Plasma Level of 13,14-Dihydro-15-keto-PGE_2 in Patients With Diabetic Ketoacidosis and in Normal Fasting Subjects

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SUMMARY

Plasma levels of 13,14-dihydro-15-keto-PGE_2, a stable derivative of PGE_2, are elevated in rats with diabetic ketoacidosis (DKA) and decrease in response to insulin therapy. In patients with insulin-dependent diabetes mellitus type I (IDDM) the plasma levels of this derivative also rise in response to insulin withdrawal and then fall in response to insulin replacement. We wished to determine whether the level of this substance is elevated acutely when patients present with DKA and to determine whether the levels fall during treatment. We also wished to identify the origin of the circulating 13,14-dihydro-15-keto-PGE_2 in patients with DKA and in normal fasting subjects. We measured the plasma level of 13,14-dihydro-15-keto-PGE_2 in five patients with DKA and in six normal subjects during a 24-h fast. In the patients with DKA before treatment, the plasma 13,14-dihydro-15-keto-PGE_2 level was threefold above normal. During therapy, the 13,14-dihydro-15-keto-PGE_2 level fell toward normal. There was a significant direct correlation between the plasma free fatty acid (FFA) level and the plasma 13,14-dihydro-15-keto-PGE_2 level before and during treatment. In addition, the inverse correlation between the plasma free-insulin level and the plasma 13,14-dihydro-15-keto-PGE_2 level approached significance (P = .06). In contrast, in the normal fasting subjects the plasma FFA level rose to values comparable to those observed in the patients with DKA, but there was no significant increase in the plasma 13,14-dihydro-15-keto-PGE_2 level. There was no quantitatively important correlation between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level. We conclude that *1*) the plasma level of 13,14-dihydro-15-keto-PGE_2 is elevated in DKA and decreases during treatment, *2*) the adipocyte is an important source of the circulating 13,14-dihydro-15-keto-PGE_2 in this disorder, and *3*) insulin inhibits the production of PGE_2 by the adipocyte and perhaps other cells in patients with DKA. The increased production of PGE_2, a potent antilipolytic substance, may moderate the accelerated lipolysis that occurs in DKA but is probably not of sufficient magnitude to produce systemic effects. The rise in the plasma FFA level without a concomitant rise in the plasma 13,14-dihydro-15-keto-PGE_2 level in normal fasting subjects suggests that cells other than the adipocyte may be the source of the circulating 13,14-dihydro-15-keto-PGE_2 in normal subjects.

DIABETES 1986; 35:1004–10.

The plasma level of 13,14-dihydro-15-keto-PGE_2, a stable derivative of prostaglandin E_2 (PGE_2), is elevated in rats with diabetic ketoacidosis (DKA) and decreases toward normal in response to treatment with insulin.[1] Similarly, in patients with insulin-dependent diabetes mellitus type I (IDDM), the plasma level of this derivative rises in response to insulin withdrawal and falls in response to insulin replacement.[2]

The elevated plasma level of 13,14-dihydro-15-keto-PGE_2 in rats with DKA appears to be derived in large part from the adipocyte mass, because rat adipocytes produce PGE_2[3–5] and because the elevated level of this derivative in rats with DKA is reduced by insulin and 5-methylpyrazole-3-carboxylic acid, two structurally unrelated antilipolytic agents.[1] The origin of the elevated plasma level of 13,14-dihydro-15-keto-PGE_2 in patients with diabetes mellitus during insulin withdrawal is unknown.[2]

Soon after the discovery that PGE_1 and PGE_2 are potent antilipolytic substances, the hypothesis was advanced that one of these substances mediates a feedback mechanism for the regulation of lipolysis within adipose tissue.[6,7] The hypothesis was later restricted to PGE_2 when it was demonstrated that PGE_2 is the principal PGE in adipose tissue.[3,4] Numerous studies have confirmed that isolated rat adipocytes also produce PGI_2 (prostacyclin) and $PGF_{2\alpha}$ during nor-

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Received for publication 30 December 1985 and in revised form 31 March 1986.

epinephrine-induced lipolysis.[5,10,11] PGE_2 is a potent antilipolytic agent in vitro[8] and in vivo.[9] However, PGI_2 is not antilipolytic in intact adipocytes,[10] and $PGF_{2\alpha}$ is produced in small quantities by the adipocyte.[10] Thus, the elevated level of 13,14-dihydro-15-keto-PGE_2 in DKA may reflect increased PGE_2 production by the adipocyte mass, which may moderate the accelerated lipolysis that is an inherent feature of that disorder.

In our study, we measured the plasma 13,14-dihydro-15-keto-PGE_2 concentration in five patients with IDDM admitted in DKA before treatment and during the first 12 h of therapy and in six normal subjects during a 24-h fast. We sought to determine whether the level of this substance is elevated acutely at the time of presentation and to determine whether the level declines during treatment in patients with DKA. We also attempted to determine the origin of the circulating 13,14-dihydro-15-keto-PGE_2 in patients with DKA and in normal fasting subjects.

MATERIALS AND METHODS

Patient population. We studied five patients with IDDM who presented to the emergency ward and were admitted to the hospital with DKA (Table 1) and six normal adult male subjects who were admitted to the General Clinical Research Center of the Massachusetts General Hospital. Informed consent was obtained from each patient with DKA (or from a close relative) and from each normal subject.

Patients with DKA were treated with conventional low-dose insulin regimens and with intravenous fluids by their physicians. Our protocol specified that our studies would not delay or alter management but would be confined to drawing blood before and during treatment.

