RESEARCH ARTICLE

The Role of High Concentration of Resistin in Endothelial Dysfunction Through Induction of Proinflammatory Cytokines Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) and Chemokin Monocyte Chemotactic Protein-1 (MCP-1)

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BACKGROUND: Many previous studies have reported that central obesity is related to inflammation and endothelial dysfunction. It has also been reported that resistin can induce proinflammatory cytokines TNF-α and IL-6, which can result in endothelial dysfunction, although the role of resistin in human remains unclear. The aim of this study was to assess the role of resistin in influencing the proinflammatory cytokines TNF-α, IL-6, and chemokin MCP-1 in nondiabetic, central obese individuals. Results of this study are hoped to be useful to make a strategy for early prevention of endothelial dysfunction especially in obese individuals.

METHOD: This was a cross-sectional study on 73 non diabetic obese male subjects (waist circumferences >90 cm). Resistin, hs-TNFα, IL-6, MCP-1, VCAM-1 were assessed by ELISA. Statistical analysis was performed using SPSS for Windows v.11.5 with significance $p<0.05$. The correlations among biomarkers were assessed using Spearman's Rho test.

RESULTS: The study results showed a significant correlation between resistin and TNF-α ($r = 0,274$, $p<0,005$), and a significant correlation between TNF-α and IL-6 ($r = 0,430$, $p<0,001$). It was found that high concentration of resistin caused the concentration of TNF-α, IL-6 and MCP-1 to increase, and affected the increase of VCAM-1 ($p = 0,0030$), A significant correlation between waist circumference and inflammation (hsCRP, $r = 0,296$ $p<0,005$, IL-6, $r = 0,374$ $p<0,001$ and HOMA IR, $r = 0,331$ $p<0,001$) was also found.

CONCLUSION: This study showed that the role of resistin in endothelial dysfunction occurred at a high concentration of resistin through induction of proinflammatory cytokines TNF-α, IL-6, and chemokin MCP-1. We suggest that inflammation in obesity starts with a positive feedback loop mechanism between resistin and TNF-α.

KEYWORDS: Obesity, Inflammation, Adipocytokines, Resistin, Tumor Necrosis Factor-α, Interleukin-6, Monocyte Chemotactic Protein-1, Vascular Cell Adhesion Molecule – 1.

Introduction

Studies by the Asia Pasific Cohort Study Collaboration have shown that populations in the various countries in the region have attributable fractions due to overweight and obesity of 0.8% to 9.2% for coronary heart disease mortality, 0.2% to 2.9% for haemorrhagic stroke mortality, and 0.9% to 10.2% for ischemic stroke mortality. These results indicate that the negative consequences of overweight and obesity for the health and economy of many of these countries tend to increase in the coming years (1).

Obesity is usually the result of the combination of genetic factors and sedentary lifestyle, characterized by excess nutrition and lack of regular physical activity. It is closely associated with the development

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of type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease, as some of the medical problems (3). The main mechanism by which obesity can cause these metabolic and vascular diseases is the development of insulin resistance. This frequent metabolic abnormality is typically defined as reduced insulin action in peripheral tissues such as skeletal muscles, adipose tissues, and liver. A state of adipose tissue excess, particularly that of visceral fat (central obesity), is associated with a continuous production of mediators that impair insulin action in skeletal muscles. All of these abnormalities result in a state of constant and progressive damage to the vascular wall, manifested by a low-grade progressive inflammatory process and endothelial dysfunction (2).

Endothelial dysfunction is defined as the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, pro-atherogenic and anti-atherogenic factors, and pro-coagulant and anti-coagulant factors that are considered as an early pivotal event in atherogenesis (2,3).

Obesity is associated with a state of chronic, low-grade inflammation, particularly in the white adipose tissue. A recent finding states that obesity is characterized by macrophage accumulation in the white adipose tissue and the overlapping biology and function of macrophages and adipocytes has added another dimension to our understanding of the development of adipose tissue inflammation in obesity. Accumulation of macrophages in the adipose tissues is due to the infiltrating macrophages, as well as transdifferentiation of preadipocytes or adipocytes. (5,6,7).

