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Insulin Inhibits Intranuclear Nuclear Factor κB and Stimulates IκB in Mononuclear Cells in Obese Subjects: Evidence for an Anti-inflammatory Effect?

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ABSTRACT

In view of the fact that insulin resistance is associated with atherogenesis and that troglitazone, an insulin sensitizer, has anti-inflammatory effects, which may be potentially antiatherogenic in the long term, we have now investigated whether insulin has potential anti-inflammatory effects. We infused 2.0 to 2.5 IU/h in 5% dextrose (100 mL/h) iv into 10 obese subjects for 4 h followed by 5% dextrose alone for 2 h. The rate of insulin infusion was varied to maintain glucose concentrations as close to the baseline as possible. Blood samples were obtained before and at 2, 4, and 6 h. Subjects were also infused with 5% dextrose without insulin and with saline on separate occasions. Intranuclear nuclear factor κB (NFκB) in mononuclear cells fell at 2 and further at 4 h, reverting toward the baseline at 6 h ($P < 0.05$). IκB increased significantly at 2 h, increasing further at 4 h and remaining elevated at 6 h ($P < 0.001$). Reactive oxygen species (ROS) generation by mononuclear cells fell significantly at 2 h and fell further at 4 h; it partially reverted to baseline at 6 h ($P < 0.005$). $p47^{phox}$ subunit, the key protein of nicotinamide adenine dinucleotide phosphate oxidase also fell at 2 h and 4 h, reverting toward the baseline at 6 h ($P < 0.05$). In addition, soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1) fell significantly following insulin infusion. Glucose or saline infusions without insulin caused no alteration in NFκB, IκB, ROS generation, $p47^{phox}$ subunit, sICAM-1, MCP-1, or PAI-1.

We conclude that insulin has a potent acute anti-inflammatory effect including a reduction in intranuclear NFκB, an increase in IκB, and decreases in ROS generation, $p47^{phox}$ subunit, plasma soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1. This acute anti-inflammatory effect, if demonstrated in the long term, may have implications for atherosclerosis and its complications. (*J Clin Endocrinol Metab* **86:** 3257–3265, 2001)

MONONUCLEAR CELLS (MNC), monocytes in particular, are cellular mediators of inflammation (1, 2). The monocyte is also the cell that initiates the process of atherosclerosis when it gets attached to abnormal/damaged endothelium (3, 4). Monocytes and MNC are known to be active in atherosclerosis and diabetes mellitus. Increased reactive oxygen species (ROS) generation by MNC and monocytes leads to increased tissue/cellular damage (5, 6), increased lipid peroxidation (7), protein oxidation (8), and DNA damage (9) in diabetic patients. Increased lipid peroxidation and increased activation of monocytes are key processes in the formation of foam cells and the pathogenesis of the fatty streak, atherosclerosis (10, 11). Atherosclerosis is now thought to be an inflammation of the arterial wall (1).

The cardinal cellular signal of inflammation is the transcription factor, nuclear factor κB (NFκB), which induces the transcription of pro-inflammatory cytokines, adhesion molecules, and enzymes generating ROS (12, 13). We have previously demonstrated that glucocorticoids inhibit ROS generation and NFκB in MNC *in vivo* (14). This action is believed to be the basis of their anti-inflammatory effect. In this study, we showed that hydrocortisone at a modest dose of 100 mg given iv induced a rapid fall in ROS generation by polymorphonuclear leukocytes (PMNL) and MNC, reduced intranuclear and total cellular NFκB and induced IκB, an inhibitor of NFκB. These effects are now thought to be the basis of the anti-inflammatory action of glucocorticoids and other anti-inflammatory drugs like aspirin (15). In view of the fact that 1) insulin resistance is associated with increased atherogenesis (16, 17), 2) troglitazone, a thiazolidinedione and an insulin sensitizer, has an inhibitory effect on NFκB and other inflammatory mediators (18 19), and 3) insulin inhibits NFκB in human aortic endothelial cells *in vitro* (20), we have now investigated whether insulin inhibits *in vivo*: 1) ROS generation and $p47^{phox}$ subunit by MNC; 2) NFκB and stimulates its inhibitor IκB in MNC; and 3) inflammatory mediators, including soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1. This study tests the hypothesis that insulin may have an anti-inflammatory and, thus, a potential antiatherogenic effect.

Subjects, Materials, and Methods

Subjects

Ten obese nondiabetic subjects (age range 29–64 yr; mean 48.3 ± 10.9 yr), all with body mass index greater than 37 kg/m^2, (body mass index mean 42.6 ± 9.1 kg/m^2) were included in this study. All patients had a fasting venous plasma glucose of less than 100 mg/dL. None of the obese subjects was on vitamin E or C or any other antioxidant therapy.

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There were eight female and two male subjects. Insulin was infused into subjects (2–2.5 IU/h) along with 5% dextrose (100 mL/h) for 4 h. The rate of insulin infusion was titrated such that glucose concentrations were maintained as close to the basal levels as possible. Blood samples were collected at baseline and at 2, 4, and 6 h. Subjects returned after 2–3 weeks and were infused with 5% dextrose (100 mL/h) only or saline (100 mL/h) only for control data. The Institutional Review Board of the State University of New York at Buffalo based at Millard Fillmore Hospital approved the study. Written informed consent was obtained from all subjects.

MNC isolation

Blood samples were collected in Na-EDTA as an anticoagulant. Three and a half milliliters of the anticoagulated blood sample were carefully layered over 3.5 mL of the PMN isolation medium (Robbins Scientific Corp., Sunnyvale, CA). Samples were centrifuged at 450 $\times$ *g*, in a swingout rotor for 30 min at 22 C. At the end of the centrifugation, two bands separate out at the top of the red blood cell pellet. The top band consists of MNC, while the bottom consists of PMNL. The MNC band was harvested with a Pasteur pipette, repeatedly washed with HBSS and reconstituted to a concentration of 4×10^5 cells/mL in HBSS. This method provides yields greater than 95% pure MNC suspension.

