Proinflammatory Cytokines, Markers of Cardiovascular Risks, Oxidative Stress, and Lipid Peroxidation in Patients With Hyperglycemic Crises

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Acute and chronic hyperglycemia are proinflammatory states, but the status of proinflammatory cytokines and markers of oxidative stress and cardiovascular risks is not known in hyperglycemic crises of diabetic ketoacidosis (DKA) and nonketotic hyperglycemia (NKH). We studied 20 lean and 28 obese patients with DKA, 10 patients with NKH, and 12 lean and 12 obese nondiabetic control subjects. We measured *1*) proinflammatory cytokines (tumor necrosis factor-α, interleukin [IL]-6, IL1-β, and IL-8), *2*) markers of cardiovascular risk (C-reactive protein [CRP], homocysteine, and plasminogen activator inhibitor-1 [PAI-1]), *3*) products of reactive oxygen species (ROS; thiobarbituric acid [TBA]-reacting material, and dichlorofluorescein [DCF]), and *4*) cortisol, growth hormone (GH), and free fatty acids (FFAs) on admission (before insulin therapy) and after insulin therapy and resolution of hyperglycemia and/or ketoacidosis. Results were compared with lean and obese control subjects. Circulating levels of cytokines, TBA, DCF, PAI-1, FFAs, cortisol, and GH on admission were significantly increased two- to fourfold in patients with hyperglycemic crises compared with control subjects, and they returned to normal levels after insulin treatment and resolution of hyperglycemic crises. Changes in CRP and homocysteine in response to insulin therapy did not reach control levels after resolution of hyperglycemia. We conclude that DKA and NKH are associated with elevation of proinflammatory cytokines, ROS, and cardiovascular risk factors in the absence of obvious infection or cardiovascular pathology. Return of these values to normal levels with insulin therapy demonstrates a robust anti-inflammatory effect of insulin. *Diabetes* 53:2079–2086, 2004

Diabetes is a chronic inflammatory state associated with insulin resistance (1–3). Hyperglycemia has been shown to induce proinflammatory cytokines and chemokine genes in monocyctic cells (4). Certain cytokines, such as tumor necrosis factor-α (TNF-α), impair insulin action in peripheral tissue (5) and have a direct role in obesity-linked insulin resistance (6). Interleukin-6 (IL-6) also influences glucose metabolism by alteration of insulin sensitivity (7). Chronic hyperglycemia has been shown to be responsible for multiple micro- and macrovascular complications as a result of hyperglycemic damage through four major biochemical processes, including advanced glycation end products (AGEs), the polyol pathway, the hexosamine pathway, and activation of protein kinase C, as described by Brownlee (8) and King and Brownlee (9). Recent studies suggest that lipid infusion in normal subjects may also result in the alteration of some of the above pathways and may induce insulin resistance (10).

Hyperketonemia in patients with type 1 diabetes has also been associated with increased plasma lipid peroxidation (11). Increased blood levels of IL-6 are also reported in type 1 diabetes without clinical evidence of micro- or macrovascular complications (12). Furthermore, abnormal markers of endothelial dysfunction and oxidative stress have been demonstrated in children with type 1 diabetes with no clinical vascular disease (13). Obesity and insulin resistance are also associated with adipose tissue secretion of IL-6 and TNF-α (14). Additionally, interaction of AGEs with cellular receptors alters the level of gene expression, which leads to the development of vascular abnormalities and activation of the transcription factor nuclear factor-κB in polymorphonuclear leukocytes (15).

Furthermore, transient hyperglycemia (16) or hyperlipidemia (17) in normal subjects results in the activation of the generation of reactive oxygen species (ROS) and the reduction of certain antioxidants. It has also been reported that normoglycemic obese individuals as well as patients with type 2 diabetes have elevated levels of C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), free fatty acid (FFA), IL-6, and TNF-α expression (18–20). Thus, these cardiovascular risk factors may play important roles in predicting diabetes and are components of insulin resistance syndrome (21).

Diabetic ketoacidosis (DKA) and nonketotic hypergly-

TABLE 1
Clinical characteristics of hyperglycemic patients on admission

Parameters	Lean DKA	Obese DKA	Obese hyperglycemia	Lean control	Obese control
Patients (n)	20	28	10	12	12
Age (years)	39 ± 2.7	38 ± 2.0	50 ± 3.7	35.7 ± 1.9	36.5 ± 3.1
Sex (M/F)	13/7	20/8	5/5	7/5	4/8
BMI (kg/m^2)	22 ± 0.6	33 ± 0.9*	30 ± 0.9*	22 ± 0.7	34 ± 1.6*
Temperature (°F)	97.3 ± 0.5	97.2 ± 0.3	98.3 ± 0.2	98.4 ± 0.1	98.5 ± 0.2
HbA$_{1c}$	12.5 ± 0.4	11.6 ± 0.4	10.8 ± 0.6	5.4 ± 0.5*	5.7 ± 0.7*
White blood cell × 10^6	14.4 ± 0.7	14.2 ± 1.3	11.8 ± 2.0	6.5 ± 0.7*	6.8 ± 0.6*

