Plasma Adiponectin and Endogenous Glucose Production in Humans

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OBJECTIVE — High plasma adiponectin is associated with reduced risk of type 2 diabetes, probably a consequence of its insulin-sensitizing properties. In vivo data in rodents suggest that the insulin-sensitization responsible for improvement of glycemia occurs in muscle and liver. Whereas associations of plasma adiponectin with muscle insulin sensitivity in humans have been examined, this has not been done for the liver.

RESEARCH DESIGN AND METHODS — We therefore analyzed the relationship between fasting plasma adiponectin and basal endogenous glucose production [EGP]-basal) and insulin-suppressed EGP (EGP-insulin, isotope dilution technique) in 143 Pima Indians (94 with normal glucose tolerance, 36 with impaired glucose tolerance, and 16 with type 2 diabetes).

RESULTS — Fasting plasma adiponectin concentrations were negatively correlated with EGP-basal and EGP-insulin before ($P = 0.006$ and $P < 0.0001$, respectively) as well as after adjustment for age, sex, percent body fat, and insulin-stimulated whole-body glucose uptake ($P = 0.007$ and $P = 0.0005$, respectively).

CONCLUSIONS — These findings are compatible with the hypothesis that adiponectin increases hepatic insulin sensitivity. Consistent with data in animals, adiponectin may have generalized insulin-sensitizing effects in humans.

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P lasma concentrations of adiponectin, unlike other adipokines, decrease with increasing adiposity (1–4) and increase after weight loss (5,6). Studies in rodents have shown that adiponectin plays an important role in modulating glucose tolerance and insulin sensitivity. Adiponectin knockout mice exhibit severe diet-induced insulin resistance (7,8), and administration of recombinant adiponectin to both lipatrophic and obese rodents is followed by improvement of insulin sensitivity and glycemia (9–11). This is thought to be due to a stimulatory effect of adiponectin on lipid oxidation and muscle and hepatic insulin signaling. In a recent study in mice, however, administration of recombinant adiponectin during a euglycemic clamp enhanced suppression of hepatic gluconeogenesis, while muscle glucose uptake remained unaltered (12).

In healthy humans, high plasma adiponectin concentrations predict a lower incidence rate of type 2 diabetes, independent of obesity (13,14). The mechanisms by which adiponectin exerts beneficial effects on glucose tolerance in humans continue to be investigated. Recent evidence suggests that high plasma adiponectin concentrations are associated with increased insulin-stimulated glucose disposal during a hyperinsulinemic-euglycemic clamp (15–17), suggesting that insulin sensitization of muscle is one of the mechanisms by which adiponectin improves glycemia. Whether plasma adiponectin concentrations are also associated with measures of hepatic insulin sensitivity has not yet been investigated.

Because plasma adiponectin concentrations are lower in individuals with impaired glucose tolerance and lowest in diabetic subjects compared with healthy individuals (15), we hypothesized that if adiponectin influences hepatic insulin sensitivity, hypoadiponectinemia may contribute to the increase in endogenous glucose production (EGP) that characterizes the progression of the disease (18). Therefore, we tested the hypothesis that fasting plasma adiponectin concentrations are negatively associated with basal- and insulin-suppressed EGP, independent of insulin-stimulated glucose uptake. For this purpose we analyzed data from hyperinsulinemic-euglycemic clamps performed in combination with isotopic determination of EGP in a large cohort of Pima Indians covering a wide range of glucose tolerance.

RESEARCH DESIGN AND METHODS
— A total of 143 subjects (Pima Indians) who were participants in ongoing studies of the pathogenesis of obesity and type 2 diabetes were included in this analysis. A subgroup of these individuals was included in previous publications describing the relationship between fasting plasma adiponectin concentrations and insulin sensitivity (15,16). All subjects were between 18 and 50 years of age, nonsmokers at the time of the study, and except for the 16 subjects with type 2 diabetes and mild fasting hyperglycemia (range 4–7 mmol/l), healthy according to a physical examination and routine laboratory tests.

