Brief Report

Selective Downregulation of the High–Molecular Weight Form of Adiponectin in Hyperinsulinemia and in Type 2 Diabetes

Differential Regulation From Nondiabetic Subjects

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OBJECTIVE—Adiponectin is an adipocyte-specific secretory protein found in circulation in several different forms and is present at significantly lower levels in the plasma of diabetic patients compared with that of insulin-sensitive individuals. We wanted to test whether insulin per se is a contributing factor toward lower plasma adiponectin concentrations and, if so, whether the splanchnic bed contributes to this phenomenon.

RESEARCH DESIGN AND METHODS—We sampled femoral artery and hepatic venous samples and measured the high–molecular weight (HMW) and low–molecular weight (LMW) fractions of adiponectin in 11 type 2 diabetic and 7 nondiabetic subjects matched for age, sex, and BMI during basal conditions and during a hyperglycemic (~9.5 mmol/l) hyperinsulinemic (~700 pmol/l) clamp.

RESULTS—Under these conditions, total arterial adiponectin, HMW, and the ratio of HMW to total adiponectin all were lower ($P < 0.01$) in the diabetic versus nondiabetic subjects, whereas the LMW form did not significantly differ. Under hyperinsulinemic conditions, total adiponectin levels dropped, primarily due to a reduction of the HMW form, whereas LMW forms were not significantly affected.

CONCLUSIONS—HMW adiponectin and the ratio of HMW to total adiponectin are lower in individuals with diabetes than in nondiabetic subjects. We conclude that HMW adiponectin is downregulated in hyperinsulinemia and type 2 diabetes. ***Diabetes*** **56:2174–2177, 2007**

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Received for publication 9 February 2007 and accepted in revised form 2 May 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 18 May 2007. DOI: 10.2337/db07-0185.

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HMW, high molecular weight; LMW, low molecular weight

Adiponectin is a secretory protein uniquely produced by adipocytes (1) and accepted as a marker for systemic insulin sensitivity, particularly as an indicator of hepatic insulin sensitivity and lipid content (2). Decreased levels correlate well with cardiovascular and atherosclerotic disease, and negative correlation with proinflammatory markers makes it one of the most promising biomarkers for the metabolic syndrome. Studies with recombinant protein and, more importantly, analysis of a number of mouse models with altered adiponectin levels have demonstrated potent hepatic insulin-sensitizing and anti-atherosclerotic activities (3,4).

Several articles have clarified relevance of the observation that adiponectin circulates as a mixture of several different complexes. The sexual dimorphism causing higher levels of adiponectin in females is primarily due to higher levels of the high–molecular weight (HMW) form, a complex of at least 18 subunits of adiponectin (5). A number of studies have taken advantage of the potent predictive potential that measurement of the HMW offers and have further strengthened the strong correlations with the metabolic syndrome and insulin sensitivity previously revealed with measurements of total adiponectin (6,7).

A small study in patients suggested that during hyperinsulinemic-euglycemic clamp, plasma adiponectin levels were significantly decreased (8). Patients carrying mutant insulin receptor genes that lead to functionally impaired receptors or subjects with anti-insulin receptor autoantibodies present with very high levels of adiponectin (9). This lends further support to the hypothesis that insulin and its receptor exert potent repressive effects on adiponectin expression. The present studies were done to examine the distribution of the different adiponectin complexes in insulin-sensitive and insulin-resistant subjects under both basal and hyperinsulinemic conditions across the splanchnic bed. The results reveal a potent repressive effect of insulin on circulating adiponectin levels, particularly the HMW form.

