Elevation of Free Fatty Acids Induces Inflammation and Impairs Vascular Reactivity in Healthy Subjects

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To test the possible acute proinflammatory effects of fatty acids, we induced an increase in plasma free fatty acid (FFA) concentrations after a lipid and heparin infusion for 4 h in 10 healthy subjects. We determined the nuclear factor-κB (NF-κB) binding activity in mononuclear cells (MNCs), the p65 subunit of NF-κB, reactive oxygen species (ROS) generation by MNC, and polymorphonuclear leukocytes (PMN). Brachial artery reactivity, using postischemic flow-mediated dilation, was also measured. NF-κB binding activity in the MNC nuclear extracts increased to 163 ± 17% and 144 ± 14% as compared with basal levels at 2 and 4 h ($P < 0.005$) and remained elevated ($P < 0.05$) at 6 h (2 h after cessation of lipid infusion). NF-κB p65 subunit protein expression in MNC homogenates also increased at 2, 4, and 6 h ($P < 0.05$). ROS generation by PMNs increased significantly at 2 and 4 h ($P < 0.005$), whereas that by MNCs increased at 4 h ($P < 0.05$). Plasma macrophage migration inhibitory factor increased at 2 ($P < 0.05$) and 4 h ($P < 0.005$), respectively, and declined to baseline at 6 h. The postischemic flow-mediated dilation of brachial artery decreased from 6.3 ± 1.1% at baseline to 4.3 ± 1.9% and 2.7 ± 2.1% ($P < 0.01$) at 2, 4, and 6 h, respectively. We conclude that an increase in FFA concentration induces oxidative stress and has a proinflammatory effect; it also impairs postischemic flow-mediated vasodilation of the brachial artery. *Diabetes* 52:2882–2887, 2003

Studies in humans as well as rodents have consistently demonstrated that an experimental elevation in free fatty acid (FFA) concentrations in healthy subjects reduces the insulin-stimulated glucose uptake (1,2). In addition, elevated FFA concentrations lead to impairment in endothelium-dependent (3) and insulin-mediated vasodilation as a result of decrease in nitric oxide (NO) production (4). The mechanisms by which FFA induces insulin resistance, however, are not clear.

Recently, anti-inflammatory agents have been shown to prevent fat-induced insulin resistance in rodents, thereby suggesting the involvement of inflammatory pathways in the pathogenesis of fat-induced insulin resistance (5,6). Inflammation is activated by the proinflammatory transcription factor nuclear factor-κB (NF-κB), usually a heterodimer of p65 (Rel A) and p50 proteins. NF-κB is located in the cytosol, where it is bound to inhibitor κB (IκB). Inflammatory signals, including endotoxin and proinflammatory cytokines, cause phosphorylation and ubiquitination of IκB, thus liberating and activating NF-κB. This allows NF-κB to translocate to the nucleus and to activate transcription of genes that are involved in the inflammatory response, such as proinflammatory cytokines, adhesion molecules, and enzymes generating reactive oxygen species (ROS). We have previously shown that thiazolidinediones (troglitazone and rosiglitazone) exert a significant anti-inflammatory effect in obese subjects and patients with type 2 diabetes (7,8) while also reducing plasma FFA concentrations.

It has recently been shown that inflammatory mechanisms may mediate insulin resistance. Obesity is associated with an increase in the proinflammatory mediators such as tumor necrosis factor-α (TNF-α) in both animal models and humans (9,10). Plasma concentration of TNF-α as well as several indexes of oxidative stress are increased in the obese and fall with weight loss (11). Weight loss is known to restore insulin sensitivity. Thus, inflammation may contribute to insulin resistance. Indeed, it has recently been shown that the inhibition of IκB kinase-β (IKK-β) by the classical anti-inflammatory drug aspirin may significantly increase the sensitivity to insulin in the *fa/fa* Zucker rat (6). IKK-β is the kinase that phosphorylates IκB, thus allowing NF-κB to translocate into the nucleus and to induce expression of proinflammatory genes.

In the present study, we hypothesized that an increase in FFA concentration in normal, healthy subjects leads to *1*) activation of proinflammatory NF-κB and an increase in ROS generation by leukocytes and *2*) an increase in proinflammatory mediators in plasma, including the macrophage migration inhibitory factor (MIF).