Data are means ± SE. *$P < 0.01$ vs. lean DKA on admission.

cemia (NKH) are two acute hyperglycemic emergencies characterized by decreased effective concentrations of insulin, leukocytosis, dehydration, elevation of counterregulatory hormones, and derangement of electrolytes and mineral metabolism, with severe alteration of protein, lipid, and carbohydrate metabolism (22). Our recent study demonstrated in vivo activation of T-cells in patients with DKA who were exhibiting de novo emergence of growth factor receptors (insulin, IGF-1, and IL-2) in association with an increased level of lipid peroxidation (thiobarbituric acid [TBA]-reacting material and ROS such as dichlorofluorescein [DCF]) (23). We had also reported earlier that in obese and lean subjects with DKA and hyperglycemia, serum leptin levels on admission are markedly decreased, and that after 6 h of insulin treatment, leptin levels return to control values (24). Because leptin is an adipokine generated by fat tissue, we hypothesized that alteration of other adipokines, such as TNF-α and IL-1β, -8, and -6, might occur in patients with obese hyperglycemic crises. Therefore, because DKA occurs in both lean and obese subjects, and obesity itself may influence the generation of proinflammatory cytokines, we undertook the present study to evaluate two conditions, obesity and ketoacidosis, with comparable levels of hyperglycemia. We studied these cytokines, as well as markers of oxidative stress and cardiovascular risk factors, in lean and obese DKA and in obese NKH patients (before and after resolution of hyperglycemia) who did not have severe infection or discernable cardiovascular pathology, and we compared them to age- and weight-matched lean and obese control subjects. Our study indicates that plasma levels of proinflammatory cytokines, markers of oxidative stress, certain cardiovascular markers, and lipid peroxidation are elevated on admission in patients with hyperglycemic crises. These values, as well as levels of counterregulatory hormones, promptly returned to control values with the administration of insulin and resolution of hyperglycemia. The constellation of these findings constitutes the first report of such events in lean and obese subjects with acute hyperglycemic crises. Demonstration of salutary and anti-inflammatory effects of low-dose insulin in these conditions supports the latter role of insulin in other clinical conditions associated with hyperglycemia (25–28).

RESEARCH DESIGN AND METHODS

A total of 58 patients admitted to the Regional Medical Center (Memphis, TN) for DKA and severe hyperglycemia were treated on a low-dose insulin protocol using intravenous infusion of insulin with the established rate of 0.1 unit · kg body wt^{-1} · h^{-1} (29). Patients with DKA had an admission blood glucose >250 mg/dl (13.9 mmol/l), pH <7.3, bicarbonate <18 mmol/l, anion gap >15 mmol/l, and positive ketonemia and ketonuria. Patients with NKH were admitted with blood glucose >400 mg/dl (22.4 mmol/l), pH >7.3, and serum bicarbonate >18 mmol/l. There was no apparent infection or other known precipitating illness for DKA and/or hyperglycemia in any of the study subjects. We excluded patients with gastrointestinal bleeding, fever, obvious endocrine disorders, history of myocardial infarction, pregnancy, congestive heart failure, history of cardiovascular disease, chronic obstructive pulmonary disease, or chronic renal failure. The criteria for resolution was defined as blood glucose <250 mg/dl (13.9 mmol/l), HCO$_3$ >18 mmol/l, pH >7.3, normal anion gap, and normal mental status. Blood was drawn on admission for diagnosis and clinical management of DKA and NKH and at 4-h intervals until total resolution of hyperglycemic crisis, which was at 20–24 h after initiation of insulin therapy. The patients were on intravenous fluids and received no food by mouth for the entire study. The total amount of insulin per kilogram of body weight was similar in all hyperglycemic patients (~90 units until resolution of hyperglycemia/ketoacidosis). Laboratory tests included a complete metabolic profile, cell blood count, arterial pH, blood glucose, blood cultures, and other routine chemistries, which were performed in the hospital laboratory. The other specified assays in this report were performed in the endocrinology laboratory at the University of Tennessee Health Science Center (UTHSC). Blood specimens for these assays were drawn in citrated tubes on admission and at resolution of hyperglycemic crisis at 20–24 h. The specimens were centrifuged at 4°C, and plasma was separated and stored at −70°C until assayed. The consent forms for the protocol, which was approved by the institutional review board at UTHSC, were obtained from the patient or next of kin.

Determination of plasma cytokines, metabolic hormones, and markers of cardiovascular risks, oxidative stress, and lipid peroxidation. Levels of proinflammatory cytokines (TNF-α and IL-1B, -6, and -8), markers of cardiovascular risks (high-sensitivity CRP and homocysteine), and metabolic hormones (growth hormone [GH], cortisol, and C-peptide) were measured in the plasma using a solid-phase, two-site sequential chemiluminescent immunometric assay on an Immulite analyzer (Diagnostic Products, Los Angeles). The coefficients of variation of the assays were all <5%. The instrument calibrations for the assays were performed as recommended by the manufacturers and were within the specifications.

