Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.59925-59929, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ADIPOCYTOKINES AND ASYMMETRIC DIMETHYLARGININE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

***,[1]Daniela Iv. Koleva, [1]Maria M. Orbetzova, [2]Julia G. Nikolova, [3]Tanya I. Deneva and [4]Iliana B. Atanasova**

[1]Clinic of Endocrinology and metabolic diseases, Sv. Georgy" University Hospital, Medical University, Plovdiv
[2]Department of Physiology, Medical University, Plovdiv
[3]Department of Clinical Laboratory, "Sv. Georgy" University Hospital, Plovdiv, Medical University, Plovdiv
[4]Clinical Centre of Endocrinology, Acad. Ivan Penchev University Hospital of Endocrinology, Medical University, Sofia

ARTICLE INFO

Article History:
Received 23rd July, 2017
Received in revised form
20th August, 2017
Accepted 16th September, 2017
Published online 31st October, 2017

Key words:

Resistin; Interleukin-6;
Endothelial dysfunction;
Polycystic ovary syndrome.

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is considered to be a prototype of metabolic syndrome (MS).
Objective: To determine the levels of resistin, interleukin-6 (IL-6) and asymmetric dimethylarginine (ADMA) as well as to assess their relationship with clinical and metabolic parameters in women with PCOS.
Materials and methods: The study included 22 overweight/obese PCOS patients and 20 metabolically healthy obese (MHO) women. Anthropometric, glucose and lipid parameters, total testosterone, resistin, IL-6, ADMA were determined. Body mass index (BMI), waist-to-hip ratio (WHR) and homeostasis model assessment insulin resistance index (HOMA-IR) were calculated.
Results: Significantly higher levels of ADMA and resistin were found in the PCOS women as compared to those in the MHO. IL-6 was positively correlated with WHR and negatively associated with HDL-cholesterol in the PCOS group.
Conclusion: Higher resistin and ADMA concentrations in PCOS women showed a state of chronic low-grade inflammation. The correlation of IL-6 with WHR and HDL-cholesterol suggested a possible role of IL-6 in the development of MS in PCOS.

Citation: Daniela Iv. Koleva, Maria M. Orbetzova, Julia G. Nikolova, Tanya I. Deneva and Iliana B. Atanasova. 2017. "Adipocytokines and asymmetric dimethylarginine in women with polycystic ovary syndrome", *International Journal of Current Research*, 9, (10), 59925-59929.

INTRODUCTION

Adipose tissue has been shown by the recent advances in science to be an active endocrine organ that synthesizes and releases a number of bioactive mediators (adipocytokines) which are involved in the maintenance of the homeostasis, blood pressure control, the lipid and carbohydrate metabolism and in the development of low-grade inflammation and atherosclerosis. They act locally or systemically altering the insulin sensitivity of the target organs (muscle, liver); their effects become manifest by neuroendocrine, autonomous and immune mechanisms. It has been found that plasma levels of the cytokines resistin, leptin, IL-6, C-reactive protein (CRP) and others, tend to be elevated in patients with obesity and diabetes (Koleva *et al.*, 2013). Metabolic syndrome (MS) is characterized by the presence of hyperinsulinemia, hypertriglyceridemia, elevated levels of LDL-cholesterol (LDL-C), reduced levels of HDL-cholesterol (HDL-C), hyperuricemia, hyperhomocysteinemia, dyslipidemia, hypertension and diabetes mellitus type 2 (T2DM). PCOS is considered to be a prototype of MS. A lot of data have proved that PCOS women have an unfavourable cardiovascular profile and their metabolic state has been often associated with android obesity, insulin resistance (IR), dyslipidemia and arterial hypertension (Legro *et al*., 1999). Some authors have described elevated serum levels of inflammatory markers in women with PCOS – a sign of a chronic low-grade inflammatory process which, in turn, is a risk factor for the development of endothelial dysfunction (ED) and cardiovascular disease (CVD). Recently, the scientific interest in the so called "metabolically healthy obesity" has been greatly increasing. It is a condition associated with the lack of typical metabolic syndrome components, namely glucose intolerance/T2DM, atherogenic dyslipidemia, arterial hypertension (Puri *et al*., 2014). It is known that MHO individuals display less visceral adipose tissue and a reduced inflammatory profile showing lower levels of circulating