The normal subjects received a balanced weight-maintenance diet for supper on the day of admission and for the first full hospital day. Thereafter, they began a total fast during which water was allowed ad lib. After a 14-h overnight postabsorptive period, blood was drawn at the following intervals during a 24-h fast: −½, 0, 1, 2, 4, 6, 8, 12, 16, and 24 h (from 0730 h to 0800 h the following day).

Analytical procedures. The plasma levels of 13,14-dihydro-15-keto-PGE_2 were measured by radioimmunoassay. Blood was collected in a heparinized tube. The plasma was separated immediately by centrifugation in the cold and stored at −20°C until assayed. The plasma (~3 or 4 ml) was extracted by the addition of 3 vol ethanol. Each ethanolic mixture was shaken every 20 min for 1 h at 25°C and then centrifuged at 800 × *g* for 15 min at 4°C. The supernatant was evaporated to dryness under negative pressure and the residue was redissolved in 1 ml Tris buffer (0.01 M Tris, 0.14 M NaCl, pH 7.4, containing 0.1% gelatin). The specimen was centrifuged to remove insoluble material and an aliquot (~100 μl) was assayed.

The radioimmunoassay system used in our study has been characterized in detail with respect to the serologic specificity of the antiserum used in the radioimmunoassay, the validity of serologic measurements of 13,14-dihydro-15-keto-PGE_2 levels in rat plasma and in Tris buffer, and the effects of the plasma free fatty acids (FFAs) on the assay system.[1] The serologic stability of 13,14-dihydro-15-keto-PGE_2 in human plasma was different than in rat plasma. In undiluted rat plasma the serologic half-life was >24 h,[1] whereas in undiluted human plasma it was 12 h at 37°C. In addition, we measured 13,14-dihydro-15-keto-PGE_2 levels in unextracted plasma and after high-performance liquid chromatography of extracted pooled plasma samples from normal rats and rats with DKA.[1] Pooled plasma both from normal rats and from rats with DKA yielded a single distinct peak of 13,14-dihydro-15-keto-PGE_2; plasma from rats with DKA produced a higher peak of immunologically active material than plasma from normal animals.[1]

Plasma glucose was measured by a glucose oxidase method. Plasma FFA levels were determined by a colorimetric method.[12] Blood β-hydroxybutyrate and acetoacetate levels were determined by enzymatic methods.[13] The total blood ketone level is the sum of the β-hydroxybutyrate and acetoacetate levels. The pH of heparinized arterial blood was measured with a Corning pH meter (model 178). Plasma free insulin was determined by radioimmunoassay after precipitation of bound insulin by polyethylene glycol.[14] Plasma glucagon was measured by radioimmunoassay with antibody 30K.[15,16]

Statistical analysis was performed with the *t* test for paired data and with linear regression, including simple correlation, to determine the degree of association between variables. Computations for regression were done with the SAS package version 79.5 at the Health Science Computing Facility of the Harvard School of Public Health (Boston, MA). In figures in which we present a simple correlation coefficient (*r*),

TABLE 1
Characteristics of patients with DKA

Patient	Sex	Age (yr)	Duration of diabetes (yr)	Usual insulin dose	Precipitating event	Associated illness
1	M	41	0	None	New onset of diabetes	None
2	M	18	10	NPH 20 U (a.m.) Reg. 10 U (a.m.) NPH 20 U (p.m.) Reg. 10 U (p.m.)	Not known	None
3	F	51	0	None	New onset of diabetes	Hypertension, angina pectoris
4	F	77	20	NPH 26 U (a.m.) Reg. 6 U (a.m.)	Reduction in insulin dose 1 wk before admission and emotional stress	Hypothyroidism (treated), hypertension, cataracts, modified left radical mastectomy for breast carcinoma 1 yr before admission
5	F	36	18	NPH 40 U (a.m.)	Omission of insulin for 6 days	None

M, male; F, female; NPH, isophane insulin; Reg., regular insulin; a.m., morning; p.m., evening.