NFκB electrophoretic mobility shift assay

DNA-binding protein extracts were prepared from MNC by the method described by Andrews et al. (21). Total protein concentrations were determined using BCA protein assay (Pierce Chemical Co., Rockland, IL). NFκB gel retardation assay was performed using NFκB-binding protein detection kit (Life Technologies, Inc., Long Island, NY). Briefly, the double-stranded oligonucleotide containing a tandem repeat of the consensus sequence for the NFκB-binding site was radiolabeled with γ-P^{32} by T4 kinase. Then 5 μg of the nuclear extract were mixed with the incubation buffer and the mixture was preincubated at 4 C for 15 min. Labeled oligonucleotide (60,000 cpm) was added, and the mixture was incubated at room temperature for 20 min. Samples were then applied to wells of 6% nondenaturing polyacrylamide gel. The gel was dried under vacuum and exposed to x-ray film. Densitometry was performed using Bio-Rad Laboratories, Inc. molecular analyst software (Hercules, CA).

Total IκB and p47^{phox} subunit Western blotting

MNC cell lysates were prepared by adding 1 mL boiling lysis buffer (1% SDS), 1 mM sodium ortho-vanadate, 10 mM Tris (pH 7.4) to MNC pellets. Total protein concentrations were determined using BCA protein assay (Pierce Chemical Co.). Sixty micrograms of total cell lysate were electrophoresed on 12% for IκB or 10% for p47^{phox} subunit. The proteins were transferred to polyvinylidene difluoride membrane, blocked for 1 h in 5% nonfat dry milk and then incubated for 1 h with polyclonal antibody against IκB (Rockland, Gilbertsville, PA) or a monoclonal antibody against p47^{phox} subunit (Transduction Laboratories, Inc., San Diego, CA). Finally, the membrane was washed and developed using super signal chemiluminescence reagent (Pierce Chemical Co.). Densitometry was performed using Bio-Rad Laboratories, Inc. molecular analyst software.

ROS generation assay

Respiratory burst activity of MNC was measured by detection of superoxide radical via chemiluminescence. Five hundred milliliters PMNL or MNC (2×10^5 cells) were delivered into a Chronolog Lumi-Aggregometer cuvette to which a spin bar was added. Fifteen milliliters of 10 mM luminol were then added, followed by 1.0 μL of 10 mM formylmethionyl leucinyl phenylalanine. Chemiluminescence was recorded for 15 min (a protracted record after 15 min did not alter the relative amounts of chemiluminescence produced by various cell samples). Our method, developed independently, is similar to that published by Tosi and Hamedani (22). In this assay system, the release of superoxide radical as measured by chemiluminescence, has been shown to be linearly correlated with that measured by the ferricytochrome C method. We further established that, in our assay system, there is a dose-dependent inhibition of chemiluminescence by superoxide dismutase and catalase as well as diphenylene iodonium (data not shown), a specific inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme responsible for the production of superoxide radicals. The specific inhibitory effect of diphenylene iodonium on NADPH oxidase has been established by Hancock and Jones (23). The

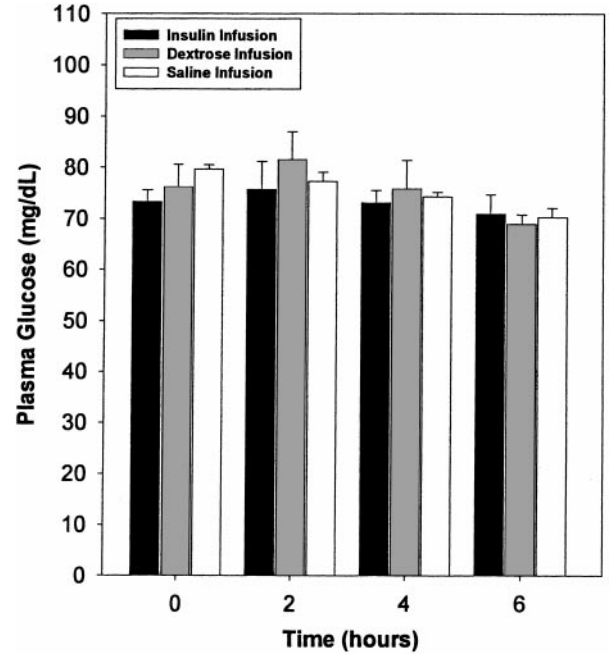

FIG. 1. Plasma glucose levels.

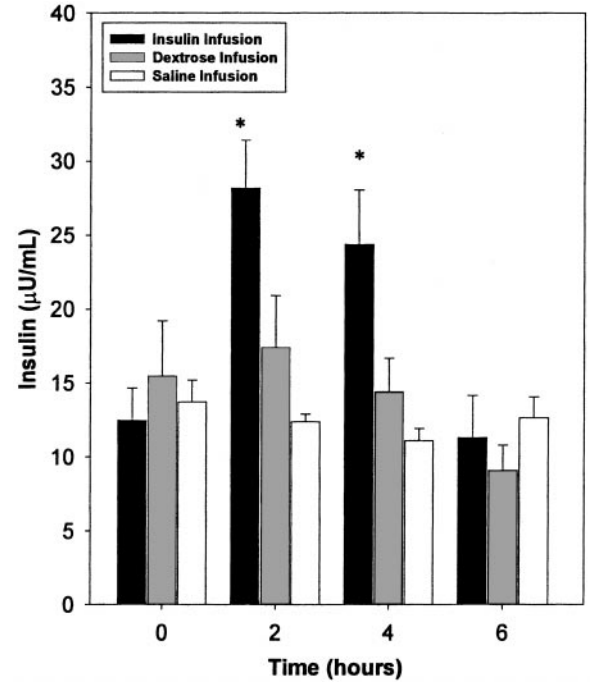

FIG. 2. Plasma insulin concentrations increased to over two times the basal in the insulin infusion group, and it decreased to a much smaller extent in the glucose infusion group. The saline infusion group did not show any change in insulin concentrations (*, $P < 0.05$).