Macrophages, upon activation, secrete numerous cytokines and chemokines, such as TNF-a, IL-1, IL-6, and MCP-1 that are known to cause insulin resistance in adipocytes These cytokines and chemokines further activate macrophages to increase lymphokine production and secretion. These amplifying signals increasingly impair the adipocyte insulin signaling process and eventually cause systemic insulin resistance (8).

The adipose tissue has long been thought as a simple energy storage organ for triglycerides. The discovery of the adipose hormone leptin in 1994 by Zhang et al. [1] has revolutionized our understanding of energy homeostasis, and has definitely confirmed that adipose tissue is an organ that actively participates in energy homeostasis. It is now generally accepted that adipose tissue secretes many bioactive substances to modulate its own or other tissues' functions in response to neural (sympathetic), hormonal and nutrient (mainly fatty acid) stimuli, known as adipokine or adipocytokine, such as leptin, TNF-α, IL-6, PAI-1, adiponektin, resistin, angiotensinogen, MCP-1, etc (9,10,11).

One of the adipokine secreted is resistin (12). Resistin was originally described as an adipocyte-derived polypeptide that provided the link between obesity and insulin resistance, but up to now the role of resistin in human is still unclear (14).

In humans, resistin is expressed strongly in macrophages, and less in monocytes, adipocytes and preadipocytes (15). Molecules of the resistin-like molecule (RELM) family are found abundantly in inflamed tissues suggesting that resistin can play a role in the inflammatory process. A study by Bokarewa et al has shown that resistin displays potent proinflammatory properties by strongly up-regulating IL-6 and TNF-α, responding to TNF-α challenge, and enhancing its own activity by a positive feedback. Proinflammatory properties of resistin are abrogated by NF-κB inhibitor, indicating the importance of NF-κB in signaling pathway for resistin-induced inflammation. (16).

Resistin can induce adhesion molecules expression in endothelial cells in vitro such as VCAM-1, MCP-1, dan ICAM-1 (3,4,12,15). This shows the role of resistin in activating endothelial cell by inducing endothelin-1 release (11).

Methods

This study was a cross-sectional study involving 73 nondiabetic obese male subjects, defined by waist circumference > 90 cm and glucose < 126 mg/dl. Hypertension (blood pressure > 140/85 mmHg), renal failure, liver failure, and those who had consumed antiinflammation in the last 3 months or with an acute inflammation, were excluded.

Resistin (Biovendor, Inc., Czech), hs-TNFα, IL-6, MCP-1, VCAM-1 (R&D Systems, Inc., Minnesota) were assessed by ELISA. Statistical analysis was done by SPSS for windows v.11.5 with a significance level at $p<0.05$. The correlations between biomarkers were assessed by Spearman's Rho test.

Results

This study was carried out exclusively on m ales subjects to p revent any biases r egarding hormonal effects found o n female subjects (17, 18). T able 1 showed general description o f subjects' baseline characteristics. Subjects aged average 37.73 years old, with waist circumferences means 98.9932 ±6.4191 cm. Subjects included in this research have no hypertension (blood pressure systolic means 115.3014 ± 7.8416 and diastolic 76.8493 ± 6.1524), no diabetes (Fasting Plasma Glucose means 87.1644 ± 9 .5743), no liver enzyme impaired (AST means 26.9589 ± 6.5774, ALT 38.3973 ± 13.7090), no renal dysfunction (creatinin means 0.9374 ± 0.1573), and n o acute inflammation (hsCRP means 2.0921 ± 1.8322).

Table 1. Description of Subjects' Baselines Characteristics.

Characteristics	Median	Means S	D
N = 73			
Age	37	37.73	7.67
Height	167	160.59	33.90
Weight	80	78.15	16.01
WC	98	98.00	6.42
SBP	120	115.30	7.84
DBP	80	76.85	6.15
FPG	86	87.16	9.57
AST	27	26.96	6.58
ALT	37	38.40	13.71
Total Bilirubin	0,74	0.76	0.24
Creatinine	0,94	0.94	0.16
Triglyceride	179	187.07	109.88
HDL	43	44.32	9.26
Insulin	8,50	10.46	6.18
Resistin	4,7425	6.16	4.24
TNF-α	5,5000	8.53	7.36
IL-6	1,5800	2.20	1.69
MCP-1	315,300	319.94	89.16
VCAM-1	680,600	688.13	151.43
hsCRP	1,6700	2.10	1.83
HOMA IR	1,846	2.33	1.65

