The Relationship Between Plasma Insulin Level, Prostaglandin Production by Adipose Tissue, and Blood Pressure in Normal Rats and Rats With Diabetes Mellitus and Diabetic Ketoacidosis

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There is a correlation between circulating insulin levels and blood pressure over a wide range of insulin levels and in a variety of clinical conditions. Production of prostaglandin (PG)E_2 (PGE_2) and prostacyclin (PGI_2), two potent vasodilators, by adipose tissue is increased in severe insulin deficiency, eg, diabetic ketoacidosis (DKA), explaining the decreased peripheral vascular resistance in DKA. Conversely, decreased production of PGE_2 and PGI_2 may mediate the relationship between hyperinsulinemia and hypertension. Although insulin inhibits PG production in normal rat adipose tissue, PG production in adipose tissue from patients or experimental animals with nonketotic diabetes mellitus (DM) and DKA has not been studied. We examined the effect of plasma insulin levels on blood pressure and on adipose tissue PG production in rats with DM and DKA and normal rats. There was a significant relationship between plasma insulin level and blood pressure in rats with DM and normal controls ($P < .021$) and in rats with DKA and normal controls ($P < .0001$). There was an inverse linear correlation between plasma insulin levels and basal 6-keto-$PGF_{1\alpha}$ production by a mixture of adipocytes and endothelial cells from epididymal adipose tissue in rats with DKA and normal rats ($P < .0252$, $R^2 = .67$). Rates of basal glycerol, PGE_2, and 6-keto-$PGF_{1\alpha}$ production by a mixture of adipocytes and endothelial cells from epididymal adipose tissue were significantly higher in rats with DKA than in normal rats. These rates were also higher in rats with DM than in normal rats, but only glycerol values were statistically significant. In rats with DM, PGE_2 production induced by epinephrine 2×10^{-5} mol/L (but not lower concentrations) was significantly greater than basal production ($P < .05$); production of 6-keto-$PGF_{1\alpha}$ was not stimulated. In rats with DKA, 6-keto-$PGF_{1\alpha}$ production induced by epinephrine 2×10^{-5} mol/L (but not lower concentrations) was significantly greater than basal production ($P < .05$); production of PGE_2 was not stimulated. We conclude the following: (1) there is a close correlation between circulating insulin level and systemic blood pressure when rats with DM and DKA are compared with controls; (2) in insulin deficiency, PGI_2 and PGE_2 production are increased in adipose tissue versus normal tissue; and (3) the correlation between insulin level and blood pressure may be mediated by the inhibitory effect of insulin on vasodilative PG production by adipose tissue.

THERE APPEARS to be a direct relationship between insulin levels and blood pressure through a wide range of insulin levels, mediated by the inhibitory effect of insulin on production of prostaglandin (PG) E_2 (PGE_2) and prostacyclin (PGI_2), two potent vasodilators, by adipose and possibly other tissues.[1] PGE_2 and PGI_2 production by normal adipose tissue in vitro is increased in the absence of insulin and decreased in a dose-response manner by insulin.[2,3] These findings and a variety of in vivo observations in rats and in humans suggest that adipose tissue is the principal source of the elevated levels[4,5] of these two potent vasodilators in diabetic ketoacidosis (DKA), a condition caused by severe insulin deficiency. Increased production of PGI_2 and PGE_2 by adipose tissue may explain the decreased vascular resistance and hypotension of DKA.[1] Conversely, decreased production of PGI_2 and PGE_2 by adipose tissue may mediate the relationship between hyperinsulinemia and hypertension.[1] In normal rat adipose tissue, epinephrine-stimulated lipolysis is under the coordinate control of PGI_2, a potent lipolytic agent, and PGE_2, a potent antilipolytic agent.[6] In nonketotic diabetes mellitus (DM) or in DKA, the coordinate control of epineprine-stimulated lipolysis in adipose tissue by PGE_2 and PGI_2 might be altered by an imbalance in the production of these two prostanoids.[6] PG production in adipose tissue of patients or experimental animals with DM and DKA has not been studied. The purpose of this study was to examine the relationship between circulating insulin level, blood pressure, and PG production by adipose tissue in rats with DM and DKA. Our findings support the hypothesis that the relationship between insulin level and blood pressure is mediated by the inhibitory effect of insulin on PG production by adipose tissue.

MATERIALS AND METHODS

Animal Model

These studies were approved by the Subcommittee on Animal Care of the Massachusetts General Hospital. Two separate studies were performed in normal rats and rats with streptozotocin-induced DM or DKA. In each experiment, 26 male rats were weighed and divided into two groups. The first group consisted of 13 normal rats. The second group consisted of 13 rats in which DM (in some experiments) or DKA (in other experiments) was induced immediately after blood pressure measurements. DM and DKA were induced in white male Sprague-Dawley (CD strain) rats weighing 150 to 175 g by subcutaneous injection of streptozotocin 70 and 150 mg/kg body weight, respectively. Control animals received the vehicle only, 50 mmol/L citrate buffer, pH 4. The animals were then allowed continuous access to water and Purina laboratory chow (St Louis, MO) for 40 hours.

