Rapid Publications

Induction of Hyperinsulinemia Combined With Hyperglycemia and Hypertriglyceridemia Increases Plasminogen Activator Inhibitor 1 in Blood in Normal Human Subjects

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Hypofibrinolysis caused by increased plasminogen activator inhibitor 1 (PAI-1) has been implicated in the vasculopathy of type 2 diabetes, typified by increased insulin, glucose, and triglycerides. However, short-term infusions of insulin have not increased PAI-1 in normal subjects. We hypothesized that induction of increased insulin accompanied by increased glucose and triglycerides would increase PAI-1. Accordingly, 30% glucose and 10% Intralipid were infused for 6 h in ten normal lean individuals (54 ± 3 years) resulting in increased insulin (42 ± 5 μU/dl), glucose (200 ± 24 mg/dl), and triglycerides (425 ± 45 mg/dl), simulating changes in type 2 diabetes. In contrast to results with infusion of saline alone (n = 16) and euglycemic-hyperinsulinemic clamps (n = 10, serum insulin = 89 ± 7 μU/dl), PAI-1 in blood increased significantly 6 h after the onset of infusion (15 ± 5 ng/ml, P <0.05 vs. baseline = 7.4± 1.1, saline 6 h = 3.4 ± 1.1, and insulin alone 6 h = 3.7 ± 0.8) and remained elevated for an additional 6 h (combined infusion = 13.8 ± 3.8 ng/ml, saline = 6.7 ± 2 ng/ml, insulin alone = 7.8 ± 1.7 ng/ml, P = 0.06). Our data suggest that combined hyperinsulinemia, hypertriglyceridemia, and hyperglycemia are likely to contribute to hypofibrinolysis of type 2 diabetes by increasing the blood levels of PAI-1. Moreover, these results underscore the potential importance of modifying insulin resistance as well as achieving glycemic and lipidemic control in individuals with type 2 diabetes. *Diabetes* 47:290–293, 1998

Subjects with insulin-resistant syndromes, including type 2 diabetes, exhibit a high prevalence of accelerated atherosclerosis (1). Although the mechanisms responsible are multiple, one factor implicated is impaired fibrinolysis, which is secondary to overexpression of the primary physiological inhibitor of plasminogen activators, plasminogen activator inhibitor type-1 (PAI-1) (2–5). We and others have shown that subjects with insulin-resistant syndromes, including obesity, type 2 diabetes (6,7), and the polycystic ovary syndrome (8), have depressed fibrinolytic activity in blood, which is secondary to elevated concentrations of PAI-1 under basal conditions and in response to a physiological stress, such as venous occlusion. Insulin-resistant syndromes are typified by compensatory elevation of concentrations in blood of insulin and frequently also by elevations of concentrations of glucose, triglycerides, and free fatty acids (9,10). Direct effects of insulin and proinsulin augmenting expression of PAI-1 have been observed not only in vitro in human hepatoma (Hep G2) cells (11,12), isolated human hepatocytes (13), and arterial segments (14), but also in vivo in anesthetized rabbits in which prolonged infusions of insulin or proinsulin increased PAI-1 concentrations in blood and PAI-1 gene expression in vessel walls under conditions of euglycemic clamping (15). Thus, the possibility exists that the hyperinsulinemia secondary to insulin resistance stimulates PAI-1 production in vivo, impairing fibrinolysis and thereby contributing to acceleration of atherosclerosis.

Despite these considerations and observations, a lack of elevation of blood PAI-1 has been reported in normal human subjects given infusions of insulin for 2 h under euglycemic conditions (16,17). However, we have found that strikingly synergistic effects occur when the Hep G2 cells are exposed to both insulin and fatty acids in vitro (18). Accordingly, we hypothesized that augmentation of PAI-1 by insulin may require concomitant elevation of lipids and glucose and perhaps other metabolites in blood. We suspect this was the case in the anesthetized rabbits previously studied (15) and is the case in human subjects with insulin-resistant states. The present study was performed to characterize effects on concentrations of PAI-1 in blood of induction of hyperinsulinemia combined with derangements in concentrations of glucose and lipids in blood simulating those seen in type 2 diabetes. Results obtained during 6 h after 6-h infusions of glucose and Intralipid were compared with those results seen with sham infusions in which normal saline was administered instead and with those seen with infusions of insulin combined only with glucose sufficient to maintain euglycemia.

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PAI-1, plasminogen activator inhibitor 1; t-PA, tissue-type plasminogen activator.

