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Central Obesity, Inflammation and Angiogenesis in Adult Men

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Abstract: Obesity is known to link with low-grade inflammation. Experimental studies revealed that inflammation was associated with angiogenesis process in adipose tissue. We investigate the correlation between inflammation and angiogenesis in Indonesian adult men with central obesity. A cross-sectional study was undertaken on 161 healthy Indonesian adult men (age: 23-53 y, waist circumference: 64-125 cm). Clinical parameters; biochemical markers; anthropometric parameters (weight, height and abdominal circumference); inflammatory biomarkers (tumor necrosis factor-α (TNF-α) and its *soluble* tumor necrosis factor receptor-2 (*s*TNFR-2), interleukin (IL)-1β); and biomarkers of angiogenesis (leptin, vascular endothelial growth factor (VEGF), angiopoietin (Ang)-1 and Ang-2 were measured. Obese II, Obese I subjects had higher concentrations of fasting blood glucose and triglycerides (P<0.005) than those Non-obese subjects. Obese II subjects had higher concentrations of hsCRP (P<0.05) than obese I subjects; and obese I subjects had higher concentration of hsCRP (P<0.05) than Non-obese subjects. *s*TNFR-2 correlated positively with leptin and Ang-2 (r*s* = 0.376, *P*< 0.001 and r*s* = 0.281, *P* = 0.003, respectively) and negatively with Ang-1 in obese subjects. High concentration of *s*TNFR-2 also correlated with increased concentrations of leptin, VEGF and Ang-2 in all subjects (*P*= 0.001, *P*= 0.033, and *P*= 0.005, respectively). In obese subjects, high concentration of *s*TNFR-2 had correlated with increased leptin and Ang-2, and decreased Ang-1 (7.4 %, 10.9% and 9.2%, respectively). This study was able to demonstrate the relations between inflammation and angiogenesis in Indonesian adult men with central obesity. Findings of this study suggest that inflammation and angiogenesis were mutually supportive processes contribute to systemic low grade inflammation in central obesity.

Keywords: inflammation; angiogenesis; central obesity.

Abbreviations

ATMϕ: adipose tissue macrophage; ALT: alanine amino transferase; AST: aspartate amino transferase; Ang-1: angiopoietin-1; Ang-2: angiopoietin-2; BMI: body mass index; BW: body weight; JNK/SAPK: CD: cluster of differentiation; c-jun N terminal kinase-1/stress activated protein kinase; DBP: diastolic blood pressure; ERK1: extracellular-regulated kinase-1/2; FBG: fasting blood glucose; FGF: fibroblast growth factor;Ht: height; HDL-C: high density lipoprotein-cholesterol; *hs*CRP: *high sensitivity* C-reactive protein; HIF-1α: hypoxia inducible factor-1α; IL-1β: interleukin-1β;ISO: International Standard Operation; LDL: low density lipoprotein; NFκB: nuclear factor kappa B; PKC: protein kinase-C; *s*TNFR-2:*soluble* tumor necrosis factor receptor-2;SBP: systolic blood pressure; SPSS: Statistical Package for the Social Sciences; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; WC: waist circumference; WHO: World Health Organization.

1 Introduction

The annual increase in the prevalence of obesity and central obesity worldwide particularly in Indonesia [1] requires serious attention. Obesity is associated with low-grade, systemic inflammation, leading to insulin resistance, type-2 diabetes and cardiovascular disease. The underlying causes of inflammation are not well understood. It has been suggested that the production of

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a number adipokines, including tumor necrosis factor-α (TNF-α) and its *soluble* tumor necrosis factor receptor-2 (*s*TNFR-2), interleukin (IL)-1β, increased during the expansion of adipose tissue mass in the obese individuals, resulting in tissue inflammation [2].

Obesity is a condition with an excess of adipose tissue, attributed to hypertrophy and hyperplasia of adipocytes [3]. Adipocyte grows larger (hypertrophy) during the development of obesity, and their size can reach t140 -180 μm in diameter. However, at a size of 100 μm there is limitation of oxygen diffusion [4]. A physiological compensation to this cellular hypoxia includes a vasculature response and increase in the number and/ or size of blood vessels in response to requirements for nutrients and oxygen supply [5]. Clearly, the increase of adipose tissue mass in central obesity is accompanied by an increased size of the microcirculations [6]. Hence, angiogenesis is required for adipogenesis and adipose tissue development [4,5,7,8].

Receptor-mediated activation of hypoxia inducible factor (HIF)-1α is largely independent of the cellular O_2 content, but both mechanisms can act cooperatively to enhance HIF-1α protein stability and, consequently, activity [9]. Nuclear factor kappa B (NFκB) is activated by hypoxia, and NFκB activation has also been shown to be required for both TNF-α–mediated and IL-1β-mediated activation of HIF-1α[10]. TNF-α and its *s*TNFR2 are expressed in adipose tissue and are possibly involved in the pathogenesis of insulin resistance [11,12].

Recent studies revealed that inflammation is associated with formation of HIF-1α, which supports angiogenesis during in adipose growth. The increase in HIF-1α expression was confirmed in adipose tissue by microarray and immunohistostaining. HIF-1α stimulates the secretion of leptin, and vascular endothelial growth factor (VEGF) from mature adipocytes in vitro [10], angiopoietin (Ang)-2, and receptor Tie2 [13,14].

