# Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 6 (4): 626-630
July-August, 2006

# Hypoadiponectinaemia in Egyptian Patients with Type II Diabetes Mellitus with Vascular Complications

[1]Nagwa Abd EL-Ghaffar and [2]Nehal Hamdy EL-Said

Adiponectin, a collagen-like plasma protein produced and secreted exclusively by adipose tissue, has been shown to have compelling anti-atherogenic, anti-inflammatory and insulin-sensitizing properties. Adiponectin has been linked to insulin resistance in obesity and diabetes. The aim of the study is to measure serum adiponectin concentration in type II obese diabetic patients with various micro and macrovascular complications, as adiponectin seems to be an important modulator for metabolic and vascular diseases. The study was carried out on 60 obese patients with type II diabetes (30 patients with macrovascular complications and 30 patients with various microvascular complications). The control group consisted of 20 healthy subjects of matched age and sex. For all subjects participated in the study, Body Mass Index (BMI), fasting (FBG) and postprandial (PPBG) blood glucose, creatinine, sodium, potassium, lipid profile and adiponectin were measured. We found a highly significant decrease in serum adiponectin levels in diabetic patients compared with control group. There was a significant negative correlation between adiponectin levels and BMI, FBG, PPBG, creatinine, sodium, potassium, triglyceride, total cholesterol and LDL-C, with significant positive correlation between adiponectin serum levels and duration of diabetes and HDL-C in diabetic patients. These results support the hypothesis that low circulating levels of adiponectin are an important determinant of risk of cardiovascular disease and that increased adiponectin levels might be associated with better glycemic control, better lipid profile and reduced inflammation in diabetic subjects. Thus, therapeutic approaches aimed at increasing concentrations or adiponectin tissue sensitivity and action could represent a novel treatment strategy for insulin resistance in type II diabetes and might have therapeutic implications as an anti-obesity drug or as anti-atherogenic plasma protein.

**Key words:** Diabetes mellitus, adiponectin, obesity

*JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.*

*For further information about this article or if you need reprints, please contact:*

Nagwa Abd EL-Ghaffar
Department of Clinical and Chemical Pathology,
National Research Center, Giza

ANSInet
Asian Network for Scientific Information

[1]Department of Clinical and Chemical Pathology, National Research Center, Giza
[2]Department of Internal Medicine, Faculty of Medicine, Cairo University, Egypt

## INTRODUCTION

Adiponectin is emerging as an important protein in the etiology of obesity and related metabolic disorders (Hanley *et al*., 2003). Adiponectin, predominantly synthesized in the adipose tissue, seems to have substantial anti-inflammatory properties and to be a major modulator of insulin resistance and dyslipidemia, mechanisms that are associated with an increased atherosclerotic risk in diabetic patients (Schulz *et al*., 2005). Adiponectin, an adipocyte-derived peptide with anti-inflammatory and anti-atherogenic effects, is known to protect against the initiation and progression of atherosclerosis (Chen *et al*., 2005).

Low plasma adiponectin concentration is associated with a decrease in whole body insulin sensitivity (Tschritter *et al*., 2003) and has been shown to be predictive of future development of diabetes in a few studies (Daimon *et al*., 2003).

Adiponectin is a novel-adipocyte specific protein, although it circulates in high concentrations, adiponectin levels are lower in obese subjects than in lean subjects (Lihn *et al*., 2005). Apart from negative correlations with measures of adiposity, adiponectin levels are also reduced in association with insulin resistance and type II diabetes, visceral adiposity has been shown to be an independent negative predictor of adiponectin (Lihn *et al*., 2005).

The aim of the study is to measure serum adiponectin concentration in type II obese diabetic patients with various micro and macrovascular complications, as adiponectin seems to be an important modulator for metabolic and vascular diseases.