RESEARCH DESIGN AND METHODS

Volunteers. A total of 20 normal adult subjects (10 men, 10 women) participated in this study and were characterized during and after a total of 36 infusions consistent with a protocol approved by the institutional review committee of The University of Vermont and after acquisition of written informed consent. Studies were performed in the Clinical Research Center (CRC) of the University of Vermont over a 2-day interval of for each infusion study. Each subject was verified to have had a normal physical examination and normal baseline laboratory results, including lipid profiles and thyroid function. Diabetes was excluded with the oral glucose tolerance test (19) and all had a normal electrocardiogram. Additional inclusion criteria required stability of body weight (within 2 kg) during the 12 months preceding the study, absence of a regimented physical fitness or dietary program, and absence of a history of current or past smoking. None of the female volunteers were taking exogenous hormonal agents.

Metabolic testing. A total of 10 subjects were assigned to protocol A in which infusions included glucose plus Intralipid, which was sufficient to induce hyperinsulinemia, hyperglycemia, and elevated triglycerides. A total of 10 subjects were assigned to protocol B in which the experimental agent infused was insulin with glucose, which was only sufficient to maintain euglycemic conditions (clamping). A total of 16 volunteers (seven studied with protocol A, nine studied with protocol B) participated in a second study (control infusion) in which the infusion was with normal saline (protocol C).

During the first day, each subject was fed a standardized meal at 5:30 P.M. A central intravenous catheter was inserted via a peripheral vein (PICC line) at 7:00 P.M. The PICC line was kept open overnight with a slow saline drip. On the second day at 8:00 A.M., two additional intravenous lines were placed (one for infusion of insulin or metabolites and the other for sampling of blood). At 9:00 A.M., baseline blood samples were obtained and an infusion was initiated and continued for 6 h. For subjects in protocol A, glucose (30%) was infused through the PICC line, and Intralipid (10%) was infused through the PICC line at 2 ml/min. By adjusting the infusion rate of glucose, the blood glucose concentration was raised to a level simulating that typical in patients with type 2 diabetes (~200 mg/dl) with the use of the hyperglycemic clamp technique (20). Blood glucose was measured every 5 min for the first 2 h and every 10 min for the last 4 h. For subjects in protocol B, a constant infusion of insulin (40 mU · m^{-2} · min^{-1}) was used to increase insulin in blood to a level typical of that seen in the postprandial state and simulating 24 h average levels in subjects with type 2 diabetes (~80 μU/ml). Glucose (30%) was infused through the PICC line to maintain euglycemia consistent with the clamp technique (20). For subjects who participated in protocol C, normal saline was infused at a rate simulating the infusion rate that had been administered to the same subject in protocol A or B. After each infusion, the central and one peripheral venous lines were withdrawn. Each volunteer was fed a late lunch that comprised 33%, a supper comprising 50%, and a snack comprising 17% of usual daily intake at 1530, 1730, and 2000 respectively. Blood samples for assay of PAI-1 were obtained at 0 time, 3, 6, and 12 h in relation to each infusion. Samples for assay of insulin and metabolites were obtained at 0 time, 1, 2, 3, 4, 5, 6, and 12 h in relation to the infusion.

Analytical methods. Glucose was measured with the use of an automated glucose analyzer (YSI Analyzer, Yellow Springs, OH) and insulin by radioimmunoassay (21). Tissue-type plasminogen activator (t-PA) and PAI-1 in blood were assayed by enzyme-linked immonsorbent assay (ELISA; for PAI-1 assays we used TintElize kits from Biopool, Umea, Sweden). The PAI-1 method detects active and latent forms of free PAI-1 and PAI-1 complexed to plasminogen activators. The t-PA method detects both free and t-PA complexed to PAI-1. The coefficient of variations of both procedures used was 9%. Each sample was assayed in duplicate. Free fatty acids were measured with the use of WAKO kits.

Statistical analysis. A three-way analysis of variance model with repeated measurements (time) was used to compare the effects of infusions of glucose and Intralipid with those seen with infusions of insulin and euglycemic clamping and with those seen with infusions of saline on concentrations of PAI-1 in blood. Once a time and treatment interaction had been identified, post hoc comparison of means was performed with Schaeffe's test. Statistical procedures were performed with the BMDP package run on a personal computer.

RESULTS

Characteristics of the subjects studied included average age 52 ± 3 years and BMI 25 ± 1.2 (mean ± SD). Because no sex-dependent differences in any of the measured end points were present, results are presented as pooled data. Blood glucose, insulin, triglyceride, and fatty acid concentrations are shown in Fig. 1.