RESEARCH DESIGN AND METHODS

Participants. Ten healthy volunteers (eight men and two women; age, 28.2 ± 4.1 years; BMI, 24.8 ± 4.7 kg/m^2) participated in the study (Table 1). All participants had normal glucose tolerance, were not on any medications, had normal blood pressure, and had normal fasting lipid profiles. All volunteers

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CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; ICAM, intracellular adhesion molecule; IκB, inhibitor κB; IKK-β, IκB kinase-β; MCP-1, monocyte chemoattractant protein; MIF, macrophage migration inhibitory factor; MNC, mononuclear cell; NF-κB, nuclear factor-κB; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; sICAM, soluble ICAM; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α.

TABLE 1
Clinical characteristic of the subjects

Male/female	8/2
Age (years)	28.2 ± 4.1
BMI (kg/m^2)	24.8 ± 4.7
Pulse	78 ± 6
BP (mmHg)	
Systolic	114 ± 13
Diastolic	78 ± 5
Cholesterol (mg/dl)	156 ± 47.4
Triglyceride (mg/dl)	75.6 ± 16
HDL cholesterol (mg/dl)	39.7 ± 8.6
LDL cholesterol (mg/dl)	114 ± 30.2

Data are mean ± SD.

gave their written, informed consent, and the study protocol was approved by the Human Research Committee of the State University of New York at Buffalo.

Protocol. Participants came to the clinical research center at 8.00 A.M., after an overnight 12-h fast. A catheter was inserted into the antecubital vein. A baseline blood sample was obtained, and a triglyceride emulsion (Liposyn, 10%; Abbott Laboratories, North Chicago, IL) and heparin (0.2 units · kg^{-1} · min^{-1}) were infused at a rate of 50 ml/h for 4 h. We used a lower concentration of Liposyn compared with most earlier studies because infusion of 20% Liposyn resulted in lipemic serum where separation of MNCs and PMNs was not possible. The triglyceride infusion was stopped after 4 h, and blood samples were collected at 0, 2, 4, and 6 h. Vascular reactivity was also measured at 0, 2, 4, and 6 h. On two different occasions, seven subjects participated in two other studies in which 0.9% normal physiological saline or 5% dextrose was infused for 4 h and samples were collected at 0, 2, 4, and 6 h.

MNC and PMN isolation. Blood samples were collected in Na-EDTA as an anticoagulant. A total of 3.5 ml of the anticoagulated blood sample was carefully layered over 3.5 ml of the PMN isolation medium (Robbins Scientific, Sunnyvale, CA). Samples were centrifuged at 450*g* in a swing-out rotor for 30 min at 22°C. At the end of the centrifugation, two bands separated out at the top of the red blood cell pellet. The top band consists of MNC, and the bottom consists of PMN. The MNC band was harvested with a Pasteur pipette, repeatedly washed with Hanks' balanced salt solution, and reconstituted to a concentration of 4×10^5 cells/ml in Hanks' balanced salt solution. This method provides yields >95% pure MNC suspension.

Measurement of ROS generation. Respiratory burst activity of PMNs and MNCs was measured by detection of superoxide radical via chemiluminescence (12). A total of 500 ml of PMNs or MNCs (2×10^5 cells) was delivered into a Lumiaggregometer (Chronolog) plastic flat-bottom cuvette to which a spin bar was added. Fifteen microliters of 10 mmol/l luminol was then added, followed by 1 μl of 10 mmol/l formylmethionylleucinylphenylalanine. 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 (13). The interassay coefficient of variation for this assay is 6%. We have further established that in our assay system, there is a dose-dependent inhibition of chemiluminescence by superoxide dismutase and catalase: superoxide dismutase inhibited chemiluminescence by 82% at 10 μg/ml, whereas catalase inhibited chemiluminescence by 47% at 40 μg/ml. Chemiluminescence is also inhibited by diphenyleneiodonium chloride (data not shown), a specific inhibitor of NADPH oxidase, the enzyme responsible for the production of superoxide radicals (14). Our assay system is exquisitely sensitive to diphenyleneiodonium chloride at nanomolar concentrations.

