS generation and other cellular markers/mediators in the mononuclear cell fraction.

Plasma insulin, glucose, FFA, triglyceride, LDL cholesterol, and HDL cholesterol concentrations

In the group receiving insulin, insulin concentrations increased by up to fourfold ($P < 0.001$) (Table 1). In the control group, insulin concentrations did not change in the first 6 h but were significantly higher than baseline at 24 h ($P < 0.05$) (Table 1). There was no significant change in glucose concentrations. Consistent with that finding, HOMA-IR increased significantly at 24 h from 1.22 ± 0.24 vs. 2.36 ± 0.39 in the control group (Table 1). After LPS injection, there was a significant increase in plasma FFA concentration. Insulin infusion prevented this increase (Table 1). Serum triglyceride concentration fell significantly in both groups (Table 1). LDL cholesterol, VLDL cholesterol, and HDL cholesterol concentrations did not change.

ROS generation by PMNLs

LPS injection induced an increase in ROS generation by PMNLs of 200 ± 42% over the basal with a peak at 1 h and another peak at 4–6 h. Insulin infusion reduced (Fig. 1*A*) ROS generation throughout the infusion period ($P < 0.05$ by two-factor RMANOVA).

Plasma TBARS concentrations

LPS injection induced a rapid increase in TBARS concentration from 1.29 ± 0.29 to 2.15 ± 0.41 μmol/l ($P < 0.01$) at 1 h, with a return to baseline at 2 h (Fig. 1*B*). This pattern was observed in each of the LPS-injected subjects. Insulin infusion totally prevented this increase.

Plasma NOM concentrations

Plasma NOM concentration increased rapidly after the injection of LPS at 1 h, peaked to 75 ± 24% over the baseline at 2 h (from 29.4 ± 2.6 to 47.7 ± 5.4 μmol/l), and declined to the baseline by 4 h. There was a secondary rise in NOM concentration at 6 h (Fig. 1*C*). With insulin infusion, the LPS-induced increase in NOM was totally prevented and in fact there was a small but significant decrease by 16 ± 10% below the baseline in plasma NOM concentrations (from 31.7 ± 2.8 to 26.9 ± 2.6 μmol/l).

Plasma MIF, TNF-α, IL-6, and CRP concentrations

Plasma concentrations of MIF increased significantly after LPS injection at 1 h with a secondary increase at 4–6 h from 727 ±

Table 1—*Changes in clinical, oxidative, and inflammatory end points after 2 ng/kg injection of LPS alone or LPS and 2 units/h insulin for 6 h in normal healthy subjects*