Assays for markers of oxidative stress and lipid peroxidation were determined by TBA assay (30), and ROS were determined by DCF assay (31). FFA (32) and β-hydroxybutyrate levels (33) were determined by the methods established in this laboratory, as previously described. PAI-1 was assayed using the Zymutest PAI-1 activity enzyme-linked immunosorbent assay (Hyphen BioMed, Andresy, France), which measures only active PAI-1 (34–37). The normal range for fasting normal subjects for this assay was established to be <5 ng/ml. Absorbances of the enzyme-linked immunosorbent assay were determined on an HTS 7000 Plus microplate reader (Perkin-Elmer, Norwalk, CT) and the HTS data analysis software.

Normal fasting values of lean control subjects established in this laboratory for the cytokine assays are as follows: TNF-α <5.0 pg/ml, IL-1β <3.0 pg/ml, IL-6 <5.0 pg/ml, and IL-8 <10 pg/ml. Normal fasting values for the other assays measured in this laboratory are listed in Tables 1, 2, and 3.. Two levels of assay controls were determined with each assay for each analyte, and all control values were within the established ranges.

Data analysis. The mean ± SE were calculated for all continuous variables. Baseline demographics and clinical characteristics between groups were compared using ANOVA and Scheffe's method for continuous variables, with log transformations when necessary. χ^2 analyses were performed for comparison of categorical variables. A two-tailed P value of <0.05 was considered statistically significant. StatView version 5.0.1 (SAS Institute, Cary, NC) was the statistical software used for the analysis.

TABLE 2
Laboratory values at admission and resolution of patients in hyperglycemic crisis

| | Lean DKA | | Obese DKA | | Obese hyperglycemia | | | |
	Admission	Resolution	Admission	Resolution	Admission	Resolution	Lean control	Obese control
Blood glucose (mg/dl)	766 ± 72	176 ± 10*	743 ± 60	184 ± 16*	690 ± 66	186 ± 22*	92 ± 1.5*	93 ± 1.7*
Serum HCO$_3$ (mEq/l)	6.0 ± .8	20.9 ± .6*	8.3 ± .6	20.5 ± .6*	25 ± .7*	25.6 ± 1.1*	26 ± 1.0*	26 ± 1.5*
Venous pH	7.09 ± .03	7.35 ± .01	7.17 ± .02	7.33 ± .01*	7.34 ± .04	7.43 ± .01	—	—
Osmolality (mOsm/kg)	310 ± 4.3	293 ± 3.1*	317 ± 5.0	294 ± 4.0*	311 ± 9.8	289 ± 5.0*	285 ± 6.0*	286 ± 5.0*
β-Hydroxybutyrate (mmol/l)	10.6 ± .7	0.8 ± .2*	8.7 ± .6	1.2 ± .2*	0.5 ± .04*	0.3 ± .03*	0.1 ± .02*	0.1 ± .03*
Cortisol (μg/dl)	46.2 ± 2.3	21.7 ± 1.1*	55.4 ± 5.8	24.6 ± 3.6*	23 ± .9*	17.2 ± 1.4*	14 ± 1.2*	13 ± 1.1*
GH (ng/ml)	12.3 ± 2.2	3.2 ± 1.0*	10.0 ± 3.1	4.0 ± 1.2*	1.6 ± .3*	0.9 ± .2*	0.8 ± .2*	0.8 ± .2*
C-peptide (ng/ml)	0.8 ± .1	0.7 ± .1	1.5 ± .2*	0.8 ± .1	2.5 ± .3*	1.3 ± .4	0.9 ± .2	1.2 ± .3

Data are means ± SE. *$P < 0.01$ vs. lean DKA on admission.

RESULTS

Table 1 shows the clinical characteristics of study subjects. The groups consisted of 28 obese DKA subjects, 20 lean DKA subjects, and 10 obese subjects with NKH. In addition, 12 obese and 12 lean nondiabetic subjects, matched for age, BMI, and ethnicity, served as control subjects. All subjects were African American. None of the subjects had elevated temperature or a white blood cell count $>16 \times 10^6$ cell/ml or a recognized precipitating cause of DKA. The mean HbA$_{1c}$ level on admission was 12.1%.

Table 2 shows the admission laboratory values before insulin therapy and at resolution of DKA or NKH, as well as baseline values in lean and obese control subjects. As previously shown (38), patients with DKA had lower levels of C-peptide than those with NKH. With insulin therapy, levels of counterregulatory hormones were significantly decreased at resolution of DKA and/or hyperglycemia.