****Corresponding author:*** **Daniela Iv. Koleva,**
Clinic of Endocrinology and metabolic diseases, Sv. Georgy" University Hospital, Medical University, Plovdiv.

proinflammatory cytokines compared to those in metabolically unhealthy obese individuals (Denis *et al*., 2013). Significantly higher plasma levels of asymmetric dimethylarginine (ADMA) – a candidate for a novel atherogenic factor, have been determined both in patients with MS and PCOS as compared to those in the clinically healthy women, while PCOS patients tend to have higher values as compared to those with MS (Diamanti-Kandarakis *et al.*, 2006; Kravariti *et al.*, 2005). The aim of the present study was to assess the levels of resistin, interleukin-6 (IL-6) and plasma asymmetric dimethylarginine (ADMA) and their relationship with clinical and metabolic parameters in women with polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS

The present study was conducted in the Clinic of Endocrinology and Metabolic Diseases at the University Hospital "Sv. Georgy", Plovdiv. It covered 22 overweight/obese women with PCOS (mean age - 26.64±3.96 years; mean BMI - 30.56±4.34 kg/m²) and 20 metabolically healthy obese (MHO) women (mean age - 26.35±5.30 years; mean BMI – 30.20±3.39 kg/m²). The diagnosis of PCOS was made according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored 2003), when two of the following three features were present: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound examination (the presence of≥12 follicles measuring 2–9 mm in diameter and/or ovarian volume >10 cm3). Metabolically healthy overweight/obese women were defined as individuals with BMI ranged from 25.0 to 39.9 kg/m², lacking the typical components of MS (Puri *et al.*, 2014). A comprehensive set of hormonal tests was performed in all study subjects (androgens, gonadotropins, estradiol, prolactin, TSH, free T4, serum cortisol at 8 and 22h) for diagnostic purposes and in order to exclude pregnancy and other available endocrine pathology: Cushing's syndrome, inherited adrenal hyperplasia, prolactinoma, hypo-/hyperthyroidism, hypopituitarism, hypogonadism, androgensecreting tumors. Venous blood samples for determination of hormonal parameters were collected in early morning at 12-hour fasting stage in the early follicular phase (days 1-5) of spontaneous bleeding.

The following measurements and laboratory tests were performed in all the women after signing the informed consent of the study: weight, height, waist and hip circumferences, fasting plasma glucose (FPG), immunoreactive fasting insulin (IRI), total testosterone, resistin, IL-6, ADMA, total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG). Waist circumference was defined in the horizontal plane midway between the lower edge of the 12th rib and the top of the iliac crests. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. Body mass index (BMI) = weight (kg)/height (m)², waist-to-hip ratio (WHR) and the homeostasis model of insulin resistance index (HOMA-IR) = FPG (mmol/l) × IRI (μIU/ml) /22.5. The cut-off values of 2.5 for HOMA-IR were used for determining IR (American Diabetes Association, 1998). Venous blood samples for determination of FPG, IRI, TC, HDL-C, TG, total testosterone and ADMA were taken to Central Clinic Laboratory, Sv. Georgy University Hospital. Venous blood samples for evaluation of resistin and IL-6 levels were conducted to the Laboratory of Clinical Centre of Endocrinology, "Acad. Ivan Pentchev" University Hospital of Endocrinology, Sofia.

