Selective Downregulation of the High Molecular Weight form (HMW) of Adiponectin in Hyperinsulinemia and in Type 2 Diabetes: Differential Regulation from Non-diabetic Subjects

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Abstract

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

Research Design and Methods: To do so, we sampled femoral artery and hepatic venous samples and measured the high molecular weight (HMW) and low molecular weight (LMW) fraction of adiponectin in 11 type 2 diabetic and 7 non-diabetic subjects matched for age, gender and body mass index 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 S_A ratio of HMW/total adiponectin all were lower (p<0.01) in the diabetic versus non-diabetic 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/total adiponectin are lower in people with diabetes as compared to non-diabetic subjects. We conclude that HMW adiponectin is downregulated in hyperinsulinemia and type 2 diabetes.
Adiponectin is a secretory protein that is 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 pro-inflammatory 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 papers 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 form (HMW)”, a complex of at least 18 subunits of adiponectin (5). A number of studies have taken advantage of the potent predictive potential that measurements of the HMW offers and further strengthened the strong correlations with the metabolic syndrome and insulin sensitivity previously revealed with the measurements of total adiponectin (6,7).

A small study in patients suggested that during hyperinsulinemic euglycemic clamp studies, 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**

**Subjects**

After approval from the Mayo Institutional Review Board, 7 non-diabetic and 11 subjects with type 2 diabetes mellitus gave informed written consent to participate in the study. This is a subset of the 14 non-diabetics 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 three weeks prior to study. Subjects were instructed to follow a weight maintenance diet for at least three days prior to the day of study. Non-diabetic and diabetic subjects were matched for age (66 ± 2 vs. 65 ± 1 yrs), body mass index (29 ± 2 vs. 32 ± 1 %), fat free mass (53 ± 3 vs. 53 ± 4 kgs), and body fat (36 ± 3 vs. 39 ± 3 %) percent (11).

**Experimental Design**

Subjects were admitted to the Mayo Clinic Research Unit (CRU) evening before the study and fed a standard 10 cal/kg meal at 1800 hours. An 18-gauge catheter was inserted into a forearm vein and an infusion of insulin was started at 1900 hrs in the diabetic subjects (100 U regular human insulin in 1 L of 0.9% saline containing five ml of 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 hours on the following morning, subjects were taken to an interventional radiology suite where femoral
artery, femoral and hepatic venous catheters were placed as previously described (13).

At ~0900, [3-5H] glucose and a hormone cocktail containing somatostatin and replacement amounts of growth hormone and glucagon were started (time 0 minutes) 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/min 0 -180 minutes, at 1.56 mU/kg lean body wt /min 181 -300 minutes and at 3.1 mU/kg lean body wt /min from 301 - 420 minutes. Dextrose containing [3-3H] glucose also was begun and the rate adjusted so as to maintain plasma glucose concentrations at ~9.3mM (~165 mg/dl) and specific activity constant over the next seven hours of study (15). These experiments offered us the opportunity to measure serum adiponectin and its HMW and LMW fractions across the splanchnic bed for the 3.1mU/kg lean body weight/min 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 analyser (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 (Hologic, Waltham, MA, SmartScan™ Version 4.6). Velocity sedimentation/gel filtration chromatography was used for separation of adiponectin complexes as previously described using a human adiponectin radioimmunoassay, LINCO (5,11). Measurement of adiponectin levels and distribution were performed with approval from the Albert Einstein Institutional Review Board.

Statistical Analysis
Data in the text and figures are expressed as mean ± SEM and rates as µmol per kg lean body mass per minute. Response during the high dose insulin infusion was determined by taking the mean of the results present respectively from 390 to 420 minutes. Student’s nonpaired t-test was used to compare results between groups (e.g. diabetic versus nondiabetic subjects) and paired t-test within a group (e.g. basal versus high dose insulin infusion). A p<0.05 was considered as statistically significant.

RESULTS
Plasma glucose, and insulin concentrations
Plasma glucose concentrations were higher (p<0.001) in the diabetic than non-diabetic subjects (Fig. 1, upper panel) 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 subjects as compared to non-diabetic subjects at baseline (48 ± 6 vs. 39 ± 5 pmol/l) and did not differ during the high dose insulin infusions (Fig.1, lower panel).

Total adiponectin, HMW adiponectin, and LMW adiponectin concentrations in femoral artery
Total adiponectin concentrations were significantly lower (p<0.001) in the diabetic subjects than non-diabetic subjects (Fig. 2, upper panel) 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 HMW (p<0.001). A similar trend was observed in the non-diabetic subjects, but failed to reach statistical significance presumably due to the small sample size. HMW adiponectin
concentrations were significantly lower \((p<0.001)\) in the diabetic subjects than non-diabetic subjects (Fig.2, middle panel) both at baseline \(3.1 \pm 0.2 \, \text{vs.} \, 11.0 \pm 2.0 \, \mu g/mL\) and during the high dose insulin infusion \(1.7 \pm 0.2 \, \text{vs.} \, 5.1 \pm 0.8 \, \mu g/mL\). Similar to total adiponectin, the levels of the HMW form were significantly lower with increasing doses of insulin in both diabetic (ANOVA \(p<0.001\)) and non-diabetic subjects (ANOVA \(p<0.01\)). Interestingly, the concentrations of the LMW fraction of adiponectin (Fig. 2, lower panel) did not differ in the diabetic and non-diabetic subjects, either in the basal state \(4.75 \pm 0.52 \, \text{vs.} \, 5.27 \pm 0.9\) or during the high dose insulin infusion \(3.1 \pm 0.4 \, \text{vs.} \, 4.7 \pm 0.8\). There was a very small but significant decrease \((p<0.05)\) in the LMW fraction adiponectin with increasing doses of insulin in the diabetic subjects but not in the non-diabetic subjects. Combined, this data suggests 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 femoral artery**

