# Low Plasma Adiponectin Concentrations Do Not Predict Weight Gain in Humans

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**Low concentrations of plasma adiponectin, the most abundant adipose-specific protein, are observed in obese individuals and predict the development of type 2 diabetes. Administration of adiponectin to rodents prevented diet-induced weight gain, suggesting a potential etiologic role of hypoadiponectinemia in the development of obesity. Our aim was to prospectively examine whether low plasma adiponectin concentrations predict future weight gain in Pima Indians, explaining the predictive effect of adiponectin on the development of type 2 diabetes. We measured plasma adiponectin concentrations in 219 nondiabetic Pima Indians (112 M/107 F, age 31 ± 9 years, body weight 96 ± 20 kg [mean ± SD]) in whom body weight and height were measured and BMI calculated at baseline and follow-up. Cross-sectionally, plasma adiponectin concentrations were negatively associated with body weight ($r = -0.28$, $P = 0.0001$). Prospectively, plasma adiponectin concentrations at baseline were not associated with change in weight or BMI before or after adjustment for time of follow-up or after additional adjustment for age at follow-up and sex (all $P > 0.3$). Our data suggest that low plasma adiponectin concentrations do not play an etiologic role in development of obesity in Pima Indians. Therefore, the predictive effect of low plasma adiponectin concentrations on the development of type 2 diabetes seems to be mediated by factors other than increased adiposity.**
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Adiponectin has been recently identified as an adipose-derived protein with important metabolic effects. It is exclusively expressed in adipose tissue and released into the circulation (1,2). Plasma adiponectin concentrations are decreased in individuals with obesity (3), insulin resistance (4), and type 2 diabetes (3). It was shown that plasma adiponectin concentrations increased after weight reduction in obese nondiabetic and diabetic subjects (5) and severely obese subjects following bariatric surgery (6). Administration of adiponectin to mice prevented diet-induced obesity (7), suggesting that low plasma adiponectin concentrations may not be the result of obesity but instead contribute to body weight gain.

We have recently shown that low plasma adiponectin concentrations predict a decrease in insulin sensitivity (8) and the development of type 2 diabetes (9). Hypothetically, this prospective relationship between low plasma adiponectin concentrations and insulin resistance and/or type 2 diabetes could be mediated, at least in part, by an effect of this hormone on body weight gain. Our aim was to examine prospectively whether low plasma adiponectin concentrations predict subsequent changes in body weight in Pima Indians, a population with one of the highest prevalence rates of obesity (10).

## RESEARCH DESIGN AND METHODS

**Subjects.** A total of 219 Pima (or closely related Tohono O'odham) Indians (Table 1) were selected from two different ongoing longitudinal studies.

Ninety-eight subjects were selected from a longitudinal study of the pathogenesis of obesity and type 2 diabetes (study 1) performed at the Clinical Diabetes and Nutrition Section of the National Institutes of Health. After a baseline examination, which included weight and waist and thigh circumference measurements and a 75-g oral glucose tolerance test (OGTT), subjects were invited back at approximately annual intervals for repeated weight measurements and OGTTs. The group of subjects with adiponectin measurements was originally selected for a cross-sectional study designed to evaluate the relationships between plasma adiponectin concentrations, adiposity, and insulin sensitivity (3). All subjects were on weight-maintaining diets at least for 2 days, nonsmokers, and healthy according to a physical examination and routine laboratory tests. Subjects were nondiabetic (11) at baseline and follow-up examinations.

One hundred twenty-one subjects were selected from the longitudinal study of diabetes and its complications (study 2) conducted in the Gila River Indian Community. Residents of this community are invited to participate every 2 years, regardless of their health status. Each participant undergoes a physical examination, which includes height, weight, and waist and thigh circumference measurements and 75-g OGTTs. The group of subjects with adiponectin measurements was originally selected for a case-control series designed to evaluate the prospective relationship between plasma adiponectin concentrations and the risk of development of type 2 diabetes (9). The subjects had normal glucose tolerance at baseline (fasting plasma glucose <110 mg/dl and 2-h plasma glucose <140 mg/dl) and were nondiabetic at follow-up (11).

The protocols were 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. All subjects provided written informed consent before participation.

