Hyperglycemia prevents the suppressive effect of hyperinsulinemia on plasma adiponectin levels in healthy humans

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Blümer RM, van der Crabben SN, Stegenga ME, Tanck MW, Ackermans MT, Endert E, van der Poll T, Sauerwein HP. Hyperglycemia prevents the suppressive effect of hyperinsulinemia on plasma adiponectin levels in healthy humans. Am J Physiol Endocrinol Metab 295: E613–E617, 2008. First published June 24, 2008; doi:10.1152/ajpendo.90288.2008.—Adiponectin is a fat-derived hormone with insulin-sensitizing properties. In patients with type 2 diabetes plasma adiponectin levels are decreased. Since these patients are characterized by high plasma insulin and glucose concentrations, hyperinsulinemia and hyperglycemia could be responsible for the downregulation of adiponectin. Insulin decreases adiponectin levels in humans. The effect of hyperglycemia is unknown. To determine the selective effects of insulin, glucose, or their combination on plasma adiponectin, clamps were performed in six healthy males on four occasions in a crossover design: 1) lower insulinemic-euglycemic clamp (100 pmol/l insulin, 5 mmol/l glucose) (reference clamp); 2) hyperinsulinemic-euglycemic clamp (400 pmol/l insulin, 5 mmol/l glucose); 3) lower insulinemic-hyperglycemic clamp (100 pmol/l insulin, 12 mmol/l glucose); and 4) hyperinsulinemic-hyperglycemic clamp (400 pmol/l insulin, 12 mmol/l glucose). Adiponectin concentrations and high-molecular-weight (HMW)-to-total adiponectin ratio were measured at the start and end of the 6-h clamps. After the 6-h study period, total plasma adiponectin levels were significantly (P = 0.045) decreased by 0.63 μg/ml in the lower insulinemic-euglycemic clamp (clamp 1). In both euglycemic groups (clamps 1 and 2) adiponectin concentrations significantly declined (P = 0.016) over time by 0.56 μg/ml, whereas there was no change in both hyperglycemic groups (clamps 3 and 4) (P = 0.420). In none of the clamps did the ratio of HMW to total adiponectin change. We conclude that insulin suppresses plasma adiponectin levels already at a plasma insulin concentration of 100 pmol/l. Hyperglycemia prevents the suppressive effect of insulin. This suggests that, in contrast to glucose, insulin could be involved in the downregulation of plasma adiponectin in insulin-resistant patients.

hypo adiponectinemia; glucose metabolism; type 2 diabetes

In addition, adiponectin-knockout mice are more insulin resistant compared to wild-type mice (20, 21). In plasma, adiponectin circulates as several different entities, including a high-molecular-weight (HMW), a hexameric (medium molecular weight), and a trimeric (low molecular weight) form. The HMW oligomer has been implicated as the major active form responsible for the insulin-sensitizing effects of adiponectin in the liver and peripherally. In line with this, the ratio of HMW to total adiponectin has been described to correlate better with insulin sensitivity than total adiponectin levels (34).

Because adiponectin is mainly synthesized and released by white adipose tissue, it may be expected that its expression in adipocytes would increase in obesity. However, plasma levels of adiponectin (1, 16, 24) have been shown to be reduced in subjects with obesity as well as in patients with type 2 diabetes and human immunodeficiency virus (HIV)-lipodystrophy, and this is considered to contribute to the degree of insulin resistance in these diseases. The factors responsible for this counterintuitive finding in these patients have not yet been fully determined. Since hyperinsulinemia and hyperglycemia are characteristic biochemical features of these patients, insulin as well as glucose could be responsible for the downregulation of adiponectin. Insulin’s inhibiting effect on adiponectin gene expression and plasma levels has been shown in several in vitro and in vivo studies (8, 11, 37). However, additional factors must be involved, because in the more advanced stages of type 2 diabetes, associated with decreased plasma insulin levels, adiponectin concentrations remain low (17). Hypothetically, hyperglycemia could be involved in the low adiponectin levels under these circumstances. Indeed, several studies associated low adiponectin concentrations with poor glycemic control and high dietary glyceric load in diabetic men (26, 27).

The effects of hyperglycemia per se and/or in combination with hyperinsulinemia on plasma adiponectin levels in human subjects have not yet been studied. We hypothesize that hyperglycemia, in addition to hyperinsulinemia, results in a (further) decline in plasma adiponectin levels. To test this hypothesis, we performed a controlled crossover study in six healthy males on four occasions with plasma levels of insulin and glucose aimed at 1) 100 pmol/l insulin, 5 mmol/l glucose, 2) 400 pmol/l insulin, 5 mmol/l glucose, 3) 100 pmol/l insulin, 12 mmol/l glucose, and 4) 400 pmol/l insulin, 12 mmol/l glucose, respectively. Total and HMW oligomer plasma ad-
ponecitin levels were measured at baseline and at the end of the 6-h clamps.

