

CLINICAL STUDY

Adiponectin is independently associated with glycosylated haemoglobin

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Abstract

Background: In humans, adiponectin has been demonstrated to circulate in inverse proportion to the degree of insulin resistance.

Objective: To investigate the association between adiponectin and glycosylated haemoglobin (HbA_{1c}) in a population-based study.

Design and methods: Two hundred and ninety-seven individuals aged 30–75 years were enrolled in a cross-sectional study. They included patients with type 2 (non-insulin-dependent) diabetes mellitus and stable, good metabolic control ($n = 32$) and individuals with glucose intolerance ($n = 54$). Adiponectin was measured using a sandwich enzyme-linked immunosorbent assay (intra-assay and interassay coefficients of variation 3.3 and 7.4% respectively).

Results: Adiponectin correlated with age ($r = 0.161$; $P = 0.006$), body mass index ($r = -0.197$; $P = 0.001$), diastolic blood pressure ($r = -0.181$; $P = 0.005$), fasting glucose and HbA_{1c} ($r = -0.251$ and $r = -0.22$ respectively; $P < 0.0001$), high-density lipoprotein cholesterol ($r = 0.442$; $P < 0.001$) and serum triglycerides ($r = -0.362$; $P < 0.001$). In multiple regression analysis, sex, age, fasting and post-load glucose, and adiponectin independently contributed to 40% of the variance in HbA_{1c}. Among individuals with normal glucose tolerance, fasting glucose ($P = 0.0033$), post-load glucose ($P = 0.0015$), age ($P = 0.001$) and adiponectin ($P = 0.0083$) independently contributed to 21% of the variance in HbA_{1c}.

Conclusion: Adiponectin is significantly associated with altered glucose metabolism and independently contributes to the variance of HbA_{1c} in a population-based manner.

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Introduction

Insulin resistance is a major feature in the aetiology of obesity and type 2 (non-insulin-dependent) diabetes mellitus, and the relationship between insulin resistance and these pathophysiological states is complex. The adipocyte is now known to secrete a variety of proteins such as tumour necrosis factor (TNF)- α , adiponectin, plasminogen activator inhibitor-1, leptin, resistin and adiponectin (1). Because these proteins appear to have structural properties similar to those of cytokines, they have been collectively called 'adipocytokines' and are implicated in a wide range of biological effects. Adiponectin (also called Acrp30 or adipoQ in mice) is a 244 amino acid protein synthesised and secreted exclusively by the adipose tissue (2, 3). Blood concentrations of adiponectin are decreased in obesity, type 2 diabetes and coronary artery disease, and adiponectin circulates in inverse proportion to the degree of insulin

resistance (4–16). ACRP30 is the rodent homologue of adiponectin, and ACRP30 concentrations are lower in mice fed a high-fat diet. Administration of ACRP30 to diabetic *db/db* mice improved insulin resistance and corrected hyperglycaemia and hyperinsulinaemia. Furthermore, administration of this protein enhances insulin sensitivity in normal mice (4–7).

Although hyperglycaemia is the diagnostic criterion and a main prognostic parameter in diabetes, early markers could assist in the evaluation of the best therapeutic approach for diabetes mellitus. Conversely, epidemiological and intervention studies have defined the target values for the control of glycaemia, and there is general consensus that antihyperglycaemic treatment should aim at reducing glycated haemoglobin (HbA_{1c}) concentrations to less than 7% (17–18).

Adiponectin possesses anti-inflammatory and anti-atherogenic properties (19–22), and long-term glycosylation could enhance atherosclerotic risk in the

presence of decreased concentrations of adiponectin. We aimed to explore the association between circulating adiponectin and glycosylated haemoglobin in a population-based study.

Methods

Participants

Two hundred and ninety-seven randomly selected individuals, aged 30–75 years, were enrolled in a population-based cross-sectional study in Asturias, Northern Spain, as described previously (23). They included 32 patients with type 2 diabetes under stable, good metabolic control, and 54 individuals with glucose intolerance.

All participants were of White origin and reported that their body weight had been stable for at least 3 months before the study. None was taking any medication or had any evidence of metabolic disease other than obesity. Inclusion criteria were: body mass index (BMI, weight in kilograms divided by the square of height in metres) $< 40 \text{ kg/m}^2$; absence of any systemic disease; absence of any infections in the previous month. The main clinical characteristics of the participants are shown in Table 1.

Informed written consent was obtained after the purpose, nature and potential risks of the study had been explained to the participants. The experimental procedure was approved by the hospital ethics committee.

