Serum resistin is related to plasma HDL cholesterol and inversely correlated with LDL cholesterol in diabetic and obese humans

Maciej Owecki [1], Elżbieta Nikisch [2], Anna Miczke [3], Danuta Pupek-Musialik [3], Jerzy Sowiński [1]

1 Department of Endocrinology, Metabolism and Internal Medicine, University of Medical Sciences in Poznań, Poland
2 Department of Informatics and Statistics, University of Medical Sciences in Poznań, Poland
3 Department of Internal Diseases, Metabolic Disturbances and Hypertension, University of Medical Sciences in Poznań, Poland

Correspondence to: Assoc. Prof. Maciej Owecki, MD., PhD.
Department of Endocrinology, Metabolism and Internal Medicine
ul. Przybyszewskiego 49, 60-355 Poznań, Poland.
TEL: +48 61 8691330; FAX: +48 61 8691682; E-MAIL: mowecki@ump.edu.pl

Submitted: 2010-06-09 Accepted: 2010-08-24 Published online: 2010-12-05

Key words: **metabolism; resistin; adipocytokines; diabetes; obesity; atherosclerosis; lipids**

Neuroendocrinol Lett 2010; **31**(5):673–678 **PMID:** 21173741 NEL310510A08

Abstract

OBJECTIVES: Plasma cholesterol, triglycerides and serum resisistin may all be influenced by diabetes and obesity, but their associations remain unclear. Therefore, we put forward a hypothesis that serum lipids might be parallel to resistin, as they all reflect the metabolic status of obese humans.

DESIGN AND SETTING: We measured the concentrations of resistin, total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG) in 134 obese non-diabetic (73 women and 61 men) and 65 obese diabetic (33 women, 32 men) humans, and examined their interrelations. Obesity was defined according to the WHO criterion (BMI, ≥30 kg/m2) The presence of diabetes was the only differentiating factor between two groups of frankly obese humans.

RESULTS: Non-diabetic vs. diabetic, median and interquartile range, respectively: resistin (ng/mL) 26.08, 16.09 vs. 22.37, 14.54, p=0.736; TC (mmol/L) 5.02, 1.39 vs. 5.16, 1.56, p=0.374; HDL-C (mmol/L): 1.10, 0.41 vs. 1.02, 0.47 p<0.05; LDL-C (mmol/L): 3.00, 1.05vs.3.00, 1.30 p=0.978; TG (mmol/L) 1.70, 1.43vs.1.95, 1.81 p<0.05. To investigate the interrelations between resistin and lipids, a simple regression analysis was used, and the results were for resistin & TC, HDL-C, LDL-C, and TG, respectively: in the whole cohort r=–0.1364, p=0.0670, r=0.1514, p=0.0437, r=–0.2573, p=0.0006, r=0.0434, p=0.5597; in non-diabetics: r=–0.2067, p=0.0213, r=0.1023, p=0.2621, r=–0.2399, p=0.0083 and r=0.0288, p=0.7497; in diabetics r=0.0280, p=0.8360, r=0.2267, p=0.0929, r=–0.2933, p=0.0298, r=0.1349, p=0.3127.

CONCLUSIONS: In diabetic and non-diabetic subjects the atherogenic LDL cholesterol shows an inverse correlation with resistin, whereas the protective anti-atherosclerotic HDL cholesterol is positively correlated with resistin.

Abbreviations:

TC	- total cholesterol	LDL-C	- low density lipoprotein cholesterol
HDL-C	- high density lipoprotein cholesterol	TG	- triglycerides
		BMI	- the body mass index