NF-κB electrophoretic mobility shift assay. NF-κB gel retardation assay was performed as previously described. DNA-binding protein extracts were prepared from MNCs by the method described by Andrews et al. (15). Total protein concentrations were determined using BCA protein assay (Pierce, Rockland, IL). NF-κB gel retardation assay was performed using NF-κB binding protein detection kit (Life Technologies, Long Island, NY). Briefly, the double-stranded oligonucleotide containing a tandem repeat of the consensus sequence for the NF-κB binding site was radiolabeled with γ-P^{32} by T4 kinase. Then, 5 μg of the nuclear extract was 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 molecular analyst software (Hercules, CA).

p47^{phox} subunit, p65 (Rel A) and IκB, and IKK-α and IKK-β Western blotting. Western blotting was carried out as previously described. Briefly, total protein concentrations were determined using BCA protein assay (Pierce). Twenty micrograms of total homogenates were used for SDS-PAGE. Western blotting was carried out using an antibody against p47^{phox} subunit purchased from Transduction Labs (San Diego, CA) and polyclonal antibodies against NF-κB p65 (Rel A) or IκB (Rockland, Gilbertsville, PA) or IKK-α and IKK-β (Biotechnology, Santa Cruz, CA). Densitometric analysis of the Western blots was carried out using Bio-Rad molecular analyst software.

IKK immunoprecipitation and in vitro kinase assay. For determining IKK activity, the whole-cell lysates were obtained in immunoprecipitation buffer (50 mmol/l HEPES [pH 7.6], 250 mmol/l NaCl, 10% glycerol, 1 mmol/l EDTA) containing 0.1% NP40 with protease and phosphatase inhibitors. A 500-μg sample of the total cell lysate was used for immunoprecipitation. The cell lysate was cleared and incubated for 2 h at 4°C with IKK-α antibody (Biotechnology), at which time the A/G agarose-conjugated beads (Biotechnology) were added to the tube and incubated overnight at 4°C. The immunoprecipitates were washed three times with immunoprecipitation buffer and once with kinase buffer (20 mmol/l Tris-HCl [pH 7.6], 10 mmol/l $MgCl_2$, 0.5 mmol/l dithiothreitol). The kinase assays were performed with glutathione S-transferase–IκB-α (1–317) protein in 20 μl of kinase buffer containing 10 μmol/l ATP, 3 μCi of $^{\gamma\text{-}32}$P ATP, and protease inhibitors. Half of the immunoprecipitated IKK-α complex was incubated in the kinase buffer with 3 μg of the substrate for 30 min at 37 C°. The reaction was stopped by boiling the samples for 5 min in 2× SDS loading buffer. Samples were resolved by 10% SDS-PAGE and autoradiography. The remaining half of the immunoprecipitated samples were run on a separate 10% SDS-PAGE and were Western blotted with the IKK-α antibody to check for equality of loading.

Plasma C-reactive protein, MIF, TNF-α, soluble intracellular adhesion molecule-1, thiobarbituric acid reactive substances, and monocyte chemoattractant protein-1 measurements. Plasma MIF, monocyte chemoattractant protein-1 (MCP-1), soluble intracellular adhesion molecule (sICAM-1), and TNF-α were assayed with enzyme-linked immunosorbent assay (ELISA) kits from R&D systems (Minneapolis, MN). C-reactive protein (CRP) ELISA kit was purchased from Diagnostic Systems Laboratories (Webster, TX). Thiobarbituric acid reactive substances (TBARS) were measured using the method described by Yagi et al. (16).

Plasma FFA, insulin, and glucose measurements. FFA levels were measured in plasma containing EDTA and lipoprotein lipase inhibitor Paraoxon (diethyl-*p*-nitrophenyl-phosphate, 0.275 mg/ml blood; Sigma, St. Louis, MO) by a colorimetric assay (Wako, Richmond, VA). Insulin levels were determined using an ELISA kit from Diagnostic Systems Laboratories. Glucose levels were measured in plasma by YSI 2300 STAT Plus glucose analyzer (Yellow Springs, OH).

Assessment of brachial arterial reactivity. Brachial artery diameter was measured by an Acuson 128XP/10 high-resolution ultrasonograph with a 7.5-MHz linear array transducer as previously described (17). The forearm was compressed 40 mm above the systolic blood pressure for 5 min, and brachial artery diameter was recorded at 15 s and again at 45 to 60 s after ischemia. Vascular reactivity was assessed at 0, 2, 4, and 6 h.