VEGF plays an important role in embryonic development and angiogenesis during wound healing and menstrual cycle in the healthy adults [15]. VEGF expression in adipose tissue is correlated with fat weight [7]. Moreover, Fukumura et al [13], recently reported that medium conditioned by endothelial cells containing VEGF accelerated adipocyte differentiation, whereas treatment with a VEGF receptor 2–blocking antibody inhibited adipocyte differentiation. Conversely, Ang-2 plays a complex role in the regulation of vascular remodeling, leading to either vessel sprouting or regression, depending on its expression in combination with the other angiogenic stimuli. Ang-1 is required for the stabilization of peri-endothelial contacts with surrounding smooth muscle cells in mature vessels [15].

The available experimental data indicate that angiogenesis and inflammation play an important role in the pathophysiology of obesity, but the mechanistic linkage is unclear. Understanding this relationship during expansion of adipose tissue will be critical to efforts to intervene in the on going pathology of obesity.

2 Materials and Methods

2.1 Subjects

This cross-sectional study involved 161 apparently healthy Indonesian male adults, aged 23–55. Subjects were recruited from medical checkup patients of Prodia Clinical Laboratory located in Jakarta, Indonesia, employees of some company and members of some community who were interested to participate in this study. No subjects had renal, hepatic, endocrine, and oncological diseases; fever, received anti-diabetic drugs, or other drugs known to affect lipoprotein metabolism for at least 3 weeks prior to the study. All subjects agreed to sign informed consent prior to the commencement of the study. The proposal and clinical protocol of this study were approved by the Medical Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. This study was the expansion of the previous one, which was published elsewhere [16].

2.2 Blood pressure measurement

Sitting blood pressure was measured using a sphygmomanometer during the course of the study. Subjects were seated for at least 5 minutes before the actual measurement was taken. The cuff was placed at the mid part of the right arm and it was ensured that air tubes were not in contact with the stethoscope. The arm to be measured and the sphygmomanometer were at the same level as the heart. As recommended by the American Heart Association, the first Korotkoff phase represented systolic blood pressure (SBP) value and the fifth Korotkoff phase (when sound just disappears) was taken as the diastolic blood pressure (DBP). Two measurements were taken with a 5-minute interval, and the average value was used in the analyses.

2.3 Anthropometric assessments

The anthropometric indicators were measured twice. Mean values were used in analyses. Body weight (BW)

was measured in kilograms to the nearest 0.1 kg, with light clothes on, using a beam scale Tanita (Tanita, Japan). Height (Ht) was measured in centimeters to the nearest 0.1 cm, in standing position with socks and shoes removed, using a microtois (stature meter). Waist circumference (WC) was measured in centimeters to the nearest 0.1 cm, using a flexible non-elastic tape made by Roche (Roche, Switzerland). The World Health Organization (WHO) recommendation for the measurement of WC was applied [17], WC was measured at the midway region between the lowest rib margin and the iliac crest, in standing position with abdomen relaxed, feet together and weight equally divided over both legs. Body mass index (BMI) was calculated by dividing body weight in kg by height in squared meter.

2.4 Biochemical assessments

Blood specimens were collected in the morning between 07:00 till 10:00 am after an overnight fast of 12 hours. Plasma and serum were separated immediately by centrifugation and aliquots were frozen at –20^{0}C for batched analysis for leptin, VEGF, Ang-1, Ang-2, TNF-α, *s*TNFR-2 and IL-1β. Routine biochemical parameters like alanine amino transferase (ALT), aspartate amino transferase (AST) and *high sensitivity* C-reactive protein (*hs*CRP), fasting blood glucose (FBG), high density lipoprotein-cholesterol (HDL-C), creatinine, total and direct bilirubin were determined immediately soon after the blood separation process. All biochemical analyses were performed at Prodia Clinical Laboratory located in Jakarta, Indonesia. Prodia Clinical Laboratory has its long-standing reputation in providing laboratory services in clinical management and research, and has been granted International Standard Operation (ISO) accreditation version 2000:9000 since 1999 and ISO version 15189 since 2007.

Measurements of ALT, AST, FBG, *hs*CRP, triglycerides, HDL-C, creatinine, total and total and direct bilirubin concentrations were performed using the methods as described in the previous paper [16].

Commercially available kits and reagents were purchased to measure serum TNF-α (Quantikine® HS *High Sensitivity ELISAs* Human TNF-α/TNFSF1A produced by R&D Systems, Inc., Minneapolis, MN, USA), TNFR-2 (Quantikine® Human *s*TNF RII/TNFRSF1B produced by R&D Systems, Inc., Minneapolis, MN, USA), IL-1β (Quantikine® HS *High Sensitivity ELISAs* Human IL-1β/IL-1F2 produced by R&D Systems, Inc., Minneapolis, MN, USA), Leptin (Quantikine® Human Leptin produced by R&D Systems, Inc., Minneapolis, MN, USA), VEGF (Quantikine® Human VEGF produced by R&D Systems, Inc., Minneapolis, MN, USA), Ang-1 (Quantikine® Human Angiopoietin-1 produced by R&D Systems, Inc., Minneapolis, MN, USA), Ang-2 (Quantikine® Human Angiopoietin-2 produced by R&D Systems, Inc., Minneapolis, MN, USA).