Marker and group	−1 h	0 h	1 h	2 h	4 h	6 h	24 h	*P* value*	*P* value†
Systolic blood pressure (mmHg)									
LPS	113 ± 11	111 ± 10	118 ± 12	129 ± 13	123 ± 12	118 ± 10	118 ± 12	0.01	NS
LPS	119 ± 11	117 ± 10	120 ± 12	135 ± 14	124 ± 13	119 ± 10	119 ± 11	0.01	
Diastolic blood pressure (mmHg)									
LPS + Ins	74 ± 7	73 ± 7	74 ± 8	74 ± 10	75 ± 10	74 ± 6	76 ± 7	NS	NS
LPS + Ins	75 ± 9	74 ± 8	76 ± 9	77 ± 10	76 ± 10	75 ± 9	76 ± 8	NS	
Temperature (°F)									
LPS	97.7 ± 0.5	97.5 ± 0.5	98.0 ± 0.7	98.4 ± 0.5	100.9 ± 0.9	99.1 ± 0.9	97.5 ± 0.5	0.001	NS
LPS + Ins	97.8 ± 0.7	97.8 ± 0.6	98.2 ± 0.7	98.6 ± 0.7	101.1 ± 1.0	99.3 ± 0.9	97.5 ± 0.6	0.001	
White blood cell count (10^3 cells/mm^3)									
LPS	4.3 ± 0.8	4.1 ± 0.7	2.8 ± 0.5	6.8 ± 0.9	10.4 ± 1.1	11.8 ± 1.2	7.0 ± 1.1	0.001	NS
LPS + Ins	4.8 ± 0.9	4.6 ± 0.8	3.4 ± 0.6	6.1 ± 0.8	9.8 ± 1.1	10.6 ± 1.0	6.8 ± 0.9	0.001	
Pulse (/min)									
LPS	63 ± 7	60 ± 7	63 ± 7	76 ± 8	88 ± 9	97 ± 9	64 ± 6	0.001	NS
LPS + Ins	65 ± 8	63 ± 6	66 ± 5	81 ± 7	91 ± 8	96 ± 9	62 ± 6	0.001	
Body aches score									
LPS	0.0	0.0	0.47 ± 0.2	1.22 ± 0.3	0.76 ± 0.3	0.34 ± 0.1	0.0	0.001	0.02
LPS + Ins	0.0	0.0	0.61 ± 0.2	0.88 ± 0.3	0.32 ± 0.1	0.25 ± 0.1	0.0	0.01	
Glucose (mg/dl)									
LPS	87.2 ± 2.6	89.9 ± 2.4	88.6 ± 4.5	85.5 ± 4.9	84.5 ± 4.2	84.5 ± 3.3	86.6 ± 3.0	NS	NS
LPS + Ins	83.6 ± 2.0	85.7 ± 2.2	81.4 ± 5.3	82.9 ± 3.2	87.3 ± 4.5	80.2 ± 4.8	85.6 ± 2.5	NS	
Insulin (μIU/ml)									
LPS	6.9 ± 1.8	6.4 ± 1.4	7.4 ± 1.6	7.2 ± 1.2	6.4 ± 1.1	5.9 ± 1.1	13.1 ± 2.2‡	NS	0.001
LPS + Ins	7.5 ± 1.5	31.1 ± 3.7	30.5 ± 5.1	24.9 ± 4.2	15.4 ± 2.8	28.9 ± 4.8	14.9 ± 3.0‡	0.004	
HOMA-IR									
LPS	1.22 ± 0.24						2.36 ± 0.39‡		