Statistical analysis. Statistical analysis was carried out using SigmaStat software (Jandel Scientific, San Rafael, CA). All data are expressed as mean ± SD. Analysis was carried out with Kruskal-Wallis ANOVA on ranks. Dunnett's method was used for all multiple comparison procedures. Data for ROS generation by MNCs, PMNs, and MIF were logarithmically transformed. Wilcoxon signed rank test was used to compare endothelium-dependent vasodilation.

RESULTS

Plasma FFA, triglycerides, and insulin concentrations. Plasma FFA concentration rose from 352 ± 62 μmol/l to 621 ± 59 μmol/l at 2 h, 732 ± 75 μmol/l at 4 h ($P < 0.005$), and was 345 ± 42 μmol/l at 6 h. Plasma triglycerides concentration rose from 75 ± 16 mg/dl to 139 ± 42 mg/dl at 2 h, 120 ± 53 mg/dl at 4 h, and declined toward baseline to 85 ± 27 mg/dl at 6 h. Plasma insulin and glucose concentrations did not change significantly (Table 2).

ROS generation by PMNs and MNCs. The mean logROS generation by PMNs in the fasting state (basal) was 4.39 ± 0.25 mV. It increased to 5.13 ± 0.25 mV ($P < 0.05$; Fig. 1) at 2 h and to 5.12 ± 0.25 at 4 h ($P < 0.05$). It declined to 4.69 ± 0.25 at 6 h. The mean logROS generation by MNCs

TABLE 2
Plasma concentrations of glucose, insulin, FFAs, triglycerides, TBARS, and cytokines

	0	2 h	4 h	6 h
Glucose (mg/dl)	78.7 ± 2.3	80.8 ± 1.8	80.7 ± 2.5	82.8 ± 2.9
Insulin (mU/l)	11.3 ± 6.5	8.7 ± 3.2	8.4 ± 5.6	7.3 ± 3.6
FFA (μmol/l)†	352 ± 62	621 ± 59	732 ± 75	345 ± 42
Triglycerides (mg/dl)‡	75 ± 16	139 ± 42	120 ± 53	88 ± 27
TBARS (μmol/l)‡	1.46 ± 0.4	1.77 ± 0.3	1.54 ± 0.3	1.56 ± 0.4
TBARS (μmol/l)/triglycerides (mmol/l)	0.85 ± 0.2	0.95 ± 0.2	1.35 ± 0.6	1.91 ± 0.3
MCP-1 (pg/ml)	124 ± 33	113.8 ± 33	114.5 ± 33	114.5 ± 48.5
ICAM (ng/ml)	221 ± 36	214 ± 26	216 ± 25.6	210 ± 19.2
TNF-α (pg/ml)	3.0 ± 0.9	3.6 ± 1.1	3.9 ± 0.7	3.5 ± 1.3
Log MIF (pg/ml)*†	7.15 ± 0.56	7.69 ± 0.58	7.99 ± 0.58	7.58 ± 0.94

Data are expressed as mean ± SD. *$P < 0.005$ baseline vs. 4 h; †$P < 0.05$, 2-h and 4-h values vs. the baseline; ‡$P < 0.05$, baseline vs. 2-h values.

in the fasting state was 4.97 ± 0.26 mV; it increased to a peak of 5.63 ± 0.26 mV at 4 h ($P < 0.05$). The protein expression of $p47^{phox}$ subunit of NADPH oxidase in MNC homogenates, however, did not change during the infusion (data not shown).

Plasma TBARS concentrations and TBARS/triglycerides ratio. Plasma TBARS concentrations increased significantly at 2 h ($P < 0.05$); the increases at 4 and 6 h were not significant. When expressed as a ratio of TBARS to triglycerides, the changes after lipid infusion were not significant (Table 2).