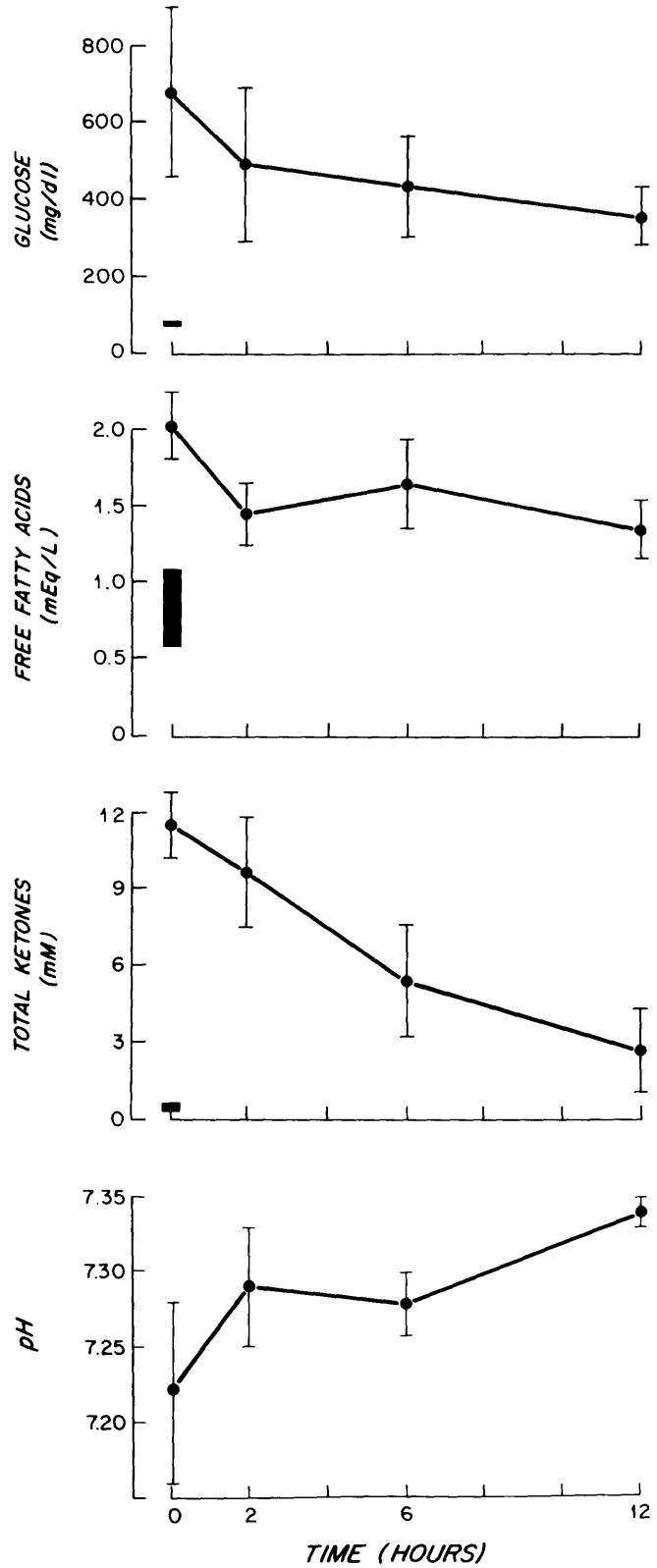

FIG. 1. Circulating levels of glucose, FFAs, total ketones, and pH in 5 patients with DKA before treatment and during first 12 h of therapy (means ± SE). *Shaded area* at time 0 in each of 3 *upper panels* represents range (mean ± SE) in 6 normal male subjects after 14-h overnight fast.

the *P* value given is that for the predictive factor in a regression model taking the effect of individuals into account. Descriptive results are expressed as means ± SE.

RESULTS

Patients with DKA. Plasma glucose, plasma FFA, total blood ketone, and arterial pH levels in five patients with DKA before treatment and during the first 12 h of therapy are depicted in Fig. 1.

The plasma levels of 13,14-dihydro-15-keto-PGE_2 in the same five subjects at the same time points are displayed in Fig. 2 and Table 2. Before treatment, the plasma level of 13,14-dihydro-15-keto-PGE_2 was 0.09 ± 0.02 ng/ml (range 0.04–0.16), threefold above normal. During therapy, the levels of 13,14-dihydro-15-keto-PGE_2 were variable but fell toward normal. In general, the 13,14-dihydro-15-keto-PGE_2 level gradually declined in each of the five patients; in each patient the level was lower at 12 h than it was before treatment. The mean value at 12 h was significantly lower than the mean value before treatment ($p < .01$). However, at 6 h there was a marked rise in the plasma 13,14-dihydro-15-keto-PGE_2 level in two patients (patient 1 and patient 3). These two values are the highest observed in the entire study. In each instance, the rise in the plasma 13,14-dihydro-15-keto-PGE_2 level was associated with a marked fall in the plasma free-insulin level, to values of 12 and 10 μU/ml in patient 1 and patient 3, respectively (Table 2). These two values are also displayed in Fig. 4, in which they conform to the general relationship depicted therein between the plasma 13,14-dihydro-15-keto-PGE_2 level and the plasma free-insulin level.

The plasma free-insulin level was 15.0 ± 5.7 μU/ml before treatment, rising to 66.6 ± 15.9 μU/ml during the first 12 h of therapy. The plasma glucagon level was 286 ± 124 pg/

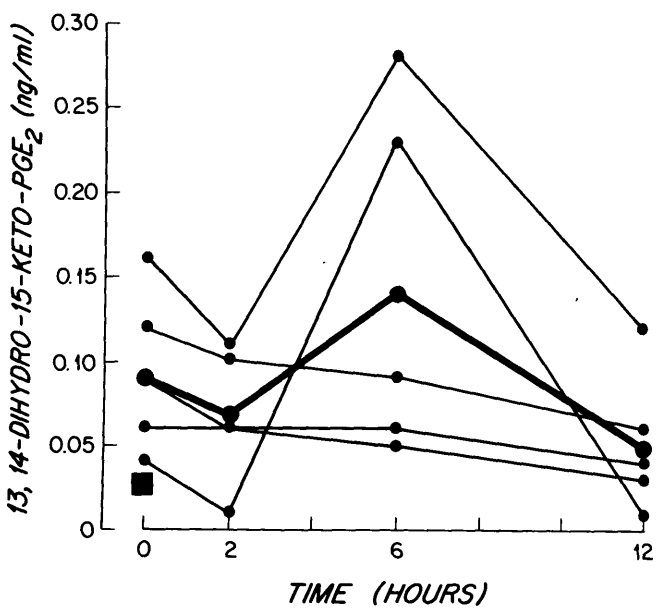

FIG. 2. Plasma levels of 13,14-dihydro-15-keto-PGE_2 in 5 patients with DKA (fuel levels depicted in Fig. 1). *Heavy line* depicts mean values. (Mean values ± SE at each time point are presented in Table 2.) *Shaded area* at time 0 represents range (mean ± SE) in 6 normal male subjects after 14-h overnight fast. Value for 13,14-dihydro-15-keto-PGE_2 is significantly lower at 12 h than at 0 h ($P < .01$).