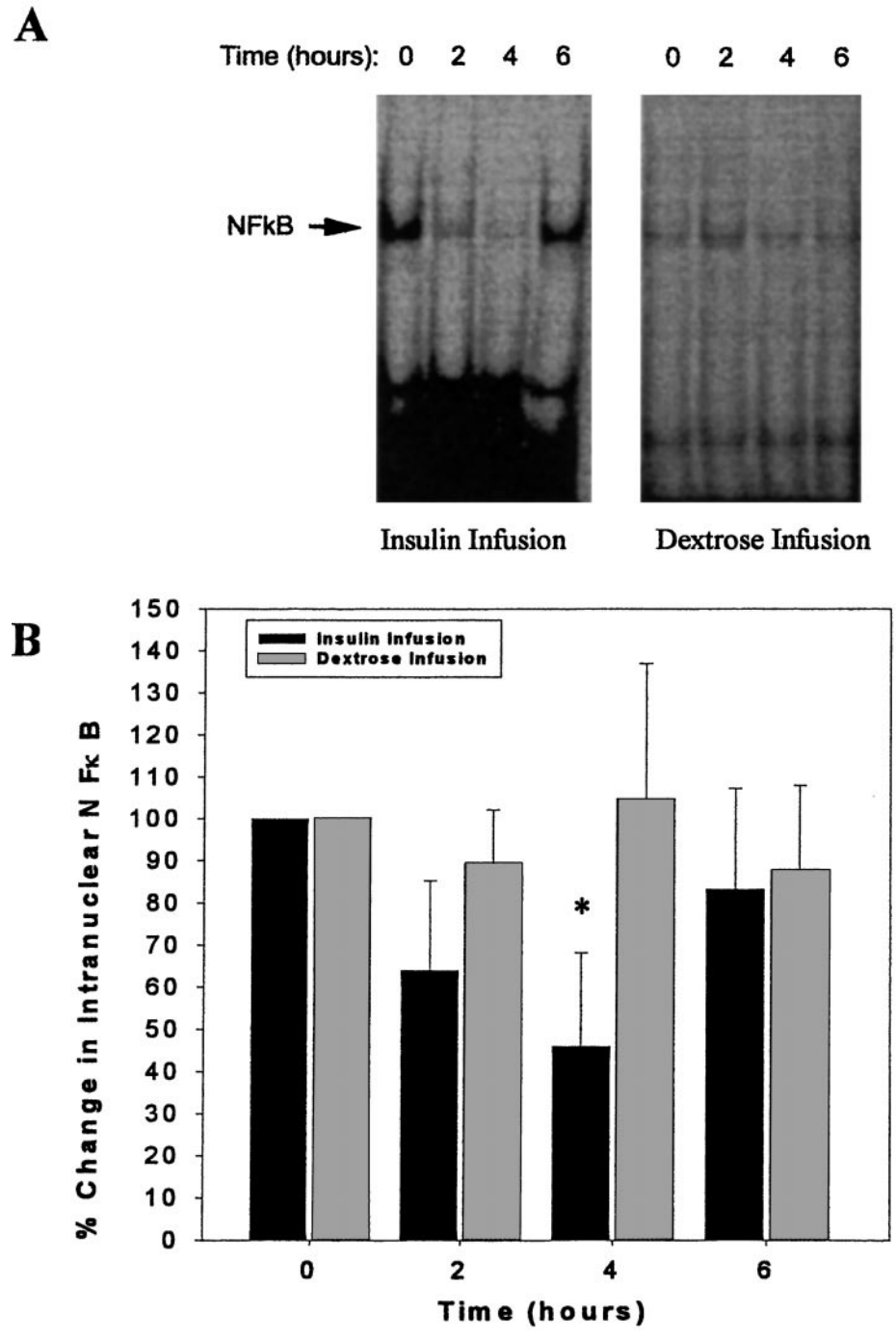

FIG. 3. A, Gel shift assay showing the relative NFκB binding to the double-stranded oligonucleotide containing NFκB DNA binding site following insulin or dextrose infusion. Band-shift assays were performed using 5 μg MNC nuclear extract for each time point. B, Relative NFκB binding to double-stranded oligonucleotide containing NFκB DNA binding site. All values were normalized to 100% for baseline levels and the following values were expressed as percent of basal. The results are presented as mean ± SE; *, $P < 0.05$.

variation of ROS generation by MNC in normal or obese subjects varies by less than 8% over a period of 2 weeks.

Plasma sICAM-1, MCP-1, and PAI-1 measurements

Plasma sICAM-1 and MCP-1 were assayed with enzyme-linked immunosorbent assay kits from R&D systems (Minneapolis, MN). Plasma PAI-1 levels were measured using a TintElize PAI-1 kit (Biopool International, Ventura, CA).

Plasma insulin and glucose measurements

Insulin was measured from fasting plasma samples using an enzyme-linked immunosorbent assay kit (Diagnostics Systems Laboratories, Inc., Webster, TX). Glucose was measured in whole blood by a Hemocue glucose analyzer (Hemocue Inc., Mission Viogo, CA).

Statistical analysis

Statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA). All data on ROS generation, MCP-1, sICAM-1, and PAI-1 were normalized to a baseline of 100% in view of the interindividual variability and are expressed accordingly as percent of the basal. Kruskal-Wallis one-way ANOVA on ranks was used to compare all the indices measured in this study. Dunnett's method was used for all pairwise comparisons. The results are expressed as mean ± SE.