Blood Pressure Measurement

Systolic blood pressure was determined by a validated method.[7] To measure systolic blood pressure, an occlusion cuff was placed at the base of the tail and connected by t-tube to an inflation bulb and Gould P23 ID pressure transducer (Orlando, FL). Immediately distal to the occlusion cuff, a cuff-contained pulse transducer

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(Buffington Clinical Devices, Cleveland, OH) was applied with a cuff inflation pressure of 40 to 50 mm Hg. Readings from the pressure and pulse transducers were displayed on a Gould multichannel recorder (model 2200S), thereby permitting the simultaneous recording of cuff pressure and arterial pulse. Indirect systolic pressure measurements were then obtained by increasing pressure in the occlusion cuff until the arterial pulse was no longer visible up to 0 to 200 mm Hg and then gradually releasing the cuff pressure. The cuff pressure at which the first pulse displacement reappeared was defined as systolic pressure. A total of six measurements were recorded per rat, and the mean of six values was taken as the value for each rat. After injection of streptozotocin or vehicle, six normal rats and six rats with DM or DKA were placed into individual restraining cages. At least 30 minutes was allowed to elapse before blood pressure was measured while warming the rat tails with direct light (ambient temperature, 35° to 40°C) to allow the rats to adjust to restraining cages and to eliminate any stress-related vasoconstriction. The remaining rats in each group were left in the animal facility. Their blood pressure was not measured, to minimize the time required for these measurements; they were used subsequently in studies of circulating insulin and fuel levels and studies of adipose tissue (which follow). Forty hours later, all 26 rats were weighed again. Blood pressure was measured in the same 12 rats (six normal rats and six rats with DM or DKA) in which it was measured previously under the same defined conditions.

Analytical Procedures

Following blood pressure measurements, all 26 rats received ether anesthesia by inhalation under a hood. Approximately 2.0 mL blood from each rat was withdrawn into a heparinized tube via cardiac puncture. Immediately thereafter, 200 μL blood was extracted into 2.0 mL 3% (vol/vol) perchloric acid on ice. These samples were vortexed vigorously and centrifuged for 5 minutes at 5,000 rpm on an International Centrifuge (size 2, model V; International Equipment, Boston, MA) in a cold room at 4°C. The supernatants were again centrifuged for 5 minutes at the same speed under the same conditions. The final supernatants were stored overnight at −20°C and assayed for β-hydroxybutyrate and acetoacetate by enzymatic methods.[8] Blood glucose levels were estimated using an Ames Blood Glucose Meter (Glucometer 3; Miles, Elkhart, IN). The remaining 1.8 mL blood was immediately centrifuged for 5 minutes on a GPR centrifuge (model 9C022; Beckman Instruments, Fullerton, CA). The plasma was stored at −20°C until needed for radioimmunoassay of insulin in the laboratory of Dr Gordon C. Weir at the Joslin Diabetes Center.[9]

Preparation of Adipocytes and Nonfat Cells From Epididymal Fat Pads

Adipocytes and nonfat cells were isolated by a previously reported method.[6,10] Epididymal fat pads of all 26 rats in each experiment were used for in vitro studies. In each experiment, cells were obtained from epididymal fat pads of 13 normal rats and 13 rats with DM or DKA. The rats were stunned by a blow to the head and killed by cervical dislocation. The distal part of each epididymal fat pad was immediately removed, and the pads from each study group (normal and DM or DKA) were collected. The tissues of four to five rats at a time were minced and disaggregated in a flask containing 10 mg collagenase in 10 mL Dulbecco's modified Eagle's medium, 4% bovine serum albumin (fatty acid–free), and 20 mmol/L sodium pyruvate. The flasks were shaken in a metabolic shaker at 130 cycles/min for 75 minutes at 37°C. The volume of Dulbecco's modified Eagle's medium and the amount of collagenase were adjusted to keep the ratios of enzyme and volume to number of fat pads constant.

Clumps were dissociated by pipetting during the collagenase digestion. To remove fragments of tissue, stroma, and blood vessels still remaining after this treatment, the suspension was filtered slowly through eight layers of gauze (4-Ply USP Gauze; Hermitage Hospital Products, Niantic, CT). The suspension was further filtered through polyethylene mesh (Spectra/Mesh macroporous filters; Spectrum, Houston, TX) with a pore diameter of 230 μm to remove pieces of microvessels. The cell suspension was centrifuged in a 15-mL polypropylene conical centrifuge tube for 2 minutes at 500 × *g*. The pelleted stromal-vascular cells (nonfat cells) and the medium were aspirated from below the floating adipocytes with a syringe, transferred to a fresh centrifuge tube, and centrifuged for 5 minutes at 500 × *g* to pellet the nonfat cells. The adipocytes were washed four more times with Krebs-Ringer phosphate buffer, pH 7.4, containing 5 mmol/L glucose and 4% bovine albumin (KRPGA). Additional nonfat cells were recovered when necessary from the first two additional washes of the adipocytes by centrifugation for 5 minutes at 500 × *g* and all the nonfat cells combined. Throughout this procedure, the cells and buffers were kept at 37°C.