NF-κB binding activity, cellular p65 (Rel A), IκB and phosphorylated IκB protein content, and IKK-α and IKK-β. NF-κB binding activity in MNCs increased to 163 ± 17% ($P < 0.005$) and 144 ± 14% ($P < 0.05$) above the basal levels (100%) at 2 and 4 h, respectively ($P < 0.005$), and remained elevated at 6 h (154 ± 15%; $P < 0.05$; Fig. 2*A*). NF-κB p65 subunit protein quantity in MNC homogenates increased at 2, 4, and 6 h (246 ± 61%, 343 ± 165%, and 223 ± 37% above the basal; $P < 0.05$; Fig. 2*B*). Neither total IκB protein expression nor phosphorylated IκB protein expression in MNC homogenates changed significantly after the lipid infusion. Also, IKK-α and IKK-β expression in the MNCs did not change after the lipid infusion.

IKK and IKK-α kinase activity in IKK immunoprecipitates. To rule out the possibility that the bioactivity might be altered even in the absence of a change in the expression of IKK-α and IKK-β contents, we performed the bioactivity assays. Neither IKK-α nor IKK-β enzymatic activity changed significantly after lipid infusion. IKK activity in MNCs was 91 ± 13% and 109 ± 37% of the basal levels (100%) at 2 and 4 h, respectively (NS), and tended to decline to 80 ± 26% at 6 h (NS). IKK-α activity in MNCs was 108 ± 12%, 116 ± 27%, and 80 ± 15% of the basal levels (100%) at 2, 4, and 6 h, respectively (NS). Correspondingly, the phosphorylated IκB-α did not change significantly: 121 ± 18, 111 ± 29, and 103 ± 18% of baseline at 2, 4, and 6 h, respectively (NS) after lipid infusion.

Plasma MIF, CRP, MCP-1, sICAM-1, and TNF-α concentrations. Plasma MIF concentration increased from 7.15 ± 0.56 pg/ml (100%) at baseline to 7.69 ± 0.58 ($P < 0.05$) at 2 h and 7.99 ± 0.58 ($P < 0.005$) at 4 h and declined to 7.58 ± 0.94 at 6 h (Fig. 3). Saline or 5% dextrose infusion did not alter the plasma MIF concentration. Plasma CRP, MCP-1, sICAM-1, and TNF-α concentrations did not change significantly after lipid infusion (Table 2).

Brachial arterial reactivity. Brachial arterial diameter did not alter during lipid infusion. The postischemic flow-mediated dilation at 0 h was 6.3 ± 1.1% of the basal. It decreased significantly to 3.7 ± 1.5% at 2 h ($P < 0.05$), to 2.1 ± 1.9% at 4 h ($P < 0.05$; Fig. 4), and to 4.7 ± 2.1% at 6 h