Figure 1A–D shows levels of inflammatory cytokines (TNF-α and IL-8, -6, and -1β) on admission and at resolution of the hyperglycemic state. The values for lean and obese control subjects are also included. All values (in pg/ml) are means ± SE. Figure 1A shows the relationship of IL-8 at admission and resolution for the three groups of patients and the control subjects. The admission and resolution levels, respectively, for the groups are lean DKA: 29.3 ± 3.4 and 10.6 ± 2.3 pg/ml; obese DKA: 27.4 ± 3.8 and 11.9 ± 2.8 pg/ml; obese hyperglycemic: 25.8 ± 3.4 and 9.3 ± 2.8 pg/ml; lean control subjects: 4.9 ± 1.4 pg/ml; and obese control subjects: 5.5 ± 1.7 pg/ml. Figure 1B shows the relationship between the admission and resolution levels, respectively, of IL-6 of the groups: lean DKA: 14.9 ± 2.6 and 3.9 ± 1.1 pg/ml; obese DKA: 12.6 ± 2.1 and

4.3 ± 0.6 pg/ml; obese hyperglycemic: 10.2 ± 1.7 and 3.3 ± 0.7 pg/ml; lean control subjects: 1.8 ± 0.2 pg/ml; and obese control subjects: 2.1 ± 0.3 pg/ml. Similarly, Fig. 1C shows this relationship for TNF-α in these groups: lean DKA: 22.7 ± 3.6 and 4.6 ± 0.9 pg/ml; obese DKA: 28.3 ± 2.8 and 5.9 ± 0.7 pg/ml; obese hyperglycemic: 24.3 ± 3.1 and 5.1 ± 1.3 pg/ml; lean control subjects: 1.7 ± 0.2 pg/ml; and obese control subjects: 3.9 ± 0.6 pg/ml. This relationship of IL-1β levels can also be seen for each group at admission and resolution in Fig. 1D: lean DKA: 9.8 ± 2.3 and 2.1 ± 0.2 pg/ml; obese DKA: 13.7 ± 2.1 and 2.4 ± 0.3 pg/ml; obese hyperglycemic: 11.4 ± 0.8 and 3.1 ± 0.4 pg/ml; lean control subjects: 1.3 ± 0.2 pg/ml; and obese control subjects: 1.9 ± 0.3 pg/ml. In all patients, levels of cytokines on admission were significantly higher than at resolution or with matched control subjects. TNF-α levels were significantly higher in the obese DKA than the lean DKA subjects on admission and were correlated significantly with their BMI ($r = 0.81$, $P < 0.05$). The other cytokines did not demonstrate significant correlation with BMI (data not shown). The cytokine levels at resolution of hyperglycemia with insulin therapy were reduced to levels not significantly different from the lean or obese control subjects. Admission levels of TNF-α and IL-8 were greater than those of IL-1β and -6.

Figure 2 shows serum levels of CRP, homocysteine, PAI-1, TBA, DCF, and FFAs on admission and at resolution of DKA and NKH. As can be seen, admission levels were significantly higher in all groups than after insulin therapy and resolution of hyperglycemia. The actual values for these parameters are presented in Table 3. After resolution, levels of DCF, TBA, PAI-1, and FFAs were reduced to near control values, whereas reductions in circulating

TABLE 3
Markers of cardiovascular risks and oxidative stress at admission and resolution of hyperglycemic crisis

| | Lean DKA | | Obese DKA | | Obese hyperglycemia | | | |
	Admission	Resolution	Admission	Resolution	Admission	Resolution	Lean control	Obese control
CRP (mg/l)	51 ± 3	28 ± 1*†	59 ± 13	34 ± 9*†	28 ± 6	13 ± 3*†	1 ± 0.2*†	2 ± 0.4*†
Homocysteine (μmol/l)	18.8 ± 0.8	14.8 ± 0.7*†	23.6 ± 3.6	21.6 ± 2.8	14.8 ± 1.6	12.4 ± 1.2*	7.2 ± 0.4*†	8.8 ± 1.2*†
FFA (mmol/l)	1.6 ± 0.1	0.6 ± 0.1*†	1.4 ± 0.1	0.7 ± 0.1*†	1.2 ± 0.2	0.8 ± 0.1*†	0.5 ± 0.1*†	0.7 ± 0.1*†
DCF (μmol/l)	8.6 ± 0.8	3.7 ± 0.5*†	8.9 ± 1.2	4.1 ± 0.7*†	7.8 ± 0.6	3.8 ± 0.5*†	2.3 ± 0.4*†	3.1 ± 0.6*†
TBA (μmol/l)	3.8 ± 0.7	1.3 ± 0.4*†	4.0 ± 0.6	1.6 ± 0.2*†	3.3 ± 0.5	1.5 ± 0.4*†	0.8 ± 0.1*†	0.9 ± 0.1*†
PAI-1 (ng/ml)	42.1 ± 12.2	4.2 ± 2.1*†	40.4 ± 12.4	13.0 ± 3.4*†	35.4 ± 9.3	7.3 ± 2.4*†	1.4 ± 0.2*†	2.5 ± 0.4*†