Calculation of Mean Surface Area and Volume of an Adipocyte Cell Population

The mean surface area of an adipocyte population was calculated by the method of Hirsch and Gallian[11] and Marcus et al.[12] The packed adipocyte cell volume was adjusted to 51% for control rats and 52% for rats with DKA. An aliquot of 10 μL of this suspension was placed in a hemocytometer (the lowest grid of which has 50-μm calibrations). The diameter of 100 consecutive cells was measured with a Spencer microscope (Westbury, NY) equipped with an Olympus ocular micrometer (Woodbury, NY). At a magnification of 100×, the caliper scale was calibrated so that the divisions had an interval of 10 μm. The cells were brought into the caliper field and aligned with the caliper scale using a systematic motion of the stage control knobs. The formula used to calculate the fat cell surface area from the mean diameter (D) was $(D^2 + SD^2) \times \pi$. In this report, the data are expressed per square micrometer of cell surface area to avoid variation due to the reduced size and cell radius of adipocytes from diabetic animals.[13]

Measurement of Lipolysis and PG Production

In the standard procedure, 1-mL samples containing 10^5 adipocytes (with or without added nonfat cells) in KRPGA (gassed with 95% O_2/5% CO_2) were added to 15-mL plastic vials containing 1 U/mL adenosine deaminase, incubated at 37°C, and gently shaken at approximately 70 cycles/min for 2 hours. Epinephrine was dissolved in Krebs-Ringer phosphate buffer, pH 7.4, just before use and added to the vials in a volume of 20 μL as indicated.

Indomethacin was dissolved in 0.1 mol/L sodium carbonate and diluted before use in KRPGA. Epinephrine and indomethacin were added simultaneously. The commercial preparation of adenosine deaminase was in the form of an ammonium sulfate suspension. Before use, this preparation was centrifuged for 2 minutes in an Eppendorf 5412 centrifuge (Brinkman, Westbury, NY) to precipitate the ammonium sulfate.

We used a fixed concentration of nonfat cells per experiment. The quantity of nonfat cells was expressed as rat equivalents, as in previous studies from this laboratory, to account for any heterogeneity in the nonfat cell population.[6,10] A rat equivalent is defined as the amount of nonfat cells obtained from the epididymal fat pads of one rat. The fraction of a rat equivalent is equal to the number of rats used per number of vials containing nonfat cells.[10] We used 0.46 rat equivalents per vial, which is approximately equivalent to 7×10^6 nonfat cells.[6] The identity of the nonfat cells was demonstrated in a previous study, which showed that angiotensin-

converting enzyme activity, a marker for endothelial cells, coeluted from a density gradient with the cell population that produced PGI_2.[10] This is consistent with the interpretation that the nonfat cells are predominantly endothelial cells. Microscopic examination revealed a nearly homogeneous population of cells with endothelial cell characteristics.[10]

An aliquot of 0.4 mL was stored at −20°C for glycerol determination.[6] An aliquot of 0.3 mL of each sample was transferred to a 1.5-mL Eppendorf tube, and the reaction was halted by addition of 1N NaOH to increase the pH to 11. The samples were then centrifuged in an Eppendorf centrifuge for 4 minutes, and the supernatants were decanted into fresh tubes. The samples were neutralized to pH 7.4 by addition of 1N HCl, kept at 4°C for 14 to 16 hours, and then assayed for 6-keto-$PGF_{1\alpha}$. An additional aliquot of 0.3 mL of each sample was centrifuged in an Eppendorf centrifuge for 30 seconds, and the infranatant was transferred to a fresh tube and then stored overnight at 4°C for use in the radioimmunoassay of PGE_2.

Measurement of 6-Keto-$PGF_{1\alpha}$ by Radioimmunoassay

The concentration of 6-keto-$PGF_{1\alpha}$, a stable derivative of PGI_2, was determined by double-antibody radioimmunoassay as described previously.[10]

Measurement of PGE_2 by Radioimmunoassay

The production of PGE_2 was determined by radioimmunoassay using a kit manufactured by NEN Research Products (Boston, MA) as described previously.[6,14]

Statistical Analysis

The data are expressed as the mean ± SE. Statistical analysis was performed using one-way ANOVA and two-way analysis of covariance and the two-tailed Student's *t* test. We performed analyses to determine whether values for the various indices were normally distributed. All data were tested for normal distribution. The Kolmogorov-Smirnov test was used. In all cases, we failed to reject the null hypothesis that the data fit a normal distribution at a *P* level of .05 or less. Therefore, the use of *t* tests was appropriate, and a nonparametric method was not used. *P* less than .05 was considered significant.