TABLE 2
Plasma levels of 13,14-dihydro-15-keto-PGE_2 and free insulin in five patients with DKA

	0 h		2 h		6 h		12 h	
Patient	$DKPGE_2$ (ng/ml)	Free insulin (μU/ml)	$DKPGE_2$ (ng/ml)	Free insulin (μU/ml)	$DKPGE_2$ (ng/ml)	Free insulin (μU/ml)	$DKPGE_2$ (ng/ml)	Free insulin (μU/ml)
1	0.04	15	0.01	75	0.23	12	0.01	70
2	0.06	36	0.06	38	0.06	28	0.04	36
3	0.16	14	0.11	65	0.28	10	0.12	32
4	0.09	5	0.06	180	0.05	90	0.03	120
5	0.12	5	0.10	10	0.09	24	0.06	75
Means ± SE	0.09 ± 0.02	15.0 ± 5.7	0.07 ± 0.02	73.6 ± 28.9	0.14 ± 0.05	32.8 ± 14.7	0.05 ± 0.02	66.6 ± 15.9

$DKPGE_2$ = 13,14-dihydro-15-keto-PGE_2.

ml before treatment, falling to 68 ± 19 pg/ml during the first 12 h of treatment.

There was a significant linear correlation between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level before and during treatment (Fig. 3).

In addition, there was a significant inverse correlation between the plasma free-insulin level and the plasma FFA level before and during treatment (Fig. 4). There was also an inverse relationship between the plasma free-insulin level and the plasma 13,14-dihydro-15-keto-PGE_2 level (P = .06) (Fig. 4). In contrast, there was little or no correlation between the plasma glucagon level and the plasma levels of FFAs or 13,14-dihydro-15-keto-PGE_2.

Normal fasting subjects. The circulating levels of glucose, FFAs, total ketones, and 13,14-dihydro-15-keto-PGE_2 in the six normal subjects after a 14-h overnight fast are depicted in the shaded areas at time 0 in Fig. 1 and 2 for comparison with the patients in DKA. The values for each normal subject at two time points (0730 and 0800 h) after the overnight postabsorptive period were averaged to obtain the postabsorptive value of each circulating level for that subject. The plasma level of 13,14-dihydro-15-keto-PGE_2 was 0.027 ± 0.007 ng/ml.

During the subsequent 24-h fast, the plasma glucose level decreased to 66 ± 3 mg/dl, the plasma FFA level rose to 1.89 ± 0.27 meq/L, and the total blood ketone level rose to 2.56 ± 0.32 mM. The plasma level of 13,14-dihydro-15-keto-PGE_2 was detectable in all subjects at all time points studied during the fast but was never significantly different from time 0. The plasma 13,14-dihydro-15-keto-PGE_2 level at 24 h was 0.047 ± 0.013 ng/ml.

In contrast to the patients with DKA, there was no quantitatively important direct correlation between the plasma FFA level and the plasma level of 13,14-dihydro-15-keto-PGE_2 during the fast (Fig. 5). Although there was a significant correlation between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level, the regression line describing the relationship between these substances was nearly horizontal. Because we used a regression model that takes the effect of individuals into account, the finding of a significant correlation may reflect the power of the statistical analysis. Although the correlation was significant, the r value of .14 indicates that this correlation can explain only ~2% of the variance in the 13,14-dihydro-15-keto-PGE_2 measurements (r^2 = .0196).

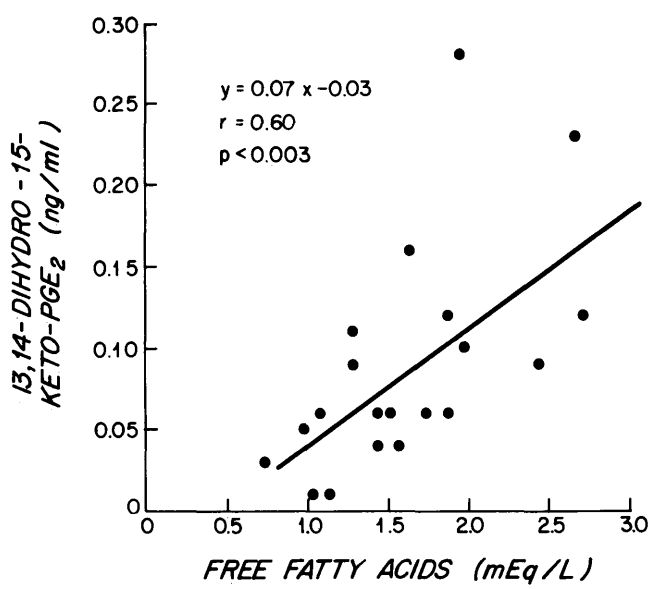

FIG. 3. Relationship between plasma FFA level and plasma level of 13,14-dihydro-15-keto-PGE_2 before treatment and during first 12 h of therapy in 5 patients with DKA. Data are those depicted in Figs. 1 and 2.