**Methods.** After a 12-h overnight fast, all subjects underwent a 75-g OGTT for assessment of glucose tolerance according to World Health Organization diagnostic criteria (11). BMI was calculated as the ratio of weight (in kilograms) divided by height (in meters) squared. Waist and thigh circumferences were measured at the umbilicus in the supine position and at the gluteal fold in the standing position. The waist-to-thigh ratio (WTR) was calculated as an index of body fat distribution.

In study 1, plasma adiponectin concentrations were measured from fasting plasma samples drawn during OGTT with prechilled syringes, transferred into prechilled EDTA tubes, and immediately placed on ice. All tubes were

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OGTT, oral glucose tolerance test; WTR, waist-to-thigh ratio.

TABLE 1
Anthropometric characteristics of the study population

| | Study 1 (72 M/26 F) | | Study 2 (40 M/81 F) | |
|---|---|---|---|---|
| | Baseline | Follow-up | Baseline | Follow-up |
| Age (years) | 29 ± 7 | 31 ± 7 | 33 ± 9 | 36 ± 10 |
| Height (cm) | 170 ± 8 | 170 ± 8 | 164 ± 7 | 164 ± 7 |
| Body weight (kg) | 94 ± 22 | 97 ± 23* | 97 ± 20 | 101 ± 21* |
| BMI (kg/m$^2$) | 33 ± 7 | 34 ± 8† | 36 ± 7 | 38 ± 7† |
| WTR | 1.65 ± 0.14 | 1.67 ± 0.17‡ | 1.66 ± 0.21 | 1.70 ± 0.20‡ |
| Body fat (%) | 30 ± 8 | 31 ± 6† | — | — |
| Adiponectin (μg/ml) | 6.9 ± 2.7 | — | 5.1 ± 1.7 | — |
| Follow-up time (years) | — | 1.8 ± 1.7 | — | 3.0 ± 2.0 |

Data are means ± SD. Significant differences between baseline and follow-up variables in the longitudinal analysis (except for age, paired *t* test, *$P < 0.0001$, †$P < 0.05$, ‡$P < 0.001$.

cold-centrifuged (4°C) within several minutes of collection and stored at –70°C until assayed. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Body composition was estimated by underwater weighing, with simultaneous determination of residual lung volume by helium dilution (12) or by total-body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) with calculations of percent body fat, fat mass, and fat-free mass as previously described (13).

In study 2, plasma adiponectin concentrations were measured from 2-h samples during OGTT. Blood samples were drawn and stored at room temperature until centrifugation (within 3 h). Samples were chilled to –20°C and stored at −70°C. Plasma glucose concentrations were determined by potassium ferrocyanide (Technicon) or hexokinase method (Chiron).

All samples were assayed for adiponectin at the Department of Internal Medicine and Molecular Sciences, Osaka University, Japan. Plasma adiponectin concentrations were determined using a validated sandwich enzyme-linked immunosorbent assay employing an adiponectin-specific antibody (intra-assay and interassay coefficients of variation, 3.3 and 7.4%, respectively). Because plasma adiponectin concentrations show no diurnal change (5) and a pilot study indicated no significant differences in adiponectin concentration in plasma samples taken fasting or 2 h after an OGTT (2 h, 96% of fasting; absolute difference, 0.26 ± 0.3 μg/ml; paired *t* test, $P = 0.44$; unpublished data, R.S.L., J.K., W.C.K.), plasma adiponectin measurements from studies 1 and 2 were pooled together.

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

The relationship between plasma adiponectin concentrations and body weight was examined by calculating Pearson correlation coefficients. The relationships between plasma adiponectin concentrations at baseline and changes in weight and/or other measures of adiposity (follow-up adjusted for baseline) were examined by multiple linear regression models to allow adjustment for covariables. Differences in anthropometric measurements between the visits were assessed by unpaired Student's *t* test.

## RESULTS

The anthropometric characteristics and plasma adiponectin concentrations of the subjects included in the study are summarized in Table 1.

Cross-sectionally, in the whole group, plasma adiponectin concentrations were negatively associated with body weight ($r = -0.28$, $P = 0.0001$), BMI ($r = -0.38$, $P = 0.0001$), and WTR ($r = -0.24$, $P = 0.0001$), but not waist circumference ($r = -0.10$, $P = 0.2$). In study 1, percent body fat ($r^2 = 0.25$, $P = 0.0001$) was the only independent determinant of plasma adiponectin concentrations. In study 2, BMI ($r^2 = 0.16$, $P = 0.0001$) and age ($r^2 = 0.01$, $P = 0.03$) were independent determinants of plasma adiponectin concentrations.