RESULTS

Blood Pressure, Blood Glucose, Plasma Insulin, and Total Ketone Levels

Systolic blood pressure in rats with DM was not significantly different from values in normal rats at 0 or 48 hours. Systolic blood pressure was not significantly different at 0 hours in normal rats (120.7 ± 1.4 mm Hg) and rats with DKA (117.9 ± 2.7 mm Hg), but it was significantly lower in rats with DKA (107.0 ± 1.9 mm Hg) than in normal rats (130.1 ± 3.0 mm Hg) 48 hours after injection of streptozotocin ($P < .001$). Values in rats with DKA 48 hours after induction of DKA were significantly lower than in the same rats before induction of DKA ($P < .05$, paired analysis). Blood glucose levels 48 hours after injection of streptozotocin were significantly higher in rats with DM (316.9 ± 19.9 mg/dL) than in concurrently studied normal rats (167.6 ± 0.8 mg/dL, $P < .001$), and were significantly higher in rats with DKA (426.7 ± 3.3 mg/dL) than in normal rats (220.3 ± 4.0 mg/dL, $P < .001$). Plasma insulin levels were significantly lower in rats with DM (120.3 ± 3.5 pmol/L) than in normal rats (255.0 ± 27.4 pmol/L) 48 hours after injection of streptozotocin ($P < .005$). Similarly, plasma insulin levels were significantly lower in rats with DKA (71.4 ± 5.9 pmol/L) than in normal rats (205.8 ± 15.7 pmol/L) 48 hours after administration of streptozotocin ($P < .001$). Blood total ketone levels were significantly higher in rats with DM (1.6 ± .20 mmol/L) than in normal rats (0.48 ± .09 mmol/L, $P < .001$) and were significantly higher in rats with DKA (7.1 ± 1.0 mmol/L) than in normal rats (0.53 ± .11 mmol/L, $P < .001$).

We analyzed measurements of systolic blood pressure and plasma insulin level in rats with DM and DKA and in normal control rats by analysis of covariance (Fig 1). There was a strong positive correlation between plasma insulin level and systolic blood pressure in rats with DM in comparison to concurrently studied normal control rats ($P < .021$; Fig 1) and in rats with DKA in comparison to concurrently studied normal control rats ($P < .0001$; Fig 1). When rats with DM are compared with rats with DKA, there is a significant correlation between systolic blood pressure and plasma insulin level ($P < .0001$; Fig 2). In addition, there was a significant inverse linear correlation between mean plasma insulin level and basal 6-keto-$PGF_{1\alpha}$ production by adipose tissue in rats with DKA as compared with concurrently studied normal control rats ($P < .0252$, $R^2 = .67$; Fig 3).

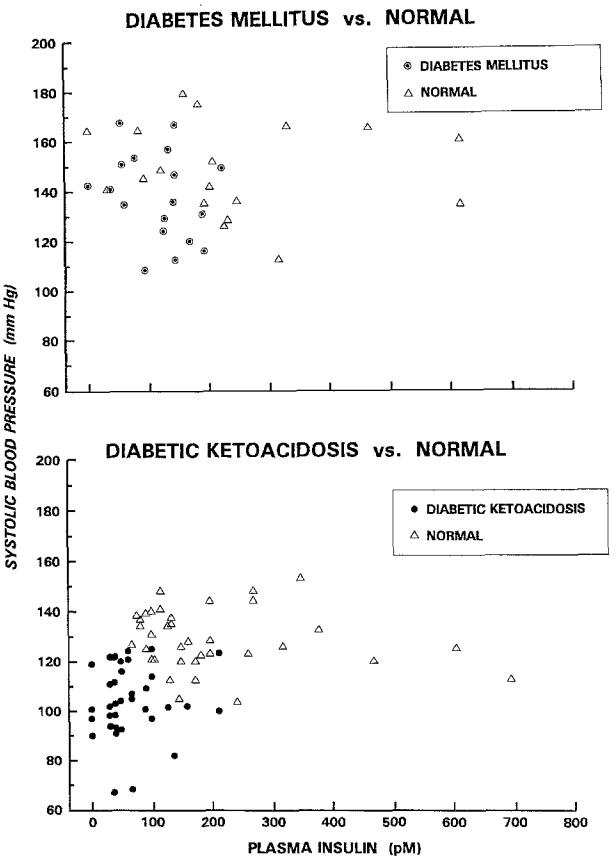

Fig 1. Relationship between plasma insulin level and systolic blood pressure in rats with DM and normal rats and in rats with DKA and normal rats.

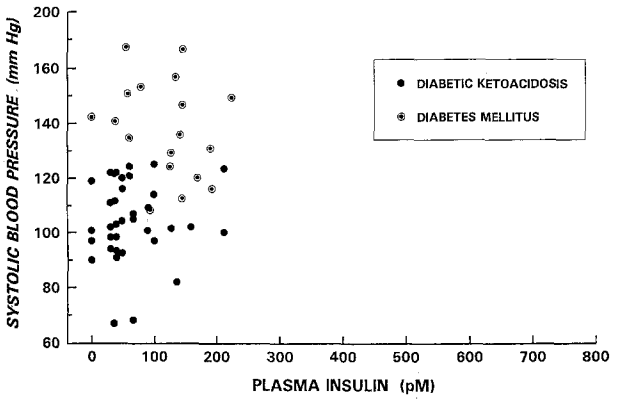

Fig 2. Relationship between plasma insulin level and systolic blood pressure in rats with DM and rats with DKA.