* Age (years old); Height (cm); Weight (Kg); WC = Waist Circumference (cm); FPG = Fasting Plasma Glucose (mg/dl); AST = Aspartate Aminotransferase (U/L); ALT = Alanine Aminotransferase (U/L) SBP = Sistolic Blood Pressure (mmHg); DBP = Diastolic Blood Pressure (mmHg); Total Bilirubin (mg/dl); Creatinine (mg/dl); Triglyceride (mg/dl); HDL-C = High Density Lipoprotein Cholesterol (mg/dl); Insulin = Fasting Insulin (uIU/ml); Resistin = pg/ml; TNF-α = Tumor Necrosis Factor – α (pg/ml); IL-6 = Interleukin 6 (pg/ml); MCP-1 = Monocyte Chemotactic Protein – 1 (pg/ml); VCAM-1 = Vascular Cell Adhesion Molecule – 1 (ng/ml).

Table 2. Correlation between Waist Circumferences and the inflammatory biomarkers.

	Resistin	TNF-α	IL-6	MCP-1	VCAM-1	hsCRP	HOMA-IR
WC	r = 0.061	r = 0.023	r = 0.374** r	= -0.214	r = 0.093	r = 0.296*	r = 0.331**
	p= 0.068	p= 0.863	p= 0.004	p= 0.068	p= 0.436	p= 0.011	p= 0.005
Resistin		r = 0.274*	r = 0.237	r = -0.008	r = -0.065		
		p= 0.034	p= 0.071	p= 0.947	p=0.588		
VCAM-1		r = -0.1	r = 0.17	r = -0.134			
		p= 0.442	p= 0.899	p= 0.258			

* Significant at p < 0.005; ** significant at p < 0.001.

The positive correlation between WC and the inflammatory markers IL-6 and hsCRP as in table 2, showed that central obesity (WC >90 cm) was associated with the state of chronic low grade inflammation that could develop into insulin resistance (9,19), while there was no significant correlation between WC with MCP-1 and VCAM-1 (table 2).

The results from table 2 also showed a significant correlation between resistin and TNF-α (r=0.274; p<0.05), and a significant correlation between TNF-α and IL-6 (r=0.430, p<0.01). A significant correlation between waist circumference and inflammation (hsCRP with r=0.296; p<0.05, IL-6 with r=0.374; p<0.001, and HOMA-IR with r=0.331; p<0.001) was also found. From figure 1 we can see that at high concentration of resistin, the concentration of TNF-α, IL-6 and MCP-1 will be increased, and affect the increase of VCAM-1 (p=0.0030).

Correlation between resistin and combination of TNF-α, IL-6, and MCP-1 at high and low concentration. It can be seen that higher levels of resistin strongly increases the inflammation state showed by higher levels of combination of TNF-α, IL-6 and MCP-1 (p=0.007).