RESEARCH DESIGN AND METHODS

After approval from the Mayo institutional review board, 7 nondiabetic and 11 type 2 diabetic subjects gave informed written consent to participate in the study. This is a subset of the 14 nondiabetic and 12 diabetic subjects studied as part of another protocol, previously published (10). In brief, all subjects were in good health and on no medications at the time of study other than either thyroxine or hormone replacement therapy. Oral hypoglycemic agents

were discontinued 3 weeks before study. Subjects were instructed to follow a weight maintenance diet for at least 3 days before the day of study. Nondiabetic and diabetic subjects were matched for age (66 ± 2 vs. 65 ± 1 years), BMI (29 ± 2 vs. 32 ± 1 kg/m^2), fat-free mass (53 ± 3 vs. 53 ± 4 kg), and body fat (36 ± 3 vs. 39 ± 3%) (11).

Subjects were admitted to the Mayo Clinic Research Unit on the evening before the study and fed a standard 10 cal/kg meal at 1800 h. An 18-gauge catheter was inserted into a forearm vein, and an infusion of insulin was started at 1900 h in the diabetic subjects (100 units regular human insulin in 1 l of 0.9% saline containing 5 ml 25% human albumin) and saline in the nondiabetic subjects. The insulin infusion rate was adjusted to maintain overnight euglycemia in the diabetic subjects (~5 mmol/l) (12).

At 0600 h on the following morning, subjects were taken to an interventional radiology suite where femoral artery and femoral and hepatic venous catheters were placed as previously described (13).

At ~0900 h, [3-^{3}H]glucose and a hormone cocktail containing somatostatin and replacement amounts of growth hormone and glucagon were started (time 0 min) and continued until the end of the study as part of a separate experiment (14). Insulin was infused at a rate of 0.78 mU · kg lean body wt^{-1} · min^{-1} from 0 to 180 min, then at 1.56 mU · kg lean body wt^{-1} · min^{-1} from 181 to 300 min, and then at 3.1 mU · kg lean body wt^{-1} · min^{-1} from 301 to 420 min. Dextrose containing [3-^{3}H]glucose was also begun and the rate adjusted so as to maintain plasma glucose concentrations at ~9.3 mmol/l (~165 mg/dl) and keep specific activity constant over the next 7 h of study (15). These experiments offered us the opportunity to measure serum adiponectin and its HMW and low–molecular weight (LWM) fractions across the splanchnic bed for the 3.1 mU · kg lean body wt^{-1} · min^{-1} infusion.

Analytical techniques. All samples were stored at −20°C until analysis. Plasma glucose was measured by a glucose oxidase method using a YSI glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin was measured by chemiluminescence with the Access ultrasensitive immunoenzymatic assay system (Beckman, Chaska, MN). Body composition and lean body mass were measured using dual-energy X-ray absorptiometry (SmartScan, version 4.6; Hologic, Waltham, MA).Velocity sedimentation/gel filtration chromatography was used for separation of adiponectin complexes as previously described using a human adiponectin radioimmunoassay (Linco) (5,11). Measurements of adiponectin level and distribution were performed with approval from the Albert Einstein institutional review board.

Statistical analysis. Data in the text and figures are expressed as means ± SEM and rates as micromol per kilogram lean body mass per minute. Response during the high-dose insulin infusion was determined by taking the mean of the results from 390 to 420 min. Student's nonpaired t test was used to compare results between groups (e.g., diabetic vs. nondiabetic subjects) and paired t test for within a group (e.g., basal vs. high-dose insulin infusion). $P < 0.05$ was considered statistically significant.

RESULTS

Plasma glucose and insulin concentrations. Plasma glucose concentrations were higher ($P < 0.001$) in the diabetic than in the nondiabetic subjects (Fig. 1*A*) at baseline (8.0 ± 0.3 vs. 5.5 ± 0.1 mmol) but did not differ during the insulin infusion. Plasma insulin concentrations were slightly but not significantly higher in the diabetic compared with the nondiabetic subjects at baseline (48 ± 6 vs. 39 ± 5 pmol/l) and did not differ during the high-dose insulin infusions (Fig. 1*B*).