Data are means ± SE. *$P < 0.01$ vs. lean DKA on admission; †$P < 0.05$ vs. admission value of each group.
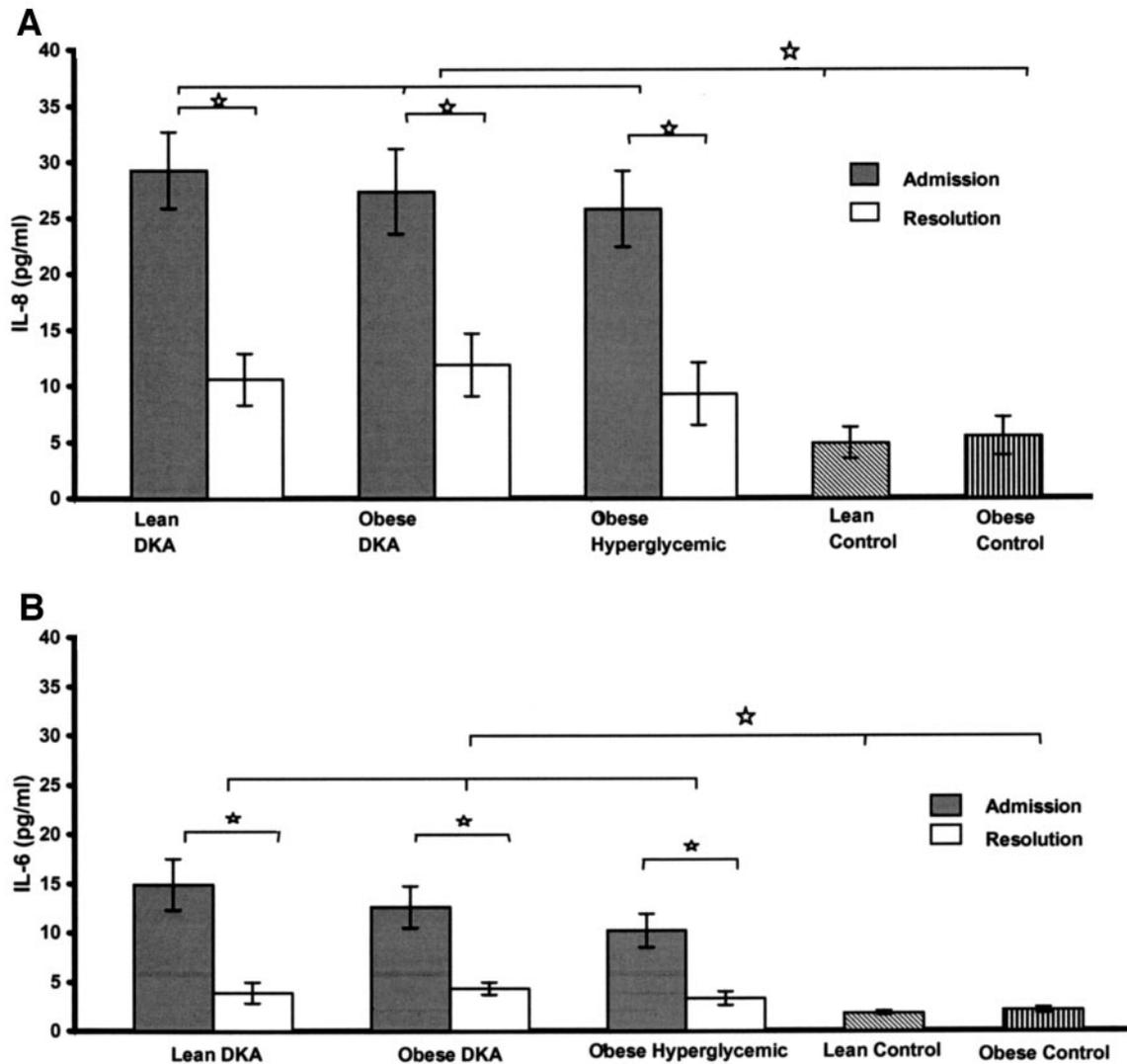
FIG. 1. The proinflammatory cytokine levels of IL-8 (*A*), IL-6 (*B*), TNF-α (*C*), and IL-1β (*D*) measured in the plasma at admission and resolution of the patients in hyperglycemic crisis. The bar graphs show the means ± SE for each of the patient groups: lean DKA (*n* = 20); obese DKA (*n* = 28); obese hyperglycemic (*n* = 10); and lean and obese control subjects (*n* = 12 each). ☆The admission and resolution levels are significantly different (*P* ≤ 0.01), and the admission levels are significantly different from the control subjects.

levels of CRP and homocysteine were not as robust and remained above control values. It is of interest that homocysteine was not decreased significantly with resolution of DKA/hyperglycemia and insulin treatment.

DISCUSSION

Hyperglycemia in subjects with or without a history of diabetes is a common finding in hospitalized patients admitted to critical care settings (39,40) and is associated with greater morbidity and mortality compared with those diagnosed with diabetes (39). The reason for greater mortality may be related to the delay in diagnosis of diabetes and/or its proper therapy (39). On the other hand, numerous prospective studies in diabetic and nondiabetic subjects admitted to critical care units have clearly shown a salutary effect of insulin, improving clinical outcome through correction of hyperglycemia and inflammation (41–45). Some of these studies have shown direct correlation between high blood glucose levels and increased mortality (45). A more recent study in such patients has

demonstrated the salutary effect of insulin to be attributable to its anti-inflammatory properties (27).