Results

Plasma glucose and insulin concentrations

Plasma glucose concentration remained steady during insulin infusion. Glucose levels were 73 ± 2.3 mg/dL at baseline, 76 ± 5.4 mg/dL at 2 h and 73 ± 2.4 mg/dL at 4 h (Fig. 1). Plasma glucose concentration remained steady also following dextrose or saline infusion. Plasma insulin concentrations increased from a basal level of 12.5 ± 2.2 μU/mL to 28.2 ± 3.3 μU/mL at 2 h and 24.4 ± 3.7 μU/mL at 4 h after insulin infusion (Fig. 2). Insulin levels decreased after insulin infusion cessation and returned to basal level (11.3 ± 2.8 μU/mL). Dextrose infusion caused a slight increase in insulin levels at 2 h and saline infusion alone did not affect insulin level as shown in Fig. 2.

NFκB levels. Intranuclear NFκB as measured by electrophoretic mobility shift assay in MNC fell significantly following insulin infusion at 2 h (64 ± 21% of the basal level) with a peak inhibitory effect at 4 h 46 ± 22% of the basal level). Intranuclear NFκB reverted toward normal at 6 h (72 ± 12% of the basal level), but it was still less than that at baseline (Fig. 3; $P < 0.05$). Glucose infusion did not cause any change in NFκB levels.

IκB levels. IκB protein levels in MNC increased significantly to 254 ± 99% at 2 h, peaked at 4 h (403 ± 266% of the basal

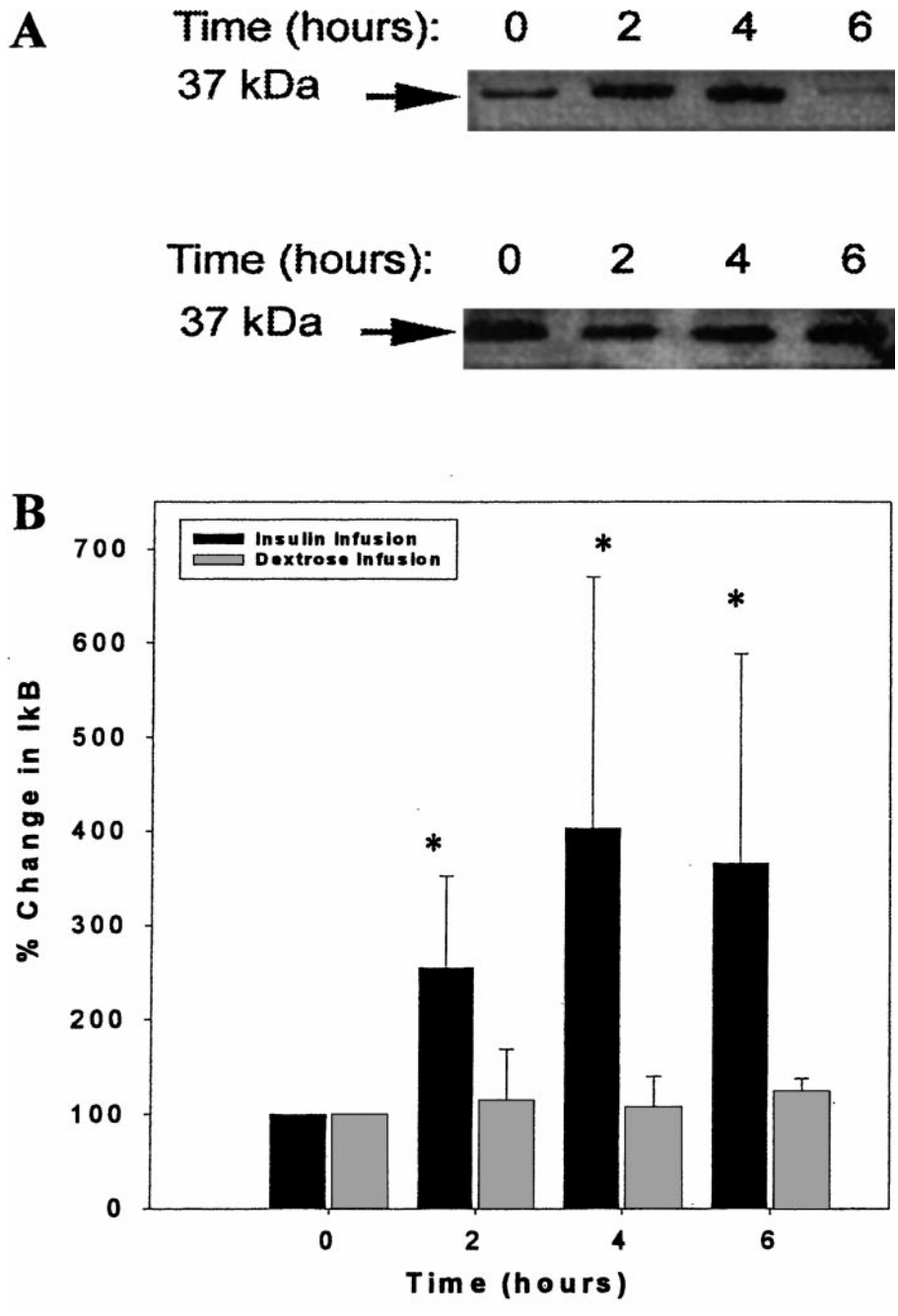

FIG. 4. A, A representative Western blot showing the increase in IκBα quantity in MNC homogenates following insulin *(upper gel)* or dextrose *(lower gel)* infusions. B, Densitometric quantitative analysis of IκB protein content (*, $P < 0.05$).

level) and declined after stopping insulin infusion to 366 ± 222% (Fig. 4). The increase was highly significant ($P < 0.001$). Dextrose infusion alone did not affect IκB protein levels.

ROS generation by MNC. Insulin infusion caused a significant fall in ROS generation by MNC from a basal level of 359 ± 58 mV (100%) to 56.8 ± 24.6% of basal at 2 h and 47.3 ± 11.2% at 4 h ($P < 0.005$). ROS generation reverted to 74.8% of basal at 6 h, 2 h after cessation of insulin infusion (Fig. 5). Glucose or saline infusions did not cause any significant change in ROS generation by MNC.