LPS + Ins	1.33 ± 0.28						2.48 ± 0.41‡		
FFA (mmol/l)									
LPS	0.39 ± 0.08	0.35 ± 0.05	0.28 ± 0.04	0.51 ± 0.09	0.62 ± 0.1	0.82 ± 0.12	0.32 ± 0.09	0.012	0.024
LPS + Ins	0.29 ± 0.07	0.29 ± 0.08	0.11 ± 0.02	0.16 ± 0.06	0.32 ± 0.08	0.36 ± 0.08	0.31 ± 0.07	0.005	
Triglyceride (mg/dl)									
LPS	141 ± 24	140 ± 24	132 ± 23	133 ± 21	108 ± 20	92 ± 18	94 ± 18	0.02	NS
LPS + Ins	125 ± 21	118 ± 19	109 ± 23	104 ± 19	106 ± 22	83 ± 17	89 ± 17	0.01	
CRP (mg/l)									
LPS	1.16 ± 0.34	1.13 ± 0.28	1.21 ± 0.23	1.13 ± 0.21	1.46 ± 0.36	3.07 ± 0.52	15.03 ± 1.1	0.001	NS
LPS + Ins	1.27 ± 0.37	1.27 ± 0.39	1.28 ± 0.29	1.21 ± 0.34	1.56 ± 0.35	3.39 ± 0.61	14.25 ± 1.3	0.001	
TNF-α (pg/ml)									
LPS	1.2 ± 0.7	1.2 ± 0.7	123 ± 29	136 ± 40	18 ± 6	8.3 ± 2.2	2.6 ± 1.1	0.002	NS
LPS + Ins	0.9 ± 0.6	0.9 ± 0.6	138 ± 34	134 ± 43	21 ± 8	8.1 ± 2.1	2.1 ± 0.8	0.001	
IL-6 (pg/ml)									
LPS	2.49 ± 0.8	2.49 ± 0.8	25.7 ± 8	48.9 ± 4.5	48.3 ± 6.1	22.8 ± 5.3	4.8 ± 1.1	0.002	NS
LPS + Ins	1.9 ± 0.8	1.9 ± 0.8	26.9 ± 7	50.7 ± 4.3	49.2 ± 1.8	24.3 ± 4.3	4.4 ± 2.8	0.003	
MCP-1 (ng/ml)									
LPS	727 ± 111	727 ± 111	1,056 ± 133	1,003 ± 117	1,081 ± 140	1,345 ± 145	828 ± 138	0.008	0.028
LPS + Ins	713 ± 102	713 ± 102	700 ± 137	863 ± 127	1,093 ± 121	1,080 ± 87	885 ± 139	0.027	
LBP (μg/ml)									
LPS	13.2 ± 2.6	13.3 ± 2.8	15.02 ± 2.8	15.7 ± 3.0	15.8 ± 3.0	17.6 ± 5.3	21.2 ± 6.8	0.021	NS
LPS + Ins	11.5 ± 2.0	11.1 ± 1.7	12.0 ± 2.2	12.5 ± 2.1	12.5 ± 1.8	14.8 ± 2.8	20.2 ± 3.8	0.019	
Resistin (ng/ml)									
LPS	7.72 ± 0.9	7.78 ± 0.7	9.57 ± 0.8	17.69 ± 1.3	23.83 ± 2.2	24.74 ± 2.4	11.69 ± 1.5	0.001	NS
LPS + Ins	8.15 ± 1.1	8.04 ± 0.9	9.86 ± 1.2	17.89 ± 1.6	21.49 ± 2.3	24.95 ± 2.6	11.35 ± 1.6	0.001	