Although previous studies have suggested an elevation of IL-6 and TNF-α in uncontrolled diabetes (46), elevation of IL-1B and -8 along with an increase in counterregulatory hormones and cardiovascular markers, to our knowledge, have not been recorded before. Of interest is our finding that these significant elevations of proinflammatory cytokines as well as cortisol, GH, TBA, PAI-1, DCF, and FFAs are all reduced to normal levels promptly in response to insulin therapy and normalization of blood glucose. The times for resolution of DKA and NKH were remarkably close, with both reaching total resolution within ≤24 h. Increased levels of these markers occurred in both ketotic and nonketotic hyperglycemic patients with similar blood glucose (i.e., >600 mg/dl.).

We must therefore conclude that hyperglycemia and ketoacidosis independently induce changes in proinflammatory cytokines, oxidative stress, and cardiovascular markers without synergistic effects of one or the other. It
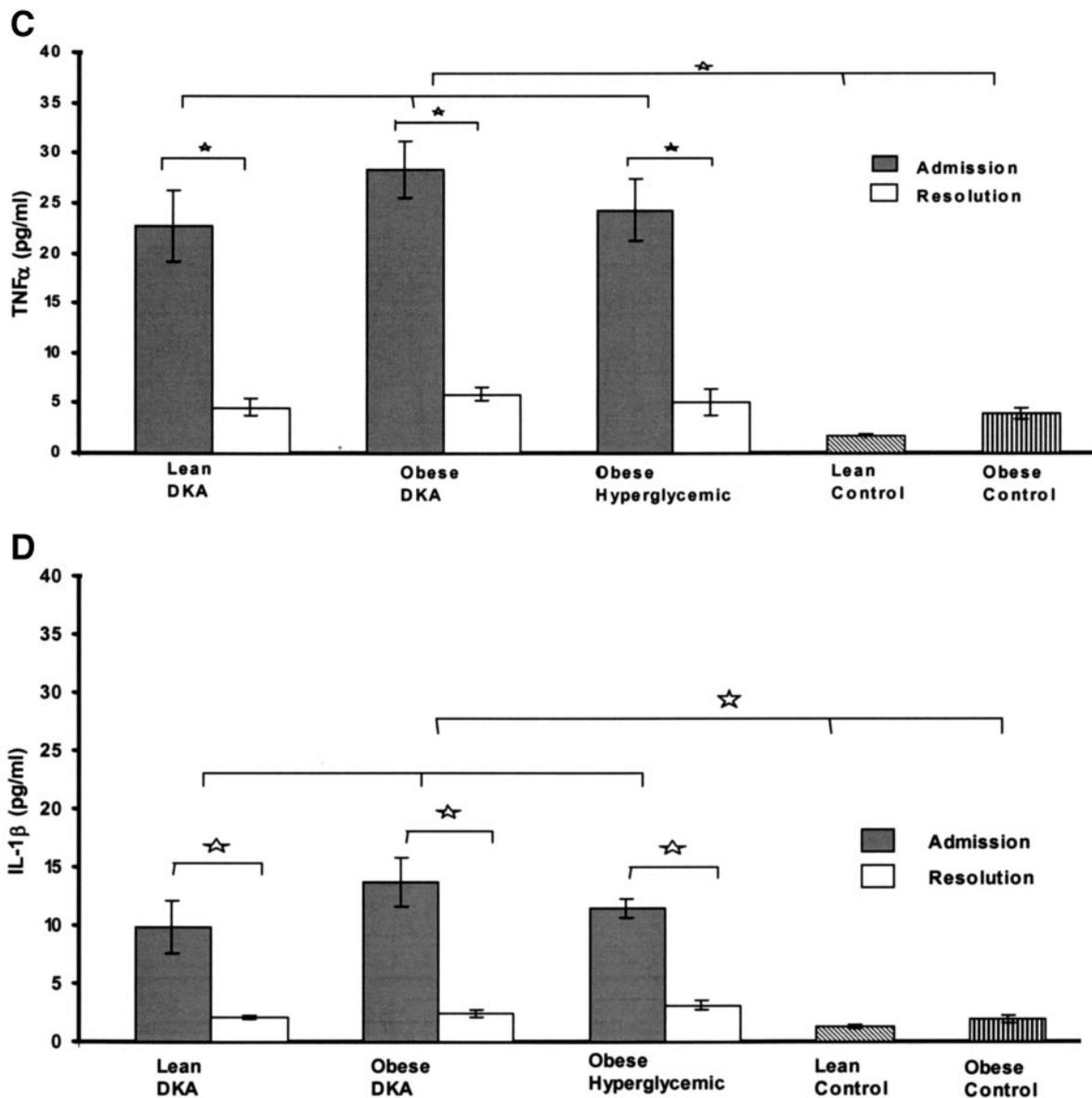
FIG. 1—*Continued.*

is of note that lean DKA patients exhibited as much of an increase in cardiovascular risk markers, oxidative stress, counterregulatory hormones, and cytokines as obese ketoacidotic patients. The only exception to this statement was the level of TNF-α, which was significantly greater in obese DKA than either lean DKA or NKH subjects. Although TNF-α values exhibited high correlation with BMI ($r = 0.81$, $P < 0.05$) in all three groups, other cytokines did not demonstrate such a correlation.