ORIGINAL ARTICLE

INTRODUCTION

Adipose tissue is a complicated endocrine organ with numerous neuroendocrine regulatory pathways, and it plays an important role in the pathogenesis of obesity and type 2 diabetes mellitus. Endocrine substances, or adipocytokines, secreted by this tissue include adiponectin, leptin, IL-6, TNFα (Spiegelman and Flier 1996; Friedman and Halaas 1998; Prins 2002; Steppan and Lazar 2002), and resistin (FIZZ3/ADSF). The latter belongs to the family of cysteine-rich resistin-like molecules (RELM) (Steppan and Brown *et al.* 2001), together with RELM-α and RELM-β. Serum concentrations of resistin are increased in obese mice and influence insulin action (Steppan and Bailey *et al.* 2001), however, although resistin might link obesity with insulin resistance and diabetes in mice models, numerous studies in rodent models have called this interesting idea into question (Juan *et al.* 2001; Le Lay *et al.* 2001; Way *et al.* 2001). In humans, the role of resistin is even more controversial. In contrast to mice, human resistin is barely detectable in adipose tissue (Nagaev and Smith 2001; Savage *et al.* 2001), and no correlation was found between resistin expression of isolated adipocytes and obesity or type 2 diabetes (Nagaev and Smith 2001; Savage *et al.* 2001; Janke *et al.* 2002). Furthermore, serum resistin levels were found to be related to body mass index (BMI) in human subjects by some authors (Azuma *et al.* 2003; Degawa-Yamauchi *et al.* 2003; Fujinami *et al.* 2004), whereas others did not demonstrate this association (Heilbronn *et al.* 2004; McTernan *et al.* 2003; Youn *et al.* 2004).

As plasma cholesterol and triglycerides influence the metabolic status and are important risk factors for cardiovascular damage in obese and diabetic humans, we hypothesized that resistin levels could be related to plasma lipids. Both plasma lipids and resistin are substances which may be influenced by diabetes and obesity. Therefore, the aim of our study was to measure resistin concentrations in non-diabetic and diabetic humans with a similar range of trunkal obesity, and to examine whether resistin concentrations were related to the levels of plasma cholesterol and triglycerides. For this purpose, we investigated two homogenous groups of obese humans, in whom the presence of type 2 diabetes mellitus was the solely differentiating factor.

SUBJECTS AND METHODS

The study group consisted of 134 obese non-diabetic (73 women and 61 men) and 65 obese diabetic (33 women, 32 men) humans recruited by local advertising. Their ages were respectively: 50, 14 years; and 55, 12 years (data shown as median and inter-quartile range). Obesity was defined according to the WHO criterion (BMI, ≥30 kg/m^2). All diabetic and non-diabetic subjects suffered from hypertension, well controlled on medication with ACE-inhibitors. Except for hypertension, all subjects were otherwise healthy according to history, clinical examination, and routine laboratory findings. In particular, none of the studied subjects had evidence of acute or chronic inflammatory disease, had a history of alcohol overconsumption, was completely sedentary, or involved in athletics. In all non-diabetics, a normal glucose tolerance was confirmed with the standard oral glucose tolerance test: 75 grams of glucose was administered orally and the plasma glucose concentration was measured in 2 hours. In the diabetes group, all subjects presented with type 2 diabetes mellitus. Diabetes was defined according to WHO criterion (fasting glucose equal or more than 126 mg/dL, or 6.99 mmol/L, on two various days). All subjects were treated with oral sulfonylureas and diabetes was controlled sufficiently: fasting plasma glucose close to the values found in the non-diabetic subgroup was the condition of entering into the study.

All subjects were examined in the morning (at 08:00 a.m.) after an overnight fast. Waist and hip circumferences where measured. Repeated bioimpedance tests were performed at the same time after an overnight fast (08:00 a.m.). Exercise, and alcohol or caffeine consumption was banned 24 hrs prior to the bioimpedance test. A venous blood sample was obtained for the measurement of plasma levels of glucose, total plasma cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) at the hospital's routine chemistry laboratory. Serum levels of resistin were measured using an ELISA method (R&D Systems, Inc., Minneapolis, MN) and were expressed as nanograms per milliliter. Resistin was measured according to the manufacturer's recommended protocol, with a sensitivity of 0.026 ng/mL. An intra-assay and inter-assay coefficients of variation were 5.25% and 6.47%, respectively.

The values are given as the median and inter-quartile range. Statistical tests included Mann-Whitney test, Kruskal-Wallis test, Dunn test (for post hoc analysis), and Spearman correlation analysis. STATISTICA 6.0 (StatSoft, Inc., U.S.A.) software was used for statistical analyses.

The study was approved by the ethics committee of the Poznań University of Medical Sciences. All subjects gave informed consent to participate.