Data are means ± SEM. *$P < 0.05$ with one-way RMANOVA. †Two-way ANOVA. ‡$P < 0.05$ with paired *t* test at 24 h compared with −1 h.

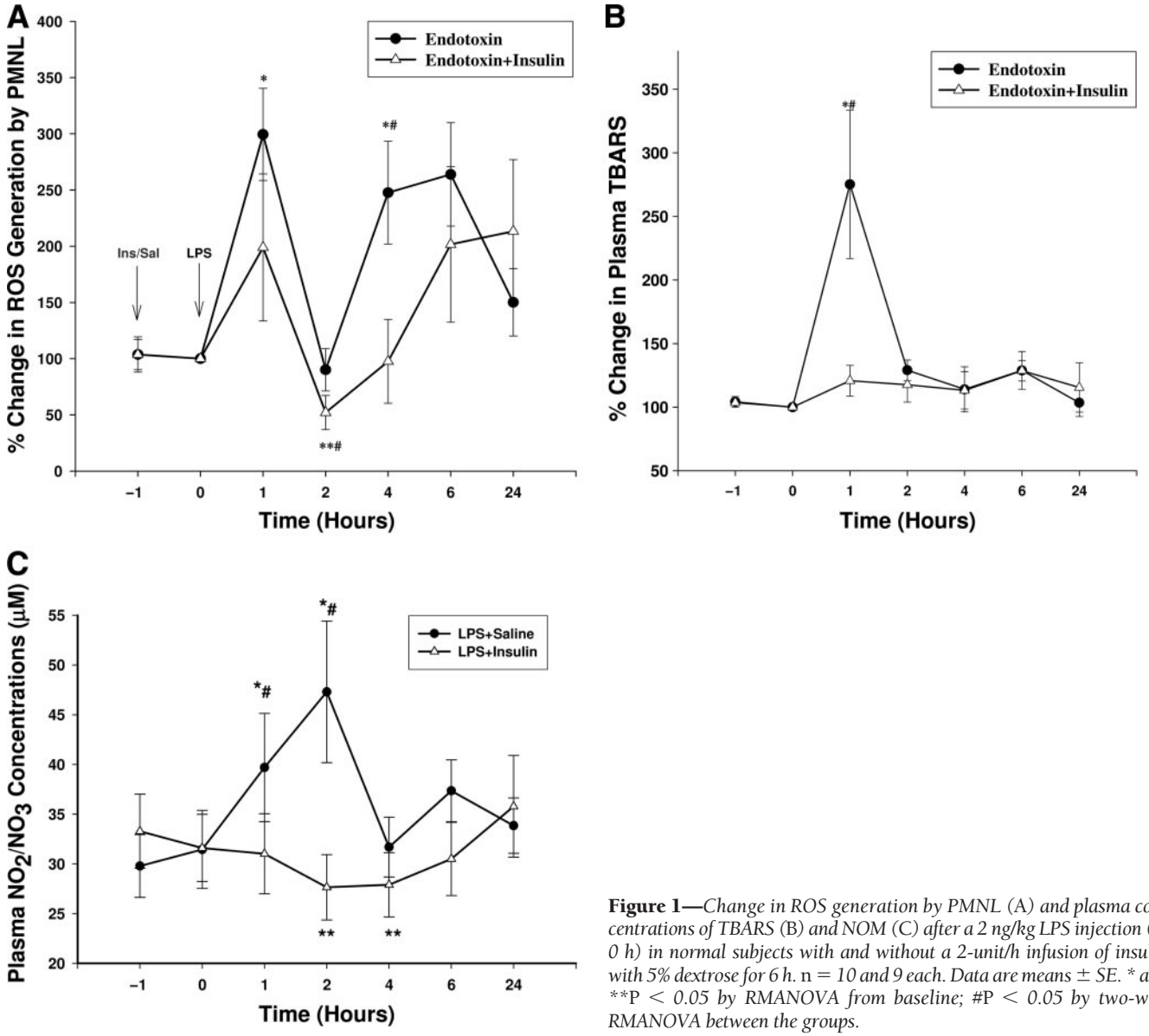

Figure 1—*Change in ROS generation by PMNL* (A) *and plasma concentrations of TBARS* (B) *and NOM* (C) *after a 2 ng/kg LPS injection (at 0 h) in normal subjects with and without a 2-unit/h infusion of insulin with 5% dextrose for 6 h.* n = *10 and 9 each. Data are means ± SE. * and* **P < *0.05 by RMANOVA from baseline;* #P < *0.05 by two-way RMANOVA between the groups.*

111 to 1,345 ± 145 ng/ml at 6 h (Fig. 2*A*). Insulin infusion with LPS prevented the LPS-induced increase of MIF during the initial increase and suppressed it significantly during the secondary increase (from 700 ± 137 to 1,080 ± 87 ng/ml at 6 h). The plasma concentration of TNF-α increased at 1 h, peaked between 1 and 2 h ($P < 0.001$, Table 1), and declined thereafter, reaching near the baseline at 24 h. IL-6 increased at 1 h, peaked between 2 and 4 h ($P < 0.001$) (Table 1), and declined toward the baseline by 24 h. CRP concentration increased at 6 h and was still elevated at 24 h (from 1.2 ± 0.2 to 15.2 ± 5.7 mg/l, $P < 0.001$) (Table 1). Insulin infusion did not alter the LPS-induced increases in TNF-α, IL-6, or CRP concentrations.

Plasma resistin, visfatin, and LBP concentrations

Plasma visfatin concentrations increased significantly after LPS injection starting at 4 h, peaked at 6 h (87 ± 37% above baseline, from 11.4 ± 1.2 to 19.2 ± 2.4 ng/ml, $P < 0.001$) (Fig. 2*B*), and was maintained at that level for 24 h. When insulin was infused before LPS injection, visfatin concentrations fell significantly from 11.9 ± 1.5 to 7.7 ± 1.1.0 ng/ml ($P < 0.001$) (Fig. 2*B*) at 4 h and were significantly different from that in the control group. LPS injection also caused a significant increase in resistin concentrations, which started at 2 h after the injection, peaked at 6 h (223 ± 25% above baseline, from 8.1 ± 1.1 to 24.7 ± 4.7 ng/ml $P < 0.001$) (Table 1) and continued to be higher than baseline at 24 h. Plasma LBP concentrations increased gradually after LPS injection and were higher by 48 ± 19% above the baseline at 24 h ($P < 0.05$) (Table 1). Insulin did not cause any significant change in the LPS-induced increases in resistin or LBP concentrations.

Plasma myoglobin and HMG-B1 concentrations

The plasma concentration of myoglobin also increased significantly from 22.3 ± 4.1 to 32.8 ± 5.3 ng/ml at 4 h and to 39 ± 5.8 ng/ml ($P < 0.05$) at 24 h after LPS injection (Fig. 3*A*). Insulin prevented the increase in myoglobin concentrations. HMG-B1 concentrations in plasma increased after LPS injection