To our knowledge, this is the first report demonstrating increased levels of GH, cortisol, cytokines, cardiovascular risk markers, and oxidative stress in obese and lean patients with DKA (in the absence of any history or evidence of cardiovascular events, trauma, or severe infection) and their prompt suppression in response to intravenous insulin. Although previous studies support the association of DKA with oxidative stress and have studied possible mechanisms for the generation of ROS (47–49), it is not clear whether there is a causal relationship between markers of oxidative stress and acute diabetes complica-

tions, since the majority of these markers have been demonstrated in plasma.

Of interest is our finding in regard to homocysteine concentration and the fact that elevated levels of this risk factor did not respond to insulin as robustly as FFA, PAI-1, TBA, and DCF, all of which returned to control levels with resolution of DKA and hyperglycemia by insulin treatment. Homocysteine is regulated by many factors, and its levels are affected by various drugs (50), but its lack of response may be similar to the observations of Fonseca et al. (51), in that homocysteine levels in diabetic patients, unlike nondiabetic subjects, do not respond to insulin because these patients are insulin resistant. Our study confirms this phenomenon and extends these findings to patients in hyperglycemic crises.

In a more systematic study in type 1 diabetes, a variety of markers of oxidative stress were measured, including TBA, organoperoxide, vitamin E, vitamin C, glutathione, and glutathione peroxidase. The results suggest that most markers are not associated with long-term complications
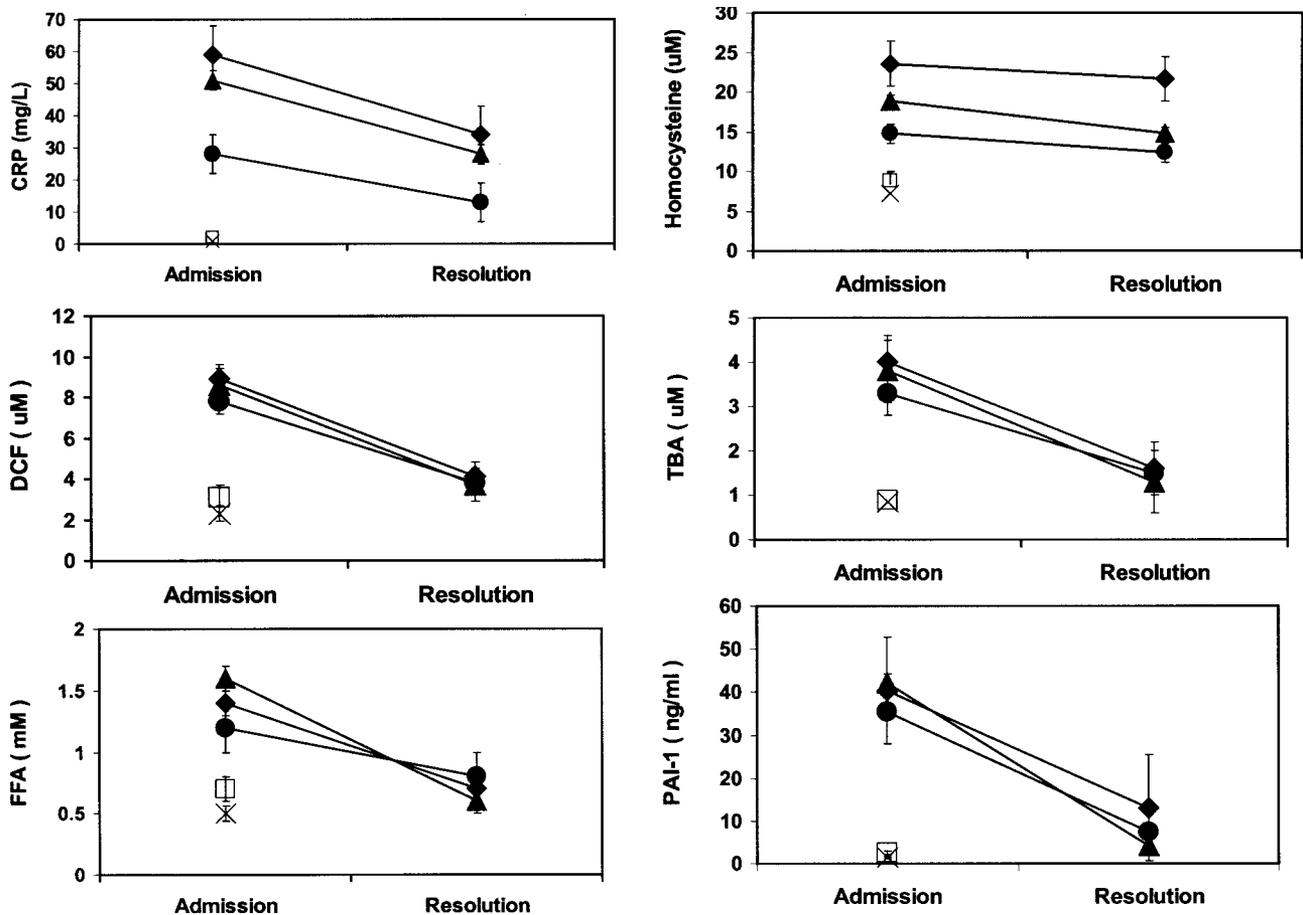
FIG. 2. Markers of oxidative stress and cardiovascular risk markers determined in the plasma of the patient groups at admission and resolution of hyperglycemic crisis. The graphs show the means ± SE for each of the patient groups for each of the markers: high-sensitivity CRP, FFAs, homocysteine, DCF reactive, PAI-1, and TBA reactive. ▲, lean DKA ($n = 20$); ♦, obese DKA ($n = 28$); ●, obese hyperglycemic ($n = 10$); and ×, lean; □, obese control subjects ($n = 12$ each). All markers were significantly different at admission compared with at resolution of hyperglycemic crisis except for homocysteine, where only the lean DKA values were significantly different.