RESULTS

The clinical data of diabetic and non-diabetic subjects are shown in Table 1, both genders, and women and men separately. Diabetic patients showed higher levels of glycosylated haemoglobin, plasma glucose and TG, and lower HDL-C. Except for that, the study groups were of similar age, and their anthropometric characteristics, percent body fat, as well as serum resistin concentrations did not differ. For both sexes compared separately, no differences were found, except plasma glucose and HbA1c (Table 1).

To investigate the influence of resistin on serum concentrations of HDL-C, LDL-C, TC and TG, correla-

tions between those lipids and resistin were calculated as follows: for all subjects grouped together, for diabetics only and for non-diabetics only. In order to evaluate the influence of sex, we also estimated all correlations for females and males separately.

In a simple regression analysis performed in the whole cohort, serum levels of resistin were correlated with the levels of HDL-C and LDL-C (r =0.1514; p=0.0437, and r=–0.2573; p=0.0006, respectively), but did not correlate with TC and TG (r=–0.1364; p=0.0670, and r=0.0434; p=0.5597, respectively). Dissimilar results were found in non-diabetics: r=–0.2067; p=0.0213, r=0.1023; p=0.2621, r=–0.2399; p=0.0083 and r=0.0288; p=0.7497 for TC, HDL-C, LDL-C and TG, respectively. In diabetics, serum resistin did not correlate with the levels of TC, HDL-C and TG, whereas an inverse correlation with LDL-C was found (r=0.0280; p=0.8360, r=0.2267; p=0.0929, r=0.1349; p=0.3127 and r=–0.2933; p=0.0298, respectively). The correlations between resistin and HDL-C, and LDL-C in non-diabetics and diabetics are shown on Figures 1 and 2, respectively.

Women. In diabetic women, serum levels of resistin did not correlate with the levels of TC, HDL-C, LDL-C and TG (r=0.0211; p=0.9152, r=0.2901; p=0.1506, and r=–0.1164; p=0.5711, r=–0.0120; p=0.9515, respectively). In non-diabetic women, serum resistin inversely correlated with LDL-C levels (r=–0.2838; p=0.0220), whereas it did not correlate with other lipids (r=–0.1005; p=0.4219, r=0.1853; p=0.1395, and r=0.1412; p=0.2545, for TC, HDL-C and TG, respectively). In all women assessed together, serum levels of resistin were not correlated with TC and TG (r=–0.0658; p=0.5287, r=0.0714; p=0.4915, respectively). In contrast, resistin correlated with HDL-C and inversely with LDL-C (r=0.2624; p=0.0120, r=–0.2458; p=0.0189, respectively) in this group.

Men. The results for TC, HDL-C, LDL-C and TG were respectively: in diabetic men: r=–0.0079; p=0.9676, r=0.2138; p=0.2565, r=–0.3606; p=0.0546, and r=0.1800; p=0.3413; in non-diabetic men: r=–0.3050; p=0.0199, r=0.0227; p=0.8667, r=–0.2172; p=0.1112, and r=–0.0898; p=0.5027. Furthermore, in all men assessed together resistin levels were not correlated with the levels of TC, HDL-C, TG, but they inversely correlated with LDL-C (r=–0.1991; p=0.0645, r=0.0772; p=0.4773, r=–0.0053; p=0.9606, and r=–0.2690; p=0.0133, respectively).

Tab 1. Clinical characteristics of non-diabetic and diabetic subjects examined. Data are shown as median and inter-quartile range, and presented separately for women, separately for men, and for both genders together.