2.5 Statistical analysis

Data analysis was done using SPSS 13.0 statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA). Distributions of continuous variables were assessed for normality using the Kolmogorov-Smirnov. For continuous variables with normal distributions, such as anthropometric and biochemical measures, descriptive statistics were presented as means ± s.d. Statistical analysis was performed using a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Spearman's correlation analyses were applied to measure associations between continuous variables with approximately normal distributions and results were described as Spearman's correlation coefficients.

The degree of central obesity was defined by the value of WC. Subjects with WC < 90 cm were defined as non-obese, subjects with 90 cm ≤ WC < 100 cm were defined as obese I, and subjects with WC ≥ 100 cm were defined as obese II. In order to understand further on the relations between pro-inflammatory and angiogenic factors, the Spearman correlation analyses between these variables were also performed in 3 different categorizations, they were total subjects (n = 161 subjects), non-obese (n = 52) vs obese (n = 109) subjects, and obese I (n = 55) vs obese II (n = 54) subjects. Comparisons were made between categories.

Using angiogenic indicators (leptin, Ang-2 and Ang-1) as dependent variables, multiple regression analysis was performed to examine contributions of pro-inflammatory indicators (TNF-α, sTNFR-2 and IL-1β) to variations of angiogenic indicators.

To demonstrate the magnitude of interaction between pro-inflammatory indicators in their contribution to angiogenic indicators, categorization of pro-inflammatory variables, especially sTNFR-2, was made using median values as cut-off to define high and low values. Table 1 shows median cut-off values of pro-inflammatory indicators which were used for the analysis. All tests were two-sided and considered significant at $P < 0.05$.

Table 1: Median cutoff values for proinflammatory and angiogenic factors.

	Median cutoff value	Unit
Proinflammatory factor		
TNF-α	2.334	pg/mL
*s*TNFR-2	2.096	µg/mL
IL-1β	0.167	pg/mL
Angiogenic factor		
Leptin	7.571	µg/mL
VEGF	0.278	µg/mL
Ang-2	1.741	µg/mL
Ang-1	52.685	µg/mL

Abbreviations: TNF-α, tumor necrosis factor-α; *s*TNFR-2, *soluble* tumor necrosis factor receptor-2; IL-1β, interleukin-1β; VEGF, vascular endothelial growth factor; Ang-2, angiopoietin-2; Ang-1, angiopoietin-1.

3 Results

Table 2 describes basic clinical and biochemical characteristics of the subjects, by obesity category. Subjects were comparable in age, height, body temperature, serum concentrations of HDL-C, TNF-α, VEGF and Ang-1. A linear increase in BMI with the degree of obesity was observed following the WC's cut-offs used to define the degree of central obesity. No subjects were identified with liver and kidney diseases. Although there were differences in serum AST, ALT and bilirubin concentrations, and glomerular filtration rate, these indicators were within desirable range and might be due to the variation of body weight. Obese subjects had higher fasting blood glucose and serum triglycerides concentrations than their non-obese counterparts, but these differences were not detected between obese subjects. We observed a linear increase of serum *hs*CRP and leptin concentrations with the degree of obesity. Obese II subjects had higher serum *s*TNFR-2 and IL-1β concentrations than those of non-obese and obese I subjects. Obese II subjects had higher serum Ang-2 concentration than their obese I counterparts.

Table 3 presents Spearman correlation coefficients between pro-inflammatory and angiogenesis biomarkers of the subjects, by obesity category. Variation of correlations was found in the study. Serum TNF-α concentration correlated positively with serum Ang-1 concentration in total (r_s = 0.200, P = 0.011) and non-obese (r_s = 0.311, P = 0.025) subjects. These correlations were not found in the obese subjects. Positive correlations were found between serum *s*TNFR-2 and leptin concentrations in total subjects (r_s = 0.223, P = 0.002), and between serum *s*TNFR-2 and Ang-2 concentrations in total (r_s = 0.196, P = 0.013), total obese (r_s = 0.281, P = 0.003) and obese II (r_s = 0.422, P = 0.001) subjects. In similar patterns, positive correlations were also found between serum IL-1β and Ang-2 concentrations in total (r_s = 0.223, P = 0.005), total obese (r_s = 0.229, P = 0.017) and obese II (r_s = 0.229, P = 0.017) subjects. On the other hand, negative associations were obtained between serum *s*TNFR-2 and Ang-1 concentrations in total (r_s = -0.253, P = 0.001), non-obese (r_s = -0.381, P = 0.005) and total obese (r_s = -0.190, P = 0.048) subjects.

Results of multiple regression analysis are shown in Table 4. It was apparent that serum *s*TNFR-2 concentration contributed 7.4%, 10.9%, and 9.2% to the variation of serum leptin, Ang-2, and Ang-1 concentrations, respectively.

Figure 1 illustrates differences of mean values of angiogenesis factors, namely leptin, VEGF, Ang-2 and Ang-1 between subjects with serum concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL in total subjects. Subjects with serum concentration of *s*TNFR-2 ≥ 2.096 µg/mL had higher serum leptin, VEGF and Ang-2 concentrations than those with serum concentration of *s*TNFR-2 < 2.096 µg/mL.