Prospectively, in the whole group, plasma adiponectin concentrations at baseline were not associated with change in weight after adjustment for time of follow-up ($r = -0.72$, $P = 0.8$) and additional adjustment for age at follow-up and sex ($r = -0.31$, $P = 0.9$). When the term for participation in study 1 or 2 was included in the model, study was not a significant determinant of the weight change after adjustment for age at follow-up, time of follow-up, and sex ($P = 0.7$). The relationship between plasma adiponectin and weight change did not differ between subjects in studies 1 and 2 after adjustment for age at follow-up, time of follow-up, and sex ($P = 0.4$ for interaction). Plasma adiponectin concentrations at baseline were not associated with change in BMI after adjustment for age at follow-up, sex, and time of follow-up ($P = 0.8$). Similarly, plasma adiponectin concentrations at baseline were not associated with change in waist circumference ($P = 0.8$) or WTR ($P = 0.2$) after adjustment for age at follow-up, sex, time of follow-up, and baseline weight.

When analysis was performed separately in study 1 and study 2, plasma adiponectin concentrations were not associated with changes in body weight (Fig. 1), BMI, or WTR (data not shown).

In 98 Pima Indians (study 1) who had measurements of percent body fat at baseline and follow-up, plasma adiponectin concentrations at baseline were not associated with change in percent body fat before and after adjustment for age at follow-up, sex, and time of follow-up (both $P = 0.9$).

Interaction between adiponectin and follow-up time was not significant in any of these models ($P > 0.05$).

## DISCUSSION

In the present study, we found that low plasma adiponectin concentrations do not predict future weight gain.

In mice, administration of adiponectin prevented the development of diet-induced obesity by increasing muscle lipid oxidation (7). We have previously shown that plasma adiponectin concentrations are not associated with fat oxidation in humans (14). In the present study, we showed that plasma adiponectin concentrations at baseline were not associated with subsequent change in weight and/or other measures of adiposity (BMI, WTR, or percent fat). This suggests that physiologic plasma concentrations of adiponectin do not seem to play an etiologic role in development of obesity in humans. Our finding is supported by two recent studies showing that body weight is not increased in adiponectin-deficient mice (15,16). It is still possible, however, that administration of adiponectin