Measurements

Each participant was studied in the examination room in the post-absorptive state. Weight and height were measured and BMI calculated. Blood pressure was measured in the right arm with the individual in a supine position after a 10 min rest; a standard sphygmomanometer of appropriate cuff size was used and the first and fifth phases were recorded. Values used

in the analysis are the average of three readings taken at 5 min intervals.

Participants underwent an oral glucose tolerance test (75 g glucose) and were classified as having: normal glucose tolerance (fasting glucose less than 126 mg/dl and 2 h glucose less than 140 mg/dl), glucose intolerance (fasting glucose less than 126 mg/dl and 2 h glucose at least 140 mg/dl but less than 200 mg/dl), or type 2 diabetes (fasting glucose greater than 126 mg/dl or 2 h glucose greater than 200 mg/dl, or both) as described previously (23).

Analytical methods

Serum glucose concentrations were measured in duplicate by a hexokinase method using a Hitachi 747 Glucose Analyzer. The coefficient of variation was less than 3%. HbA_{1c} was measured by high pressure liquid chromatography using a Jokoh HS-10 autoanalyser; the normal range among 774 individuals with normal glucose tolerance was $4.71 \pm 0.46\%$. Total serum cholesterol was measured by the cholesterol esterase–cholesterol oxidase–peroxidase reaction. High-density lipoprotein (HDL) cholesterol was quantified after precipitation with polyethylene glycol and α -cyclodextrin sulphatase at room temperature (Hitachi 747). Serum total triglycerides were measured with the enzymatic lipase glycerol kinase method with colourimetric determination (Hitachi 747); low-density lipoprotein LDL cholesterol was calculated by the Friedewald formula, excluding those individuals with serum triglyceride concentrations greater than 400 mg/dl. Serum insulin concentrations were measured in duplicate by monoclonal IRMA (Medgenix Diagnostics, Fleunes, Belgium). Intra-assay and interassay coefficients of variation of this technique have previously been reported to be less than 7%.

Insulin resistance was measured by the homeostasis model of assessment (HOMA) using baseline glucose and insulin values. HOMA correlates well with insulin sensitivity derived from the glucose clamp technique ($r = -0.82$, $P < 0.0001$), and this strong correlation appears to be independent of sex, age, BMI, diabetes and blood pressure. In our hands, HOMA values also correlate well with insulin sensitivity as measured by the frequently sampled intravenous glucose tolerance test and minimal model analysis ($r = -0.72$, $P < 0.0001$, $n = 46$; unpublished observations).

Circulating adiponectin was measured by a validated sandwich enzyme-linked immunosorbent assay (intra-assay and interassay coefficients of variation were 3.3 and 7.4% respectively) as described previously (11). All adiponectin samples were measured in the same assay.

Statistical methods

Descriptive results of continuous variables are expressed as means \pm S.D. Before statistical analysis,

Table 1 Clinical variables in the study patients.

| | |
|---|------------------|
| Sex (Male/Female) | 137/160 |
| Glucose intolerance/diabetes (<i>n</i>) | 54/32 |
| Age (years) | |
| Men | 53.9 \pm 13.1 |
| Women | 51.4 \pm 13.6 |
| Weight (kg) | |
| Men | 78.5 \pm 11.4 |
| Women | 66.7 \pm 10.9 |
| Body mass index (kg/m^2) | |
| Men | 27.76 \pm 3.77 |
| Women | 27.10 \pm 4.37 |
| Systolic blood pressure (mmHg) | |
| Men | 133.8 \pm 22.1 |
| Women | 127.9 \pm 18.9 |
| Diastolic blood pressure (mmHg) | |
| Men | 83.8 \pm 14.1 |
| Women | 79.8 \pm 12.6 |

normal distribution and homogeneity of the variances were tested. Parameters that did not fulfil these tests (adiponectin, HOMA value) were log-transformed. The relations between variables were analysed by simple correlation (Pearson's r) and multiple regression in a stepwise manner. We included in the multivariate analysis all those variables that were significantly associated with HbA_{1c} in univariate analysis. The level of statistical significance was set at $P < 0.05$.