Figure 2 illustrates differences of mean values of angiogenesis factors, namely leptin and VEGF, in non-obese and obese subjects with serum concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL. Obese subjects with serum concentration of *s*TNFR-2 ≥ 2.096 µg/mL had higher serum leptin than those with serum concentration of *s*TNFR-2 < 2.096 µg/mL.

Figure 3 illustrates differences of mean values of angiogenesis factors, namely leptin and VEGF, in obese I and obese II subjects with serum concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL. Obese II subjects with serum concentration of *s*TNFR-2 ≥ 2.096 µg/mL had higher serum leptin and VEGF concentrations than those with serum concentration of *s*TNFR-2 < 2.096 µg/mL.

4 Discussion

Cohort studies have provided evidence on the relations between angiogenesis and inflammation in the development of obese mice [18]. However, until now, similar studies in humans are not available. The current cross-sectional study provides *an analysis of a single time point* in a large number of patiens that begins to explore the relationship between biomarkers of angiogenesis and inflammation in obese individuals.

This study applied waist circumference to classify the degree of central obesity. It has been well documented that

Table 2: Clinical and biochemical characteristics of the subjects, by obesity category.

Subject	Unit	Degree of obesity		
		Non-obese	Obese I	Obese II
n		52	55	54
Age	year	34.35 ± 7.58	36.87 ± 5.86	37.41 ± 6.98
Clinical indicators				
Waist circumference	cm	79.08 ± 7.44[ab]	94.05 ± 2.95[ac]	106.72 ± 5.94[bc]
Height	cm	167.27 ± 5.83	167.99 ± 6.92	167.25 ± 5.32
Body weight	kg	63.71 ± 8.60[ab]	76.52 ± 7.49[ac]	90.71 ± 11.11[bc]
BMI	kg/m^2	22.74 ± 2.62[ab]	27.09 ± 1.72[ac]	32.43 ± 3.67[bc]
Body temperature	^{0}C	35.94 ± 0.59	35.99 ± 0.51	36.09 ± 0.62
Systolic blood pressure	mmHg	107.85 ± 9.30[ab]	114.16 ± 8.15[a]	112.69 ± 8.05[b]
Diastolic blood pressure	mmHg	72.11 ± 7.27[ab]	77.49 ± 5.73[a]	78.17 ± 6.43[b]
Biochemical indicators				
AST	U/L	21.10 ± 3.93[b]	22.29 ± 5.87[c]	26.20 ± 8. 80[bc]
ALT	U/L	21.46 ± 8.89[ab]	30.00 ± 11.54[ac]	40.07 ± 20.64[bc]
Total bilirubin	mg/dL	0.73 ± 0.22	0.78 ± 0.30	0.78 ± 0.22
Direct bilirubin	mg/dL	0.19 ± 0.07[a]	0.22 ± 0.08[a]	0.22 ± 0.07[b]
Fasting blood glucose	mg/dL	82.85 ± 7.25[ab]	86.18 ± 6.35[a]	87.69 ± 8.10[b]
HDL-C	mg/dL	43.62 ± 7.26	42.91 ± 7.11	42.83 ± 6.68
Triglycerides	mg/dL	113.25 ± 76.76[ab]	140.38 ± 69.21[a]	150.96 ± 63.56[b]
*hs*CRP	µg/dL	1.31 ± 1.63[ab]	1.99 ± 1.93[ac]	3.34 ± 2.03[bc]
Glomerular filtration rate	mL/mnt	103.44 ± 21.50[ab]	120.59 ± 24.46[ac]	147.29 ± 30.66[bc]
Proinflammatory indicators				
TNF-α	pg/mL	3.45 ± 3.75	2.84 ± 1.34	3.26 ± 1.43
*s*TNFR-2	µg/mL	2.19 ± 0.73[b]	2.03 ± 0.39[c]	2.49 ± 0.64[bc]
IL-1β	pg/mL	0.35 ± 0.64[b]	0.24 ± 0.43[c]	0.68 ± 1.05[bc]
Angiogenic indicators				
Leptin	µg/mL	2.97 ± 2.65[ab]	7.10 ± 3.11[ac]	13.43 ± 6.35[bc]
VEGF	µg/mL	0.30 ± 0.21	0.29 ± 0.18	0.33 ± 0.31
Ang-2	µg/mL	1.95 ± 0.59[b]	1.73 ± 0.47	1.81 ± 0.53[b]
Ang-1	µg/mL	55.61 ± 13.97	55.67 ± 15.11	54.97 ± 10.41

Data are presented in means ± s.d. Abbreviations: n, number of subjects; ALT, alanine amino transferase; AST, aspartate amino transferase; HDL-C, high density lipoprotein-cholesterol; *hs*CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; *s*TNFR-2, *soluble* tumor necrosis factor receptor-2; IL-1β, interleukin-1β; VEGF, vascular endothelial growth factor; Ang-2, angiopoietin-2; Ang-1, angiopoietin-1, s.d., standard deviation. Differences among groups were determined by one-way analysis of variance; [a] significantly different between non-obese and obese I subjects ($P < 0.05$); [b]significantly different between non-obese and obese II subjects ($P < 0.05$); [c]significantly different between obese I and obese II subjects ($P < 0.05$).

Table 3: Correlations between pro-inflammatory and angiogenesis biomarkers of the subjects, by obesity category.