ADMA plasma concentrations were determined using ELISA assay (DLD Diagnostica GMBH, Germany) with the following major characteristics of analytic reliability: intra assay imprecision - CV < 8.2%, inter assay imprecision - CV < 11.2%; accuracy - d% < 8.0%; and recovery - 92.7%. Serum levels of resist in were detected by (Human) EIA method using a commercial kit of PHOENIX PHARMACEUTICAL INC, USA with the following characteristics: sensitivity - 1.16 ng/ml; inter assay variation, CV% < 5.0; intra assay variation, CV% < 14. IL-6 was tested by means of (Human) ELISA (a commercial kit of DRG, Germany) with sensitivity - 2.0 pg/ml; inter assay variation, CV% < 7.7; intra assay variation, CV% < 4.2. Serum glucose levels were determined by a standard enzymatic colorimetric (GOD-POD) method with the following haracteristics: dilution recovery – ≤ 20 mmol/L; inter assay variation, CV from 2.40 to 2.94%, intra assay variation, CV% < 1.2. Insulin was tested using a commercial kit for quantitative determination of immunoreactive insulin on the basis of microparticular immunoenzyme analysis (MEIA) by means of AxSYM system (ABBOTT, USA) with the following characteristics: sensitivity, ≤ 0.8 mIU/ml; inter assay variation, CV% < 2.9; intra assay variation, CV% < 5.3. Total cholesterol was determined by ChOD, PAP, triglycerides by GPO, PAP, and HDL-cholesterol by MgSO4-dextran SO4 praecipitation, Schneiders Analysers; Netherlands test; Delta Kone Autoanalyser. Serum levels of glucose were determined by standard GOD-POD method. The hormonal tests were performed on an Ax-SYM^{TM} System (Abbott Diagnostics, Abbott Park, USA) using commercial kits. The statistical analysis was performed by SPSS version 21.0 for Windows. The distribution of the data was tested using the Kolmogorov-Smirnov test for normality. Correlation analyses were made using Pearson correlation coefficient. The results are presented as mean ± standard deviation (SD). Statistical significance was taken at level of P<0.05.

RESULTS

Table 1 shows the clinical and anthropometric characteristics in the studied groups of women, namely, 22 overweight/obese women with PCOS and 20 age- and BMI-matched metabolically healthy women (MHO). There were no statistically significant differences between the groups in terms of age, weight, BMI, waist and hip circumferences, WHR. We found comparable levels of FPG, TC, HDL-C, TG and IL-6 (P>0.05, Table 2).

Table 1. Clinical and anthropometric characteristics in the studied groups of women

Parameters	Groups of women	
	PCOS n=22	MHO n=20
Age (years)	26.64±3.96	26.35±5.30 NS
Weight (kg)	84.44±12.55	83.21±14.49 NS
BMI (kg/m²)	30.56±4.34	30.20±3.39 NS
Waist (cm)	89.90±6.48	90.90±5.51 NS
Hip (cm)	104.55±7.39	103.12±9.92 NS
WHR	0.86±0.08	0.85±0.08 NS

NS – non-significant difference, P>0.05

Higher values of IRI, HOMA-IR, testosterone were determined in the patients with PCOS. Significantly higher plasma levels of ADMA (0.80±0.31, respectively 0.55±0.28 μmol/l, P<0.05) and resistin (5.65±1.99, respectively 4.36±1.80 ng/ml, P<0.05) were found in women with PCOS as compared to those in the MHO (Table 2). No correlation between ADMA and the other

parameters in the women with PCOS was found. IL-6 was positively correlated with WHR (r=0.741) and negatively associated with HDL-C (r=-0.523) in the PCOS group.

Table 2. Metabolic and hormonal parameters in the studied groups of women

Parameters	Groups of women	
	PCOS	MHO
	n=22	n=20
FPG (mmol/l)	5.19±0.65	4.82±0.58 NS
IRI (μIU/ml)	11.39±4.93	5.99±1.50***
HOMA-IR	2.74±1.31	1.29±0.38**
TC (mmol/l)	4.63±0.58	4.91±0.69 NS
HDL-C (mmol/l)	1.14±0.26	1.26±0.30 NS
TG (mmol/l)	1.00±0.55	0.85±0.30 NS
TESTOSTERONE (ng/ml)	0.68±0.16	0.46±0.15**
RESISTIN (ng/ml)	5.65±1.99	4.36±1.80*
IL-6 (pmol/l)	1.57±1.11	1.21±0.76 NS
ADMA (μmol/l)	0.80±0.31	0.55±0.28*

NS – non-significant difference, P>0.05
* - presence of significant difference, P<0.05
** - presence of significant difference, P<0.01
*** - presence of significant difference, P<0.001