METHODS

Subjects. We studied six healthy, nonsmoking male volunteers (age 21.7 ± 1.2 yr, weight 73.2 ± 4.8 kg, body mass index 21.8 ± 0.9 kg/m²). None of the volunteers used medication or had a positive family history of diabetes. All volunteers had normal plasma values of fasting glucose (4.6 ± 0.2 mmol/l) and insulin (33 ± 9 pmol/l), erythrocyte sedimentation rate, complete blood count, lipid profile, and renal and hepatic function, and all had a normal oral glucose tolerance test, according to American Diabetes Association criteria (3). The study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam, and all subjects gave written informed consent. The present study was part of a study on effects of insulin and hyperglycemia on different parameters (31, 32).

The data in the present study, except for the glucose and insulin levels, have not been published previously.

Study design. The study protocol had a crossover design with a washout period of 4 wk. The sequence of the four clamps was chosen at random in all subjects. Each volunteer served as his own control and was studied on four occasions with plasma levels of insulin and glucose aimed at 1) lower insulinemic-euglycemic clamp (100 pmol/l insulin, 5 mmol/l glucose) (reference clamp), 2) hyperinsulinemic-euglycemic clamp (400 pmol/l insulin, 5 mmol/l glucose), 3) lower insulinemic-hyperglycemic clamp (100 pmol/l insulin, 12 mmol/l glucose), and 4) hyperinsulinemic-hyperglycemic clamp (400 pmol/l insulin, 12 mmol/l glucose), respectively.

For 3 days before the study, all volunteers consumed an isocaloric diet containing at least 250 g of carbohydrates with a maximum of 20% disaccharides. After an overnight fast subjects were admitted to the clinical research unit and confined to bed. The study started with placement of a catheter into an antecubital vein for infusion. Another clamp protocol was started as well. All infusions were started as follows: Somatostatin-ucb, UCB Pharma, Breda, The Netherlands) and glucose, mmol/l from euglycemia or hyperglycemia were started as well. All infusions were started with somatostatin (250 pmol/h; GlucaGen, Novo Nordisk, Alphen aan den Rijn, The Netherlands) were started to suppress endogenous insulin secretion, respectively. Concurrently, infusions of insulin (Actrapid/l, Novo Nordisk) at a rate of 10 or 40 mU·m⁻² body surface area⁻¹·min⁻¹ (aimed at plasma insulin levels of 100 and 400 pmol/l, respectively) and 10% or 20% glucose at a variable rate to obtain euglycemia or hyperglycemia were started as well. All infusions were administered by calibrated syringe pumps (Perfusor fm, Braun, Melsungen, Germany). To maintain glucose concentrations at 5 or 12 mmol/l from t = 0 until t = 6 h, every 5 min plasma glucose concentration was measured on a Beckman glucose analyzer 2 (Beckman, Palo Alto, CA). At t = 0 and every 10 min from t = 5:40 until t = 6:00, blood samples were drawn for determination of insulin levels. Blood samples were drawn for measurement of concentrations of cortisol, catecholamines, glucagon, free fatty acids (FFA), growth hormone, and total adiponectin as well as HMW-to-total adiponectin ratio immediately before and at the end of the clamps (t = 0 and t = 6 h, respectively). Blood samples were kept on ice immediately after collection and subsequently centrifuged for 10 min at 3,000 rpm and 4°C. All plasma samples were stored below −20°C.

Assays. Plasma insulin, cortisol, glucagon, catecholamines, and FFA levels were measured as described previously (6). Plasma adiponectin concentrations were measured in duplicate by RIA (Lino Research, St. Charles, MO); intra-assay coefficient of variation (CV) 4–6%; interassay CV 6–9%; detection limit 0.5 µg/ml. The HMW-to-total adiponectin ratio was measured in duplicate by gel electrophoresis and Western blot (30). Human growth hormone (somatotropin) was determined with a chemiluminescent immunometric assay (Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA).