Table 1—*Anthropometric and metabolic characteristics of the study population*

	Normal glucose tolerance	Impaired glucose tolerance	Diabetes	P*
n (male/female)	94 (73/21)	33 (20/13)	16 (5/11)	
Age (years)	28 ± 7	33 ± 8	30 ± 5	0.002
Body fat (%)	30 ± 8	35 ± 6	40 ± 6	<0.0001
Fasting glucose (mmol/l)	4.58 ± 0.50	5.10 ± 0.55	5.78 ± 0.72	<0.0001
2-h glucose (mmol/l)	5.54 ± 1.21	8.79 ± 0.98	12.82 ± 1.72	<0.0001
Fasting insulin (pmol/l)	232 ± 123	330 ± 126	387 ± 116	<0.0001
2-h insulin (pmol/l)	846 ± 726	2,076 ± 1,344	2,466 ± 1,764	0.0005
M (mg · kg EMBS^{-1} · min^{-1})	3.00 ± 1.42	2.08 ± 0.39	1.93 ± 0.20	<0.0001
EGP-basal (mg · kg EMBS^{-1} · min^{-1})	1.94 ± 0.24	1.98 ± 0.27	2.14 ± 0.21	0.01
EGP-insulin (mg · kg EMBS^{-1} · min^{-1})	0.31 ± 0.36	0.58 ± 0.32	0.82 ± 0.34	<0.0001
Adiponectin (μg/ml)	7.05 ± 2.70	5.44 ± 2.23	5.12 ± 1.75	0.0004

Data are means ± SD. EMBS, estimated metabolic body size. *ANOVA for statistical differences.

Subjects included in this analysis covered a wide range of glucose tolerance: 94 with normal glucose tolerance, 36 with impaired glucose tolerance, and 16 with type 2 diabetes, according to the 1997 World Health Organization diagnostic criteria (19). Subjects with diabetes, all newly diagnosed at the time of their visit, were not taking any diabetes medications. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation.

Subjects were admitted for 8–10 days to the National Institutes of Health Clinical Research Unit in Phoenix, Arizona, where they were fed a weight-maintaining diet (50% of calories as carbohydrate, 30% as fat, and 20% as protein) and abstained from strenuous exercise. After at least 3 days on the diet, subjects underwent a series of tests for the assessment of body composition, glucose tolerance, and insulin sensitivity.

Body composition was estimated by underwater weighing with determination of residual lung volume by helium dilution (20) or by total body dual-energy X-ray absorptiometry (Lunar Corporation, Madison, WI) (21,22). Percent body fat, fat mass, and fat-free mass were calculated as previously described (23), and a conversion equation (22) was used to make measurements comparable between the two methods.

After a 12-h overnight fast, subjects underwent a 75-g oral glucose tolerance test. Baseline blood samples were drawn for the determination of fasting plasma glucose, insulin, and adiponectin concentrations. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and was also measured at 2-h after glucose ingestion for assessment of glucose tolerance. Plasma insulin concentrations were determined by an automated radioimmunoassay analyzer (Concept 4, Horsham, PA). Blood samples for the measurement of fasting plasma adiponectin concentrations were drawn with prechilled syringes, transferred into prechilled EDTA tubes, and immediately placed on ice. All tubes were cold centrifuged (+4°C) within several minutes of collection and stored at −70°C until assayed at the Department of Internal Medicine and Molecular Sciences, Osaka University, Japan. Fasting plasma adiponectin concentration was determined using a validated sandwich enzyme-linked immunosorbent assay using a mouse monoclonal adiponectin-specific antibody (intra-assay and interassay coefficients of variation 3.3% and 7.4%, respectively) (5).

Insulin action was assessed at physiological insulin concentrations during a hyperinsulinemic-euglycemic glucose clamp as previously described (18,24). In brief, after an overnight fast, a primed continuous intravenous insulin infusion

was administered for 100 min at a constant rate of 40 mU · m^{-2} body surface area · min^{-1} leading to a steady-state plasma insulin concentration. Plasma glucose concentration was maintained at ~5.5 mmol/l with a variable infusion of a 20% glucose solution. The rate of total insulin-stimulated glucose disposal (*M*) was calculated for the last 40 min of insulin infusion. *M* was also corrected for EGP. The rate of EGP was measured by a primed (1.1 MBq), continuous (0.11 MBq per min) 3-^{3}H-glucose infusion in the basal state (EGP-basal) and during the hyperinsulinemic-euglycemic clamp (EGP-insulin), calculated by the Steele equation (25) and normalized to estimated metabolic body size (estimated metabolic body size = fat-free mass + 17.7 kg). The *M* value was additionally adjusted for the steady-state plasma glucose and insulin concentrations as previously described and normalized to estimated metabolic body size.