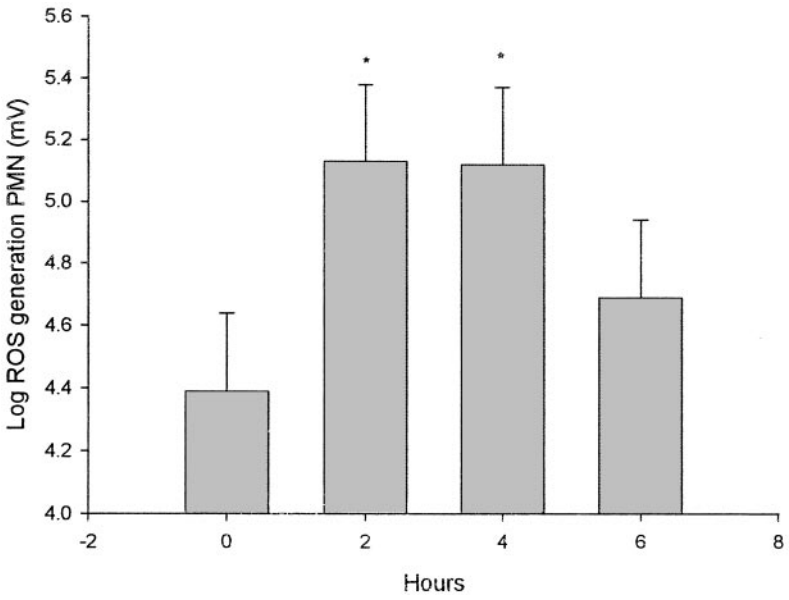
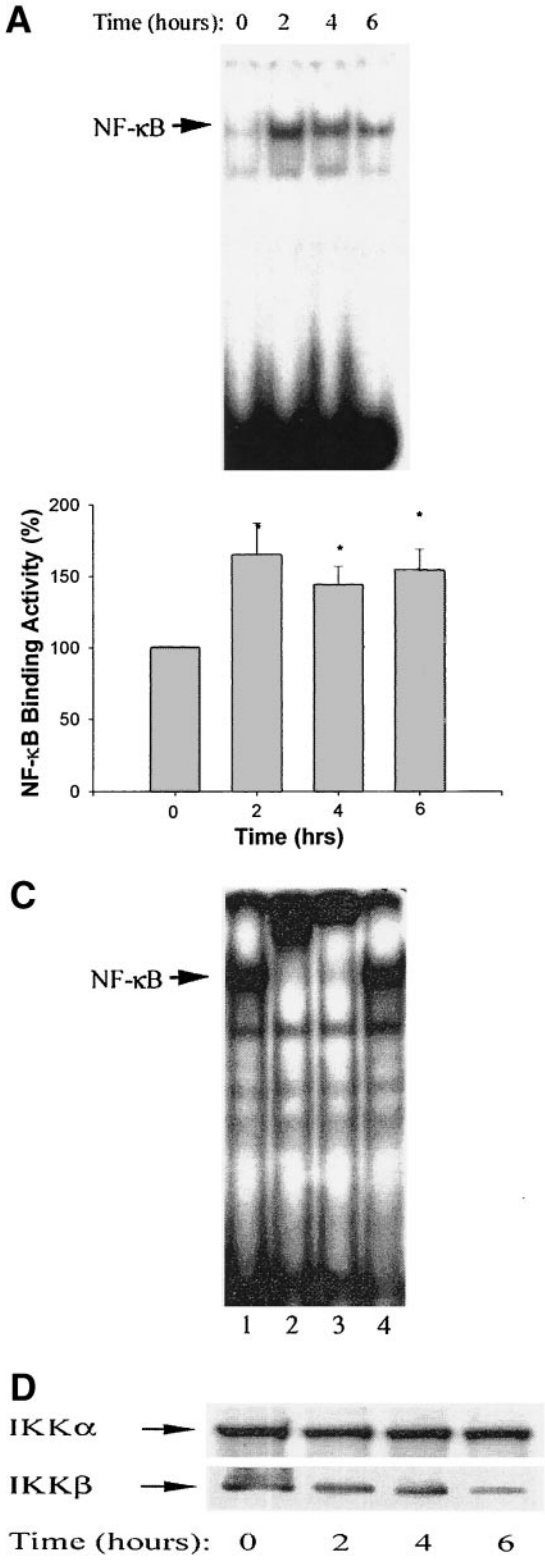


FIG. 1. ROS generation by PMNs before and after lipid infusion at 2, 4, and 6 h. Note that ROS generation increased significantly at 2 and 4 h.