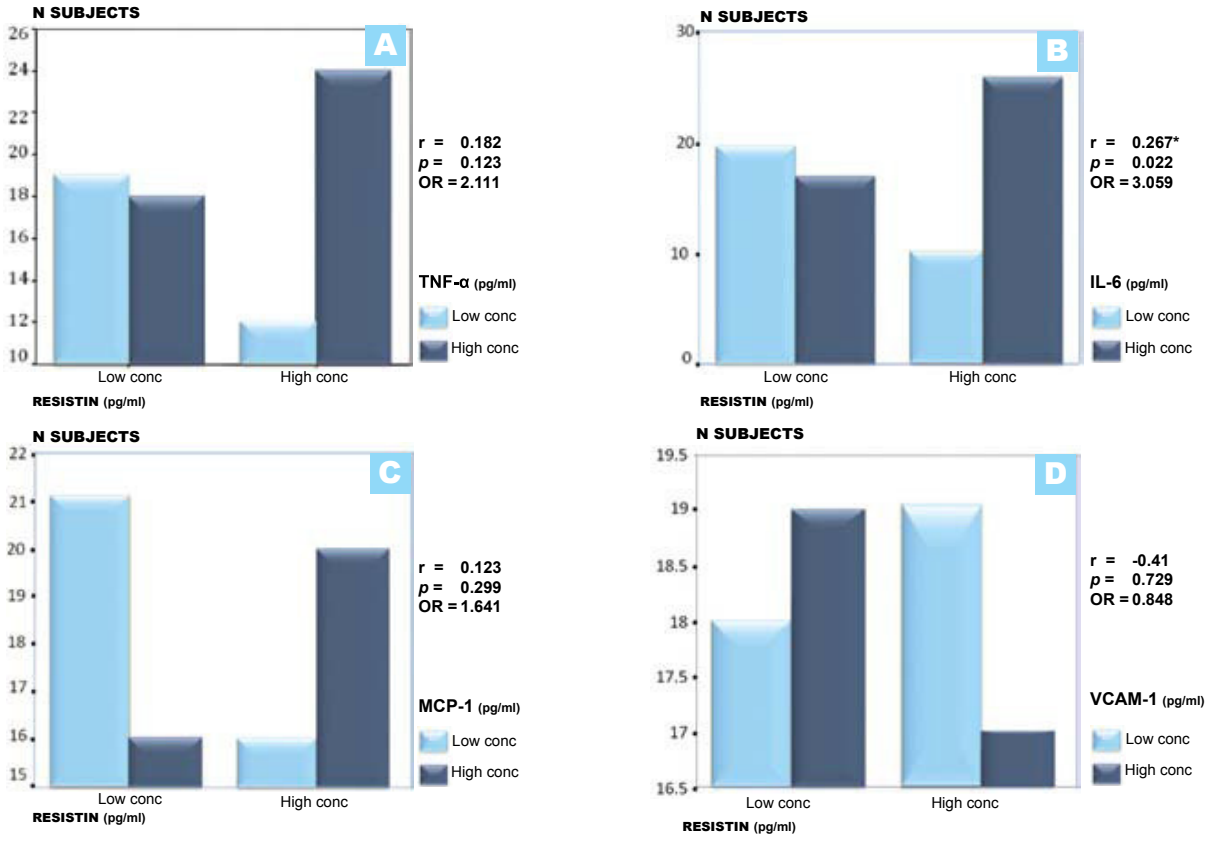

Fig1. Correlation between Resistin and TNF-α, IL-6, MCP-1, VCAM-1

At high levels of resistin, higher level of TNF-α was found (OR = 2.111 as compared with lower level of resistin (fig. 1A). Higher levels of resistin significantly increase IL-6 levels (OR = 3.059) (fig. 1B). At higher concentration of resistin, higher levels of MCP-1 was found (OR = 1.641) (fif. 1C). The absence of any correlation between resistin and VCAM-1 suggests that resistin does not affect VCAM-1 directly (fig. 1D).

Fig 2 showed us that at high levels of three variables (TNF-α, IL-6, and MCP-1). VCAM-1 doesn't increase significantly. VCAM-1 increased at subjects with high TNF-α and IL-6 but low MCP-1 ($p<0.05$). This suggests a compensate mechanism from MCP-1 at elevating TNF-α and IL-6 to avoid endothelial cells death (27, 28).