of diabetes (52,53) and that intracellular determination of these markers may be a better method of assessment of oxidative stress.

Our study confirms the well-known phenomenon of leukocytosis in hyperglycemic crises, without obvious infection, on febrile events. Although the mechanism of this finding is not known, the proinflammatory state demonstrated here and elsewhere certainly could bring about such an event, as well as stimulation of sympathetic nervous system, secondary to stress of hyperglycemia. It is well known that in normal subjects, increased sympathetic activity results in leukocytosis and elevation of TNF-α and IL-6 (54). The latter cytokines, along with IL-1β, may regulate production of acute-phase protein by raising body temperature (55), and the organism may be undergoing a compensatory mechanism in the immune and hypothalamic-pituitary-adrenal axis (56). It is of interest that despite leukocytosis and elevation of cytokines and other signs of oxidative stress, the body temperature in DKA remains hypothermic in the absence of florid infection (23). After insulin treatment, however, the temperature returns to normal, concomitant with a reduction of leukocytosis. The mechanisms of this interesting phenomenon are not fully understood, but coupled with the present demonstration of a dramatic reduction of cytokines and mediators of oxidative stress with insulin, they deserve further investigation.

Our recent studies in patients with DKA demonstrated in vivo activation of T-cells, which led us to hypothesize that hyperglycemia and/or ketosis through production of ROS and generation of proinflammatory cytokines may result in de novo emergence of growth factor receptor insulin, IGF-1, and IL-B (24). In the present study, we report elevated levels of proinflammatory cytokines and ROS as markers of oxidative stress in conjunction with elevated levels of FFAs and glucose in our patients with DKA as well as NKH (Table 3).

Based on our present and previous studies as well as other work from other laboratories (10,17,57), we may expand our hypothesis by proposing that the initial activating event for T-cells may be the presence of high levels of both FFAs and glucose in patients with hyperglycemic crises, which may result in the generation of ROS through diacylglycerol/PKC-activated NAD(P)H, supporting earlier findings on the role of FFAs on human muscle cells (10). However, further studies are needed to establish the presence of several hypothetical intermediates, including diacylglycerol, protein kinase C, and activated NAD(P)H in activated T-cells in hyperglycemic patients. To our knowledge, however, the presence of these biochemical steps and intermediary metabolites have not been demonstrated in T-cells of DKA or NKH patients.

Multiple studies have now demonstrated not only the

anti-inflammatory effect of insulin in vitro and in vivo, but also its profibrinolytic activity (25); its suppressive effects on endothelial growth factor, metaloproteinase-9 (26), plasma tissue factor, and PAI-1 (25); and its effect on activation of endothelial cells (58) and polymorphonuclear leukocytes (59). To our knowledge, however, studies on the effect of insulin on subpopulations of polymorphonuclear leukocytes such as CD-4 and CD-8 T-cells, where their in situ activation was demonstrated in DKA (23), has not been reported.

In conclusion, our study clearly demonstrates a hitherto undescribed phenomenon of anti-inflammatory effect of insulin in hyperglycemic crises concomitant with the reduction of multiple cytokines, markers of cardiovascular risk and oxidative stress, and counterregulatory hormones. Our findings thus extend the previous observation on the robust and prompt anti-inflammatory effect of insulin and other conditions not associated with DKA and NKH (25–28,58,59).

The present study, however, does not permit us to draw any definitive conclusion regarding cause and effect of these events because time-related assessment of these factors and isolation of the intermediate products were not planned in this protocol.

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