$p47^{phox}$ subunit protein levels. Immunoblots for MNC homogenates showed a fall in $p47^{phox}$ subunit at 2 h (81 ± 15% of the basal level), which progressively decreased further to a nadir at 4 h (75 ± 9% of the basal level). At 6 h, it returned toward the baseline (Fig. 6; $P < 0.05$). A fall in $p47^{phox}$ subunit expression was observed in 8 out of 10 subjects. Neither dextrose nor saline infusion alone changed $p47^{phox}$ subunit protein levels significantly.

Plasma MCP-1 and sICAM-1 concentrations. Both plasma MCP-1 and sICAM-1 concentrations decreased significantly at 2 h after insulin infusion, continued to be inhibited at 4 h, and reverted toward the baseline at 6 h (Figs. 7 and 8). MCP-1 levels decreased from a basal level of 161.4 ± 14.1 pg/mL (100%) to 89.4 ± 9.9% at 2 h, 79.1 ± 4.8% and 93.0 ± 7.2% at 6 h. sICAM-1 levels decreased from 292 ± 20 ng/mL (100%) to 85 ± 4.7 at 2 h and remained inhibited at 6 h (88 ± 1.4%).

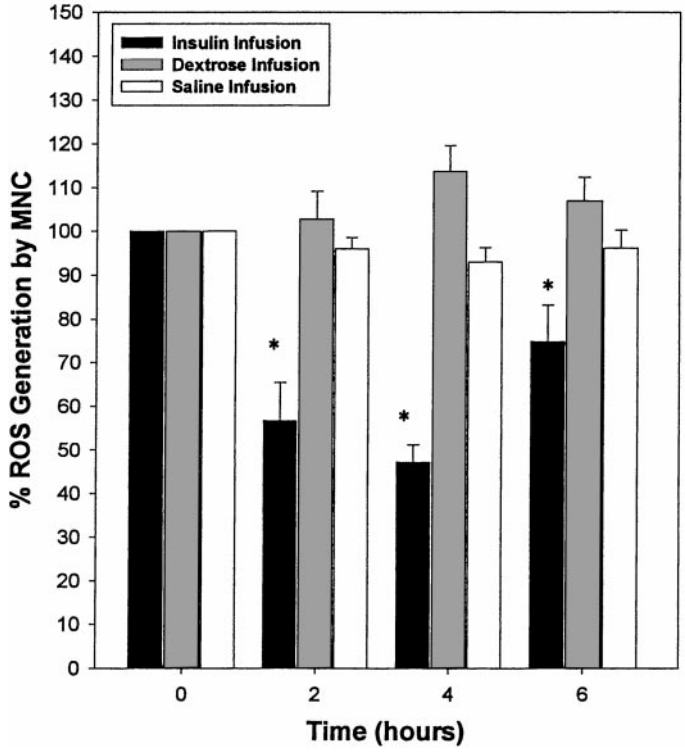

FIG. 5. ROS generation by MNC in obese subjects following insulin or dextrose or saline infusions. Note that ROS generation decreased significantly at 2 h and continued to be inhibited and reached a nadir at 4 h ($P < 0.005$). ROS generation started to increase thereafter after insulin infusion was stopped. Dextrose or saline infusions did not cause any significant change in ROS generation (*, $P < 0.05$).

It started to increase thereafter at 6 h (92.9 ± 1.4%). The fall in MCP-1 and sICAM-1 was statistically significant as compared by Kruskal-Wallis one-way ANOVA on ranks ($P < 0.05$). Neither dextrose nor saline infusion alone caused any significant changes in plasma MCP-1 or sICAM-1 concentrations.

Plasma PAI-1 concentrations. PAI-1 level was 84 ± 9.6 ng/mL. PAI-1 levels decreased significantly after insulin infusion at 2 h (57 ± 6.7% of the basal level) and at 4 h (58 ± 8.3% of the basal level). PAI-1 levels started to increase after insulin infusion was stopped as shown in Fig. 9. The inhibition of PAI-1 level was highly significant ($P < 0.001$). PAI-1 levels did not change following dextrose or saline infusion alone.

Discussion

Our data show for the first time that insulin infusion results in a fall in NFκB and an increase in IκB in MNC. These changes are characteristic of an anti-inflammatory effect at the cellular and molecular level. NFκB is a proinflammatory transcription factor that leads to the transcription of proinflammatory cytokines and adhesion molecules and to enzymatic mechanisms that cause ROS generation (12, 13). IκB binds to cytosolic NFκB and prevents its translocation into the nucleus (24, 25). The decrease in intranuclear NFκB, $p47^{phox}$ subunit and the increase in IκB occurred at 2 h and peaked at 4 h. Although NFκB returned to baseline level by 6 h, the increase in IκB persisted even at 6 h. These changes are consistent with an acute anti-inflammatory effect of insulin and are suggestive that in the long term, a persistent effect of this kind would indicate a potential antiatherogenic action of this hormone.

ROS generation by MNC fell in parallel with the fall in NFκB. ROS generation in our system mainly measures the superoxide radical (O_2^-), which is formed from molecular oxygen (O_2) by the enzyme NADPH oxidase (26). Our observations are thus consistent with a suppression of NADPH oxidase by insulin. Because ROS, including O_2^-, stimulate NFκB mediated inflammation (27), it is possible that the suppression of O_2^- contributes to the fall in NFκB. NFκB also stimulates ROS generation, induces adhesion molecules and proinflammatory cytokines in MNC, and thus promotes inflammation (12, 13). The suppression of NFκB would thus reduce ROS generation, the expression of adhesion molecules and proinflammatory cytokines and reduce inflammation. Consistent with these changes, the expression of $p47^{phox}$ subunit, the key protein of the NADPH oxidase complex (26), falls significantly following insulin; this is indicative of a fall in NADPH oxidase activity and O_2^- generation. It is noteworthy that $p47^{phox}$ subunit also falls after hydrocortisone administration in parallel with the fall in ROS generation (28, 29).