	Total subjects n=161		Non Obese n=52		Obesity category					
					Total obese n=109		Obese I n=55		Obese II n=54	
	r_s	P-*value*	r_s	P-*value*	r_s	P-*value*	r_s	P-*value*	r_s	P-*value*
TNF-α vs										
Leptin	0.057	0.474	-0.185	0.190	0.053	0.583	-0.089	0520	-0.057	0.681
VEGF	-0.042	0.599	-0.024	0.865	-0.078	0.421	-0.013	0.926	-0.109	0.432
Ang-2	0.063	0.428	0.029	0.840	0.098	0.313	0.047	0.733	0.143	0.300
Ang-1	0.200	0.011	0.311	0.025	0.128	0.185	0.021	0.142	0.099	0.478
s**TNFR-2** vs										
Leptin	0.223	0.002	-0.236	0.092	0.376	0.000	0.199	0.155	0.219	0.111
VEGF	0.129	0.103	0.249	0.075	0.062	0.542	-0.003	0.980	0.125	0.367
Ang-2	0.196	0.013	0.090	0.500	0.281	0.003	0.080	0.562	0.422	0.001
Ang-1	-0.253	0.001	-0.381	0.005	-0.190	0.048	-0.248	0.068	-0,164	0.237
IL-1β vs										
Leptin	0.113	0.153	-0.159	0.261	0.177	0.066	-0.137	0.318	0.148	0.284
VEGF	0.031	0.698	0.055	0.696	0.066	0.828	0.102	0.457	-0.056	0.689
Ang-2	0.223	0.005	0.225	0.109	0.229	0.017	0.090	0.514	0.333	0.014
Ang-1	-0.006	0.938	-0.143	0.311	0.078	0.422	-0.036	0.794	0.270	0.048

Notes: n, number of subjects; r_s, Spearman's correlation coefficient; *P,value* of significance.

Abbreviations: TNF-α, tumor necrosis factor-α; sTNFR-2, soluble tumor necrosis factor receptor-2; IL-1β, interleukin-1β; VEGF, vascular endothelial growth factor; Ang-2, angiopoietin-2; Ang-1, angiopoietin-1.

Table 4: Determinants of angiogenesis using linear regression analysis.

	B	SE	R	R^2	P-value	CI (95%)
Dependent variable:leptin						
TNF-α	-0.247	0.197	-0.098		0.212	-0.637 – 0.143
*s*TNFR-2	2.512	0.792	0.258	0.074	0.002	0.947 – 4.077
IL -1β	0.250	0.644	0.032		0.698	-1.021 – 1.522
Dependent variable: Ang-2						
TNF-α	0.027	0.017	0,124		0.108	-0.006 - 0.061
*s*TNFR-2	0.234	0.068	0.274	0.109	0.001	0.099 - 0.369
IL -1β	0.023	0.055	0.033		0.082	-0.087 - 0.132
Dependent variable: Ang-1						
TNF-α	0.644	0.424	0.124		0.131	-0.193 - 1.481
*s*TNFR-2	-6.240	1.700	0.274	0.092	0.000	-9.598 - -2.881
IL -1β	-0.177	1.382	0.033		0.898	-2.906 - 2.552

Notes: CI, confidence interval for B; B, parameter estimate; r, correlation coefficient.

Abbreviations: TNF-α, tumor necrosis factor-α; sTNFR-2, soluble tumor necrosis factor receptor-2; IL-1β, interleukin-1β; VEGF, vascular endothelial growth factor; Ang-2, angiopoietin-2; Ang-1, angiopoietin-1.