Results

Biochemical variables for the patients included in the study are summarised in Table 2. Circulating adiponectin concentrations were significantly decreased in patients with type 2 diabetes compared with non-diabetic individuals (7.11 ± 4 mg/l compared with 8.67 ± 3.6 mg/l; $P = 0.012$) and in obese individuals (BMI > 30 kg/m²) compared with those who were not obese (7.5 ± 3.4 kg/m² compared with 8.7 ± 3.6 kg/m²; $P = 0.012$), but were similar in hypertensive and non-hypertensive individuals (8.4 ± 4 mg/l and 8.4 ± 3.5 mg/l respectively; $P = 0.9$). HbA_{1c} values were $4.8 \pm 0.48\%$ among normotolerant participants, $5.2 \pm 0.54\%$ in participants with glucose intolerance and $6.2 \pm 1.5\%$ in those with type 2 diabetes (analysis of variance: $P < 0.0001$). Adiponectin correlated with age ($r = 0.161$; $P = 0.006$), BMI ($r = -0.197$; $P = 0.001$), diastolic blood pressure ($r = -0.181$; $P = 0.005$), fasting glucose (Fig. 1) and HbA_{1c} ($r = -0.25$ and $r = -0.22$ respectively, $P < 0.0001$), HDL cholesterol ($r = 0.442$; $P < 0.001$) and serum triglycerides ($r = -0.362$; $P < 0.001$), but not with post-load glucose concentrations ($r = -0.03$, NS). After controlling for age, however, this last association became significant ($r = -0.14$, $P = 0.02$).

We constructed a multiple regression analysis to predict HbA_{1c}. In the first model, BMI, age, fasting glucose, post-load glucose, adiponectin and sex independently contributed to 40% of the variance in HbA_{1c} (Table 3). Variables that did not reach statistical significance were BMI, HDL-cholesterol, HOMA value and triglyceride concentrations (Table 3). We then performed the multiple regression analysis in normotolerant participants and patients with altered glucose tolerance separately. Among participants with normal glucose

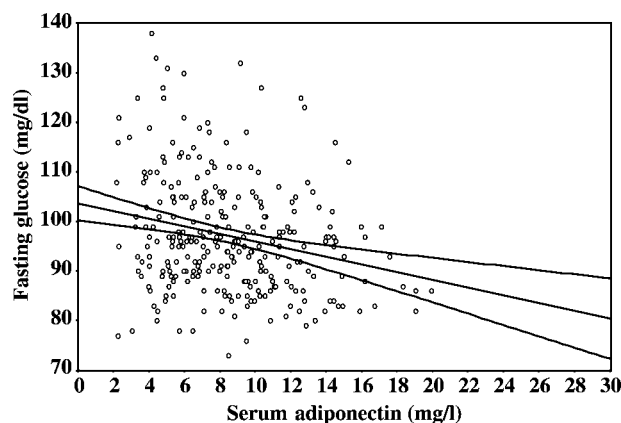


Figure 1 Linear correlation analysis between circulating adiponectin and fasting glucose concentrations.

tolerance, fasting glucose ($P = 0.0033$), post-load glucose ($P = 0.0015$), age ($P = 0.001$) and adiponectin concentration ($P = 0.0083$) independently contributed to 21% of the variance in HbA_{1c}. Excluded variables were BMI, sex, HDL-cholesterol, triglycerides, diastolic blood pressure and HOMA value. In patients with altered glucose tolerance, fasting glucose ($P < 0.00001$), sex ($P = 0.0027$) and age ($P = 0.023$) contributed to 42% of the variance in HbA_{1c}. Excluded variables were adiponectin ($P = 0.09$), BMI, HDL-cholesterol, triglyceride concentrations and HOMA value.

Discussion

In this article we report that, in a population-based study, adiponectin was found to circulate in proportion to HbA_{1c}, independently of fasting and post-load glucose, sex and age. In fact, all these parameters contributed to 40% of HbA_{1c} concentration. Interestingly, this result was maintained in individuals with normal glucose tolerance. This finding indicates that adiponectin could constitute an early marker that could assist in evaluation of the initial stages of worsening of glucose metabolism. It could be proposed that measurement of adiponectin concentration would aid the identification of high-risk individuals (those with HbA_{1c} concentrations above a particular threshold), independently of serum glucose concentration.

Despite the known relationship between adiponectin and insulin resistance, we have not found previous studies aimed at examining this association. However, the relationship of adiponectin with HbA_{1c} fits well with the observation that treatment with glitazones led to increased adiponectin and decreased HbA_{1c} concomitantly (24, 25). After treatment with troglitazone, a significant correlation was found between relative changes in adiponectin concentrations and fasting glucose ($r = -0.67$, $P < 0.05$) (25). The relationship with HbA_{1c} was not specifically addressed in that study.

Table 2 Laboratory variables in the study patients.

| | |
|-------------------------------|------------|
| Cholesterol (mg/dl) | 230.9±42.8 |
| LDL-cholesterol (mg/dl) | 152.3±38.2 |
| HDL-cholesterol (mg/dl) | 57.3±14.8 |
| Fasting triglycerides (mg/dl) | 107.2±61.3 |
| Fasting glucose (mg/dl) | 96.2±10.8 |
| Adiponectin (mg/l) | |
| Men | 4.5–8 |
| Women | 7.8–12.2 |

Values are means ± s.d. or interquartile range.