Total, HMW, and LMW adiponectin concentrations in femoral artery. Total adiponectin concentrations were significantly lower ($P < 0.001$) in diabetic than in nondiabetic subjects (Fig. 2*A*) both at baseline (8.2 ± 0.6 vs. 14.4 ± 2.8 μg/ml) and during the high-dose insulin infusion (5.0 ± 0.6 vs. 9.8 ± 1.4 μg/ml). There was a significant decrease in total adiponectin concentrations with increasing doses of insulin in the diabetic subjects (ANOVA $P < 0.001$), primarily due to a decrease in the HMW form ($P < 0.001$). A similar trend was observed in the nondiabetic subjects but failed to reach statistical significance, presumably because of the small sample size. HMW adiponectin concentrations were significantly lower ($P < 0.001$) in the diabetic than in the nondiabetic subjects (Fig. 2*B*) both at baseline (3.1 ± 0.2 vs. 11.0 ± 2.0 μg/ml) and during the high-dose insulin infusion (1.7 ± 0.2 vs. 5.1 ± 0.8 μg/ml).

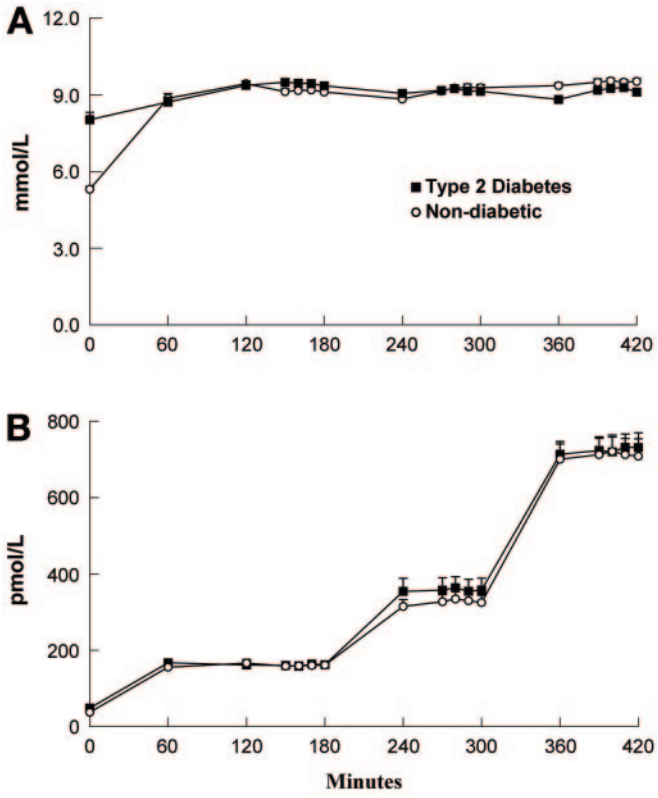

FIG. 1. Plasma glucose (*A*) and insulin (*B*) concentrations observed in the type 2 diabetic and nondiabetic subjects at baseline and during a hyperglycemic clamp. The insulin, somatostatin, glucagon, and growth hormone infusions started at time 0.

Similar to total adiponectin, the levels of the HMW form were significantly lower with increasing doses of insulin in both the diabetic (ANOVA $P < 0.001$) and nondiabetic subjects (ANOVA $P < 0.01$). Interestingly, the concentrations of the LMW fraction of adiponectin (Fig. 2*C*) did not differ in the diabetic and nondiabetic subjects either in the basal state (4.75 ± 0.52 vs. 5.27 ± 0.9 μg/ml) or during the high-dose insulin infusion (3.1 ± 0.4 vs. 4.7 ± 0.8 μg/ml). There was a very small but significant decrease ($P < 0.05$) in the LMW fraction adiponectin with increasing doses of insulin in the diabetic but not in the nondiabetic subjects. Combined, these data suggest that diabetic subjects have lower levels of adiponectin, predominantly due to differences at the level of the HMW form. Hyperinsulinemia has a significant negative impact on total adiponectin levels, primarily due to a decrease in the HMW form. This further highlights the relevance of the HMW form as the more sensitive of the circulating complexes.