No change in t-PA concentrations in blood was evident over time under any of the conditions used. Mean values at all sampling intervals regardless of the nature of the infusion were between 5.3 and 8.6 ng/ml. No significant differences were evident between values at any time compared with those at baseline nor between values at any time with one type

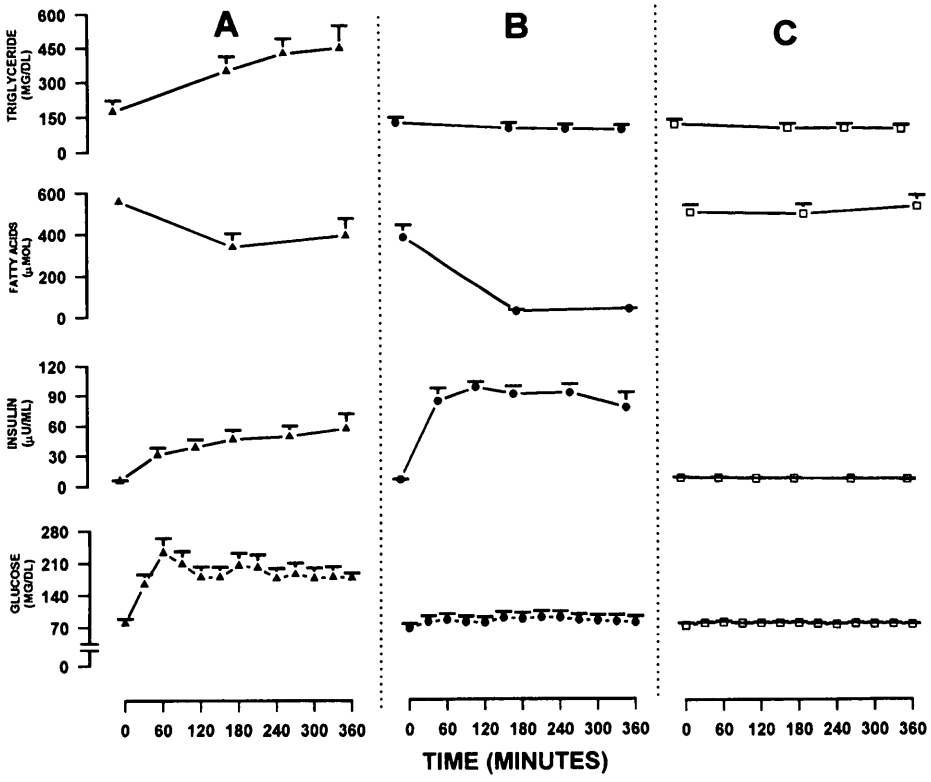

FIG. 1. Serum insulin and substrate concentrations. Blood concentrations of triglycerides, free fatty acids, insulin, and glucose in response to the infusions are depicted from top to bottom. *A* displays the results in individuals who received the combined infusion (glucose + Intralipid), *B* the results in individuals who received insulin and glucose to maintain euglycemia, and *C* the results of those given sham infusions (saline). Of note are elevations in plasma glucose, insulin, and triglycerides in the glucose- and Intralipid-infused group that closely resembled changes seen in type 2 diabetes. Free fatty acid concentrations dropped to almost undetectable levels in those given insulin and glucose, and no demonstrable changes were found in the saline-infused studies.

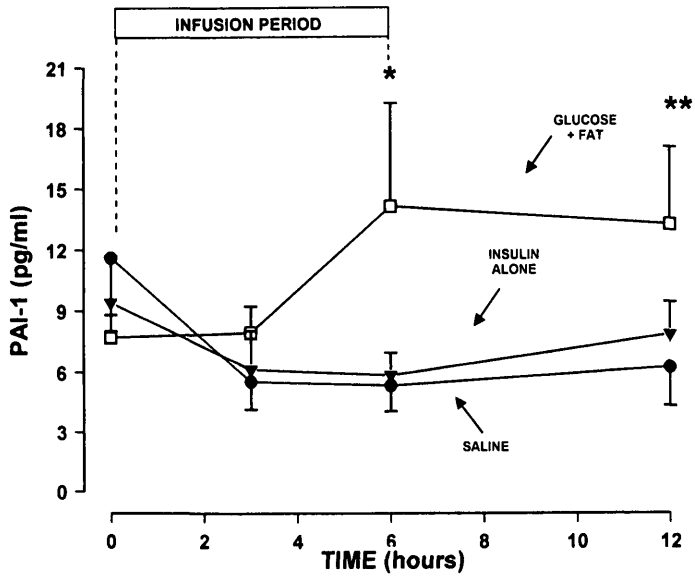

FIG. 2. Blood PAI-1 concentrations. ●, the concentrations in blood of PAI-1 with saline infusion; ▲, with euglycemic-hyperinsulinemic clamp conditions; □, PAI-1 values in response to the combined infusion. *P = 0.017; **P = 0.06.