	Non-diabetics			Diabetics			*p*-value		
	Women	**Men**	**Both genders**	**Women**	**Men**	**Both genders**	**Women**	**Men**	**Both genders**
Number	73	61	134	33	32	65	73 vs. 33	61 vs. 32	134 vs. 65
BMI (kg/m^2)	37.30; 10.40	33.10; 9.10	35.35; 10.30	36.30; 7.40	34.05; 9.25	36.20; 8.40	0.583	0.739	0.906
Waist (cm)	110.5; 21.50	112.0; 24.0	111.0; 20.0	111.0; 13.0	116.0; 17.5	115.0; 14.0	0.213	0.177	0.074
Hip (cm)	123.5; 21.50	110.0; 22.0	117.0; 22.0	120.0; 20.0	115.5; 16.5	117.0; 20.0	0.590	0.396	0.958
Glucose (mmol/L)	5.4; 1.10	5.60; 0.80	5.50; 0.90	7.73; 3.07	6.70; 1.20	6.75; 2.13	**<0.05**	0.054	**<0.01**
HbA1c	5.9; 0.90	5.9; 0.80	5.90; 0.90	7.45; 2.25	7.25; 1.25	7.40; 1.40	**<0.0001**	**<0.0001**	**<0.0001**
Total cholesterol (mmol/L)	4.93; 0.98	5.28; 1.88	5.02; 1.39	5.18; 1.42	5.12; 1.82	5.16; 1.56	0.216	0.904	0.374
HDL cholesterol (mmol/L)	1.17; 0.40	1.01; 0.36	1.10; 0.41	1.13; 0.58	0.94; 0.36	1.02; 0.47	0.215	0.115	**<0.05**
LDL cholesterol (mmol/L)	3.0; 0.70	3.00; 1.10	3.00; 1.05	3.45; 1.30	2.74; 1.50	3.00; 1.30	0.161	0.245	0.978
Triglicerydes (mmol/L)	1.36; 1.02	2.18; 1.72	1.70; 1.43	1.72; 1.25	2.54; 2.91	1.95; 1.81	0.066	0.221	**<0.05**
Resistin (ng/mL)	26.88; 15.25	25.47; 16.86	26.08; 16.09	20.75; 15.69	27.43; 12.14	22.37; 14.54	0.208	0.486	0.736

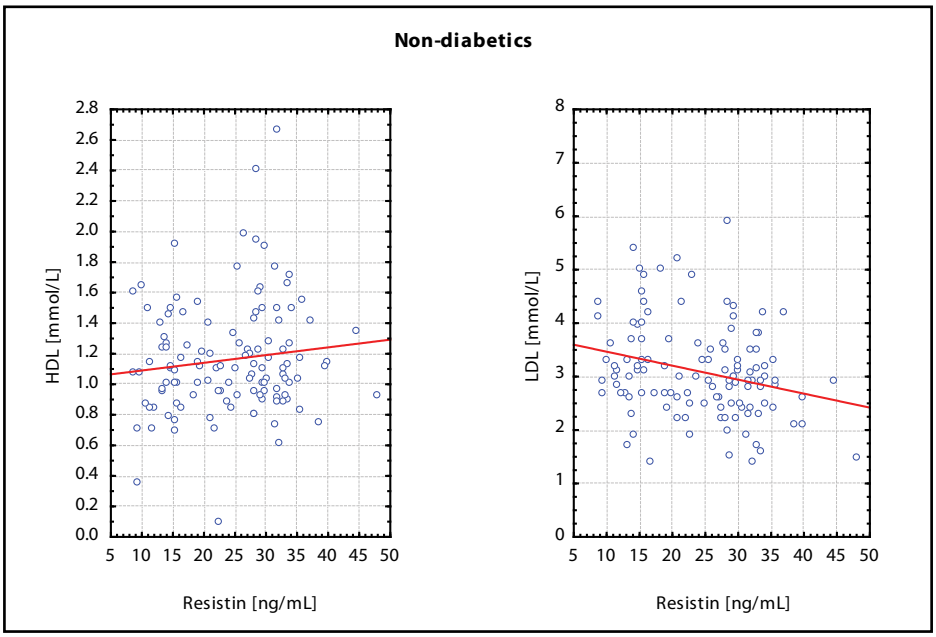

Fig. 1. Correlations between serum resistin and HDL cholesterol (r=0.1023, *p*=0.2621), and between resistin and LDL cholesterol levels (r=–0.2399, *p*=0.0083) in non-diabetic subjects.