Statistical analyses

Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are given as means ± SD. Fasting plasma adiponectin concentrations, insulin concentrations, and *M* were logarithmically transformed to approximate a normal distribution.

Relationships between variables were examined with the calculation of Pearson correlation coefficients. Because some of the subjects were related to each other, all analyses were performed after adjustment for family membership in generalized estimating equation regression models (PROC GENMOD) of the SAS procedure that account for nuclear family membership and, thus, allow analyses with all individuals in a sibship (26). In these models, fasting and 2-h plasma glucose concentrations and fasting plasma adiponectin concentrations were adjusted for age, sex, percent body fat, and *M*. EGP-basal was also adjusted for these covariates, and the associations were also tested after replacing *M* by fasting insulin concentrations in the model. In some individuals, EGP-insulin was zero (100% suppression of EGP during the clamp). This creates a nonnormal distribution of the data. We present these data, therefore, as a dichotomous trait (i.e., individuals either suppressed EGP by 100% or not) as well as a quantitative variable. Logistic regression was used to determine the effect
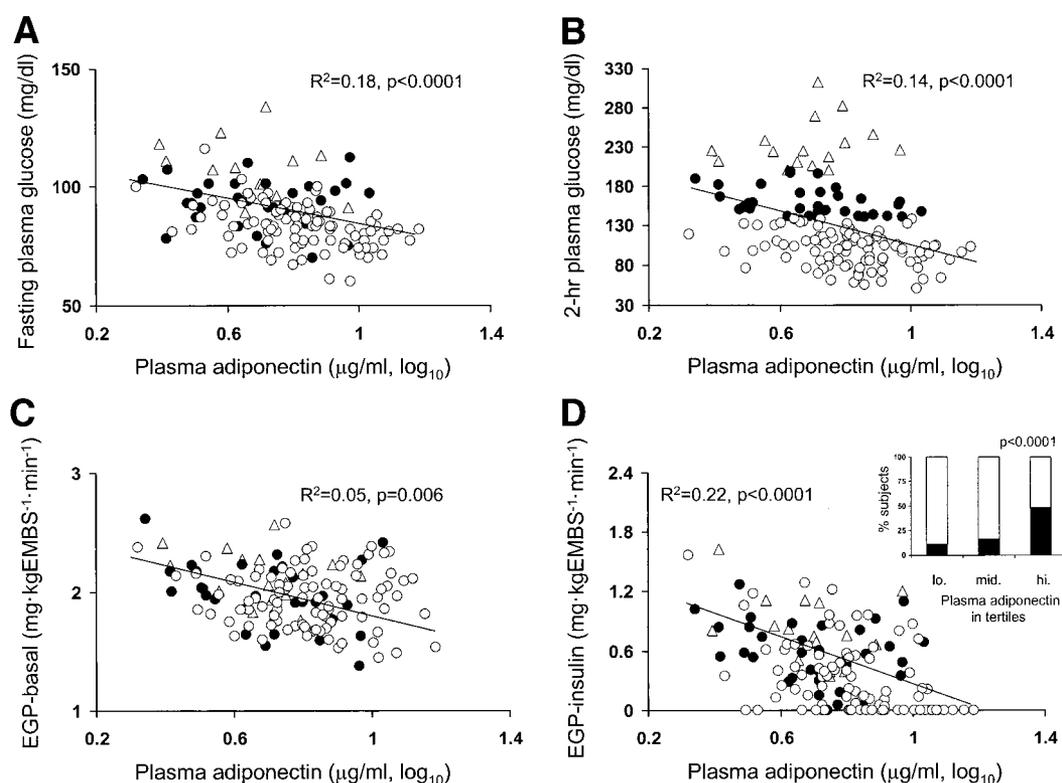
Figure 1—*Relationship of plasma adiponectin with fasting (A) and 2-h (B) plasma glucose concentrations, basal EGP (EGP-basal) (C), and EGP during the clamp (EGP-insulin) (D). ○, normal glucose tolerance; ●, impaired glucose tolerance; △, type 2 diabetes. The insert (D) shows the percentage of subjects who suppressed EGP during the clamp by 100% (black area) and the percentage of those who did not suppress EGP by 100% (white area) by tertiles of plasma adiponectin concentration (n = 23, 7.1–15.3 μg/ml compared with the middle [n = 8, 5.2–7.1] and lower [n = 5, 2.1–5.1] tertile range). The P value indicates the significance for the different distributions in a χ² test. Data were not adjusted for covariates.*