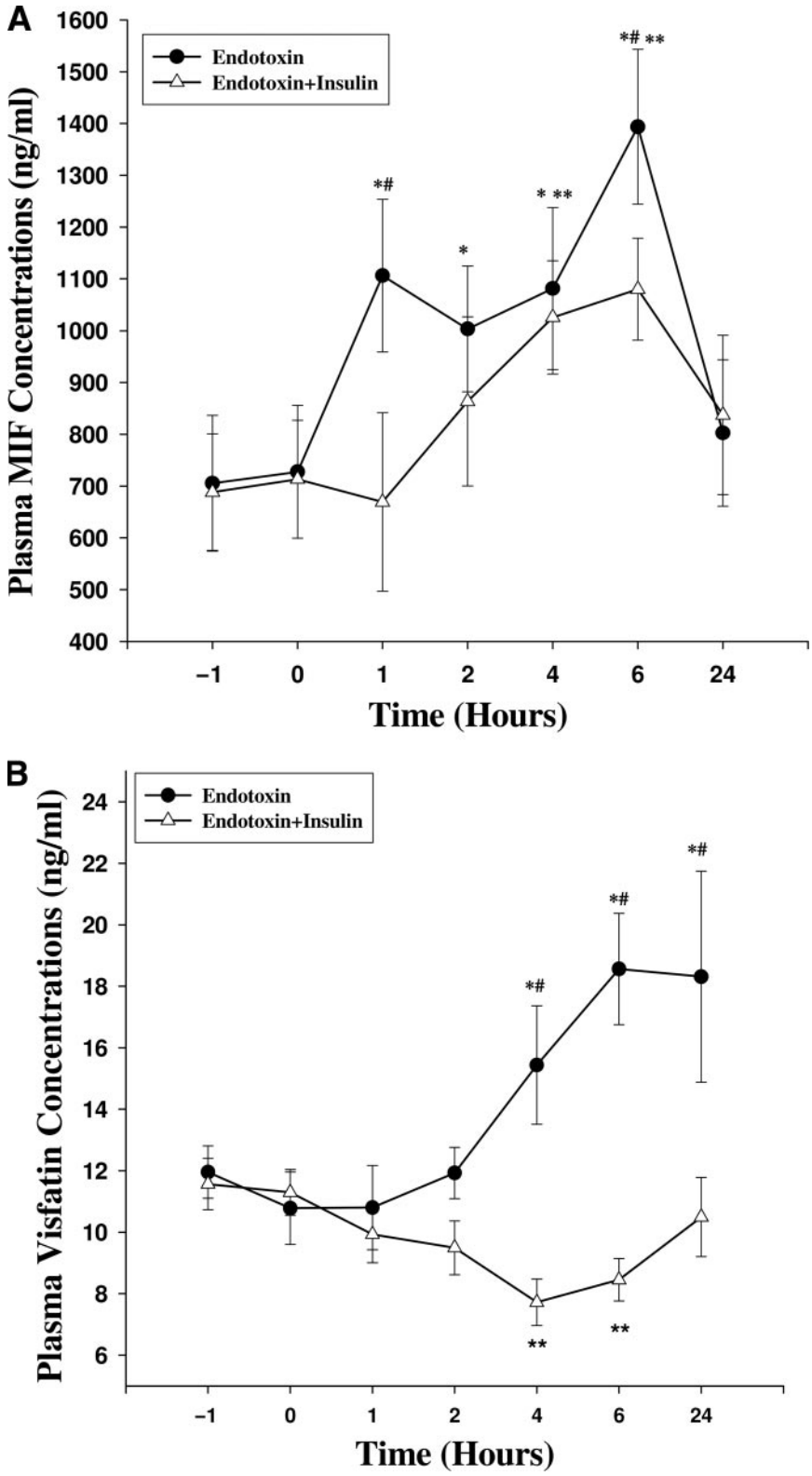

Figure 2—*Change in plasma concentrations of MIF* (A) *and visfatin* (B) *after a 2 ng/kg LPS injection (at 0 h) in normal subjects with and without a 2-unit/h infusion of insulin with 5% dextrose for 6 h.* n = *10 and 9 each. Data are means ± SE. * and **P < 0.05 by RMANOVA from baseline; #P < 0.05 by two-way RMANOVA between the groups.*

starting at 1 h and peaked at 6 h (Fig. 3*B*), whereas insulin infusion had no effect on the LPS-induced increase in HMG-B1concentrations.

CONCLUSIONS — Our data indicate several novel observations on the effects of LPS and insulin. They demonstrate for the first time the increase in ROS generation, TBARS, NOM, resistin, visfatin, myoglobin, and HMG-B1 concentrations in humans in vivo after an LPS injection. They also show for the first time that insulin infusion reduces or totally prevents the LPS-induced increases in ROS generation and the concentrations of TBARS, NOM, MIF, visfatin, and myoglobin. The relevance of each of these novel effects is discussed below.

The LPS-induced increase in ROS generation by PMNL and TBARS concentration is evidence of marked oxidative stress. Insulin suppressed the increase in ROS generation significantly while eliminating the increase in TBARS altogether. After LPS, NOM peaked at 2 h and returned to the baseline by 4 h. The insulin infusion eliminated the increase in the NOM concentration. These actions of insulin were independent of any change in glucose concentrations.