## MATERIALS AND METHODS

The study was carried out on 60 obese patients with type II diabetes with microvascular and macrovascular complications selected from outpatient clinics, at Kasr EL-Aini Hospital, Cairo University. They were divided into 2 groups: Group I consisted of 30 patients with type II diabetes with macrovascular complications (ischemic heart disease, cerebrovascular disease) with mean age of 54.3±10.7 years (12 males and 18 females). Group II consisted of 30 patients with type II diabetes with various microvascular complications (diabetic nephropathy, neuropathy and retinopathy) with mean age of 53.3±9.8 years (19 males and 11 females). Group III consisted of 20 healthy subjects of matched age and sex (52.8±11.4 years, 9 males and 11 females).

All subjects participated in the study were subjected to the following:

- Detailed clinical examination including Body Mass Index (BMI).
- Routine laboratory investigations: Fasting and Postprandial Blood Glucose (FBG and PPBG, respectively), lipid profile and kidney function.
- Adiponectin in fasting sample.

Ten milliliter of fasting venous blood samples (12 h fast) were taken from each subject participating in the study, the blood was left to clot, centrifuged at approximately 2000-3000 xg for 15 min at 4°C, the fasting blood glucose was determined immediately and the rest of the serum was stored at -20°C for the routine investigations and adiponectin determination. Two hours after meal, a venous blood sample (about 2 mL) were taken on fluoride for the determination of postprandial blood glucose.

**Kidney function tests:** Colorimetric techniques were used for the determination of creatinine using alkaline picrate method (Höuöt, 1985) and sodium and potassium were measured using flame emission spectrophotometry (Velapoldi *et al*., 1978). Blood glucose was carried out by glucose oxidase method (Siest *et al*., 1981).

**Lipid profile:** Total cholesterol was carried out using Trinder`s reaction (Allian *et al*., 1974). Triglyceride was carried out using enzymatic hydrolysis of glycerol (Fossati and Prencipe, 1982). High Density Lipoprotein Cholesterol (HDL-C) was determined using phosphotungestic acid precipitation (Lopes-Virella *et al*., 1977). Low Density Lipoprotein Cholesterol (LDL-C) was determined according to the method described by Steinberg (1981)

All the above kits were supplied by Sentinel CH (Principe Eugenio 5-20155 Milan- Italy).

**Adiponectin determination:** By quantitative sandwich enzyme immunoassay technique and the kit was supplied by LINCO Research Inc. (6 Research Park Drive, St Charles, Missouri, USA) (Faraj, 2003).

## STATISTICAL ANALYSIS

The results were expressed as mean±SD. Data was statistically analyzed using SPSS package for windows, version 7.5 (f-test and r-coefficient).

## RESULTS

There was a highly significant increase in the BMI of diabetic patients compared with control group Table 1.

There was a highly significant increase in fasting and postprandial blood glucose, triglyceride, total cholesterol and low-density lipoprotein cholesterol serum levels with highly significant decrease in high-

density lipoprotein cholesterol concentration in diabetic patients compared with control group. A significant increase was found in serum creatinine, with significant decrease in sodium and potassium serum levels in diabetic patients compared with control group Table 2.

Table 3 revealed that there was a highly significant decrease in serum adiponectin level in diabetic patients in comparison to control group.

In-group I and II diabetic patients, there was a significant negative correlation between serum adiponectin level and BMI, FBG, PPBG, creatinine, sodium, potassium, triglyceride, total cholesterol and LDL-C with significant positive correlation between serum adiponectin and duration of diabetes and HDL-C Table 4.