of plasma adiponectin on this binomial variable after adjustment for age, sex, percent body fat, and M.

RESULTS — The anthropometric and metabolic characteristics of the subjects included in the analysis are summarized in Table 1.

Plasma adiponectin was negatively associated with fasting and 2-h plasma glucose (Fig. 1A and B), EGP-basal, and EGP-insulin (Fig. 1C and D). These associations were independent of age, sex, percentage body fat, and M (Tables 2 and 3). The association between plasma adiponectin and EGP-basal was similar whether we used M or fasting insulin as a covariate. The associations between adiponectin and EGP were also independent of glucose tolerance status (normal glucose tolerance, impaired glucose tolerance, or diabetes), which was included in the model as a categorical variable, or 2-h glucose, which was included as a continuous variable (data not shown). When subjects who were normal glucose toler-

ant were analyzed separately, plasma adiponectin was negatively associated with fasting ($R^2 = 0.17$, $P < 0.0001$) and 2-h (0.10, $P = 0.002$) plasma glucose and EGP-insulin (0.19, $P < 0.0001$), but the association with EGP-basal was not statistically significant (0.01, $P = 0.30$). These associations were independent of age, sex, percentage body fat, and M ($P = 0.001$, $P = 0.03$, and $P = 0.008$, respectively).

Because of the skewed distribution of EGP-insulin, we categorized subjects into suppressors and nonsuppressors according to whether EGP-insulin was completely suppressed or not. The total number of suppressors in the whole group of 143 subjects was 36. A total of 107 subjects did not suppress EGP completely. We divided plasma adiponectin concentrations in the whole group into tertiles and found more suppressors in the upper ($n = 23$; adiponectin concentration, 7.1–15.3 μg/ml) compared with the middle (8; 5.2–7.1) and the lower tertiles (5; 2.1–5.1) (range, $P < 0.0001$, χ^2) (Fig. 1D, insert). In a logistic regression, low

plasma adiponectin concentrations were associated with less suppression of EGP during insulin infusion before ($P < 0.03$) and after adjustment for age, sex, percentage body fat, and M (Table 2).

To determine the relative contribution of adiponectin to glycemia, we investigated whether adjustment of M, EGP-basal, and EGP-insulin for plasma adiponectin affected the strength of the correlation of these variables with fasting and 2-h plasma glucose by comparing R^2 values. Fasting plasma glucose was negatively associated with M ($R^2 = 0.18$, $P < 0.0001$) and positively with EGP-basal (0.08, $P = 0.0005$). Two-hour plasma glucose was also negatively associated with M (0.28, $P < 0.0001$) and positively with EGP-insulin (0.18, $P < 0.0001$). Upon adjusting for plasma adiponectin, the associations of EGP-basal and M with fasting plasma glucose ($R^2 = 0.05$, $P = 0.007$; $R^2 = 0.04$, $P < 0.02$) and M and EGP-insulin with 2-h plasma glucose (0.13, $P < 0.0001$; 0.08, $P = 0.0006$) were less strong, which suggests

Table 2—*Determinants of fasting plasma glucose concentrations and EGP-basal (linear model)*

Variable	Estimate	P
Fasting glucose		
Intercept	88.7720	<0.0001
Age	0.1974	0.11
Sex	−2.7404	0.22
Body fat	0.3387	0.03
M ($\log_{10}$)	−13.3038	0.04
Adiponectin ($\log_{10}$)	−13.3978	0.02
EGP-basal		
Intercept	1.9247	<0.0001
Age	−0.0025	0.36
Sex	−0.0713	0.24
Body fat	0.0075	0.05
M ($\log_{10}$)	0.4884	0.001
Adiponectin ($\log_{10}$)	−0.3381	0.007

that adiponectin may act on glycemia through these parameters.