Ratio of HMW to total adiponectin concentrations in the femoral artery. The ratio of HMW to total adiponectin is used because it is considered a better index of insulin sensitivity than either total adiponectin levels or absolute levels of HMW (Fig. 3). This ratio is termed the adiponectin sensitivity index (S_A). S_A was significantly lower in the diabetic than in the nondiabetic subjects ($P < 0.01$) at baseline (0.4 ± 0.04 vs. 0.71 ± 0.05) and during the high-dose insulin infusion (0.36 ± 0.04 vs. 0.52 ± 0.02; $P < 0.01$). Importantly, hyperinsulinemia had less of an effect on S_A in the diabetic than in the nondiabetic subjects. This indicates that in insulin-sensitive individuals, there is a disproportionate loss of the HMW form relative to total levels.

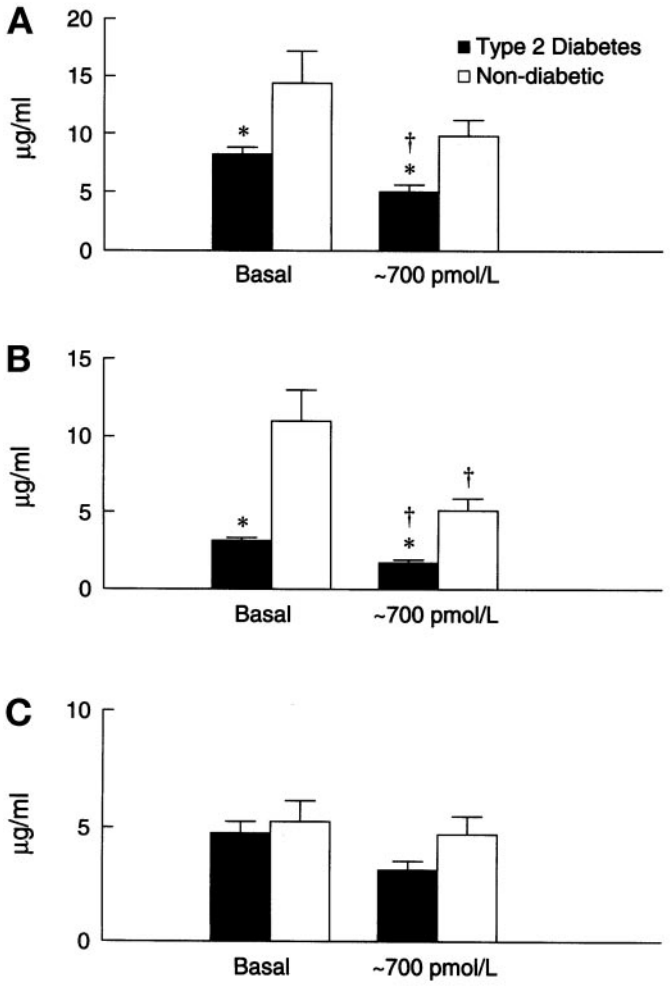

FIG. 2. Total (*A*), HMW (*B*), and LMW (*C*) adiponectin concentrations observed in the diabetic and nondiabetic subjects in the femoral artery at baseline and during the high-dose insulin infusion. **P* < 0.01 vs. diabetic subjects; †*P* < 0.001 vs. basal.