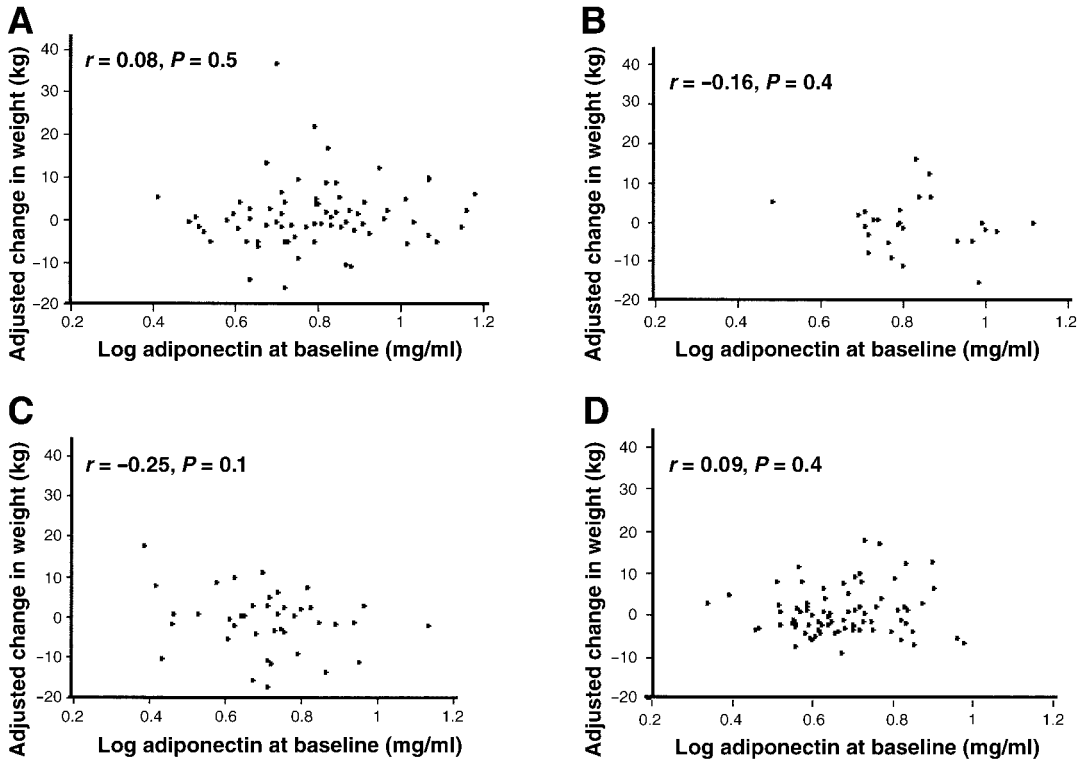


**FIG. 1. Relationship between plasma adiponectin at baseline and change in body weight (follow-up adjusted for baseline, adjusted for age at follow-up, sex, and time of follow-up) in 98 Pima Indians in study 1 (*A*, males; *B*, females) and 121 Pima Indians in study 2 (*C*, males; *D*, females).**

may decrease body weight in humans. This cannot be resolved in an observational study.

Other studies indicate that plasma adiponectin concentrations increase after weight reduction. Hotta et al. (5) showed that plasma adiponectin concentrations increased after weight reduction in both obese nondiabetic and diabetic subjects after 2 months on a calorie-restricted diet. Moreover, Yang et al. (6) showed that plasma adiponectin concentrations increased in severely obese subjects following bariatric surgery during an average follow-up of 8 months. These studies together with ours suggest that low plasma adiponectin concentrations are most likely the consequence rather than the cause of obesity.

Low plasma adiponectin concentrations predict decrease in insulin sensitivity and the development of type 2 diabetes in Pima Indians (8,9), a population with a high prevalence of type 2 diabetes (17). Because obesity is an important risk factor for the development of insulin resistance (17) and obesity is associated with low plasma adiponectin concentrations, adiponectin might influence insulin sensitivity via change in adiposity. The present study showed that low plasma adiponectin concentrations are not associated with weight gain in Pima Indians, suggesting that the relationship between plasma adiponectin concentrations and insulin sensitivity and/or type 2 diabetes is mediated by factors other than increased adiposity. Our observation is consistent with the association between low plasma adiponectin levels and insulin resistance in patients with generalized lipodystrophies (18).

Because information about adiponectin variation over time is scarce in humans, a limitation of our study is that our observation is based on a single fasting plasma adiponectin measurement. However, Hotta et al. (5) have shown no meal-related or circadian changes in plasma adiponectin concentrations.

In conclusion, low plasma adiponectin concentrations do not appear to have an etiologic role in development of obesity in Pima Indians.

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## REFERENCES

1. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
2. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K: cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 221:286–289, 1996
3. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close

association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
4. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
5. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
6. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86:3815–3819, 2001
7. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001
8. Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS, Youngren JF, Havel PJ, Pratley RE, Bogardus C, Tataranni PA: Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes* 51:1884–1888, 2002
9. Lindsay RS, Funahashi T, Hanson R, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin protects against development of type 2 diabetes in Pima Indian population. *Lancet* 360:57–58, 2002
10. Knowler WC, Pettitt DJ, Saad MF, Charles MA, Nelson RG, Howard BV, Bogardus C, Bennett PH: Obesity in the Pima Indians: its magnitude and relationship with diabetes. *Am J Clin Nutr* 53:1543S–1551S, 1991
11. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
12. Siri WE: Body composition from fluid spaces and density: analysis of methods. In: *Techniques for Measuring Body Composition*. Brozek J, Hanschels A, Eds. Washington, DC, National Academy of Sciences, 1961, p. 223–244
13. Tataranni PA, Ravussin E: Use of dual-energy X-ray absorptiometry in obese individuals. *Am J Clin Nutr* 62:730–734, 1995
14. Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Ravussin E, Weyer C, Tataranni P: Plasma adiponectin levels are not associated with fat oxidation in humans. *Obes Res*. In press
15. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
16. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002
17. Knowler WC, Pettitt DJ, Saad MF, Bennett PH: Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 6:1–27, 1990
18. Haque WA, Shimomura I, Matsuzawa Y, Garg A: Serum adiponectin and leptin levels in patients with lipodystrophies. *J Clin Endocrinol Metab* 87:2395, 2002