CONCLUSIONS— The main finding of the present study was the negative correlation between plasma adiponectin and both basal- and insulin-suppressed EGP. This relationship was independent of sex, percentage body fat, and, most importantly, insulin-stimulated glucose uptake. In this population with a wide range of glucose tolerance, basal EGP was a strong determinant of fasting plasma glu-

Table 3—*Determinants of 2-h plasma glucose (oral glucose tolerance test, linear model) and EGP-insulin (logistic regression)*

Variable	Estimate	P
2-h glucose		
Intercept	165.2289	<0.0001
Age	0.7986	0.04
Sex	−12.9401	0.29
Body fat	0.7918	0.20
M ($\log_{10}$)	−109.9040	<0.0001
Adiponectin ($\log_{10}$)	−43.1072	0.03
EGP-insulin		
Intercept	6.1780	0.73
Age	−0.0239	0.97
Sex	−12.9401	0.29
Body fat	0.0444	0.25
M ($\log_{10}$)	−6.6874	0.02
Adiponectin ($\log_{10}$)	−3.8987	0.03

cose, and insulin-suppressed EGP was likewise a determinant of 2-h plasma glucose. Adjusting both basal EGP and insulin-suppressed EGP for plasma adiponectin rendered the associations substantially weaker, although it did not completely abolish them. This suggests that the association of high plasma adiponectin with reduced risk of diabetes is, at least in part, secondary to its association with lower EGP.

These findings are compatible with the hypothesis that adiponectin renders the liver more insulin sensitive. The identification of the adiponectin receptor in liver (and muscle) generally supports the concept of a direct effect of adiponectin (27). To our knowledge, other human data examining relationships between adiponectin and hepatic glucose metabolism are not available. A recent study in Caucasians, nevertheless, demonstrated a strong positive correlation between plasma adiponectin and serum HDL cholesterol, which was independent of insulin sensitivity of glucose disposal measured by the euglycemic-hyperinsulinemic clamp (17). Because hepatic HDL synthesis is an insulin-sensitive process (28), this may be interpreted as indirect evidence for greater hepatic insulin sensitivity accompanying high-plasma adiponectin and provides indirect support for an insulin-sensitizing effect in the liver.

In rodents, recombinant adiponectin not only lowered basal- and insulin-suppressed glucose production and gluconeogenesis but also altered expression and activity of key gluconeogenic enzymes in the liver. This was indicative of an insulin-sensitizing or insulin-mimetic effect of adiponectin in the liver. However, no effect on peripheral glucose uptake was observed in this study (12). A more unifying concept emerges from in vivo studies of mice treated with adiponectin. In both muscle and liver, increased cAMP-dependent protein kinase activity was observed (29). This enzyme has a pivotal role in the regulation of both key gluconeogenic enzymes (e.g., phosphoenolpyruvate kinase and glucose-6-phosphatase) and enzymes involved in the regulation of fatty acid oxidation (coenzyme A carboxylase) in muscle, ultimately favoring insulin action in these tissues.

It is important to note that thus far we only have circumstantial evidence for di-

rect effects of adiponectin on insulin sensitivity of peripheral tissues in humans. As insulin was shown to suppress plasma adiponectin concentrations in humans (30), it is still possible that adipose tissue remains selectively sensitive to the inhibitory effects of insulin on adiponectin and that the association of hypoadiponectinemia and insulin resistance is correlative due to hyperinsulinemia and not causative. In the study by Yu et al. (30), however, supraphysiological concentrations of insulin were maintained for 5 h, and the inhibitory effect of insulin measured at the end of this period was relatively small. Therefore, a definitive clarification on the role of adiponectin in human physiology will only be achieved when adiponectin for human administration will be available.

In conclusion, plasma adiponectin is negatively and independently associated with both basal- and insulin-suppressed EGP in humans. These findings are compatible with the hypothesis generated by animal studies that adiponectin increases hepatic insulin sensitivity. Consistent with data in animals, adiponectin may have generalized insulin-sensitizing effects in humans.

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