Calculations and statistical analysis. Data were checked for normal distribution (Shapiro-Wilk test) and equal variances (Levene’s test) using the residuals. Depending on the results of these tests, data were analyzed either parametrically or nonparametrically. Results are presented as means ± SD. To analyze differences in basal plasma glucose and insulin concentrations among the four clamps, a repeated-measures ANOVA was used. Glucose and insulin levels at the end of the 6-h study period were compared among clamps by a repeated-measures ANOVA with correction for baseline levels. To test whether plasma adiponectin levels changed between baseline and after 6 h of infusion in clamp 1, a paired t-test was used. The effects of hyperinsulinemia, hyperglycemia, and their interaction on the change of adiponectin levels between t = 0 and t = 6 h were analyzed by a repeated-measures ANOVA with correction for baseline adiponectin concentrations. Changes in plasma adiponectin levels over time are presented as mean change [95% confidence interval (CI)]. Correlations between changes in plasma adiponectin levels and changes in the other parameters between t = 0 and t = 6 h were analyzed by Spearman’s correlation coefficient. A sample size of six subjects would suffice to have 80% power to detect an absolute difference in adiponectin levels of 0.55 µg/ml (~10%), assuming a standard deviation of the mean difference of 0.4 µg/ml and using a paired t-test with a two-sided α of 0.05. SPSS statistical software version 12.0.1 (SPSS, Chicago, IL) was used to analyze the data.

RESULTS

Glucose and insulin levels. Basal plasma glucose and insulin concentrations were not significantly different among the four clamps (Table 1). In all four clamps target levels of glucose and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached. During the first hour glucose levels and insulin were reached.
Adiponectin levels. After 6 h of infusion, total plasma adiponectin concentrations were significantly decreased compared with basal levels by 0.63 μg/ml (P = 0.045, 95% CI: 0.02–1.24; 10% decrease) in the lower insulinemic-euglycemic clamp (reference clamp = clamp 1) (Fig. 1, Table 1). The HMW-to-total adiponectin ratio did not change significantly during clamp 1 (0.04, 95% CI: −0.15 to 0.06). The mean decline in total plasma adiponectin levels over time was not different between the relatively low-insulin and high-insulin groups (0.42 vs. 0.32 μg/ml, mean difference of 0.10, 95% CI: −0.31 to 0.52). During the 6-h clamp, there was a significant decrease (P = 0.016) in total plasma adiponectin concentrations of 0.56 μg/ml in both euglycemic groups (clamps 1 and 2) (95% CI: 0.10–1.01), whereas there was no difference (P = 0.420) in both hyperglycemic groups (clamps 3 and 4) (0.19 μg/ml, 95% CI: −0.27 to 0.64). During the clamp, there was no significant change in the HMW-to-total adiponectin ratio in both euglycemic groups (0.05, 95% CI: −0.06 to 0.15) or in both hyperglycemic groups (0.03, 95% CI: −0.07 to 0.14).

Glucoregulatory hormones and FFA. In all clamps, there were no significant correlations between the changes over time in plasma adiponectin levels and the changes over time in plasma concentrations of glucagon (r = −0.13, P = 0.6), growth hormone (r = −0.20, P = 0.4), norepinephrine (r = 0.08, P = 0.7), epinephrine (r = −0.11, P = 0.6), cortisol (r = 0.09, P = 0.7), or FFA (r = 0.21, P = 0.3).

DISCUSSION

The low plasma adiponectin levels in patients with type 2 diabetes and HIV-lipodystrophy are unexplained. Since these patients often have hyperinsulinemia as well as hyperglycemia, insulin, glucose, or the combination of both could be responsible for this decline. The present study for the first time describes the influence of hyperglycemia, hyperinsulinemia, and their combination on plasma adiponectin concentrations in healthy male humans, in a study design in which each subject served as his own control. Insulin lowered plasma adiponectin levels, while hyperglycemia prevented this decline in healthy humans.

Several studies have investigated the effects of insulin on adiponectin production and secretion in vitro. Most, but not all (11), in vitro studies found a stimulating effect on adiponectin gene expression (15, 28, 33) or secretion (7, 25) in 3T3-L1 adipocytes as well as in human adipocytes. Additionally, during hyperinsulinemic-euglycemic clamps in rats and healthy human subjects, the expression of adiponectin in visceral and subcutaneous fat, respectively, was moderately increased as well (10, 35). In contrast to these in vitro data on adiponectin expression and secretion, insulin lowered human adiponectin levels in plasma. Hyperinsulinemic-euglycemic clamp studies in lean subjects reduced total plasma adiponectin levels by 10–20% (8, 23, 37). The increased total plasma adiponectin concentrations in patients with type 1 diabetes, specifically in those who are C-peptide negative, could be in line with this inhibiting effect of insulin (13, 18). Congruent with these human studies, we showed a 10% drop in total plasma adiponectin levels after 6 h of infusion in clamp 1 with insulin levels aimed at 100 pmol/l. Since the mean decrease in plasma adiponectin levels over time was not different between the low- and the high-insulin groups, our data suggest that insulin has a maximal inhibiting effect on total adiponectin levels at concentrations of ≥100 pmol/l. A dose-independent negative effect on plasma adiponectin levels of insulin >100 pmol/l has been described previously (8). Recently, a study reported that in nondiabetic subjects hyperinsulinemia resulted in a reduction of HMW adiponectin levels as well as the HMW-to-total adiponectin ratio (4). However, a distinction between the selective effects of insulin and glucose on adiponectin levels was impossible to make because only hyperinsulinemic-hyperglycemic clamps were performed in that study. In the present study, we did not find a difference in the HMW-to-total adiponectin ratio after 6 h of infusion in the low insulinemic-euglycemic clamp. This indicates that in healthy subjects hyperinsulinemia per se does not primarily affect HMW adiponectin levels.