Elevated plasma NOM concentrations and inducible nitric oxide synthase expression in the liver have previously been shown to be suppressed by insulin infusions in patients in ICUs (15). In this study, the plasma concentrations of NOM in the highest quartile were associated with seven times greater mortality than those in the lowest quartile. Thus, the NOM concentration could be an important predictor of morbidity and mortality in the ICU setting (16). Whether this effect on mortality is directly related to an excess of nitric oxide generation or whether the increased NOM levels are merely markers of the intensity of systemic inflammation is not clear. Either way, the rapid induction of an increase in NOM by LPS and its total prevention by insulin are important and relevant observations.

The biphasic increase in MIF after LPS was reduced by insulin infusion. On the other hand, insulin infusion did not prevent LPS-induced increases in plasma TNF-α, IL-6, intercellular adhesion molecule-1, and MCP-1 concentrations. This observation is in contrast to our previous observation in patients with obesity and type 2 diabetes in whom insulin suppressed these mediators. CRP concentra-

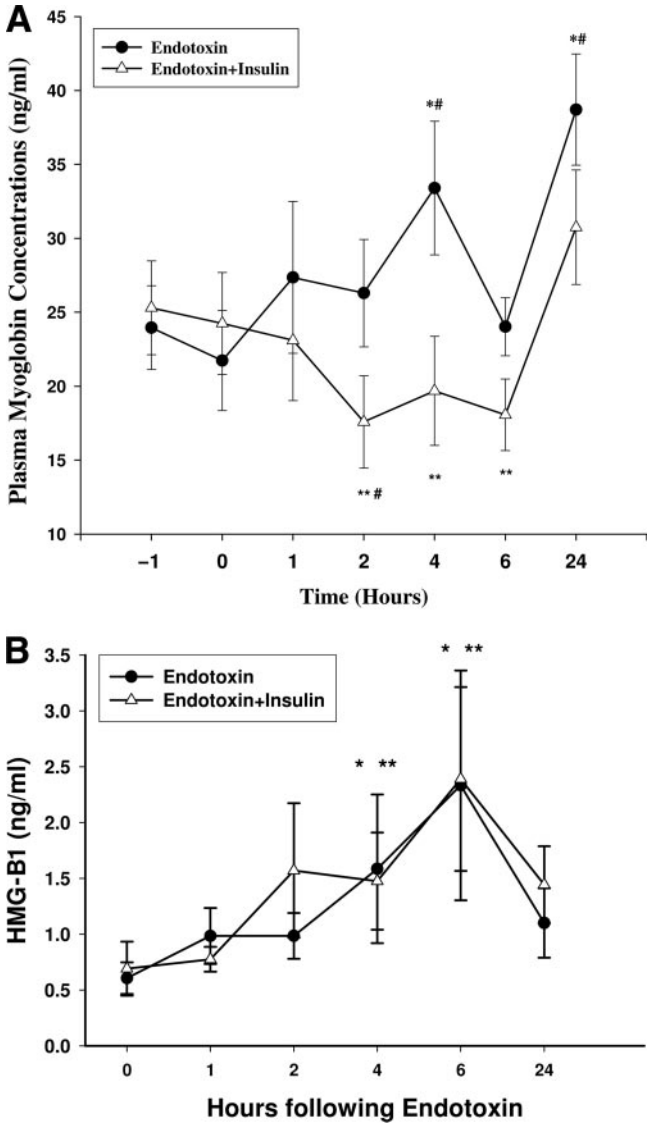

Figure 3—*Change in plasma concentrations of myoglobin* (A) *and HMG-B1* (B) *after a 2 ng/kg LPS injection (at 0 h) in normal subjects with and without a 2-unit/h infusion of insulin with 5% dextrose for 6 h.* n = *10 and 9 each. Data are presented as means ± SE. * and ***P < *0.05 by RMANOVA from baseline; #*P < *0.05 by two-way RMANOVA between the groups.*

tions began to increase at 6 h after the LPS injection at which time the infusion of insulin ended. Clearly, further studies with higher doses and for longer periods of insulin infusion are required.

LPS also induced an increase in plasma FFA concentrations within 1 h, which continued for 24 h, consistent with a potent lipolytic effect of LPS. This increase was totally inhibited by insulin. The suppressive effect of insulin FFA is important because FFAs may induce oxidative and inflammatory stress (17).