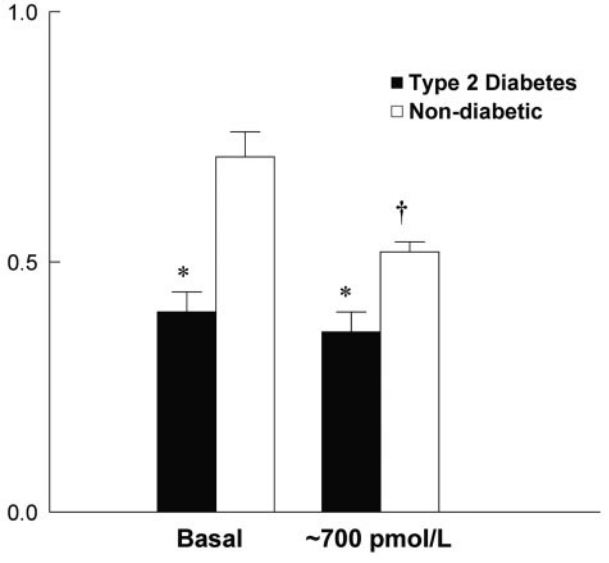

FIG. 3. The ratio of the HMW fraction and total adiponectin concentrations observed in the diabetic and nondiabetic subjects in the femoral artery at baseline and during the high-dose insulin infusion. **P* < 0.01 vs. diabetic subjects; †*P* < 0.001 vs. basal.

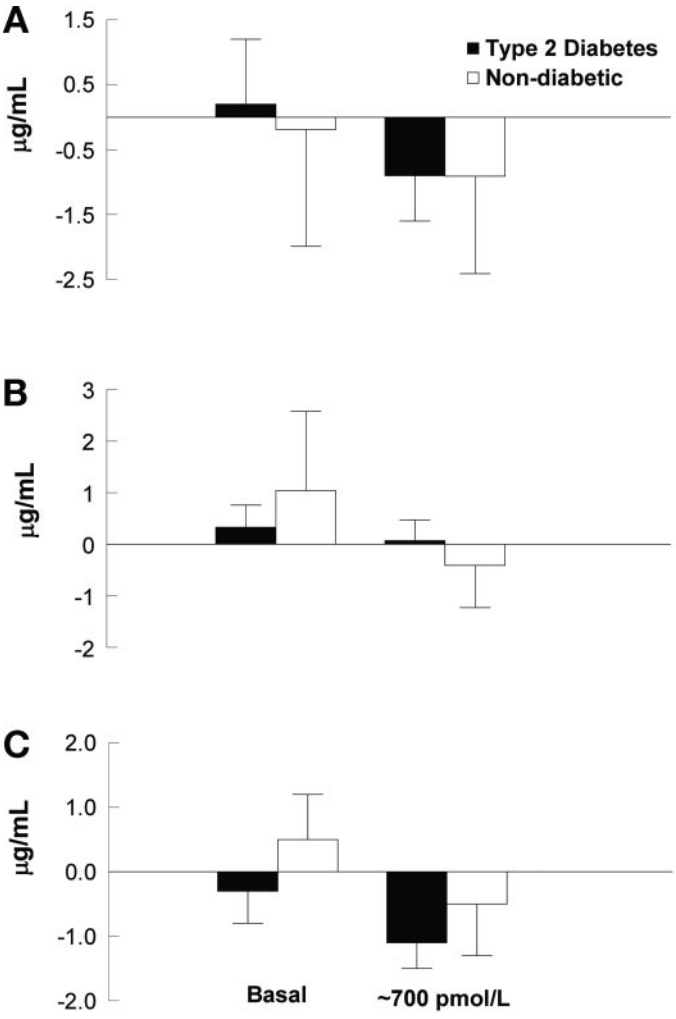

FIG. 4. Total (*A*), HMW (*B*), and LMW (*C*) adiponectin concentration gradients observed across the splanchnic bed (i.e., femoral artery minus hepatic vein) in diabetic and nondiabetic subjects at baseline and during the high-dose insulin infusion. No significant differences were observed.