Basal Lipolysis, 6-Keto-PGF$_{1\alpha}$ Production, and PGE$_2$ Production

Basal (unstimulated) glycerol release was significantly higher in rats with DM than in normal rats both in the absence of indomethacin ($P < .01$) and in the presence of indomethacin ($P < .02$; Fig 4A). Similarly, basal glycerol release was significantly higher in rats with DKA than in normal rats both in the absence of indomethacin ($P < .05$) and in the presence of indomethacin ($P < .05$; Fig 4B).

There was no difference in the basal PGE_2 production rate by a mixture of adipocytes and nonfat cells when rats with DM were compared with normal rats both in the absence and presence of indomethacin (Fig 5A). However, basal PGE_2 production was significantly higher in rats with DKA than in normal rats in the absence of indomethacin ($P < .05$; Fig 5B), but not in the presence of indomethacin (Fig 5B).

There was no significant difference in the 6-keto-PGF$_{1\alpha}$ production rate between rats with DM and normal rats both in the absence and presence of indomethacin (Fig 6A); mean values in rats with DM are approximately twice as high as those in normal rats in both instances. The basal 6-keto-PGF$_{1\alpha}$ production rate was significantly higher in rats with DKA than in normal rats both in the absence of indomethacin ($P < .001$) and in the presence of indomethacin ($P < .05$; Fig 6B).

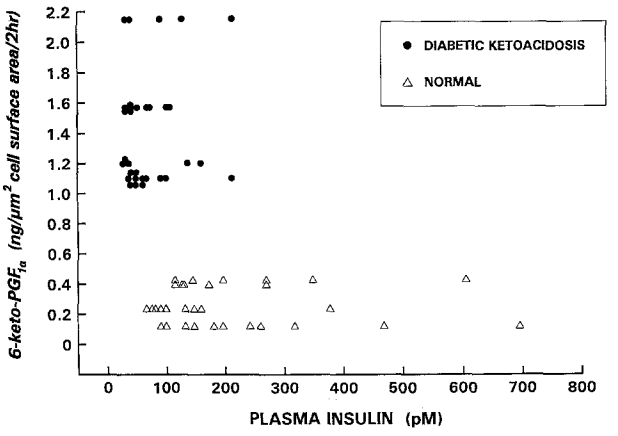

Fig 3. Relationship between plasma insulin level and basal production of 6-keto-PGF$_{1\alpha}$ by a mixture of adipocytes and nonfat cells from adipose tissue in rats with DKA and normal rats.

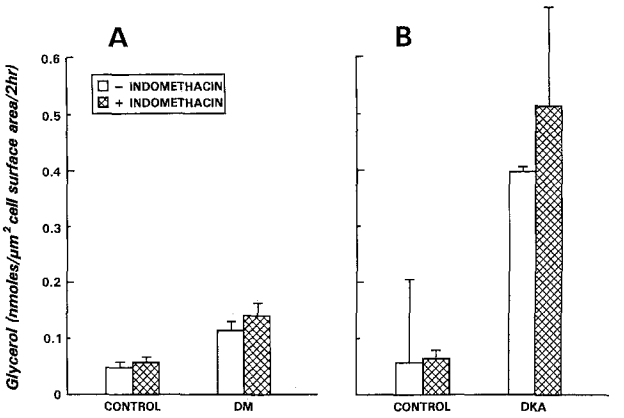

Fig 4. Basal rates of glycerol production by a mixture of adipocytes and nonfat cells in the presence and absence of indomethacin (2×10^{-5} mol/L) in rats with DM and normal rats (A) and in rats with DKA and normal rats (B). A mixture of adipocytes and nonfat cells (0.46 rat equivalents) was prepared from rat fat pads and incubated in the presence of 1 U/mL adenosine deaminase for 2 hours at 37°C (n = 5 separate experiments).

Epinephrine-Induced Lipolysis, PGE$_2$ Production, and 6-Keto-PGF$_{1\alpha}$ Production

We analyzed the effect of DM and DKA on epinephrine-stimulated lipolysis, PGE_2 production, and 6-keto-PGF$_{1\alpha}$ production in the absence and presence of indomethacin (Figs 7 and 8; note differences in ordinate scales in these two figures).

In rats with DKA, epinephrine-induced lipolysis was significantly different from basal at concentrations of 2×10^{-8} to 2×10^{-5} mol/L ($P < .05$). Epinephrine-induced 6-keto-PFG$_{1\alpha}$ production was significantly different from the basal level only at 2×10^{-5} mol/L ($P < .05$). Epinephrine-induced PGE_2 production was not significantly different from basal production.

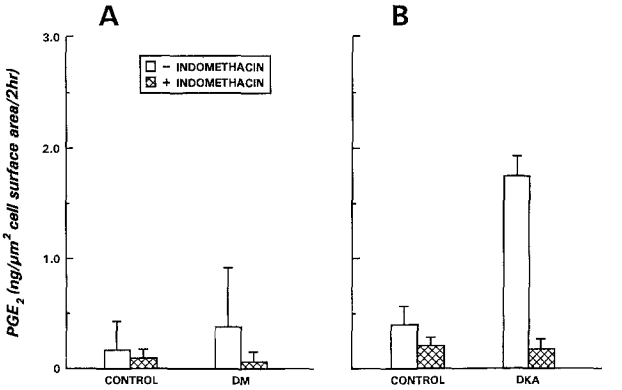

Fig 5. Basal rates of PGE_2 production in a mixture of adipocytes and nonfat cells in the presence and absence of indomethacin (2×10^{-5} mol/L) in rats with DM and normal rats (A) and in rats with DKA and normal rats (B). A mixture of adipocytes and nonfat cells (0.46 rat equivalents) was prepared and incubated as described in Fig 4 (n = 5 separate experiments).