Our data also show for the first time that LPS injection in the human induced an increase in plasma concentrations of resistin and visfatin. Insulin infusion resulted in the suppression of the increase in visfatin but not resistin. The LPS-induced increase in visfatin and the prevention of this increase with insulin are of interest in terms not only of the acute LPS-induced inflammation but also of the chronic inflammation in atherosclerotic plaques because such plaques are known to contain LPS- and TLR-4-expressing macrophages (18). Such plaques also express visfatin, which may be secreted locally in response to the LPS-TLR-4 interaction (10). It is, therefore, of interest that insulin suppresses the LPS-induced increase in visfatin and has previously been shown to suppress TLR-4 expression (5). Resistin is also known to stimulate the secretion of proinflammatory cytokines, and the evidence that its concentration increases after LPS in the human in vivo establishes it as a proinflammatory mediator (19).

Plasma concentrations of LBP also increased after LPS injection, demonstrated for the first time. The increase started late, at 6 h, like that of CRP and the previously described increase in procalcitonin (20) and continued overnight at 24 h. The increase in the concentration of LBP after LPS is important because LBP facilitates the binding of LPS to its receptor, TLR-4. As with CRP, the increase in LBP was not affected by insulin, possibly because both increased at 6 h and the insulin infusion was stopped at that time.

It is of interest that although plasma glucose concentrations did not alter significantly, insulin concentrations and HOMA-IR increased significantly 24 h after LPS injection in concert with the induction of profound inflammation. This result is consistent with the recent observation that the injection of LPS (3 ng/kg) in normal subjects induced insulin resistance as measured by frequently sampled intravenous glucose tolerance and HOMA-IR (21).

Our data also show for the first time that LPS induces an increase in HMG-B1 concentration in humans. Insulin did not alter this increase. HMG-B1 is a nuclear protein that binds to histones to promote proinflammatory gene transcription. It can be released from damaged, necrotic tissues. Circulating HMG-B1 acts like a proinflammatory cytokine through its binding to the receptor for advanced glycation end products (22).

The increase in plasma myoglobin concentrations after LPS and its inhibition by insulin is important because it signifies damage to the skeletal muscle and possibly the myocardium. Consistent with this observation, we have previously shown a reduction in the increase in myoglobin concentrations in patients with myocardial infarction treated with intravenous insulin infusions. This finding is suggestive of a cytoprotective effect of insulin.

The mechanisms underlying the effects of insulin observed in this report are probably related to several of our previous observations. Insulin suppresses the expression of TLR-4, the receptor for LPS, to reduce the activity of the major proinflammatory transcription factor, NFκB (3,5). Insulin has also been shown to suppress ROS generation and the expression

of the p47 subunit of NADPH oxidase. Insulin has been shown previously to suppress inducible nitric oxide synthase expression and the plasma NOM concentration (15).

Consistent with our observations are those of Jeschke et al. (23), who demonstrated that compared with control subjects, patients with severe burns given insulin had lower MIF and other proinflammatory cytokines and CRP concentrations with a tendency toward higher IL-10 concentrations. Insulin infusion with maintenance of normoglycemia has been shown to reduce mortality and morbidity in patients in a surgical ICU (24) and in patients in a medical ICU (25), whose stay in the ICU is for longer than 3 days.

In summary, the injection of LPS in the human induces an immediate increase in ROS generation by PMNLs and in plasma concentrations of TBARS, NOM, FFA, MIF, resistin, visfatin, LBP, and myoglobin. The concomitant infusion of insulin induces a significant reduction in ROS generation and the total prevention of the increase in TBARS, NOM, and FFA concentrations. These actions were associated with a significant reduction in the magnitude of increase in MIF, myoglobin, and visfatin concentrations independently of any change in plasma glucose concentrations. On the other hand, insulin was not able to prevent or reduce the magnitude of increase in plasma concentrations of proinflammatory cytokines like TNF-α, IL-6, or MCP-1. Clearly, the effect of more prolonged infusions and higher doses of insulin needs to be investigated.

Acknowledgments— P.D. is supported by the National Institutes of Health (grants R01-DK-069805 and R01-DK-075877) and the American Diabetes Association (grant 08-CR-13) and by Merck, sanofi-aventis, Amylin, GlaxoSmithKline, and Solvay Pharmaceuticals.

No other potential conflicts of interest relevant to this article were reported.

P.D. developed the hypothesis, analyzed and interpreted data, and wrote the manuscript. H.G. analyzed samples, performed statistical analysis of laboratory data, provided the diagrammatic presentation of data, and wrote the manuscript. A.B. developed the hypothesis and performed clinical experiments and patient care. K.K. performed laboratory measurements and analyzed laboratory data. C.L.S. analyzed laboratory data. S.D. provided clinical care and analyzed data. A.C. provided clinical care, analyzed data, and wrote the manuscript.