In control experiments conducted with glucose infusions without insulin, there was a consistently small but insignificant increase in ROS generation. We have previously described an increase in ROS generation by leukocytes following a 75-g glucose challenge (30). Thus, our current observation with a 30-g glucose challenge over a period of 6 h is consistent with our previous observation. The small increase in ROS generation induced by glucose further em-

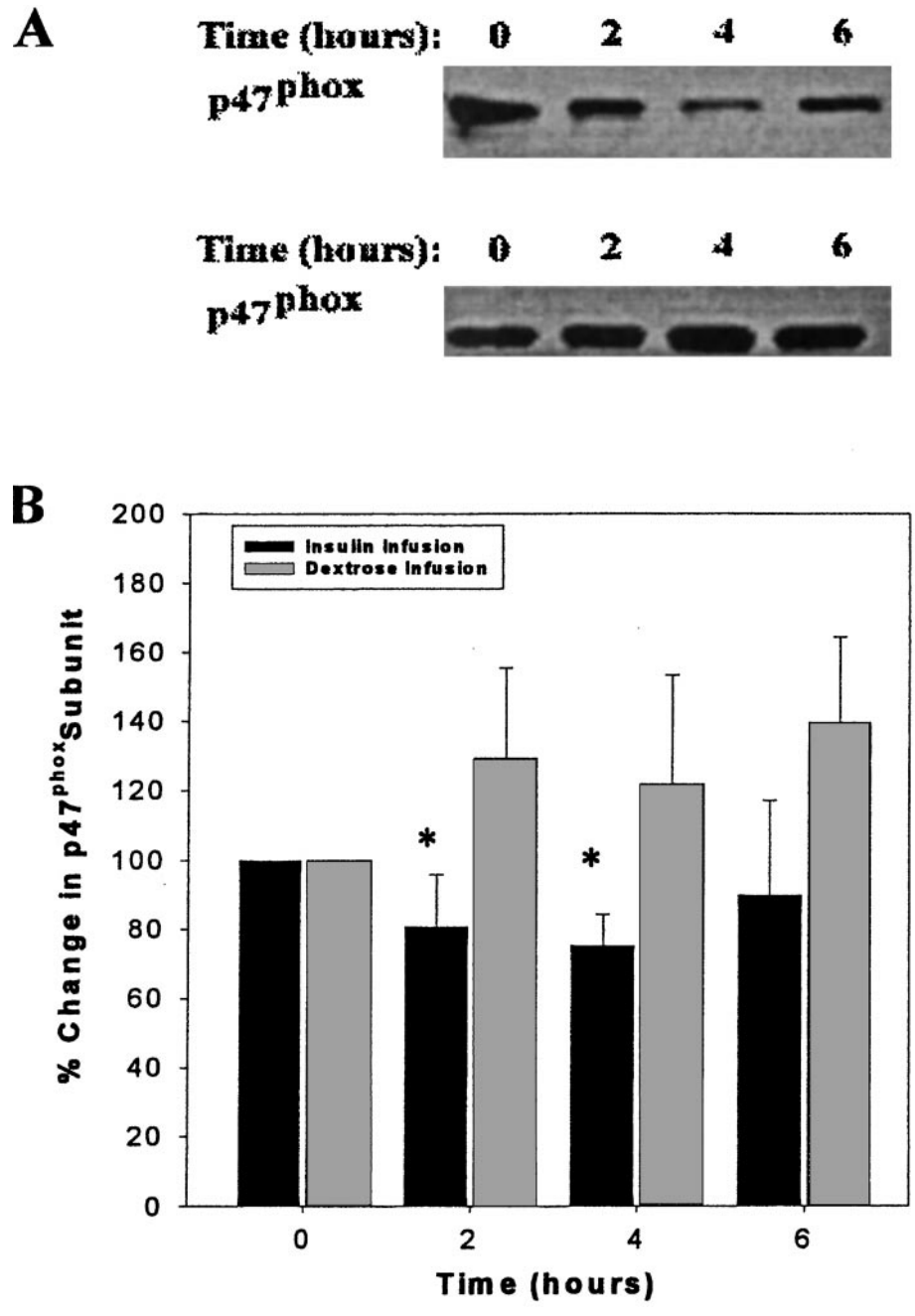

FIG. 6. A, A representative Western blot showing the relative expression of $p47^{phox}$ subunit in MNC following insulin infusion (upper gel) or dextrose infusion (lower gel). Dextrose infusion caused an increase in $p47^{phox}$ subunit protein content. This increase was not statistically significant. B, Densitometric quantitative analysis of $p47^{phox}$ subunit protein contents in MNC showing a significant decrease in the insulin treated group. The results are presented as mean ± SE ($P < 0.05$).

phasizes the inhibitory effect of insulin shown by our experiments. Glucose infusion did not cause a significant change in NFκB or IκB levels or $p47^{phox}$ subunit expression.

It is of interest that these effects of insulin were observed at physiologically relevant concentrations: 28.2 ± 3.3 μU/mL at 2 h and 24.4 ± 3.7 μU/mL at 4 h. These concentrations are comparable to those having effect on hepatic glucose production but are less than those required for glucose uptake by skeletal muscle (31).