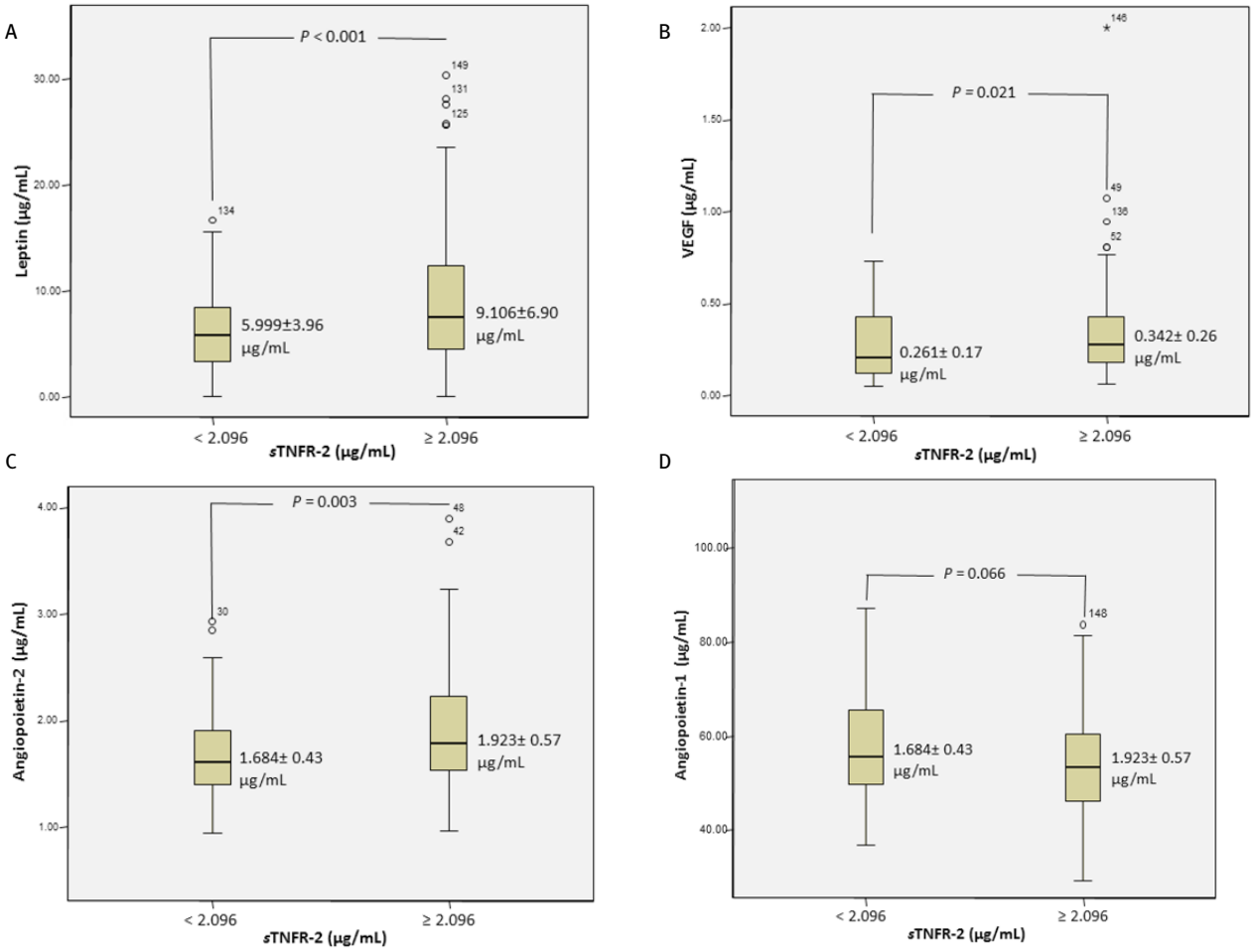

Figure 1: Differences in angiogenesis factors [A, leptin; B, VEGF; C, Angiopoietin-2; and D, Angiopoietin-1) between subjects with concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL.

waist circumference represents compartmentalization of body fatness and correlates with visceral fatness. Furthermore, visceral fatness has been demonstrated to be associated with inflammation and micro-inflammation [19].

This study was unable to prove a link between proinflammatory factors TNF-α and IL-1β with angiogenesis factors, except *s*TNFR-2. It has been well documented that TNF-α is quickly bound in the circulation and neutralized by TNF-α receptor, leading to very low serum concentration of TNF-α [20]. TNF-α expression in experimental research using human adipose tissue cultures reached its peak concentration after a 4-hour incubation, then lowered to half peak concentration after 24 hours [21]. On the other hand, TNFR-mRNA is expressed more in obese individuals than their non-obese counterparts, and, therefore, *s*TNFR-2 was found in the serum obese individuals [20].

Furthermore, in this study, IL-1β did not entirely show a significant association with angiogenic factors leptin, VEGF, Ang-2 and Ang-1. These findings might be related to high activity of IL-1β receptors. By the time IL-1β binds to its receptor, IL-1β receptors rapidly internalize their ligands, which, in turn, down-regulate IL-1β [22,23]. Hence, similar to TNF-α, IL-1β is low. It could be understood that serum concentrations of TNF-α and IL-1β in this study did not reflect the real state of inflammation. Therefore, we decided to use *s*TNFR-2 as a proinflammatory factor for further analysis.

Vascular endothelial cells play a role in the body's signals when there was inflammation and angiogenesis. The balance between these two conditions is located on two angiogenesis regulators, Ang-1 and Ang-2. Ang-1 reduces and Ang-2 increases inflammatory response [24,25]. Ang-1 is a ligand for the Tie-2 receptor, and is thought to act as a paracrine fashion to control the changes