References

1. Manthous CA, Hall JB, Samsel RW. Endotoxin in human disease. Part 1: Biochemistry, assay, and possible role in diverse disease states. Chest 1993;104:1572–1581
2. Zhang G, Ghosh S. Molecular mechanisms of NF-κB activation induced by bacterial lipopolysaccharide through Toll-like receptors. J Endotoxin Res 2000; 6:453–457
3. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S. Insulin inhibits intranuclear nuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 2001;86:3257–3265
4. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the proinflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. J Clin Endocrinol Metab 2002;87:1419–1422
5. Ghanim H, Mohanty P, Deopurkar R, Sia CL, Korzeniewski K, Abuaysheh S, Chaudhuri A, Dandona P. Acute modulation of toll-like receptors by insulin. Diabetes Care 2008;31:1827–1831
6. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin [retracted in: Science 2007;318: 565]. Science 2005;307:426–430
7. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. Nature 2001; 409:307–312
8. Boström EA, Tarkowski A, Bokarewa M. Resistin is stored in neutrophil granules being released upon challenge with inflammatory stimuli. Biochim Biophys Acta 2009;1793:1894–1900
9. Nagaev I, Bokarewa M, Tarkowski A, Smith U. Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. PLoS One 2006;1:e31
10. Dahl TB, Yndestad A, Skjelland M, Øie E, Dahl A, Michelsen A, Damås JK, Tunheim SH, Ueland T, Smith C, Bendz B, Tonstad S, Gullestad L, Frøland SS, Krohg-Sørensen K, Russell D, Aukrust P, Halvorsen B. Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation 2007;115:972–980
11. Aono K, Isobe K, Kiuchi K, Fan ZH, Ito M, Takeuchi A, Miyachi M, Nakashima I, Nimura Y. In vitro and in vivo expression of inducible nitric oxide synthase during experimental endotoxemia: involvement of other cytokines. J Cell Biochem 1997; 65:349–358
12. Hagiwara S, Iwasaka H, Hasegawa A, Asai N, Noguchi T. Hyperglycemia contributes to cardiac dysfunction in a lipopolysaccharide-induced systemic inflammation model. Crit Care Med 2009; 37:2223–2227
13. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, Dandona P. Increase in intranuclear nuclear factor κB and decrease in inhibitor κB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. Am J Clin Nutr 2004;79:682–690
14. Dandona P, Karne R, Ghanim H, Hamouda W, Aljada A, Magsino CH Jr: Carvedilol inhibits reactive oxygen species generation by leukocytes and oxidative damage to amino acids. Circulation 2000;101:122–124
15. Langouche L, Vanhorebeek I, Vlasselaers D, Vander Perre S, Wouters PJ, Skogstrand K, Hansen TK, Van den Berghe G. Intensive insulin therapy protects the endothelium of critically ill patients. J Clin Invest 2005;115:2277–2286
16. Dandona P, Mohanty P, Chaudhuri A, Garg R, Aljada A. Insulin infusion in acute illness. J Clin Invest 2005;115:2069–2072
17. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 2003;52:2882–2887
18. Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation 2001;104:3103–3108
19. Jung HS, Park KH, Cho YM, Chung SS, Cho HJ, Cho SY, Kim SJ, Kim SY, Lee HK, Park KS. Resistin is secreted from macrophages in atheromas and promotes atherosclerosis. Cardiovasc Res 2006;69: 76–85
20. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, Bohuon C. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605–1608
21. Mehta NN, McGillicuddy FC, Anderson PD, Hinkle CC, Shah R, Pruscino L, Tabita-Martinez J, Sellers KF, Rickels MR, Reilly MP. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. Diabetes 2010;59: 172–181

22. van Zoelen MA, Yang H, Florquin S, Meijers JC, Akira S, Arnold B, Nawroth PP, Bierhaus A, Tracey KJ, van der Poll T. Role of Toll-like receptors 2 and 4, and the receptor for advanced glycation end products in high-mobility group box 1-induced inflammation in vivo. Shock 2009;31:280–284
23. Jeschke MG, Klein D, Herndon DN. Insulin treatment improves the systemic inflammatory reaction to severe trauma. Ann Surg 2004;239:553–560
24. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001;345:1359–1367
25. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. N Engl J Med 2006;354:449–461