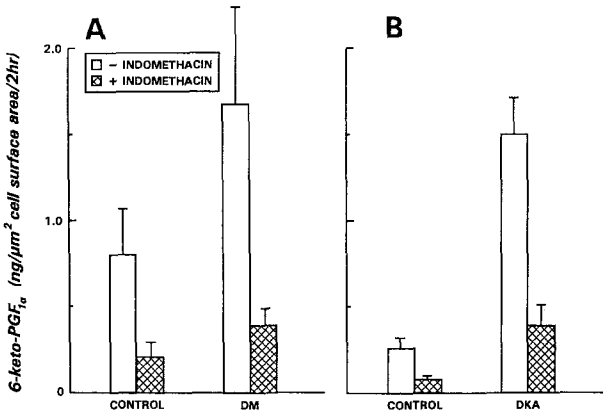

Fig 6. Basal rates of 6-keto-$PGF_{1\alpha}$ production in a mixture of adipocytes and nonfat cells in the presence and absence of indomethacin (2×10^{-5} mol/L) in rats with DM and normal rats (A) and in rats with DKA and normal rats (B). A mixture of adipocytes and nonfat cells (0.46 rat equivalents) was prepared and incubated as described in Fig 4 (n = 5 separate experiments).

In rats with DM, epinephrine-induced lipolysis was significantly different from basal at levels of 2×10^{-7} to 2×10^{-5} mol/L. There was no significant difference for levels of 6-keto-$PGF_{1\alpha}$. Epinephrine-induced PGE_2 production was significantly different from basal only at 2×10^{-5} mol/L ($P < .05$).

We also analyzed the stimulation in control rats. In the controls for DM experiments, epinephrine-stimulated 6-keto-$PGF_{1\alpha}$ production was significantly different from basal only at 2×10^{-5} mol/L epinephrine ($P < .05$). In the controls for DKA experiments, none of the 6-keto-$PGF_{1\alpha}$ values were significantly different from basal. In DM and DKA experiments, epinephrine-stimulated PGE_2 production was not significantly different from basal.

Indomethacin had no effect on epinephrine-stimulated glycerol production in all three states tested (data not shown). Indomethacin prevented any epinephrine-induced increment in PGE_2 production or 6-keto-$PGF_{1\alpha}$ production in adipose tissue from rats with DM and DKA and from normal rats (data not shown).

Circulating Glucose Levels, PGE_2 Production, and 6-Keto-$PGF_{1\alpha}$ Production

We performed an analysis of covariance to test for the correlation between 6-keto-$PGF_{1\alpha}$ production and blood glucose level and between PGE_2 production and blood glucose level in control animals and diabetic animals. The two groups (before and after induction of DM or DKA) were treated as a classification variable in the analysis of covariance. A significant positive linear correlation ($P < .05$) was observed between 6-keto-$PGF_{1\alpha}$ production and blood glucose level and between PGE_2 production and blood glucose level ($R^2 = .89$ and .81, respectively).

DISCUSSION

We examined the effect of plasma insulin level on systolic blood pressure and on adipose tissue PG production in rats with DM and DKA and in normal rats. Our study demonstrates a significant direct relationship between plasma insulin level and systolic blood pressure in rats with DKA and concurrently studied normal rats and in rats with DM and concurrently studied normal rats. Our experimental findings are consistent with epidemiologic studies indicating a direct correlation between circulating insulin level and blood pressure in a wide range of clinical settings, including non-obese, obese, nondiabetic, diabetic, nonhypertensive, and hypertensive subjects.[15-18]

Our study also reveals a relationship between circulating insulin level and several measures of PG production by adipose tissue. There was a significant inverse linear rela-