We have recently also demonstrated that troglitazone, a thiazolidinedione, known to be an insulin sensitizer, also causes an inhibition of ROS generation, an inhibition of NFκB, an induction of IκB and a suppression of $p47^{phox}$ subunit (18, 19). These changes are associated with a fall in oxidative damage and an improvement in vascular reactivity: postischemic vasodilatation of the brachial artery (18). The insulin-induced anti-inflammatory changes described in this paper are almost identical. Insulin is known to be a vasodilator (32, 33) and exerts this effect through nitric oxide release (34) and the induction of endothelial nitric oxide synthase (35). Nitric oxide is also known to have profound effects on indices associated with inflammation, *e.g.*, ICAM-1 (36, 37). Furthermore, insulin causes an inhibition of ICAM-1 expression by human aortic endothelial cells (38). It is also of interest that long-term insulin therapy appears to improve endothelium mediated vasodilatation (39).

The reduction in ROS generation, O_2^- in particular, also has implications for lipid peroxidation since ROS modulate the

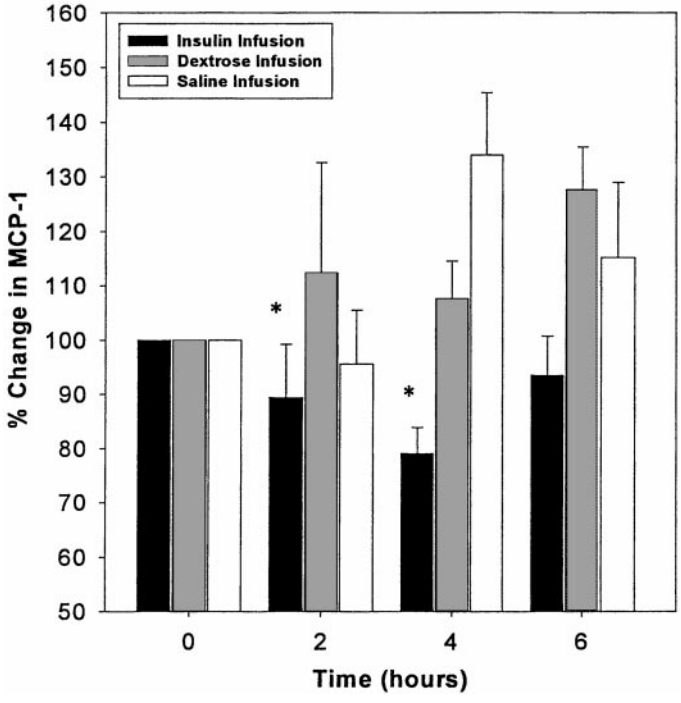

FIG. 7. Percent change in plasma MCP-1 concentrations following insulin or decrease or saline infusions. Plasma MCP-1 decreased significantly following insulin infusion ($P < 0.05$). This decrease was significant at 4 h. Dextrose and saline infusions caused a slight but nonsignificant increase in MCP-1 levels ($P < 0.05$).

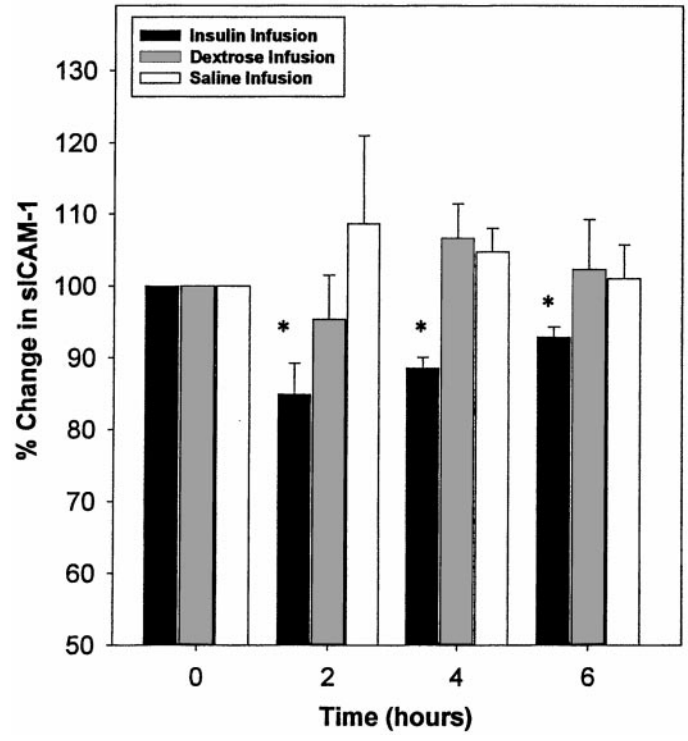

FIG. 8. Percent change in plasma sICAM-1 concentrations following insulin or dextrose of saline infusion. Note that sICAM-1 fell at 2 h following insulin infusion and continued to be inhibited at 4 h sICAM-1 started to increase thereafter following insulin infusion cessation. Neither dextrose nor saline infusions had any significant changes in sICAM-1 levels ($P < 0.05$).

oxidative damage to lipids, including low-density lipoprotein (7). Our observations have implications for our recent demonstration that glucose intake is associated with a marked increase in ROS generation and an increase in the expression of $p47^{phox}$ subunit (30). In that study, we could not decide whether this was an effect caused by glucose or by insulin. Our present data show that insulin has an inhibitory effect on ROS generation. Thus, the marked increase in ROS generation following glucose intake is probably due to glucose itself rather than endogenous insulin.

The fall in sICAM-1 over the short period of insulin infusion is consistent with the suppression of intranuclear NFκB and the transcription of proinflammatory adhesion molecules, known to be modulated by NFκB. The rapid fall at 4 h and recovery at 6 h of sICAM-1 suggests a high turnover of sICAM-1. We have recently demonstrated that insulin suppresses the expression of sICAM-1 by human aortic endothelial cells *in vitro* (38); this indicates that this effect of insulin is a direct one on the endothelium.

It is relevant that in a recent study, we demonstrated that troglitazone, an insulin sensitizer of the thiazolidinedione class of drugs, also causes an increase in IκB and a fall in NFκB (both intranuclear and total cellular), as well as a fall in the proinflammatory cytokine tumor necrosis factor α and the chemokine MCP-1 and the proinflammatory adhesion molecule sICAM-1 (19). Troglitazone also caused a reduction in ROS generation and oxidative damage of lipids and amino acids (18). Thus, insulin and insulin sensitizers exert an anti-inflammatory effect that in the long term may prove to be antiatherogenic.