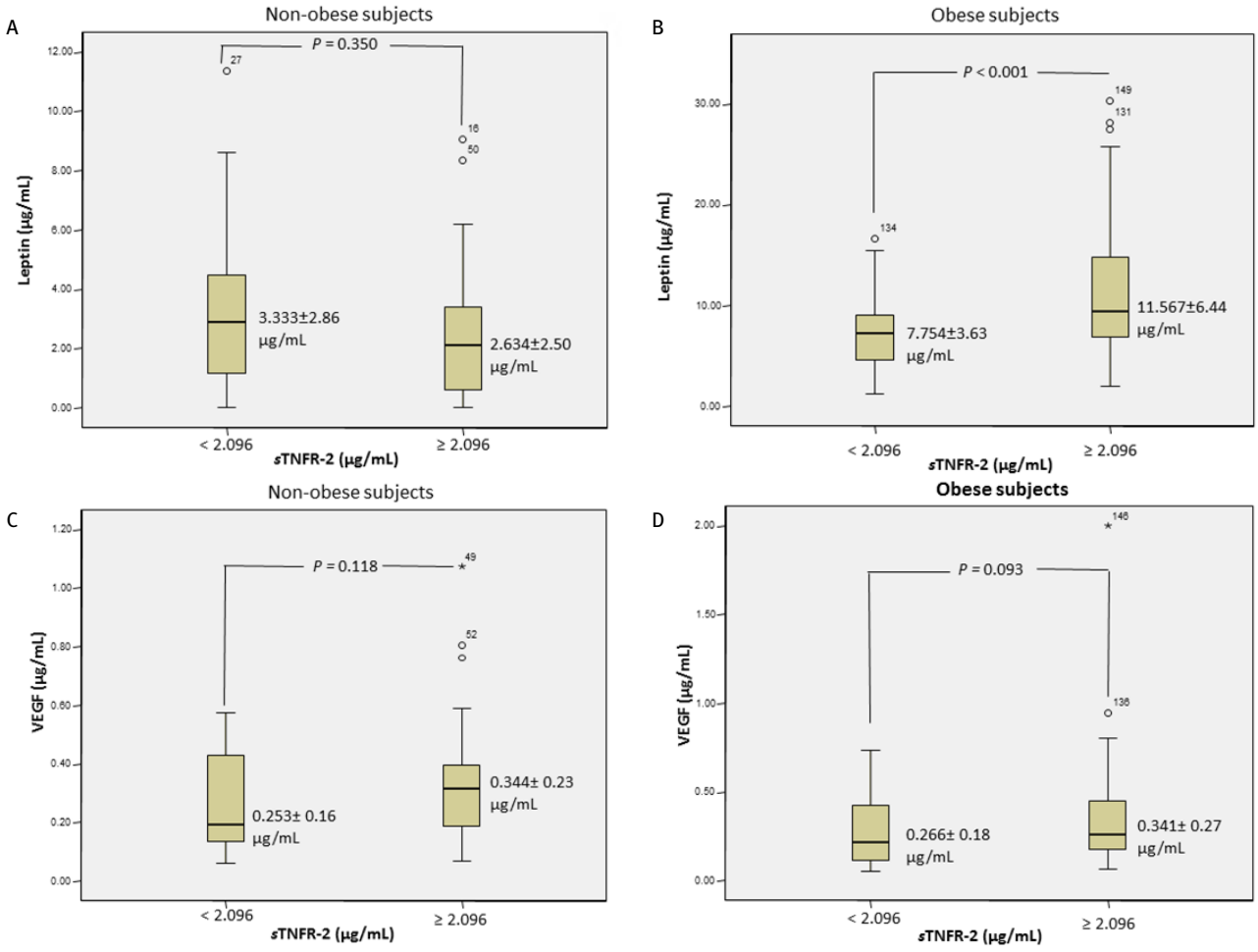

Figure 2: Differences in mean leptin (A and B) and VEGF (C and D) concentrations between subjects with concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL, by obesity category.

from inflammatory or angiogenic endothelium to resting endothelium [18], Ang-2 serves as a key paracrine mediator that controls the change from a static state of endothelial become active, and lower Tie-2 phosphorylation, leading to the plasticity of endothelial cells in blood vessels [26].

Our study found that the degree of obesity was related to serum concentrations of pro-inflammatory factors IL-1β, and angiogenesis factors leptin and Ang-2. These relations can be explained by the previous experimental study [18,27-29]. Adipocytes with increased IL-1β and IL-6 [27], led to the increased synthesis of TNF-α in adipose tissue [18], and the increase of TNF-α was accompanied by the increased activation of adipose tissue macrophage (ATMф), which, in turn, produced pro-inflammatory factors and chemokines [28,29]. These conditions may be considered as a link between angiogenesis and inflammation in obesity.

VEGF concentrations in the state of increased proinflammatory factors, as indicated by sTNFR-2, in obese II subjects were higher than obese I subjects. The explanation for this finding was probably related to the state of hypoxia in subjects with obese II. Subjects with central obesity, as indicated by high WC, are usually accompanied by hyperplasia and hypertrophy of adipocytes, in a disproportionate dimension beyond the capacity of oxygen diffusion, leading to hypoxic state [30]. State of hypoxia in experimental studies has been shown to induce the expression of angiogenic factors VEGF and Ang-2, resulting in the process of angiogenesis [9].

It was therefore reasonable to assume that obese individuals had additional size or number of adipocytes derived from adipocyte precursors (adipogenesis) [14,31]. It was very likely that in subjects with obese II, hypoxia occurred due to the addition of the size and number of adipocytes, and, consequently the expression of HIF-1α [30]. HIF-1α is known to be the initiator of angiogenesis that further induce the expression of several kinds of