Total adiponectin, HMW adiponectin, and LMW adiponectin gradients across the splanchnic bed. There were no significant differences in total adiponectin concentrations across the splanchnic bed; i.e., adiponectin levels in the femoral artery were comparable with those measured in the hepatic vein in the either diabetic or nondiabetic subjects at baseline (change [Δ] = 0.20 ± 1.0 vs. −0.19 ± 1.8 μg/ml) and during the high-dose insulin infusion (Δ = −0.9 ± 0.7 [diabetic] vs. −0.91 ± 1.5 μg/ml [nondiabetic]), indicating that there was no net release or uptake of adiponectin in the splanchnic bed in either group (Fig. 4*A*). On measuring the different complexes, the differences in the HMW forms did not reach statistical significance in the diabetic or the nondiabetic subjects at baseline (Δ = 0.33 ± 0.43 vs. 1.04 ± 1.54 μg/ml, respectively) or during the high-dose insulin infusion (Δ = 0.07 ± 0.4 vs. −0.41 ± 0.82 μg/ml, respectively) (Fig. 4*B*). Small but nonsignificant changes were seen for the LMW form at baseline for both the diabetic and nondiabetic subjects (Δ = −0.3 ± 0.5 vs. 0.5 ± 0.7 μg/ml, respectively) and during the high-dose insulin infusion (Δ = −1.1 ± 0.4 vs. −0.5 ± 0.8 μg/ml, respectively) (Fig. 4*C*). This suggests that in both the basal and insulin-stimulated states, the

splanchnic bed does not make any significant net contributions toward systemic changes.

DISCUSSION

We report that the HMW form of adiponectin is prone to be reduced under hyperinsulinemic conditions, particularly among insulin-sensitive nondiabetic individuals. This has profound physiological implications. Hyperinsulinemia is frequently an indicator of insulin resistance. Hypoadiponectinemia is not only frequently associated with insulin resistance (16) but also may be directly causative for reduced insulin sensitivity (17). This suggests a vicious cycle during the initial stages of hyperinsulinemia, whereby high insulin levels lead to a downregulation of adiponectin levels, which in turn decreases insulin sensitivity further, prompting an even higher level of circulating insulin to maintain glucose homeostasis. Previous experiments in rodents (5) have demonstrated that the impact on HMW levels is primarily mediated through insulin and not hyperglycemia.

We have recently demonstrated (18) that the endoplasmic reticulum chaperone pair ERp44 and Ero1 is critically involved in the assembly pathway of adiponectin higher-order complexes. The levels of these chaperones are subject to tight regulation, are lowered in diabetes, and are induced by peroxisome proliferator–activated receptor-γ agonists. The differential response is likely due to a differential impact of insulin on the levels of these chaperones in adipocytes from diabetic and nondiabetic subjects. Our observations that stimulation with peroxisome proliferator–activated receptor-γ agonists leads to an increase of circulating adiponectin, primarily due to an increase in the HMW form (11), have originally highlighted the potential importance of the HMW form. This HMW form of adiponectin is in many instances much more prone to regulation than other adiponectin complexes (19–22). While we failed to detect net differences of adiponectin levels across the splanchnic bed, we cannot rule out contributions of the visceral fat depots toward a unique local profile of adiponectin complexes that subsequently are efficiently extracted by the liver.

The methodology used in these studies is able to effectively separate the HMW form from the other adiponectin forms; however, the assay is not designed to separate the hexameric from the trimeric forms (5,11). A number of recent articles have compared the correlation coefficients of various parameters with either absolute levels of HMW or the ratio of HMW to total adiponectin concentrations. It depends on the specific parameter examined as to which of the two adiponectin measurements prevails with better correlation coefficients. The fact that under a number of circumstances the ratio of HMW to total adiponectin concentration is a superior read out indeed suggests that a competitive relationship may exist between HMW and the other adiponectin forms. It is yet unknown whether these complexes individually bear any physiological meaning, and future experiments will be required to separate these forms.

ACKNOWLEDGMENTS

This study was supported by U.S. Public Health Service Grants DK 29953, RR-00585, and R03-EY014935; a Novo Nordisk research infrastructure grant; the Mayo Foundation; New York Regional Obesity Center Grant P30DK026687-25 (adipocyte physiology core); an American Diabetes Association mentor-based fellowship (to R.B.); and National Institutes of Health Medical Scientist Training Grant T32-GM07288 (to U.B.P.).

We thank B. Dicke and L. Heins for technical assistance, R. Rood for assistance with graphics, and J. Feehan and B. Norby for assistance in performing the studies.

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