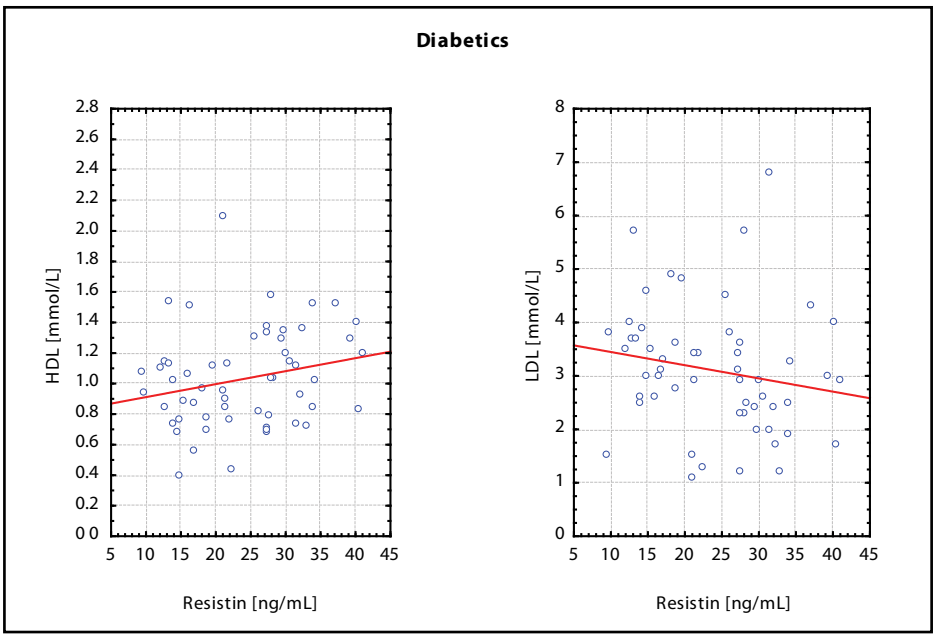

Fig. 2. Correlations between serum resistin and HDL cholesterol (r=0.2267, *p*=0.0929), and between resistin and LDL cholesterol levels (r=–0.2933, *p*=0.02980) in diabetic subjects.

DISCUSSION

Findings from animal model studies suggested that resistin could be an important factor in the development of obesity and type 2 diabetes (Steppan and Bailey *et al.* 2001; Pang and Le 2006; Muse *et al.* 2007). However, the link between resistin and obesity in humans is less clear, and conflicting results were reported. Most of the human studies failed to show this link (Nagaev *et al.* 2001; Savage *et al.* 2001; Patel *et al.* 2003), and only a few studies demonstrated that resistin serum concentrations were elevated in obese patients and could be related to obesity (McTernan PG *et al.* 2002; McTernan CL *et al.* 2002).

Even less is known about the relationships between resistin and plasma lipids, which prompted us to investigate this problem. Since plasma lipids are a strong risk factor for the development of atherosclerosis which is the major cause of cardiovascular system damage in diabetes and obesity, it seemed logical to us that the serum resistin might be parallel to plasma lipids. These parallel concentrations occurred indeed, unexpectedly however, what we demonstrated in this study was that in both diabetic and non-diabetic subjects the atherogenic LDL cholesterol showed an inverse correlation with resistin, whereas the protective anti-atheroscle-

rotic HDL cholesterol was positively correlated with resistin. This is in contrast to what found Osawa *et al.* (2007). Furthermore, a correlation between total cholesterol and resistin was found only in the whole cohort, and disappeared in separated groups. The fact that LDL cholesterol was inversely correlated with resistin seemed surprising at first sight. Indeed, we expected a positive correlation as both resistin and LDL cholesterol are known as atherosclerosis risk factors. Resistin mRNA and protein were demonstrated in atherosclerotic lesions in the aorta of mice, and resistin showed a relationship with coronary heart disease in cross-sectional studies (Burnett *et al.* 2005; Reilly *et al.* 2005; Ohmori *et al.* 2005). The explanation of this inverse correlation might be difficult, particularly because the sources of resistin differ between rodents and humans. The adipocyte is presumably the sole source of resistin in mice, whereas in humans the majority of resistin is secreted by macrophages and monocytes, and only little is produced by adipocytes. Obviously, our clinical study was not aimed at the explanation of this unexpectedly inverse correlation, and further research is necessary. Still, we might propose some theoretical explanation: presumably, a high LDL cholesterol concentration inhibits resistin secretion by the macrophages and monocytes. The fact that resistin molecules were found in atherosclerotic lesions, as were LDL-C molecules, might point to some competitive mechanisms between LDL-C and resistin. This hypothesis necessitates further research.