We have recently described glucocorticoid-mediated changes similar to those with insulin as described above (14). Thus, an injection with 100 mg hydrocortisone causes a fall in intranuclear NFκB and an increase in IκB. The magnitude of fall in NFκB and the increase in IκB were similar after an injection of 100 mg hydrocortisone as those after insulin infusion were in our experiments. Thus, insulin appears to exert several of the effects now known to mediate the anti-inflammatory actions of glucocorticoids. The parallels between the anti-inflammatory effects of glucocorticoids and insulin are quite remarkable, considering that glucocorticoids cause hyperglycemia and increase insulin resistance. Our data would suggest that the mechanism underlying the induction of insulin resistance and hyperglycemia by glucocorticoids is distinct from the anti-inflammatory effects of these drugs.

The acute anti-inflammatory effects of insulin may explain at least in part the benefits observed previously with insulin-glucose infusion treatment in patients with acute myocardial infarction as observed in the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction study (40). It has hitherto been believed that insulin improves the metabolic state of patients by inhibiting lipolysis and reducing FFA concentrations. FFAs produce a prothrombotic state (41). Our study warrants future investigation on the potential anti-inflammatory effect of insulin infusions in patients with acute coronary syndromes. Whether a part of this effect is exerted through FFA inhibition and whether FFAs exert a proinflammatory effect requires further investigation.

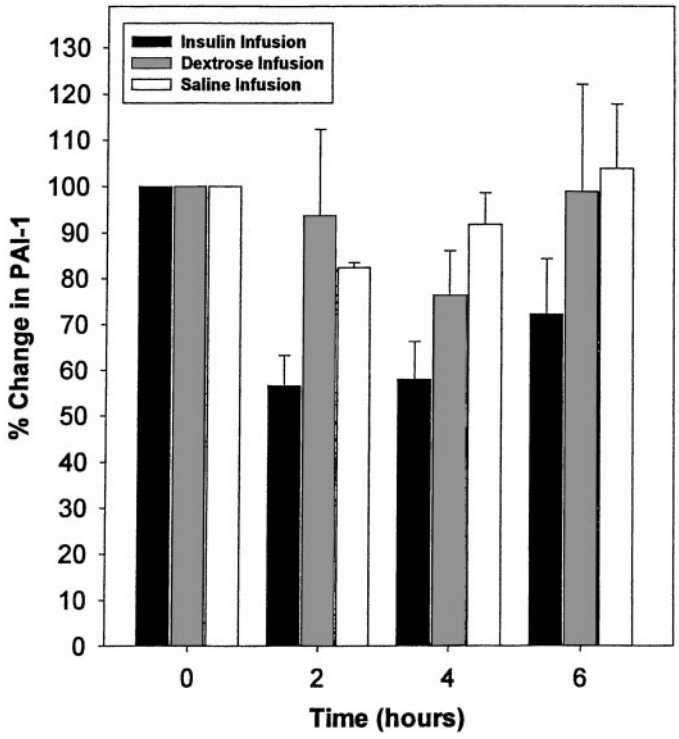

FIG. 9. Percent change in plasma PAI-1 concentrations following insulin, dextrose, or saline infusions. Note that PAI-1 levels decreased at 2 h following insulin infusion and continued to be inhibited at 4 h. PAI-1 levels increased thereafter following insulin cessation (*$P <$ 0.05). Dextrose infusion caused a slight but nonsignificant decrease in PAI-1 levels at 4 h. Saline infusions did not cause any significant changes in PAI-1 levels.

Our observations also bear relevance to the use of insulin in the treatment of type 2 diabetes. In view of the association of hyperinsulinemia and insulin resistance to type 2 diabetes and the fact that type 2 diabetics experience accelerated atherosclerosis and its clinical complications (16, 42), it has been suggested that treatment with exogenous insulin may promote further hyperinsulinemia and may promote further the cardiovascular risk (43, 44). Our data on the anti-inflammatory effect of insulin provide a challenge to this concept and suggest that insulin therapy may actually be potentially beneficial. Indeed, a recent paper by Vehkavaara *et al.* (39) shows that chronic insulin therapy may improve endothelium mediated vasodilatation. Furthermore, the United Kingdom prospective diabetes study, conducted over a protracted period, produced no evidence that insulin produced an increase in the incidence of atherosclerosis related complications (45).

Although our current observations were obtained in obese insulin resistant subjects, it is likely that insulin also exerts similar effects in normal subjects; indeed, our preliminary data demonstrate this. This fact raises questions about the mechanisms underlying the similarities in the actions of insulin and thiazolidenediones; these issues require further elucidation. These issues need to be addressed especially because obese patients are known to have increased oxidative damage (46) and also have evidence of increased C-RP (47), an index of inflammation.

Our observations also raise questions about new targets of insulin action. The circulating MNC are known to have insulin receptors (48) and the question of whether insulin promotes glucose uptake by these cells has been debated. It would appear that insulin probably exerts, hitherto, unknown and unexpected effects on the MNC. Although our study does not rule out the possibility that insulin may exert those effects through another mediator, *in vivo*, it is possible that it is a direct effect. The pharmacodynamics of this anti-inflammatory effect is similar to those of glucocorticoids.

In conclusion, our data demonstrate for the first time an acute stimulatory effect on IκB expression by MNC and suppressive actions of insulin on NFκB, ROS generation and $p47^{phox}$ subunit. Furthermore, insulin inhibits sICAM-1, MCP-1, and PAI-1 concentrations. These actions, similar to those of glucocorticoids, are consistent with an acute anti-inflammatory effect of insulin and therefore, are suggestive of a potential antiatherogenic effect of this hormone in the long term.

Acknowledgments

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