Table 3 Multiple regression analysis. (Multiple *R*: 0.63159; *R*²: 0.39890; Adjusted *R*²: 0.38805; Standard error: 0.45660).

| Variables included in the equation | Beta | T | Sig T |
|--------------------------------------|--------|--------|--------|
| Fasting glucose | 0.282 | 4.498 | 0.0000 |
| Post-load glucose | 0.243 | 3.81 | 0.0002 |
| Sex | 0.128 | 2.3 | 0.0182 |
| Log ₁₀ adiponectin | -0.178 | -3.1 | 0.0016 |
| Age | 0.274 | 5.2 | 0.0000 |
| (Constant) | | 11.215 | 0.000 |
| Variables excluded from the equation | | T | Sig T |
| BMI | | 1.421 | 0.1564 |
| HDL.chol | | -0.251 | 0.8017 |
| HOMA | | -0.33 | 0.7402 |
| Triglycerides | | -0.85 | 0.39 |

Recent findings have demonstrated that the glycation of the insulin molecule is critical for its full biological activity (26). Our findings might imply that glycosylation could alter the plasma half-life or action, or both, of the adiponectin molecule, leading to increased HbA_{1c} concentration. Current goals aimed at identifying patients with glucose intolerance or diabetes or those patients who will develop chronic complications are under discussion. Evaluation and quantification of the usefulness of adiponectin as a simple test for increased HbA_{1c} concentrations should be performed in further epidemiological studies. Current findings suggest that adiponectin is a candidate molecule for this goal.

In summary, adiponectin is significantly associated with altered glucose tolerance and independently contributes to the HbA_{1c} concentration in a population-based manner.

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References

- Fernández-Real JM & Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocrine Reviews* 2003 **24** 278–301.
- 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). *Biochemical and Biophysical Research Communications* 1996 **221** 286–289.
- Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry* 1995 **270** 26746–26749.
- Combs TP, Berg AH, Obici S, Scherer PE & Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *Journal of Clinical Investigation* 2001 **108** 1875–1881.
- Berg AH, Combs TP, Du X, Brownlee M & Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Medicine* 2001 **7** 947–953.
- Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nature Medicine* 2001 **7** 941–946.
- Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT *et al*. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *PNAS* 2001 **98** 2005–2010.
- Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H *et al*. Diet-induced insulin resistance in mice lacking adiponectin. *Nature Medicine* 2002 **8** 731–737.
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J *et al*. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications* 1999 **257** 79–83.
- Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE *et al*. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1930–1935.
- Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS *et al*. 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* 2002 **51** 1884–1888.
- Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL *et al*. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3815–3819.
- Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H *et al*. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003 **361** 226–228.
- Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA *et al*. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2001 **360** 57–58.
- Hotta K, Funahashi T, Bodkin NL, Ortmeier HK, Arita Y, Hansen BC *et al*. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001 **50** 1126–1133.
- Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y *et al*. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis and Thrombosis Vascular Biology* 2000 **20** 1595–1599.
- Gerstein HC. Fasting versus postload glucose levels: why the controversy? *Diabetes Care* 2001 **24** 1855–1857.
- Del Prato S. In search of normoglycaemia in diabetes: controlling postprandial glucose. *International Journal of Obesity and Related Metabolic Disorders* 2002 **26** (Suppl 3) S9–S17.
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y *et al*. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999 **100** 2473–2476.
- Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H *et al*. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 2000 **102** 1296–1301.
- Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J *et al*. Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry* 2002 **277** 25863–25866.

- 22 Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N *et al.* Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000 **96** 1723–1732.
- 23 Botas Cervero P, Delgado Alvarez E, Castano Fernandez G, Diaz De Grenu C, Prieto Santiago J & Diaz Cadorniga FJ. Prevalence of diabetes mellitus and glucose intolerance in the population aged 30 to 75 years in Asturias, Spain. *Revista Clínica Española* 2002 **202** 421–429.
- 24 Hirose H, Kawai T, Yamamoto Y, Taniyama M, Tomita M, Matsubara K *et al.* Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism* 2002 **51** 314–317.
- 25 Phillips SA, Ciaraldi TP, Kong AP, Bandukwala R, Aroda V, Carter L *et al.* Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes* 2003 **52** 667–674.
- 26 Hunter SJ, Boyd AC, O'Harte FP, McKillop AM, Wiggam MI, Mooney MH *et al.* Demonstration of glycosylated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans. *Diabetes* 2003 **52** 492–498.

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