In addition, we would like to comment on the approach we used in this study. Here we investigated two groups of obese patients, in whom the presence of type 2 diabetes was the only differentiating factor. However, we decided to examine only diabetic subjects with a correct, or nearly correct fasting plasma glucose achieved on medication. One may argue about this limitation, which is in contrast to many other studies. This approach diminished our sample but gave us the opportunity to rule out the influence of imbalanced diabetes on the secretion of resistin. In other words, we analyzed a strictly limited group of obese diabetic and euglycemic individuals, with the diabetic subgroup well balanced on medication. Also, because gender may be a confounding factor, we studied both men and women, and evaluated them separately. Including only women and only men into the analyses yielded comparable results as including all individuals.

Based on our results, we show here that resistin levels are paradoxically correlated with the plasma LDL and HDL cholesterol levels in obese humans. Importantly, this correlation occurs for LDL cholesterol both in diabetic and non-diabetic obese patients. The findings from our study may be another piece of information to the discussion about the role of resistin in human metabolism.

ACKNOWLEDGEMENTS

This work was supported by grant No. 501-02-02221355-08350 from the Poznań University of Medical Sciences, Poznań, Poland.

REFERENCES

1 Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, Saruta T (2003). Correlation between serum resistin level and adiposity in obese individuals. Obes Res. **11**: 997–1001.
2 Burnett MS, Lee CW, Kinnaird TD, Stabile E, Durrani S, Dullum MK, et al (2005). The potential role of resistin in atherogenesis. Atherosclerosis. **182**: 241–248.
3 Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ (2005). Resistin is an inflammatory marker of atherosclerosis in humans. Circulation. **111**: 932–939.
4 Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, et al (2003). Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab. **88**: 5452–5455.
5 Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals (1998). Nature. **395**: 763–770.
6 Fujinami A, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H, et al (2004). Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. Clin Chim Acta. **339**: 57–63.
7 Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, et al (2004). Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocrinol Metab. **89**: 1844–1848.
8 Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM (2002). Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res. **10**: 1–5.
9 Juan CC, Au LC, Fang VS, Kang SF, Ko YH, Kuo SF, et al (2001). Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. Biochem Biophys Res Commun. **289**: 1328–1333.
10 Le Lay S, Boucher J, Rey A, Castan-Laurell I, Krief S, Ferré P, et al (2001). Decreased resistin expression in mice with different sensitivities to a high-fat diet. Biochem Biophys Res Commun. **289**: 564–567.
11 McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S (2002). Resistin, central obesity, and type 2 diabetes. Lancet. **359**: 46–47.
12 McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL, et al (2003). Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. J Clin Endocrinol Metab. **88**: 6098–6106.
13 McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, et al (2002). Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab. **87**: 2407–2410.
14 Muse ED, Lam TK, Scherer PE, Rossetti L (2007). Hypothalamic resistin induces hepatic insulin resistance. J Clin Invest. **117**: 1670–1678.
15 Nagaev I, Smith U (2001). Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem Biophys Res Commun. **285**: 561–564.
16 Ohmori R, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, et al (2005). Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. J Am Coll Cardiol. **46**: 379–380.
17 Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, et al (2007). Plasma resistin, associated with single nucleotide polymorphism-420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. Diabetes Care 2007. **30**: 1501–1506.

18 Pang SS, Le YY (2006). Role of resistin in inflammation and inflammation-related diseases. Cell Mol Immunol. **3**: 29–34.
19 Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al (2003). Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun. **300**: 472–476.
20 Prins JB (2002). Adipose tissue as an endocrine organ. Best Pract Res Clin Endocrinol Metab. **16**: 639–651.
21 Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al (2001). Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator activated receptor-gamma action in humans. Diabetes. **50**: 2199–2202.
22 Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ (2003). Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol. **149**: 331–335.
23 Spiegelman BM, Flier JS (1996). Adipogenesis and obesity: rounding out the big picture. Cell. **87**: 377–389.
24 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al (2001). The hormone resistin links obesity to diabetes. Nature. **409**: 307–312.
25 Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, et al (2001). A family of tissue-specific resistin-like molecules. Proc Natl Acad Sci USA. **98**: 502–506.
26 Steppan CM, Lazar MA (2002). Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab. **13**: 18–23.
27 Way JM, Görgün CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, et al (2001). Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J Biol Chem. **276**: 25651–25653.
28 Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, et al (2004). Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. J Clin Endocrinol Metab. **89**: 150–156.