of infusion compared with another. Concentrations of PAI-1 in blood in response to infusions with saline, insulin, or glucose plus Intralipid are shown in Fig. 2. A time and treatment interaction effect on concentrations of PAI-1 was evident. Thus, hyperinsulinemia, hyperglycemia, and hypertriglyceridemia induced by the combined infusion were associated with significant increases in PAI-1 with values markedly increased 6 h after the onset of infusion and persistently elevated 6 h later after its discontinuation ($P < 0.05$ vs. baseline). The elevations in PAI-1 were significantly different compared with results seen with infusions of insulin under conditions of euglycemia and with those seen with infusions of saline ($P < 0.05$ for each comparison) at the same sampling intervals. By contrast, concentrations of PAI-1 decreased 3 and 6 h after onset of infusions of insulin with euglycemic clamping or saline alone although the differences from baseline were not statistically significant.

DISCUSSION

The results of this study indicate that hyperinsulinemia, when associated with hypertriglyceridemia and hyperglycemia, increases concentrations of PAI-1 in blood for as long as 6 h after cessation of the perturbation inducing these phenomena. We and others have previously shown that insulin increases PAI-1 gene expression in diverse cells in culture, arterial segments in vitro, and vessel walls in vivo, and in experimental animals (12–15,18,22,23). Thus, the increased PAI-I associated with type 2 diabetes appears to be attributable to hyperinsulinemia combined with changes in concentrations in blood of glucose, triglycerides, and free fatty acids typical of this condition. The failure of previous studies (16,17) to demonstrate elevations of PAI-1 secondary to exogenous insulin infused with triacylglycerol and glucose may be attributable to the brevity of the infusion (with hyperinsulinemia induced for 2 h). The present results and those in previous studies (16,17) indicate that induction of isolated hyperinsulinemia with euglycemia and without elevation of glucose, triglycerides, and free fatty acids in blood is not a sufficient condition for elevation of PAI-1 in normal human subjects. However, hyperinsulinemia accompanied by the elevated concentrations in blood of metabolites typical of those seen in type 2 diabetes appears to be a necessary condition for elevation of PAI-1 in such subjects.

Increased concentrations of PAI-1 have been shown to presage cardiac events in nondiabetic subjects with coronary artery disease (24,25). Over expression of PAI-1 and hence attenuation of fibrinolytic capacity in blood and vessel walls has been implicated strongly in acceleration of atherosclerosis and thrombotic vascular occlusion (1–5). Accordingly, the increased PAI-1 seen with type 2 diabetes and other insulin-resistant states is likely to contribute to the accelerated atherosclerosis so typically encountered.

The increased PAI-1 observed in our study associated with hyperinsulinemia, combined with hyperglycemia and hypertriglyceridemia, indicates that treatment designed to reduce insulin resistance in muscle and adipose tissue is likely not only to improve glycemic control and reduce hyperinsulinemia but also to restore fibrinolytic capacity by decreasing expression of PAI-1. Consistent with this possibility we have found that weight loss accomplished by caloric restriction and sufficient to ameliorate insulin resistance decreased, by >50%, the otherwise elevated concentrations of PAI-1 in blood in elderly obese nondiabetic subjects with the decline in PAI-1 correlating closely with the decrease in concentrations of triglycerides (7). In addition, we have found that subjects with the polycystic ovary syndrome treated with a thiazolidinedione (troglitazone) for 3 months to decrease insulin resistance exhibited a profound decline of markedly elevated PAI-1 in blood (8).

Important limitations apply to this study. Our observations indicate that a combination of metabolic derangements typical of type 2 diabetes including hyperglycemia, hypertriglyceridemia, and hyperinsulinemia induces elevations of PAI-1 in blood. Our observations do not address which of the components, alone or in combination, is sufficient to induce the phenomenon. Thus, additional studies will be needed to delineate the extent to which elevation of any one constituent or any given combination of elevations is sufficient to induce the phenomenon.

Despite those limitations, our results suggest that hyperinsulinemia combined with hypertriglyceridemia and hyperglycemia are likely to contribute to the impaired fibrinolysis and potentially consequent acceleration of atherosclerosis associated with insulin-resistant states. They suggest that normalization of fibrinolytic capacity in such individuals will require amelioration of hyperinsulinemia and hypertriglyceridemia, and in the case of subjects with type 2 diabetes, lipidemic as well as glycemic control.

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