Table 1: The different clinical parameters of all studied group (mean±SD)

| | Groups | | | |
|---|---|---|---|---|
| Parameters | III | II | I | p* |
| Age (years) | 52.8±11.4 | 54.3±10.7 | 53.3±9.8 | <0.05 |
| Sex (males) | 9 | 12 | 19 | - |
| (Females) | 11 | 18 | 11 | - |
| Duration (years) | - | 8.4±6.6 | 6.0±9.1 | - |
| BMI (Kg $m^{-2}$) | 22.55±2.65 | 30.8±3.1 | 31.9±4.0 | <0.01 |

p*<0.05: Significant, p<0.01: Highly significant, Group I: Type II diabetic patients with macrovasculr complications, Group II: Type II diabetic patients with microvascular complications, Group III: Control group

Table 2: The different biochemical parameters of all studied groups (mean±SD)

| | Groups | | | |
|---|---|---|---|---|
| Parameters | III | II | I | p* |
| FBG (mg $dL^{-1}$) | 89.25 ±9.6 | 139.3±30.7 | 152.3±62.7 | <0.001 |
| PPBG (mg $dL^{-1}$) | 100.35±7.8 | 278.5±101.5 | 230.0±60.1 | <0.001 |
| Creatinine (mg $dL^{-1}$) | 0.83±0.13 | 1.05±0.06 | 0.94±0.46 | <0.05 |
| Sodium (mmol $L^{-1}$) | 141.2±2.95 | 135.4±8.6 | 133.3±7.7 | <0.05 |
| Potassium (mmol $L^{-1}$) | 4.15±0.58 | 3.3±0.46 | 3.5±0.69 | <0.05 |
| Triglyceride (mg $dL^{-1}$) | 95.1±35.45 | 198.9±140.1 | 189.8±70.4 | <0.001 |
| Total cholesterol (mg $dL^{-1}$) | 185.35±21.1 | 246.8±40.9 | 290.47±46.17 | <0.01 |
| HDL-C (mg $dL^{-1}$) | 52.95±10.05 | 33.4±2.5 | 32.2±8.6 | <0.001 |
| LDL-C (mg $dL^{-1}$) | 112.78±6.68 | 173.62±12.18 | 220.20±23.49 | <0.01 |

Table 3: The mean level of serum adiponectin in different studied groups (mean±SD)

| | Groups | | | |
|---|---|---|---|---|
| Parameters | III | II | I | p |
| Adiponectin (μg $mL^{-1}$) | 15.35±3.51 | 10.0±4.8 | 9.0±5.1 | <0.001 |

Table 4: The correlation between serum adiponectin and different clinical and biochemical parameters of diabetic patients

| | Adiponectin | | | |
|---|---|---|---|---|
| | Macrovascular | | Microvascular | |
| Parameters | r | p | r | p |
| BMI | - 0.47 | <0.001 | -0.49 | <0.001 |
| Duration | 0.63 | <0.05 | 0.69 | <0.05 |
| FBG | -0.56 | <0.01 | -0.59 | <0.01 |
| PPBG | -0.61 | <0.01 | -0.68 | <0.01 |
| Creatinine | -0.53 | <0.05 | -0.58 | <0.05 |
| Sodium | -0.63 | <0.05 | -0.65 | <0.05 |
| Potassium | -0.71 | <0.05 | -0.69 | <0.05 |
| Triglyceride | -0.39 | <0.001 | -0.48 | <0.001 |
| Total cholesterol | -0.30 | <0.01 | -0.35 | <0.01 |
| HDL-C | 0.45 | <0.001 | 0.49 | <0.001 |
| LDL-C | -0.25 | <0.01 | -0.29 | <0.01 |

## DISCUSSION

Adiponectin is a novel and important member of the adipokine family, which has regulatory functions in the glucose and lipid metabolism (Palomer *et al*., 2005).

Adiponectin stimulates fatty acid oxidation, reduces triglycerides and improve glucose metabolism by increasing the insulin sensitivity, in addition, adiponectin inhibits the inflammatory process that accompanies atherogenesis, as it reduces expression of endothelial adhesion molecules, macrophage to foam cell transformation, tumor necrosis factor expression in macrophage and adipocyte and smooth muscle cell proliferation (Palomer *et al*., 2005).