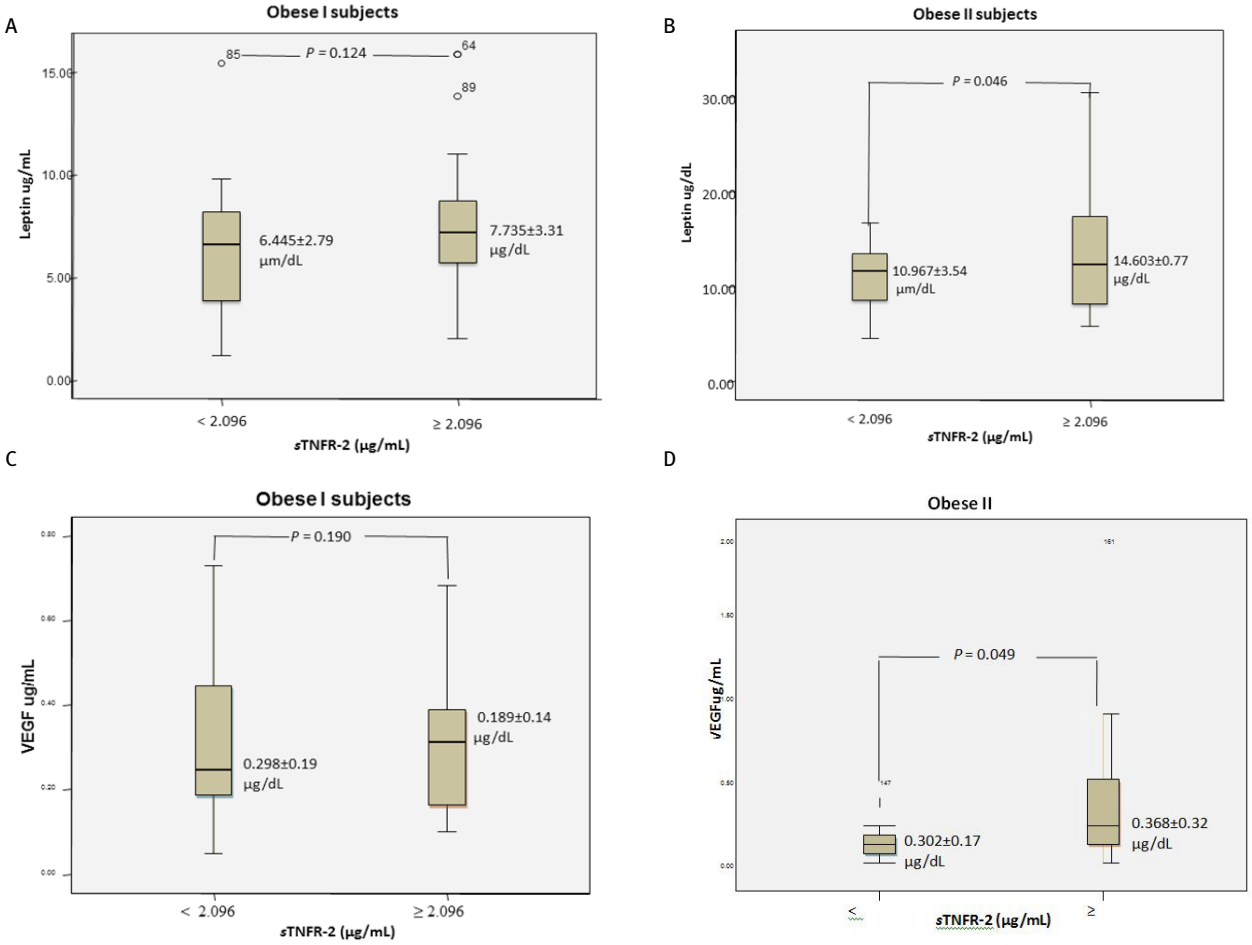

Figure 3: Differences in mean leptin (A and B) and VEGF (C and D) concentrations between subjects with concentrations of *s*TNFR-2 < 2.096 µg/mL and ≥ 2.096 µg/mL, by obesity I and II category.

angiogenic factors, including leptin, VEGF and Ang-2 [8,32]. VEGF is required for the formation of new blood vessels that can keep cells survive [33]. Adipocyte in visceral fat tissue is also a source of VEGF [33,34]. Conversely, VEGF may also mediate inflammation, and this had been proven by experimental study in rats given VEGF and fibroblast growth factor (FGF) [35].

Based on the above explanation, it is paucible to indicate that although extreme obesity with increased proinflammatory can induce hypoxia, they were not sufficient to stimulate the expression of angiogenesis factors leptin and VEGF. Whenever continuous development of adipose tissue occurred, angiogenesis was required to avoid adipocyte cells death [33,36]. Continuous development of adipose tissue in obese individuals can lead to systemic inflammation, insulin resistance, type 2 diabetes and cardio-vascular disease [37].

Overall, our study confirmed the previous ones that there were relationships between inflammation and angiogenesis, and led to a reasonable notion that a selective inhibition of angiogenesis may contribute to apparent changes in the status of obesity as well as improvement of insulin resistance [8,14,38-41].

5 Conclusions

As one of the pro-inflammatory factors, *s*TNFR-2 demonstrated high contribution to the increase ofangiogenic factors, leptin and Ang-2, and to the decline of Ang-1. Increased proinflammatory factors *s*TNFR-2 and IL-1β at the same time greatly correlated with the increased serum concentrations of leptin, VEGF and Ang-2, and decreased serum concentration of Ang-1. Clearly, in comparison with other pro-inflammatory factors, *s*TNFR-2 was the most useful one to indicate the relation between inflammation and angiogenesis in adult men with central obesity.

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Conflict of interest statement: During doctorate candidacy, Cynthia Retna Sartika was an employee of PT Prodia Widyahusada. Cynthia Retna Sartika is currently the Director of PT Prodia StemCell Indonesia. Andi Wijaya is the founder of PT Prodia Widyahusada, and he is now Commissioner of PT Prodia Widyahusada.

All authors declare no other relationships or activities that could appear to have influenced the submitted work.

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