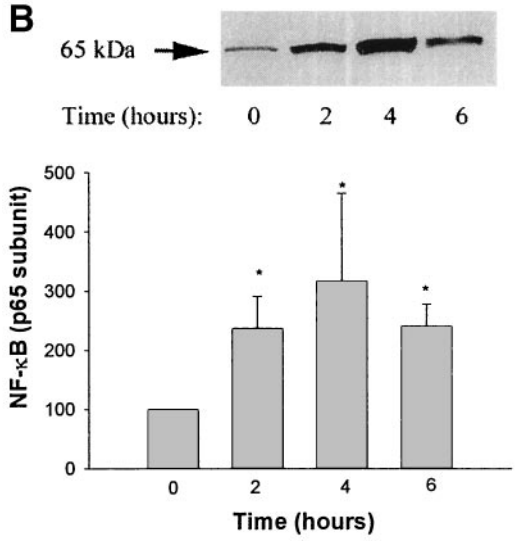


FIG. 2. ***A*****: A representative electrophoretic mobility shift assay showing the relative NF-κB binding activity in MNC nuclear extracts. Note that NF-κB binding activity is induced at 2 h after lipid infusion. This plot is representative of four different experiments with four different subjects and densitometric semiquantitative analysis of NF-κB binding activity in MNCs. All values were normalized to 100% for baseline time point, and the values were expressed as percentage of basal level (*$P < 0.05$). *B*: Western blot showing the relative expression of NF-κB p65 subunit in MNC homogenates. Note that p65 subunit is induced at 2 h after lipid infusion. This plot is representative of four different experiments with four different subjects. Densitometric semiquantitative analysis of NF-κB p65 subunit in MNCs. All values were normalized to 100% for baseline time point, and the values were expressed as percentage of basal level (*$P < 0.05$). *C*: *Lane 1*, NF-κB binding activity in the baseline sample nuclear extract for one of the subjects; *lane 2*, supershift using an antibody against p50 subunit of NF-κB; *lane 3*, supershift using an antibody against p65 subunit of NF-κB; *lane 4*, p75 (c-Rel) of NF-κB. This suggests that the band detected consists of p50:p65 complex. *D*: *Lane 1*, IKK-α; *lane 2*, IKK-β activity before and after the Liposyn infusion in the nuclear extract for one of the subjects; note that neither IKK-α nor IKK-β changed significantly after the Liposyn infusion.**

(NS). Normal saline or a 5% dextrose infusion did not cause change in any of the above parameters.

DISCUSSION

We have shown for the first time that an increase in plasma FFA concentrations acutely causes an increase in the intranuclear NF-κB binding activity and p65 expression in circulating MNCs, as well as ROS generation by MNCs and PMNs. Clearly, therefore, an increase in FFA concentration is associated with the induction of proinflammatory changes and oxidative stress. The infusion of physiological saline or a low dose of dextrose into control subjects did not produce any changes in ROS generation (18). Our data provide the first link between FFA and an increase in NF-κB, a cardinal step in the induction of inflammation. In addition, our data demonstrate that an increase in FFA also induces an increase in MIF, a proinflammatory cytokine secreted by monocytes and adrenocorticotrophs in the pituitary gland (19,20).

Because elevated FFA concentrations have been shown previously to inhibit glucose uptake by insulin responsive organs such as the skeletal muscle, it has been suggested that FFA may be the major mediators of insulin resistance in insulin-resistant states (21). Recent observations have shown that an increase in FFA causes the induction of protein kinase-θ, which in turn inhibits phosphatidylinositol 3-kinase, the key enzyme that mediates the action of insulin in skeletal muscles (22).

It is noteworthy that Itani et al. (23) have recently shown that elevation of FFA leads to decreased IκB-α in the skeletal muscles of healthy subjects. However, no data on NF-κB were provided in that study.

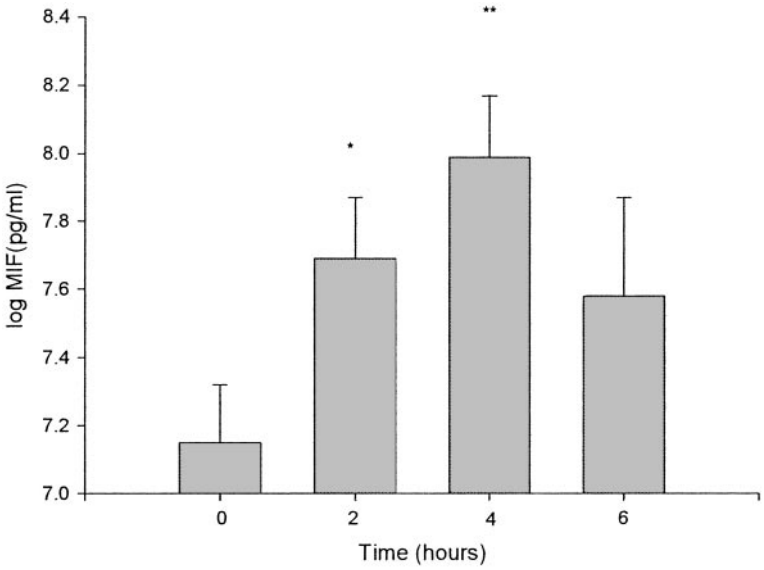


FIG. 3. Changes in plasma MIF before and after lipid infusion at 2, 4, and 6 h. Note that MIF concentrations increased significantly at 2 ($P < 0.05$) and 4 ($P < 0.005$) h.