DISCUSSION

Our research data showed significantly higher plasma levels of ADMA in the women with PCOS in comparison with those in the MHO group. In recent years, there have been a great deal of interest on ADMA, occurring a physiological regulator of the biosynthesis of nitric oxide (NO) - a critical factor in the pathogenesis and progression of atherosclerosis. Inactivation and/or impaired NO synthesis is common in patients with existing risk factors for cardiovascular disease (CVD) and may lead to the development of endothelial dysfunction (ED), hypertension, thrombosis and atherogenesis. Asymmetric dimethylarginine (ADMA), a byproduct of cellular protein turnover, is an endogenous competitive inhibitor of endothelial NO synthase (eNOS). It is believed that increased serum levels of ADMA promote the process of atherosclerosis, leading to the occurrence of ED and CVD (Teerlink *et al.*, 2009; Davids *et al.*, 2012). High levels of ADMA are associated with the pathophysiology of hypercholesterolemia, hypertension, T2DM, heart failure, kidney failure and liver failure (Teerlink *et al*., 2009, Abbasi *et al.*, 2001). ADMA is perceived as a marker of ED, a candidate for a new cardiovascular risk factor or an independent risk factor for cardiovascular morbidity and mortality (Lu *et al*., 2003).

We conducted another cross-sectional study in the Clinic of Endocrinology and Metabolic Diseases at the "Sv. Georgy" University Hospital which aimed to determine the plasma levels of ADMA in women with insulin resistance' states (24 women with MS, 38 women with PCOS and 24 age-matched clinically healthy women). The highest plasma levels of ADMA were found in the women with PCOS (0.91±0.32 μmol/l), as the differences for women with MS were not significant (0.82±0.37 μmol / l, P>0.05). The ADMA levels in the subjects with MS and PCOS were significantly higher than those in the healthy women (0.65±0.35 μmol/l, P <0.05). It wasn't found a statistically significant correlation between the levels of ADMA and BMI, HOMA-IR and lipid parameters neither in the women with MS nor in those with PCOS (Koleva *et al.*, 2014). Dennis Heutling *et al.* investigated ADMA levels among other cardiovascular, metabolic, and hormonal parameters in women with PCOS and the effect of metformin treatment on these parameters. Eighty-seven women with PCOS and 39 clinically healthy women were included and the effect of metformin after 6 months of treatment in 21 women with PCOS was studied. It was found that the serum levels of ADMA were significantly higher in the women with PCOS compared to those in the control group. HOMA-IR, fasting IRI, glycosylated hemoglobin, TC, LDL-C, TG and thickness of the intima-media layer (IMT) were significantly higher in the women with PCOS. Treatment with metformin decreased ADMA levels. In the PCOS patients ADMA was found to be positively correlated with BMI, WHR, parameters of insulin sensitivity and IMT. No relationship was found between markers of inflammation - CRP and IL-6 and levels of ADMA (Heutling *et al*., 2008).

Plasma levels of ADMA were found to be higher in women with obesity and IR as compared to those in obese women without IR. ADMA concentrations decreased in response to a reduction of body weight, accompanied by an improvement of insulin sensitivity (McLaughlin *et al.*, 2006). A strong correlation between the presence of ED, serum levels of androgens and the degree of IR in women with PCOS was detected (Cooke *et al.*, 2004). Our study didn't demonstrate the presence of a statistically significant correlation between plasma levels of ADMA and the investigated clinical and metabolic parameters in the PCOS women. However, the presence of a significant correlation does not always indicate causality, and vice versa. Elevated plasma levels of ADMA might be only a secondary phenomen (epiphenomenon) accompanying some abnormalities in a pre-diabetic state. These abnormalities can include higher glucose levels, a well-recognized cause of elevated ADMA via depressed dimethylarginine dimethylaminohydrolase (DDAH) activity due to a higher intracellular oxidative stress (Lin *et al*., 2002). DDAH plays a central role in the degradation of ADMA, which means that any disease, leading to reduced DDAH activity would increase concentrations of ADMA levels. Resistin is a member of a class of cysteine-rich proteins collectively termed resistin-like molecules. It is produced and secreted mainly by peripheral blood mononuclear cells. This recently discovered adipose tissue hormone is thought to represent a link between obesity and diabetes. Some researchers found the resistin levels in obese patients to be significantly higher than those in normal weight patients, while others found no significant differences. Contradiction exists also in the data concerning statistically significant correlations between the levels of resistin, weight, adipose tissue, IR, and these in combination in basal conditions and after weight loss. Secretion of resistin is beneficially affected by the proinflammatory cytokines TNF-α and IL-6. Resistin has been found to induce the expression of TNF-α and IL-6 in white adipose tissue and peripheral mononuclear cells (Koleva *et al.*, 2013; Lee *et al.,* 2003).