Besides insulin, other factors could have regulated adiponectin levels as well. Since there are no data on the effects of somatostatin, we cannot exclude a role for somatostatin in the decreased adiponectin levels. No significant correlations were found between changes over time in the levels of plasma adiponectin and changes over time in the plasma levels of glucagon, growth hormone, norepinephrine, epinephrine, cortisol, or FFA. Therefore, these hormones do not seem to be involved in the decrease of adiponectin. Because adiponectin does not have a circadian rhythm, it is unlikely that this phenomenon is responsible for our findings in the euglycemic clamps (29). Therefore, high insulin concentrations in patients...
with type 2 diabetes may be involved in downregulating plasma adiponectin, although the effect of insulin on the long run remains unknown.

Between the initiation and the end of the 6-h study period, plasma adiponectin concentrations significantly declined in both euglycemic groups, whereas there was no significant change in the hyperglycemic groups. These data suggest that the inhibiting effect of hyperinsulinemia on total plasma adiponectin levels is counteracted by hyperglycemia. The effect of hyperglycemia per se on adiponectin levels in human subjects has not been described previously. One study reported on the effects of hyperglycemia on adiponectin expression and plasma concentrations in rats (10). A 5-h clamp was performed with infusion of dextrose as well as somatostatin to achieve high glucose and low insulin levels, respectively. This clamp resulted in increased adiponectin expression in visceral adipose tissue, whereas plasma levels remained constant. In addition to the study in rats, we investigated the effect of hyperglycemia on plasma adiponectin levels during hyperinsulinemia, known to inhibit these levels. The common denominator in both studies is the absence of an absolute increase in plasma adiponectin levels. Therefore, it can be hypothesized that hyperglycemia per se does not increase plasma adiponectin but only compensates in case of decreased adiponectin levels. Although the effects of hyperglycemia in the absence of hyperinsulinemia remain unknown in human subjects, the relative increase in adiponectin levels during the hyperglycemic clamps could function as an adaptive mechanism to restrain glucose levels. In accordance with this hypothesis are the increased plasma adiponectin levels in human subjects with an acute severe infection, which is associated with insulin resistance and hyperglycemia (5, 19). The mechanisms by which acute hyperglycemia prevents the suppressive effect of hyperinsulinemia on plasma adiponectin levels remain to be elucidated.

There was no difference in the HMW-to-total adiponectin ratio during the euglycemic or hyperglycemic clamps in the present study. This result is partly in contrast to the above-described study, which reported a decline in the HMW-to-total adiponectin ratio during a hyperinsulinemic-hyperglycemic clamp in nondiabetic subjects (4). The reason for this difference in study results may be related to the differences in study design as insulin levels (700 pmol/l in Ref. 4 vs. 400 pmol/l in the present study) and duration of the clamp (7 h vs. 6 h).

In the present study, we investigated the acute (6 h) regulation of plasma adiponectin levels by insulin and glucose in healthy, insulin-sensitive subjects. Our data cannot fully explain the low adiponectin levels in diabetic patients, because those levels are usually much lower than can be explained by the degree of suppression by insulin found in our study. Additionally, in those subjects the concomitant hyperglycemia should have (partly) prevented the suppressive effect of insulin. Apparently, other regulatory mechanisms are involved as well in the low adiponectin levels in the chronic abnormalities of glucose regulation.

In conclusion, insulin suppresses plasma adiponectin levels already maximally at a plasma insulin concentration of 100 pmol/l. Hyperglycemia prevents the suppressive effect of insulin on plasma adiponectin levels. This suggests that, in contrast to hyperglycemia, hyperinsulinemia could be involved in the downregulation of plasma adiponectin in insulin-resistant patients.

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