Although our study has demonstrated an increase in NF-κB, no change was observed in IκB protein quantity. Because phosphorylation of IκB causes a dissociation of NF-κB from IκB, we also measured phosphorylated IκB, which did not alter either. Also, the IKK-α and IKK-β did not change after the lipid infusion. To investigate further the possibility that the IKK bioactivity might have been altered without the expression of IKK-α and IKK-β, we performed the enzymatic activity assays, and again there was no significant change with the lipid infusion. It is possible, therefore, that the activation of NF-κB in MNCs may be through a mechanism independent of IκB phosphorylation and ubiquitination, possibly through a direct increase in the expression of p65 (RelA), which was impressive at 300% when compared with the basal (100%). This could lead to an increase in NF-κB. These data are similar to our preliminary data on the effect of oral fat intake; it also induces an increase in ROS generation and p65 expression without a change in IκB.

The observation that FFA increase induces NF-κB binding is relevant to the action of thiazolidinediones, which reduce FFA concentrations and have profound anti-inflammatory effects while enhancing insulin sensitivity in humans in vivo (24). Consistent with these observations is the recent finding that insulin is an anti-inflammatory hormone that also suppresses plasma FFA concentrations and NF-κB, the key proinflammatory transcription factor (18).

Our study showed a strong stimulatory effect of FFA on plasma levels of MIF. Macrophages contain a significant amount of preformed MIF within intracellular pools that can be rapidly released on stimulation. It inhibits the ability of glucocorticoids to induce IκB synthesis in lipopolysaccharide-stimulated MNCs, thus promoting the translocation of NF-κB into the nucleus (25). MIF also promotes TNF-α expression and recently has been shown to be associated with insulin resistance (26,27). It is interesting that plasma TNF-α levels did not change in our study. This may be related to the fact that de novo mRNA generation and synthesis of TNF-α is required before secretion is observed. Also, MIF secretion from macrophages requires a lesser lipopolysaccharide stimulus than for TNF-α. It is possible that FFA infusion for a prolonged period or at a higher concentration of FFA could have resulted in an induction of plasma cytokines other than MIF.

Endothelium-dependent flow-mediated vasodilation of the brachial artery was significantly impaired within 2 h of

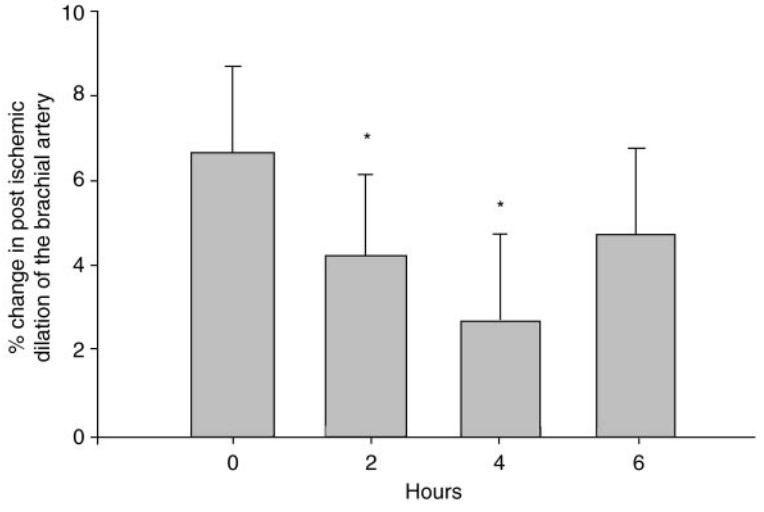


FIG. 4. Changes in the postischemic dilation in brachial artery. Results are expressed as percentage change over the baseline artery diameter after 5 min of distal occlusion. Note that it was significantly decreased after 2 and 4 h ($P < 0.05$).

lipid infusion and persisted for 4 h. Our results are consistent with the previous observations of Steinberg et al. (3,4,28), who also demonstrated impaired acetylcholine-induced NO-dependent lower limb blood flow within 2 h of lipid infusion. The increased formation of ROS including superoxide may quench NO released from endothelium and thus result in decreased vasodilatory action of NO at the level of vascular smooth muscle.

In conclusion, an increase in FFA concentration in plasma results in an acute increase in the intranuclear and total cellular NF-κB and ROS generation with a significant increase in MIF. These actions indicate a proinflammatory effect in association with an increase in oxidative stress. These effects provide a potential link between inflammation and insulin resistance.

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