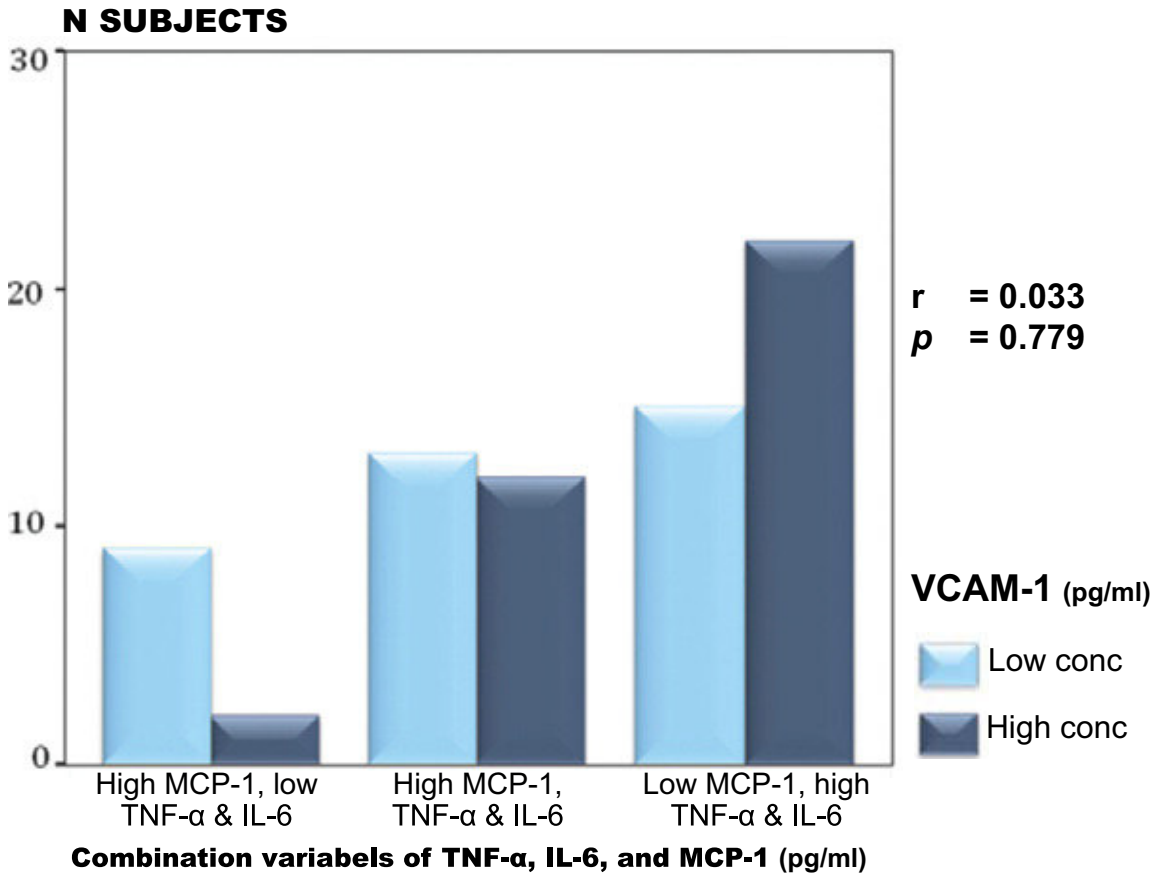

Fig 2. Correlation between combination of variables TNF-α, IL-6, MCP-1, with VCAM-1 at high and low concentration.

Discussion

In our present study we found that waist circumference was significantly correlated with IL-6 and HOMA-IR and slightly correlated with hsCRP. This shows that increased waist circumference of more than 90 cm in men is related to chronic low – grade inflammation (20). Obese adipose tissue is characterized by macrophages infiltration and these macrophages are an important source of inflammation in the tissue by secreting many proinflammatory cytokines, which will impair insulin action in the vasculatures (20).

Insulin signaling regulates GLUT4 translocation and glucose uptake in the skeletal muscle and NO production and vasodilatation in the vascular endothelium. This insulin action facilitates glucose uptake by vasodilating blood vessels via IRS-1 induction, which can activate eNOS and induces

vasoconstrictor ET-1 (21,22,23). This explains how cytokines impaires insulin action that will subsequently induce endothelial dysfunction.

We also found in this study that there was no crrelation between WC and resistin and TNF-α, as well as with MCP-1 or VCAM-1. It was known that 30% of circulating IL-6 was secreted from adipocytes (19), meanwhile TNF-α and resistin in human were mainly secreted from macrophages (6,7). With this finding, we suggest that most of IL-6 in the circulation was secreted from adipocytes in visceral adipose tissues, which could stimulate CRP secretion from the liver. However, the inflammation process that occured was not strong enough, hence not enough number of macrophages infiltrated into the adipose tissue. This suggests that the level of TNF-α and resistin secreted from the adipose tissues was not enough, so we didn't find a significat correlation between both cytokines and WC. The low HOMA IR (1.846) found in the study subjects also supported this suggestion that the inflammation that occured in the study subjects was still very low to induce insulin resistance, and the "mild" chronic inflammation might be found in younger subjects (mean age 37,73 ± 7.67), where 69.9% of the participants' age were between 30 – 40 years.