Data about resistin circulating levels in women with PCOS are rather scanty and controversial. We found significantly higher serum levels of resistin in the women with PCOS as compared to those in the controls. There are few studies which have proved elevated serum levels of resistin in patients with PCOS (Munir *et al.,* 2005). Most of the studies in this field show a lack of statistically significant difference of resistin levels between women with PCOS and clinically healthy women (Olzanecka-Glinianowicz *et al*., 2011; Zhang *et al.,* 2011). A study of F. Farshchian *et al.* aimed to compare the serum levels of resistin and visfatin in 40 women with PCOS and 40 clinically healthy women matched for age and BMI. They all

were subcategorized into obese and normal-weight according to their BMI. The investigators found higher serum levels of resistin among both obese and normal-weight women with PCOS in comparison with the controls (2.36 ng/ml and 1.58 ng/ml in normal-weight women with PCOS and controls, respectively; and 2.10 ng/ml and 1.91 ng/ml in obese women with PCOS and controls, respectively, $p<0.05$) (Farshchian *et al.,* 2014). In contrast of our data about the presence of a significant inverse correlation between the levels of resistin and total testosterone, Munir *et al*. confirmed an existing positive relationship between these two parameters. Furthermore, they proved a synergistic stimulating effect of resistin and insulin on 17α-hydroxylase mRNA expression in cultured human theca cells and on androgen production, respectively (Munir *et al*., 2005). IL-6 is a biologically active molecule – one of the proinflammatory cytokines that has been associated with obesity and IR. IL-6 secretion is two or three times higher in visceral than in subcutaneous adipose tissue. The expression of IL-6 in human adipose tissue as well as its circulating levels were found to correlate positively with the presence of obesity, impaired glucose tolerance and IR. It is proved that parenteral administration of IL-6 may lead to the development of hyperlipidemia, IR and hyperglycemia both in human and experimental animal models (Eder *et al*., 2009; Roytblat *et al*., 2000). In concordance with these data we determined a strong positive correlation between serum IL-6 levels and WHR and a negative one between IL-6 levels and HDL-C. In their study G.Zuliani *et al*. provided the epidemiological evidence that besides triglycerides, fasting insulin, and age, IL-6 is one of the main correlates of low HDL-C levels in older individuls (Zuliani *et al*., 2007). Elevated plasma levels of IL-6 predict the development of T2DM and future cardiovascular diseases. IL-6 decreases insulin signaling in peripheral tissues suppressing the expression of genes encoding components of insulin signaling system and inducing the expression of a suppressor of cytokine-3 (SOCS-3) – an inhibiting regulator of leptin and insulin action. The aforementioned effects of IL-6 may confirm its role in the process of adipogenesis and IR. Weight loss significantly decreases Il-6 levels in both adipose tissue and serum (Pittas *et al.*, 2004).

Conclusion

Higher resistin and ADMA concentrations in PCOS women showed a state of chronic low-grade inflammation. The correlation of IL-6 with WHR and HDL-cholesterol suggested a possible role of IL-6 in the development of MS in PCOS.

Declaration of interest: The authors declare no potential conflicts of interest.

REFERENCES

Abbasi F, Asagami T, Cooke JP *et al*. 2001. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am J Cardiol,* 88:1201–3.

American Diabetes Association. 1998. Diabetes Care, 21:310-14.

Cooke JP. 2004. Asymmetrical dimethylarginine: the uber marker? Circulation, 109:1813-18.