DISCUSSION

Our study demonstrates that the plasma level of 13,14-dihydro-15-keto-PGE_2 is elevated in patients with DKA before therapy is initiated and decreases during the course of treatment. The variability in the plasma 13,14-dihydro-15-keto-PGE_2 level during therapy is attributable to variations in the plasma free-insulin level. This is due in turn to the fact that each patient was treated by his or her own physician; our protocol specified that our studies would not delay or alter management but would be limited to drawing blood before and during treatment. In fact, the variability of the plasma 13,14-dihydro-15-keto-PGE_2 level at 6 h is due to a dramatic increase in this value in two patients. In each instance, this was associated with a marked fall in the plasma free-insulin level. This interpretation is supported by the observation that much of the variability was eliminated when the plasma 13,14-dihydro-15-keto-PGE_2 level was correlated with the plasma free-insulin level. In other words, the plasma 13,14-dihydro-15-keto-PGE_2 levels and the plasma free-insulin levels in the

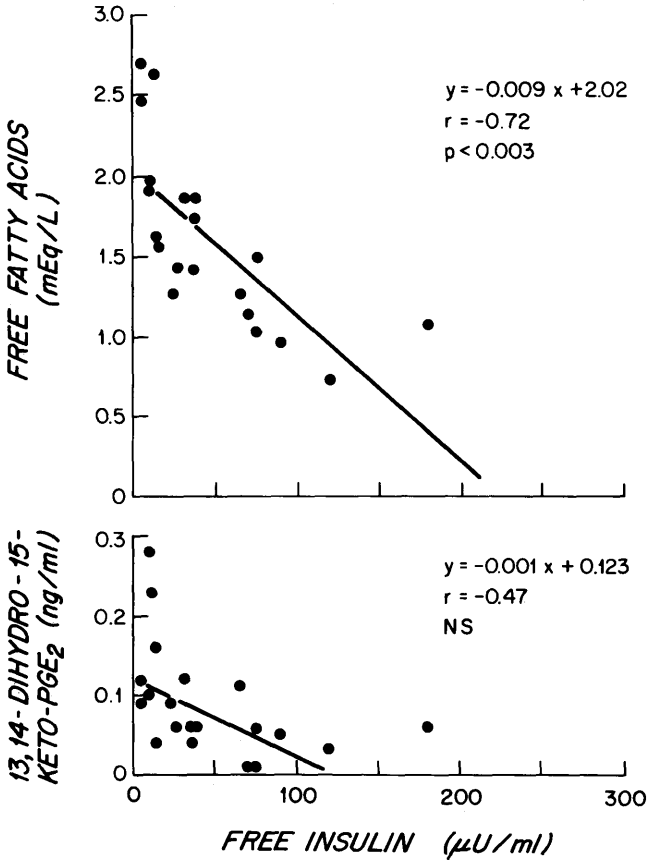

FIG. 4. Relationship between plasma free-insulin level and plasma level of FFAs (*top panel*) and 13,14-dihydro-15-keto-PGE_2 (*bottom panel*) before treatment and during first 12 h of therapy in 5 patients with DKA (fuel and PG levels depicted in Figs. 1 and 2). $P = .06$ for correlation between plasma free-insulin level and plasma 13,14-dihydro-15-keto-PGE_2 level.

two patients who displayed increases in the level of the PGE_2 derivative at 6 h conform to the general relationship between these two variables. The lability in the level of the PGE_2 derivative was not associated with a corresponding lability in the levels of the circulating fuels or the pH. This finding suggests that the plasma 13,14-dihydro-15-keto-PGE_2 level is especially labile and especially sensitive to the plasma free-insulin level in patients with DKA.

Our findings are comparable both qualitatively and quantitatively to our observations in rats with streptozocin-induced DKA.[1] Our observations are also consistent with those of McRae et al,[2] who found that the plasma level of 13,14-dihydro-15-keto-PGE_2 rises in response to insulin withdrawal and falls in response to insulin replacement in patients with IDDM. Our data indicate that the circulating levels of this PGE_2 derivative are not only elevated under the controlled circumstances of an insulin-withdrawal study, as shown by McRae et al., but are also elevated acutely in patients who present to the emergency ward in DKA.

The significant correlations we observed between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level are consistent with the view that the elevated plasma levels of this prostaglandin derivative are derived from the adipocyte cell mass. These findings in humans are consistent with the evidence that isolated rat adipocytes produce PGE_2[3-5] and that the elevated plasma levels of this derivative in rats with DKA are decreased by insulin and 5-methylpyrazole-3-carboxylic acid, two structurally unrelated antilipolytic agents.[1] On the basis of our present findings and our previous observations, it is likely that the elevated plasma levels of this derivative are attributable in large part to increased production of PGE_2 by the adipocyte cell mass. However, a contribution from other cells or from decreased clearance of these derivatives cannot be excluded.

Our observations support the view that insulin inhibits the production of PGE_2 by the adipocyte and perhaps other cells in patients with DKA. During the treatment of DKA with insulin and intravenous fluids, the plasma level of 13,14-dihydro-15-keto-PGE_2 fell toward normal. In addition, there was a significant inverse correlation between the plasma free-insulin level and the plasma FFA level before and during treatment. There was also an inverse relationship between the plasma free-insulin level and the plasma 13,14-dihydro-15-keto-PGE_2 level ($P = .06$). Although this relationship failed to achieve significance at the conventional level of $P < .05$, it still suggests the existence of a biologic relationship. These results are consistent with our earlier findings that insulin decreases the elevated levels of 13,14-dihydro-15-keto-PGE_2 in rats with DKA.[1] These changes are probably not attributable to the effects of intravenous fluid administration, because decreases in the plasma levels of this PGE_2 derivative were also observed in rats with DKA treated with insulin and saline but not in rats treated with saline alone.[1]