The aim of the study is to measure serum adiponectin concentration in type II obese diabetic patients with various micro and macrovascular complications, as adiponectin seems to be an important modulator for metabolic and vascular diseases. The study was done on two groups of diabetic obese patients, group I (those with macrovascular complications) and group II (those with microvascular complications) and a control group (group III). The results of this study showed a highly significant decrease in adiponectin levels in diabetic patients compared with control group (p<0.001), with lower levels in-group I compared to group II patients. These results agreed with several researchers, Chen *et al*. (2005) measured plasma adiponectin concentrations in patients with ischemic cerebrovascular disease with and without type II diabetes and they found statistically lower levels of plasma adiponectin in those patients especially with type II diabetes.

Matsuda *et al*. (2004) studied the relationship between adiposity and plasma adipocytokine levels (adiponectin, leptin, resistin and tumor necrosis factor-alpha) and investigated the clinical significance of adiposity and plasma adipocytokine levels on diabetic micro- and macro-angiopathy in type II diabetic subjects, they found that plasma adiponectin levels was negatively correlated with both visceral and subcutaneous fat areas.

Also the result of this study agreed with Anthony *et al*. (2003), Yilmaz *et al*. (2004) and Schulze *et al*. (2005) they all demonstrated low serum adiponectin levels in type II diabetic patient with various diabetic angiopathy.

The results of this study revealed that the mean serum adiponectin levels were negatively correlated with BMI, fasting and postprandial blood glucose, creatinine, total cholesterol and LDL-C, however; adiponectin level was positively correlated with HDL-C

and duration of diabetes in both diabetic groups. The mechanisms by which adiponectin may affect blood lipids are largely unknown. Effects of adiponectin on hepatic lipase activity, which is increased in central obesity and insulin resistance, are suspected (Cnop *et al*., 2003). Hyperglycemia acutely increases circulating cytokine concentrations (Morohoshi *et al*., 1996; Esposito *et al*., 2002), HDL-cholesterol down regulates expression of adhesive molecules on the surface of vascular endothelium (Garner *et al*., 1998) and inhibits platelet aggregation (Nofer *et al*., 1998) and thus has anti-inflammatory and antithrombotic properties

These results agreed with Schulze *et al*. (2005) who found an inverse association between adiponectin and BMI in type II diabetic patients and they also suggested that increased adiponectin levels are associated with a moderately decreased coronary heart disease risk in diabetic man. This suggests that although adiponectin might in part mediate effects of body fat on lipoproteins, other unrecognized pathways controlling its production might be important as well. Furthermore, the associations were largely independent of fasting insulin concentration (Zietz *et al*., 2003; Valsamakis *et al*., 2003) and indexes of insulin resistance assessed by the homeostasis model assessment (Shand *et al*., 2003; Yamamoto *et al*., 2002), the euglycemic-hyperinsulinemic clamp and the oral glucose tolerance test (Tschritter *et al*., 2003). This suggests that mechanisms other than effects on insulin resistance and hepatic lipase activity most likely mediate the association between adiponectin and blood lipids.

Various authors stated that circulatory adiponectin levels are lower in obese subjects than in lean subjects (Lihn *et al*., 2005) and weight reduction has resulted in increases in adiponectin (Anthony *et al*., 2003). Also adiponectin levels are reduced in association with insulin resistance. Several insulin-resistant states such as obesity and type II diabetes or cardiovascular diseases, have been found to be associated with low levels of plasma adiponectin (Palomer *et al*., 2005).

Gottsater *et al*. (2004) had prospectively follow concentrations of plasma adiponectin and serum advanced glycation end products in relation to plasma lipids and retinopathy over 3 years in type II diabetic patients, they found that plasma adiponectin levels correlated inversely with fasting C-peptide and low density lipoprotein cholesterol and directly with high density lipoprotein cholesterol and they concluded that both plasma adiponectin and advanced glycated end products increased during the 3 years follow up study period and that this increase was explained by improvements in insulin sensitivity and dyslipidemia.