Davids M. and Teerlink T. 2012. Asymmetric dimethylarginine (ADMA) and cardiovascular disease. Ned Tijdschr Klin Chem Labgeneesk, 37:10-14.

Denis GV, Obin MS. 2013. Metabolically healthy obesity: origins and implications. *Molec Asp of Med*, 34(1):59-70.

Diamanti-Kandarakis E, Alexandraki K, Piperi C, *et al*. 2006. Inflammatory and endothelial markers in women with polycystic ovary syndrome. *Eur J Clin Invest,* 36:691–7.

Eder K, Baffy N, Falus A, *et al*. 2009. The major inflammatory mediator interleukin-6 and obesity. *Inflamm Res,* 58(11):727-36.

Farshchian F, Tehrani FR and Soltani A.2014. Visfatin and resistin levels in normal-weight and obese womwn with polycystic ovary syndrome. *Int J Endocrinol Metab,* 12(3):e15503.

Heutling D, Schulz H, Nickel I, *et al*. 2008. Asymmetrical Dimethylarginine, Inflammatory and Metabolic Parameters in Women with Polycystic Ovary Syndrome before and after Metformin Treatment. *J Clin Endocrinol Metab*, 93(1):82–90.

Koleva D, Orbetzova M, Deneva T. 2014. Asymmetric dimethylarginine in women with metabolic syndrome and polycystic ovary syndrome. Endocrinologia, 19 (3):154-9.

Koleva DI, Orbetzova MM, Atanassova PK. 2013. Adipose Tissue Hormones and Appetite and Body weight Regulators in Insulin Resistance. *Folia Medica Journal,* 55(1):25-32.

Kravariti M, Naka KK, Kalantaridou SN, *et al*. 2005. Predictors of endothelial dysfunction in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab,* 90:5088–95.

Lee JH, Chan JL, Yiannakouris N, *et al*. 2003. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab,* 88(10):4848-56.

Legro RS, Kunselman AR, Dodson WC, *et al*. 1999. Prevalence and predic ors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab,* 84:165–9.

Lin KY, Ito A, Asagami T, *et al*. 2002. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethy largininedimethylaminohydrolase. Circulation, 106:987–92.

Lu TM, Ding YA, Lin SJ, *et al*. 2003. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. *Eur Heart J,* 24:1912-9.

McLaughlin, T., Stühlinger, M., Lamendola, C., *et al*. 2006. Plasma asymmetric dimethylarginine concentrations are elevated in obese insulin-resistant women and fall with weight loss. *J Clin Endocrinol Metab,* 91:1896–1900.

Munir I, Yen HW, Baruth T, *et al*. 2005. Resistin stimulation of 17alpha-hydroxylase activity in ovarian theca cells in vitro: relevance to polycystic ovary syndrome. *J Clin Endocrinol Metab,* 90(8):4852-7.

Olzanecka-Glinianowicz, M., Kuglin, D., Dabkowska-Huc, A., *et al*. 2011. Serum adiponectin and resistin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol,* 154(1):361-6.

Pittas, A.G., Joseph, N.A., Greenberg, A.S. 2004. Adipocytokines and Insulin Resistance. The Journal of *Clinical Endocrinology & Metabolism*, 89(2):447–52.

Puri, R. 2014. Is it Finally Time to Dispel the Concept of Metabolically-Healthy Obesity? *J Am Coll Cardiol*, 63(24):2687-8.

Roytblat, L., Rachinsky, M., Fisher, A., *et al*. 2000. Raised interleukin-6 levels in obese patients. *Obes Res,* 8(9):673-5.

Teerlink T, Luo Z, Palm F, *et al*. 2009. Cellular ADMA: regulation and action. *Pharmacol Res,* 60:448–60.

Zhang J, Zhou I, Tang L, *et al*. 2011. The plasma level and gene expression of resistin in polycystic ovary syndrome. *Gynecol Endocrinol,* 27(12):982-7.

Zuliani G, Volpato S, Bie A *et al*. 2007. High interleukin-6 plasma levels are associated with low HDL-C levels in community-dwelling older adults: The InChianti Study. *Atherosclerosis,* 192(2):384-92.

****