We also studied six normal subjects during a 24-h fast. Although the plasma FFA level rose to values comparable to those observed in the patients with DKA, the plasma level of 13,14-dihydro-15-keto-PGE_2 did not rise comparably. The regression line describing the relationship between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level was nearly horizontal in the normal subjects, in contrast to the findings in patients with DKA. This dissociation of the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level indicates that FFAs do not cross-react in the radioimmunoassay for 13,14-dihydro-15-keto-PGE_2, consis-

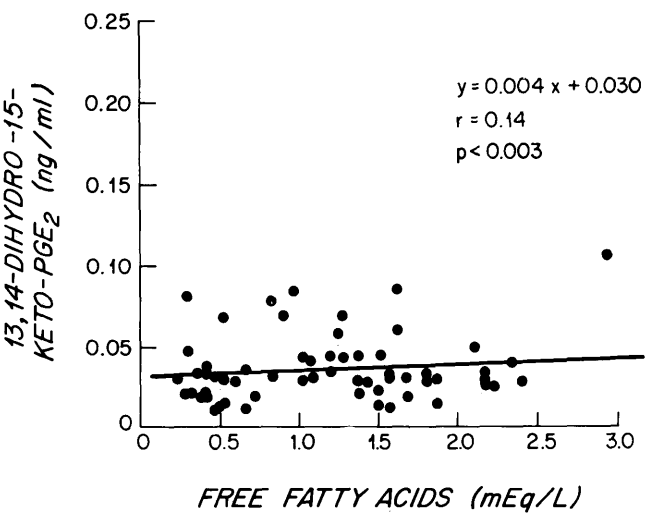

FIG. 5. Relationship between plasma FFA level and plasma level of 13,14-dihydro-15-keto-PGE_2 in 6 normal subjects during 24-h fast.

tent with in vitro studies of serologic specificity (see MATERIALS AND METHODS).

Although the evidence suggests that the adipocyte is an important source of the elevated plasma level of 13,14-dihydro-15-keto-PGE_2 in the insulin-deficient state, the rise in the plasma FFA level without a concomitant rise in the plasma level of the PGE_2 derivative in normal fasting humans suggests that other cells may be more important sources of this derivative in the circulation when insulin is present.

Furthermore, these findings indicate that triglyceride lipolysis can be dissociated from PGE_2 production in normal fasting subjects, in contrast to patients with DKA. The significant correlation between the plasma FFA level and the plasma 13,14-dihydro-15-keto-PGE_2 level in patients with DKA suggests that triglyceride lipolysis and PGE_2 release are activated concomitantly in the insulin-deficient state. This finding is consistent with the observation that norepinephrine stimulates both triglyceride lipolysis and PGE_2 production by isolated rat adipocytes in the absence of insulin.[10] The dissociation between triglyceride lipolysis and PGE_2 production in the fasting subjects, in contrast to the association between these events in DKA, is unexplained. It may reflect the difference in the degree of adrenergic activation in DKA and fasting. Catecholamine levels are elevated in DKA,[17] but sympathetic nervous system activation is decreased during fasting.[18,19] In more general terms, the dose-response relationship between the plasma free-insulin level and the inhibition of PGE_2 production by the adipocyte may be modified by the level of sympathetic activity or by other hormonal or metabolic factors that distinguish DKA from fasting.

PGE_2 may mediate a feedback mechanism for the regulation of lipolysis in adipose tissue.[3,4,6,7] This theory is supported by the following evidence: lipolytic agents increase the production of cyclic AMP in fat cells;[4] agents that increase lipolysis and cyclic AMP production also promote PGE_2 synthesis in adipose tissue;[4] PGE_2 is a potent antilipolytic agent in vitro[8] and in vivo;[9] PGE_2 binds to human adipocytes;[20,21] the PGE_2-binding sites have a dissociation constant that is comparable to the IC_{50} for the antilipolytic effect of this PG; and PGE_2 inhibits adenylate cyclase in adipocytes at physiologic concentrations,[22] an effect that appears to mediate the antilipolytic activity of this substance.[22,23]

Studies of the effects of dietary manipulation on PG synthesis and lipolysis in adipose tissue also support the view that PGE_2 is a negative-feedback regulator of lipolysis. In rats with essential fatty acid deficiency, the release of PGE_2 is decreased[3] and basal and stimulated lipolysis are increased, both in vitro[3,24,25] and in vivo.[24-26] In one study, basal lipolysis was enhanced in rats with essential fatty acid deficiency when glycerol release was expressed on the basis of cellular lipid but not when expressed on the basis of cellular protein;[27] however, stimulated lipolysis was not studied. Adipose tissue from normal rats fed a polyunsaturated-fat diet has a lower basal release rate of FFAs and glycerol than adipose tissue from rats fed a saturated-fat diet.[28] These observations are consistent with the view that an increased polyunsaturated-fat diet provides increased substrate for PGE_2 synthesis, which then results in decreased lipolysis. On the other hand, in another study of the effect of dietary fat on PG synthesis, norepinephrine-stimulated production of PGE_2 and $PGF_{2\alpha}$ increased as the ratio of polyunsaturated-to-saturated dietary fat increased, but basal and norepinephrine-stimulated lipolysis was not affected by the degree of polyunsaturation of dietary fat.[29] In that study, a large and presumably maximal lipolytic concentration of norepinephrine (10 μM) was used, which may have obscured the antilipolytic effect of PGE_2.