Many researchers had demonstrated the adiponectin levels were reduced prior to the development of type II diabetes and that decreased serum levels of adiponectin are a risk factor for the progression of type II diabetes (Tschritter *et al*., 2003; Daimon *et al*., 2003; Lihn *et al*., 2005) and they stated that administration of adiponectin has been accompanied by lower plasma glucose level as well as increased insulin sensitivity.

Furthermore, reduced expression of adiponectin has been associated with some degree of insulin resistance in animal studies indicating a role of hypoadiponectinemia in relation to insulin resistance (Lihn *et al*., 2005). The primary mechanism by which adiponectin enhance insulin sensitivity appears to be through fatty acid oxidation and inhibition of hepatic glucose production.

In conclusion this study showed that serum adiponectin levels were lower in type II diabetic patients with various angiopathy or vascular complications and this may indicate the possible anti-atherogenic affect for adiponectin, which appears to be an insulin enhancer with the potential use as a new pharmacological treatment for metabolic syndrome. Thus, therapeutic approaches aimed at increasing concentrations or adiponectin tissue sensitivity and action could represent a novel treatment strategy for insulin resistance in type II diabetes and might have therapeutic implications as an anti-obesity drug or as anti-atherogenic plasma protein.

## REFERENCES

Allian, C., C. Luucy, S. Poon, S. Cicely, G. Chen, W. Richmed and C.F.U. Paul, 1974. Enzymatic determination of total cholesterol. Clin. Chem., 20: 470-475.

Anthony, H., C. Philip, H. Stewart and Z. Bernard, 2003. Adiponectin in a native Canadian population experiencing rapid epidemiological transition. Diabetes Care, 26: 3219-3225.

Chen, M.P., J.C. Tsai, F.M. Chung, S.S. Yang, L.L. Hsing, S.J. Shis and Y.J. Lee, 2005. Hypoadiponectinemia is associated with ischemic cerebrovascular disease. Arteisoscler Thromb. Vasc. Biol., 25: 821-826.

Cnop, M., P.J. Havel, K.M. Utzschneider, D.B. Carr, M.K. Sinha and E.J. Boyko *et al*., 2003. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: Evidence for independent roles of age and sex. Diabetologia, 46: 459-469.

Daimon, M., T. Oizumi, T. Saitoh, W. Kameda, A. Hirata, H. Yamaguchi and H. Ohnuma, 2003. Decreased serum levels of adiponectin are risk factor for the progression to the type 2 diabetes in a Japanese population. Diabetes, 52: A327.

Esposito, K., F. Nappo, R. Marfella, G. Giugliano, F. Giugliano and M. Ciotola *et al*., 2002. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. Circulation, 106: 2067-2072.

Faraj, M., P.J. Havel, S. Phelis, D. Blank, A.D. Sniderman and K. Cianflone, 2003. Plasma acylation-stimulating protein, adiponectin, leptin and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. J. Clin. Endocrinol. Metab., 88: 1594-1602.

Fossati, P. and L. Prencipe, 1982. Serum triglyceride determination calorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.

Garner, B., P.K. Witting, A.R. Waldeck, J.K. Christison, M. Raftery and R. Stocker, 1998. Oxidation of high-density lipoproteins. I. Formation of methionine sulfoxide in apolipoproteins AI and AII is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol. J. Biol. Chem., 273: 6080-6087.

Gottsater, A., B. Sazelag, M. Kangro, M. Wroblewski and G. Sundkvist, 2004. Plasma adiponectin and serum advanced glycated end products increase and plasma lipid concentrations decrease with increasing duration of type 2 diabetes. Eur. J. Endocrinol., 151: 361-366.

Hanley, A., P. Connelly, S. Harris and B. Zinman, 2003. Adiponectin in a native Canadian population. Experiencing rapid Epidemiological transition. Diabetes Care, 26: 3219-3225.