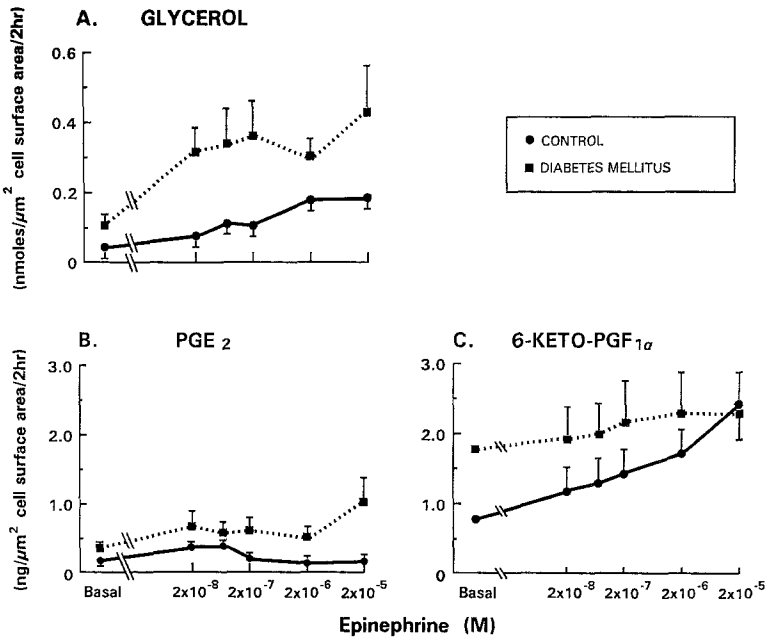

Fig 7. Basal and epinephrine-stimulated production of glycerol (A), PGE_2 (B), and 6-keto-$PGF_{1\alpha}$ (C) in a mixture of adipocytes and nonfat cells from normal rats and from rats with DM in the absence and presence of indomethacin. A mixture of adipocytes and nonfat cells (0.46 rat equivalents) was prepared from rat epididymal fat pads and incubated at the epinephrine concentrations indicated in the presence and absence of indomethacin (2×10^{-5} mol/L) and in the presence of 1 U/mL adenosine deaminase for 2 hours at 37°C (n = 5 separate experiments).

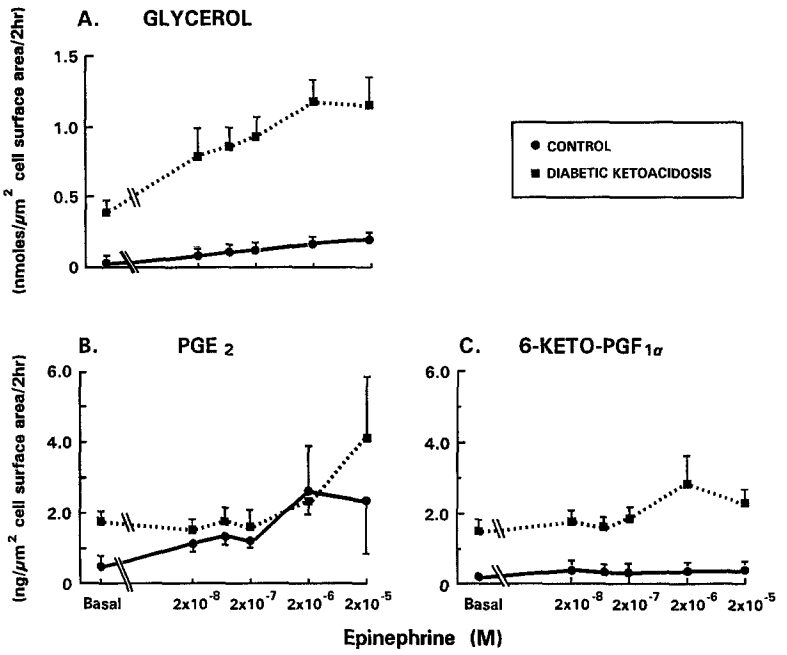

Fig 8. Basal and epinephrine-stimulated production of glycerol (A), PGE_2 (B), and 6-keto-$PGF_{1\alpha}$ (C) in a mixture of adipocytes and nonfat cells from normal rats and rats with DKA. A mixture of adipocytes and endothelial cells (0.46 rat equivalents) was prepared and incubated as described in Fig 7 (n = 5 separate experiments).

tionship between circulating insulin level and basal 6-keto-$PGF_{1\alpha}$ production by adipose tissue in rats with DKA as compared with normal rats. Basal PGE_2 production and 6-keto-$PGF_{1\alpha}$ production by a mixture of adipocytes and nonfat cells (predominantly endothelial cells) was significantly higher in rats with DKA than in normal rats. Basal PGE_2 and 6-keto-$PGF_{1\alpha}$ production rates by mixed cells of adipose tissue in rats with DM were approximately twice as high as those in normal rats, but these differences were not statistically significant. Basal glycerol release by the adipose tissue mixture, a measure of lipolysis, was significantly higher in rats with DM and in rats with DKA than in the respective normal rats.

Systolic blood pressure decreased in rats with DKA but not in rats with DM, but basal production of 6-keto-$PGF_{1\alpha}$ by a mixture of adipocytes and endothelial cells was comparable in rats with DM and rats with DKA. This may reflect the risks inherent in making comparisons between nonconcurrent experiments, especially in vitro experiments. For rats with DM, the appropriate comparison is to the concurrently studied normal rats (Fig 6A). Similarly, for rats with DKA, the appropriate comparison is to the concurrently studied normal rats (Fig 6B). In addition, factors other than adipose tissue production rates, such as decreased clearance or altered space of distribution of PGI_2, may modify the net circulating level of PGI_2 in vivo. Also, we assayed the stable derivative of PGI_2, 6-keto-$PGF_{1\alpha}$. It is possible that the levels of the short-lived biologically active compound, PGI_2, would provide a more clear-cut difference in production of PGI_2 by mixed adipocytes and endothelial cells if it were possible to measure such levels.