By Spearman correlation test, we found a positive correlation between resistin and TNF-α. This agreeswith the statement of Bokarewa et al that there was a positive feedback loop mechanisms existing between resistin and TNF-α, where the dimer form of resistin upregulated macrophages to secrete more TNF-α, meanwhile resistin expression was induced by TNF-α (16).

Data grouping based on median divided the parameters into two categorial groups: lower concentration group and higher concentration group. From this grouping we could assess the parameters' roles and correlations at higher and lower concentrations.

Correlation tests on all categorial variables TNF-α, IL-6, and MCP-1 showed that at higher concentration of resistin, TNF-α, IL-6 and MCP-1 concentrations increased. A study by Bokarewa et al and Silswal et al showed that resistin was a strong proinflammatory cytokine, where resistin could strongly induce TNF-αand IL-6 expression in vitro (16,24). Categorial correlation test between resistin and the combination variables of TNF-α, IL-6 and MCP-1 showed higher increase of resistin concentration. This indicated that TNF-α, IL-6 and MCP-1 concentration increased with increase of resistin concentration, and at the same time amplifying the inflammation process. TNF-α and IL-6 can mediate the inflammation process in an endocrinal manner, this alsocan stimulate hepatic acute phase of protein expression such as CRP. CRP then induces endothelial adhesion molecules expression (2).

TNF-α induces adhesion molecules and chemokines expression via NF-κβ cascades (25). Increased MCP-1 will attract more macrophages and secreted more cytokines like resistin and TNF-α, and these process finally will strongly increase the low grade chronic inflammation in obesity (8).

There is no correlation between resistin and VCAM-1 ; indicated that resistin did not influence VCAM-1 directly, but through a median variables.

Highest concentration of VCAM-1 was found in the subjects having higher concentrations of TNF-α and IL-6 but lower MCP-1 concentration. This suggests that in higher TNF-α and IL-6 concentrations, MCP-1 concentration will increase as a compensation mechanism to protect the endothelial cells, otherwise there will be an endothelial dysfunction shown by significantly increased VCAM-1.

In the initiation stage of endothelial dysfunction, adhesion molecules such as VCAM-1 attract monocytes in the circulation to adhere to the endothelial cells. At the next progression stage, MCP-1 restrains the rolling monocytes and attracts monocytes to migrate into the intima (26). MCP-1 has an angiogenic property to prevent hypoxia in leukocytes migration to protect the cells from death (27).

Significant increase of VCAM-1 concentration without increase of MCP-1 in the subjects population occurs when the inflammation process is not strong enough, so the impaired endothelial function is still at the initiation phase.

This study was carried out limited on only male subjects to prevent any hormonal biases. A study by Chen et al has shown that resistin concentration is higher in female subjects(17,18).

From the above description, we can conclude that the processes that occur in obesity are related to resistin. In a condition of low grade chronic inflammation such as obesity, resistin showed its proinflammatory properties by a positive feedback loop mechanism with TNF-α. This induction is purposed to attract more macrophages intp the adipose tissue. TNF-α as the "master cytokine" will stimulate IL-6 expression and then stimulate another proinflammatory cytokines and chemokines from the macrophages, including MCP-1 that also attracts even more macrophages. In chronic inflammation, as shown by increased

proinflammatory cytokines being secreted by macrophages, the adhesion molecules will increase, which finally results in endothelial dysfunction. MCP-1 increases along with the increase of TNF-α and IL-6 in order to protect cells from hypoxia, but this also increases the proinflammatory cytokines, so at the end the inflammation process becomes stronger and causes endothelial dysfunction.

Conclusion

Our present study showed that the role of resistin in endothelial dysfunction occurred at a high concentration of resistin through induction of proinflammatory cytokines TNF-α, IL-6 and chemokin MCP-1. We suggest that the inflammation in obesity started with a positive feedback loop mechanism between resistin and TNF-α, which then affect the other cytokines and chemokines resulting in endothelial dysfunction. Elevated MCP-1 is suggest to be a compensate mechanism to protect endothelial cells from hypoxia.

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