The ratio of HMW/total adiponectin is used because it is considered a better index of insulin sensitivity than either total adiponectin levels or absolute levels of HMW. This ratio is termed the adiponectin sensitivity index \((S_A)\). \(S_A\) was significantly lower in diabetics compared to non-diabetic subjects \((p<0.01)\) at baseline \(0.4 \pm 0.04 \, \text{vs.} \, 0.71 \pm 0.05\) and during the high dose insulin infusion \(0.36 \pm 0.04 \, \text{vs.} \, 0.52 \pm 0.02; \, p<0.01\). Importantly, hyperinsulinemia had less of an effect on \(S_A\) in the diabetics than in the non-diabetics. This indicates that in insulin-sensitive individuals, there is a disproportionate loss of the HMW form relative to total levels.

**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 to the levels measured in the hepatic vein in either diabetic or non-diabetic subjects at baseline \((\Delta=0.20 \pm 1.0 \, \text{vs.} \, \Delta=-0.19 \pm 1.8)\) and during the high dose insulin infusion \((\Delta=0.9 \pm 0.7 \, \text{diabetic} \, \text{vs.} \, \Delta=-0.91 \pm 1.5 \, \text{non-diabetic})\), indicating that there was no *net* release or uptake of adiponectin in the splanchnic bed in either group (Fig. 4, upper panel). Upon measuring the different complexes, the differences in the HMW forms did not reach statistical significance, neither in diabetics nor in non-diabetic subjects at baseline \((\Delta=0.33 \pm 0.43 \, \text{vs.} \, \Delta=1.04 \pm 1.54)\) or during the high dose insulin infusion \((\Delta=0.07 \pm 0.4 \, \text{diabetic} \, \text{vs.} \, \Delta=-0.41 \pm 0.82 \, \text{non-diabetic})\) (Fig. 4, middle panel). Small, but non-significant changes were seen for the LMW form at baseline \((\Delta=-0.3 \pm 0.5 \, \text{diabetic} \, \text{vs.} \, \Delta=0.5 \pm 0.7 \, \text{non-diabetic})\) and during the high dose insulin infusion \((\Delta=-1.1 \pm 0.4 \, \text{diabetic} \, \text{vs.} \, -0.5 \pm 0.8 \, \text{diabetic})\) (Fig. 4 lower panel). This suggests that both in the basal and in the insulin-stimulated state, the splanchnic bed does not make any significant net contributions towards systemic changes.

**DISCUSSION**

We report that the HMW form of adiponectin is prone to be reduced under hyperinsulinemic conditions, particularly amongst insulin-sensitive non-diabetic 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 induced by PPAR agonists. The differential response is likely due to a differential impact of insulin on the levels of these chaperones in adipocytes from diabetics and non-diabetics. Our observations that stimulation with PPAR γ agonists leads to an increase of circulating adiponectin, primarily due to an increase in the HMW form (11) has originally highlighted the potential importance of the HMW. This high molecular weight 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 towards a unique local profile of adiponectin complexes that subsequently are efficiently extracted by the liver.

The methodology employed in these studies is able to effectively separates 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 papers have compared the correlation coefficients of various parameters with either absolute levels of HMW or HMW/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, HMW/total is a superior read out indeed suggests that a competitive relationship may exist between HMW and the other adiponectin forms. It is yet unknown if these complexes individually bear any physiological meaning and future experiments will be required to separate these forms.

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REFERENCES


FIGURE LEGENDS

Figure 1
Plasma glucose and insulin concentrations observed in the diabetic and non-diabetic subjects at baseline and during a hyperglycemic clamp. The insulin, somatostatin, glucagon and growth hormone infusions started at time 0.

Figure 2
Total, high molecular weight and low molecular weight adiponectin concentrations observed in the diabetic and non-diabetic subjects in the femoral artery at baseline and during the high dose insulin infusion. # p<0.05 and * p<0.01 vs. DM and † p<0.001 vs. basal.

Figure 3
The ratio of the high molecular weight fraction and total adiponectin concentrations observed in the diabetic and non-diabetic subjects in the femoral artery at baseline and during the high dose insulin infusion. * p<0.01 vs DM and † p<0.001 vs basal.

Figure 4
Total, high molecular weight and low molecular weight adiponectin concentration gradient observed across the splanchnic bed (i.e. femoral artery minus hepatic vein) in diabetic and non-diabetic subjects at baseline and during the high dose insulin infusion. No significant differences were observed.
Figure 1
Figure 2

**Total Adiponectin**

- Type 2 Diabetes
- Non-diabetic

**High Molecular Weight Adiponectin**

**Low Molecular Weight Adiponectin**
Figure 3