We also analyzed maximal epinephrine-induced lipolysis, PGE_2 production, and PGI_2 production by the adipose tissue cell mixtures in the same rats. A further increase was not detected in instances in which basal production was already increased. This may indicate that basal production was maximal or nearly maximal, such that a further increase in response to epinephrine could not occur.

Previous studies have suggested that eicosanoid production by endothelial and other cells may be increased by exposure to high glucose concentrations in vitro.[19-22] In some of these investigations, a high concentration of glucose had no effect on arachidonic acid metabolism; an effect could be demonstrated only after pretreatment with A23187.[19,20] Furthermore, while one group of investigators observed an effect of glucose on the release of arachidonic acid and 15-HETE, the release of both radiolabeled and unlabeled prostanoids was equal at normal (5.2 mmol/L) and high (15.6 mmol/L) concentrations.[19] In another report, 44 mmol/L glucose had no effect on basal or acetylcholine-stimulated release of 6-keto-$PGF_{1\alpha}$ and PGE_2.[21] Williams and Schrier[22] demonstrated a concentration-dependent stimulation of PGE_2 and 6-keto-$PGF_{1\alpha}$ production by cultured rat glomerular mesangial cells, but did not study endothelial cells, adipocytes, or other cells. Nevertheless, we analyzed the relationship between blood glucose level and production of PGE_2 and 6-keto-$PGF_{1\alpha}$ by mixtures of adipocytes and endothelial cells before and after induction of DM and DKA. We observed a significant positive linear correlation between blood glucose level and PGE_2 production and between blood glucose level and 6-keto-$PGF_{1\alpha}$ production. These findings suggest that glucose may also stimulate eicosanoid production by adipose tissue, but a more plausible explanation is that both the hyperglycemia and the increases in production of PGE_2 and 6-keto-$PGF_{1\alpha}$ reflect the deficiency of insulin. This view is supported by the failure of high glucose concentrations to stimulate prostanoid production by endothelial cells except under special circumstances such as pretreatment with A23187.[19-21]

Our studies of PG production by adipose tissue were

performed using a mixture of isolated adipocytes and the nonadipocyte cellular components of adipose tissue, which are predominantly endothelial cells.[6,10] Previous investigation in this laboratory demonstrated that catecholamine-stimulated PGE_2 and PGI_2 production by adipose tissue requires a cell-cell interaction between the adipocyte and the endothelial cell in which the adipocyte provides the arachidonic acid substrate to the endothelial cell, which transforms it into PGE_2 and PGI_2.[6,10] Neither the adipocyte alone nor the endothelial cell alone produce PGI_2 or PGE_2 in response to catecholamines. Our method of mixing isolated adipocytes and nonfat cells in a quantitative manner makes it possible to study quantitatively the effects of catecholamines, insulin, and other hormones on PG production by adipose tissue. Using this system, we have demonstrated that catecholamine-induced lipolysis is under the coordinate control of PGE_2, a potent antilipolytic agent, and PGI_2, a potent lipolytic agent.[6]

Our studies support the hypothesis that the effect of insulin on blood pressure is mediated by an effect on PG production by adipose tissue, and perhaps other tissues as well. Physiological concentrations of insulin decrease the catecholamine-induced production of PGE_2 and PGI_2, two potent vasodilators, by adipose tissue, one of the largest organs in the body.[1] This finding suggests that hyperinsulinemia increases peripheral vascular resistance and blood pressure by opposing the stimulatory effect of adrenergic agonists (and perhaps other agonists) on PGE_2 and PGI_2 production in adipose tissue (and possibly other tissues). This concept is supported by evidence that PGE_2 and PGI_2 modulate vascular reactivity in health and disease.[1] For example, in severe insulin deficiency, ie, in DKA, PGE_2 and PGI_2 production by adipose tissue are increased and peripheral vascular resistance and blood pressure are decreased.[3-5,23] This hypothesis is also supported by evidence that blood flow through rat and human adipose tissue is decreased in obesity[24,25] and that insulin decreases blood flow through adipose tissue in non-obese rats.[26-28] It has been reported that insulin stimulates the production of vasodilative PGs from perfused rat muscle.[29] In the fasting state, these changes were small and were demonstrated only with supraphysiologic concentrations of insulin (200 and 20,000 μU/mL). In exercised animals, these insulin concentrations inhibited production of 6-keto-$PGF_{1\alpha}$ and had no effect on production of PGE_2. Physiologic concentrations of insulin produced forearm vasodilation in normal human volunteers during a 1-hour infusion,[30] but there is no clear relevance of these short-term studies to the pathophysiology of DM or DKA.

Thus, insulin appears to regulate PGI_2 and PGE_2 production by adipose tissue (and possibly other tissues) through a wide range of concentrations, with important physiological and clinical consequences. The present study provides direct evidence that there is a close correlation between circulating insulin level and systolic blood pressure when rats with DM and DKA are compared with appropriate controls, and that PGE_2 and PGI_2 production by adipose tissue are increased in the insulin-deficient state. These findings are consistent with the concept that the correlation between insulin level and blood pressure is mediated by the inhibitory effect of insulin on the production of vasodilative PGs by adipose tissue.

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