Despite the evidence just cited in support of the hypothesis that PGE_2 modulates the hormonal regulation of lipolysis in a negative-feedback manner, this idea remains controversial. The principal objection to the hypothesis arises from studies with indomethacin. If the hypothesis is correct, inhibition of endogenous synthesis of PGE_2 by an inhibitor of the fatty acid cyclooxygenase, such as indomethacin, should result in enhancement of lipolysis. The initial report of the effect of indomethacin on epinephrine-stimulated lipolysis in isolated fat cells did, in fact, demonstrate enhancement.[30] Although a similar study with another inhibitor of cyclooxygenase demonstrated enhanced lipolysis,[31] other investigators were unable to reproduce these results with indomethacin and concluded that endogenous PGs are of little or no importance as feedback inhibitors of lipolysis in adipose tissue.[32-34]

These negative studies are not convincing. They exhibit the widely known problems of biochemical studies with inhibitors, i.e., failure to demonstrate inhibition and the presence of additional effects of a supposedly specific inhibitor. PGE_2 was not measured in any of the studies with indomethacin to determine the adequacy of blockade of PGE_2 synthesis. Furthermore, indomethacin is not a specific inhibitor of the fatty acid cyclooxygenase and has a wide variety of additional effects.[34-36]

Thus, the weight of the evidence supports the concept that PGE_2 modulates the rate of lipolysis in the adipocyte. Consequently, increased PGE_2 production by the adipocyte may exert a moderating effect on the accelerated lipolysis that occurs in DKA. Confirmation of this interpretation will require evidence that inhibition of PGE_2 production by adipocytes in vivo enhances the rate of triglyceride lipolysis in diabetic patients.

PGE_2 causes hypotension, tachycardia, nausea, and abdominal pain in humans.[37-38] Nevertheless, the plasma 13,14-dihydro-15-keto-PGE_2 level in the patients with DKA suggests that PGE_2 does not play an important role in the pathogenesis of the hemodynamic and gastrointestinal features of this disorder. Using the same antiserum against 13,14-dihydro-15-keto-PGE_2 that we used in our study, other investigators found that the infusion of PGE_2 in two subjects at 30 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} for 60 min, a dose that does not cause systemic effects, resulted in peak plasma levels of 2.75 and 3.50 ng/ml by the end of the infusion.[39] Because these values are more than 30-fold higher than those observed in our study, the increase in PGE_2 production in DKA is probably not sufficient to contribute to the hemodynamic or gastrointestinal findings in patients with DKA. The increased production of PGE_2 may modulate the rate of lipolysis in uncontrolled diabetes locally in the adipose bed where it is produced without necessarily reaching the systemic arterial circulation in sufficient quantity to cause systemic effects.

ACKNOWLEDGMENTS

We are indebted to the patients and control subjects who participated in our study, to the house officers and physicians

of the Massachusetts General Hospital who permitted us to study their patients, and to the nursing staff of the General Clinical Research Center for devoted nursing care. We are also indebted to Dr. Lawrence Levine for performing the radioimmunoassay of 13,14-dihydro-15-keto-PGE_2, to Dr. Robert Lew for statistical advice, to Colleen A. Ryan for excellent technical assistance, and to Carol Bovest and Martha Chambers for typing the manuscript.

This work was supported by NIH grant AM-26475. L.A. is the recipient of a Research Career Development Award from NIH (AM-01047). P.J.B. is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

[1] Axelrod, L., and Levine, L.: Plasma prostaglandin levels in rats with diabetes mellitus and diabetic ketoacidosis. Diabetes 1982; 31:994–1001.

[2] McRae, J. R., Day, R. P., Metz, S. A., Halter, J. B., Ensinck, J. W., and Robertson, R. P.: Prostaglandin E_2 metabolic levels during diabetic ketoacidosis. Diabetes 1985; 34:761–66.

[3] Christ, E. J., and Nugteren, D. H.: The biosynthesis and possible function of prostaglandins in adipose tissue. Biochim. Biophys. Acta 1970; 218:296–307.

[4] Dalton, C., and Hope, W. C.: Cyclic AMP regulation of prostaglandin biosynthesis in fat cells. Prostaglandins 1974; 6:227–42.

[5] Axelrod, L., Minnich, A. K., and Ryan, C. A.: Stimulation of prostacyclin production in isolated rat adipocytes by angiotensin II, vasopressin, and bradykinin: evidence for two separate mechanisms of prostaglandin synthesis. Endocrinology 1985; 116:2548–53.

[6] Bergström, S.: Prostaglandins: members of a new hormonal system. Science 1967; 157:382–91.

[7] Shaw, J. E., and Ramwell, P. W.: Release of prostaglandin from rat epididymal fat pad on nervous and hormonal stimulation. J. Biol. Chem. 1968; 243:1498–503.

[8] Axelrod, L., Trzepacz, P. T., Zusman, R. M., and Martin, D. B.: Antilipolytic effect of prostaglandin E_2 analogues: therapeutic implications. Life Sci. 1976; 18:627–32.

[9] Axelrod, L., Slowman, S. D., Lloyd-Jones, P., and Martin, D. B.: Persistence of ketosis despite suppression of lipolysis by 16,16-dimethylprostaglandin E_2 in experimental diabetic ketoacidosis in rats. Diabetes 1979; 28:905–13.

[10] Axelrod, L., and Levine, L.: Prostacyclin production by isolated adipocytes. Diabetes 1981; 30:163–67.