Höuöt, O., 1985. Interpretation of Clinical Laboratory Tests. Siest, G., J. Henny, F. Schile and D.S. Yang (Ed.) Biochemical Publications, pp: 220-234.

Lihn, A.S., S.B. Pedersen and B. Richslsen, 2005. Adiponectin: Action, regulation and association to insulin sensitivity. Obes. Rev., 6: 13-21.

Lopes-Virella M.F., P. Stone, S. Ellis and J. Collwell, 1977. Cholesterol determination in high-density lipoprotein by three different methods. Clin. Chem., 23: 882-884.

Matsuda, M., F. Kawasaki, K. Yamada, Y. Kanda, M. Saito, M. Eto, M. Matsuki and K. Kaku, 2004. Impact of adiposity and plasma adipocytokines on diabetic angiopathy in Japanese type 2 diabetic subjects. Diabet, Med., 21: 881-888.

Morohoshi, M., K. Fujisawa, I. Uchimura and F. Numano, 1996. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes *in vitro*. Diabetes, 45: 954-959.

Nofer, J.R., M. Walter, B. Kehrel, S. Wierwille, M. Tepel, U. Seedorf and G. Assmann, 1998. HDL3-mediated inhibition of thrombin-induced platelet aggregation and fibrinogen binding occurs via decreased production of phosphoinositide-derived second messengers 1,2-diacylglycerol and inositol 1,4,5-tris-phosphate. Arterioscler Thromb. Vasc. Biol., 18: 861-869.

Palomer, X., A. Perez and F. Blanco-Vaco, 2005. Adiponectin: A new link between obesity, insulin resistance and cardiovascular disease. Med. Clin. (Barc), 19; 124: 388-395.

Schulze, M.B., I. Shai, E.B. Rimm, T. Li, N. Rifai and F.B. Hu, 2005. Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes, 54: 534-539.

Shand, B.I., R.S. Scott, P.A. Elder and P.M. George, 2003. Plasma adiponectin in overweight, nondiabetic individuals with or without insulin resistance. Diabetes Obes. Metab., 5: 349-353.

Siest, G., J. Henny and F. Schiele, 1981. Interpretation Des Examens Laboratories. Kargered, pp: 206-223.

Steinberg, D., 1981. Metabolism of lipoproteins at the cellular level in relation to atherogenesis. In lipoproteins Atherosclerosis and Coronary Heart Disease. Elsevier North Holland, 1: 31-48.

Tschritter, O., A. Fristsche, C. Thamer, M. Haap, F. Shirkavard and S. Rahe 2003. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes, 52: 239-243.

Valsamakis, G., R. Chetty, P.G. McTernan, N.M. Al-Daghri, A.H. Barnett and S. Kumar, 2003. Fasting serum adiponectin concentration is reduced in Indo-Asian subjects and is related to HDL cholesterol. Diabetes Obes. Metab., 5: 131-135.

Velapoldi, R.A., R.C. Paule and R. Schaffer *et al*., 1978. A reference method for the determination of sodium and potassium in serum. Washington, DC National Bureau of Standards; US Department of Commerce, NBS Special Publication, pp: 260-263.

Yamamoto, Y., H. Hirose, I. Saito, M. Tomita, M. Taniyama and K. Matsubara *et al*., 2002. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin. Sci., (Lond), 103: 137-142.

Yilmaz, M.I., A. Sonmez, C. Acikel, T. Celik, N. Bingol, M. Pinar, Z. Bayraktar and M. Ozato, 2004. Adiponectin may play a part in the pathogenesis of diabetic angiopathy. Eur. J. Endocrinol., 151: 135-40.

Zietz, B., H. Herfarth, G. Paul, A. Ehling, U. Muller-Ladner, J. Scholmerich and A. Schaffler, 2003. Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. FEBS Lett., 545: 103-104.