[11] Axelrod, L., and Levine, L.: Inhibitory effect of insulin on prostacyclin production by isolated rat adipocytes. Prostaglandins 1983; 25:571–79.

[12] Itaya, K., and Ui, M.: Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. 1965; 6:16–20.

[13] Williamson, D. H., Mellanby, J., and Krebs, H. A.: Enzymic determination of D−-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem. J. 1962; 82:90–98.

[14] Kuzuya, H., Blix, P. M., Horwitz, D. L., Steiner, D. F., and Rubenstein, A. H.: Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977; 26:22–29.

[15] Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H.: Effects of starvation on plasma pancreatic glucagon in normal man. Diabetes 1969; 18:717–23.

[16] Weir, G. C., Turner, R. C., and Martin, D. B.: Glucagon radioimmunoassay using antiserum 30K: interference by plasma. Horm. Metab. Res. 1973; 5:241–44.

[17] Christensen, N. J.: Plasma norepinephrine and epinephrine in untreated diabetics, during fasting and after insulin administration. Diabetes 1974; 23:1–8.

[18] Landsberg, L., and Young, J. B.: Fasting, feeding and regulation of the sympathetic nervous system. N. Engl. J. Med. 1978; 298:1295–301.

[19] O'Dea, K., Esler, M., Leonard, P., Stockigt, J. R., and Nestel, P.: Noradrenaline turnover during under- and over-eating in normal weight subjects. Metabolism 1982; 31:896–99.

[20] Richelsen, B., Eriksen, E. F., Beck-Nielsen, H., and Petersen, O.: Prostaglandin E_2 receptor binding and action in human fat cells. J. Clin. Endocrinol. Metab. 1984; 59:7–12.

[21] Day, R. P., Little, S. A., and Robertson, R. P.: Characterization of prostaglandin E_2 binding to isolated human adipocytes. Diabetes 1985; 34:161–65.

[22] Kather, H., and Simon, B.: Biphasic effects of prostaglandin E_2 on the human fat cell adenylate cyclase. J. Clin. Invest. 1979; 64:609–12.

[23] Dalton, C., Hope, H., and Martikes, L.: Prostaglandin inhibition of cyclic-AMP accumulation and rate of lipolysis in fat cells. Prostaglandins 1974; 7:319–26.

[24] Bergstrom, S., and Carlson, L. A.: Lipid mobilization in essential fatty acid deficient rats. A preliminary note. Acta Physiol. Scand. 1965; 64:479–80.

[25] Bizzi, A., Veneroni, E., Garattini, S., Puglisi, L., and Paoletti, R.: Hypersensitivity to lipid mobilizing agents in essential fatty acid (EFA) deficient rats. Eur. J. Pharmacol. 1967; 2:48–52.

[26] DePury, G. G., and Collins, F. D.: A raised level of free fatty acids in serum of rats deficient in essential fatty acids as a contributing cause of their fatty livers. Biochim. Biophys. Acta 1965; 106:213–14.

[27] Demeyer, D. I., Tan, W. C., and Privett, O. S.: Effect of essential fatty acid deficiency on lipid metabolism in isolated fat cells of epididymal fat pads of rats. Lipids 1974; 9:1–7.

[28] Powar, S. S., and Tidwell, H. C.: Effect of prostaglandin and dietary fats on lipolysis and esterification in rat adipose tissue in vitro. Biochim. Biophys. Acta 1968; 164:167–71.

[29] Lipinski, B. A., and Mathias, M. M.: Prostaglandin production and lipolysis in isolated rat adipocytes as affected by dietary fat. Prostaglandins 1978; 16:957–63.

[30] Illiano, G., and Cuatrecasas, P.: Endogenous prostaglandins modulate lipolytic processes in adipose tissue. Nature New Biol. 1971; 234:72–74.

[31] Lefebvre, P. J., and Luyckx, A. S.: Effect of L8027, a new potent inhibitor of prostaglandin biosynthesis, on the metabolism and response to glucagon of rat adipose tissue. Biochem. Pharmacol. 1974; 23:2119–25.

[32] Dalton, C., and Hope, H. R.: Inability of prostaglandin synthesis inhibitors to affect adipose tissue lipolysis. Prostaglandins 1973; 4:641–51.

[33] Fain, J. N., Psychoyos, S., Czernik, A. J., Frost, S., and Cash, W. D.: Indomethacin, lipolysis and cyclic AMP accumulation in white fat cells. Endocrinology 1973; 93:632–39.

[34] Fredholm, B. B., and Hedqvist, P.: Indomethacin and the role of prostaglandins in adipose tissue. Biochem. Pharmacol. 1975; 24:61–66.

[35] Flower, R. J.: Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 1974; 26:33–67.

[36] Dunn, M. J., and Hood, V. L.: Prostaglandins and the kidney. Am. J. Physiol. 1977; 233:F169–84.

[37] Carlson, L. A., Ekelund, L.-G., and Oro, L.: Clinical, metabolic and cardiovascular effects of different prostaglandins in man. Acta Med. Scand. 1970; 188:553–59.

[38] Karim, S. M. M., Somers, K., and Hillier, K.: Cardiovascular and other effects of prostaglandins E_2 and $F_{2\alpha}$ in man. Cardiovasc. Res. 1971; 5:255–59.

[39] Metz, S. A., Rice, M. G., and Robertson, R. P.: Application and limitations of measurement of 15-keto,13,14-dihydro-prostaglandin E_2 in human blood by radioimmunoassay. Prostaglandins 1979; 17:839–61.