 DOI: 10.18231/2394-6377.2017.0036

Serum vascular endothelial growth factor-New scientific role in obesity

Shilpa K. Shet[1,*], H.L. Vishwanath[2]

[1]P.G. Student, [2]Professor & Head, Dept. of Biochemistry, Bangalore Medical College and Research Institute, Bengaluru, Karnataka

***Corresponding Author:**
Email: drshilpakshet@gmail.com

Abstract

Background and Objectives: Obesity and related ailments are a major hassle across the world for many decades now. As per the published data from various sources overweight population between 1976 to 2002, [body mass index (BMI) ≥ 25 kg/m] in the USA has seen a steady increase from 46 to 66% along with the increase in that of obesity (BMI ≥ 30 kg/m^2) from 15 to 31%.(1) Vascular endothelial growth factor (VEGF) is known factor in regulation of angiogenesis and tissue remodeling. It has been evident through various publications, the potential role VEGF plays in the control of energy metabolism and adipose tissue.(2) The current study is planned to be carried out in order to investigate clinically the correlation between blood serum VEGF concentrations with BMI and waist circumference in obese and lean subjects. The study was carried out on 30 obese subjects (overweight BMI between 25-29 kg/m^2 and obese BMI >30 kg/m^2) and 30 matching age and gender lean subjects (BMI < 25 kg/m^2). Sandwich ELISA method was used to measure Serum VEGF. The measured values depicted mean and standard deviation of serum VEGF levels as 99.6±5. 1ng/mL in obese and 51.9 ± 7.2ng/mL in lean subjects [p value <0.005]. It was observed that increased levels of vascular growth factors were present in obese subjects compared to lean subjects. This may help protect against obesity and insulin resistance. With this new scientific evidence, we understand the importance of VEGF on the design of new treatment for obesity, obesity-related disorders andinsulin resistance.

Keywords: Adipose tissue; Body Mass Index; Waist circumference; Vascular Endothelial Growth Factor.

Introduction

Obesity is a medical condition which signifies accumulation of body fat across the body and this condition could have negative effect on one's health.(3) It is a general practice to classify an individual obese when their body mass index (BMI) which is a measurement obtained by dividing a person's weight in kg by the square of the person's height in cm, is over 30 kg/m^2, and those in the range of 25–30 kg/m^{2}are addressed as overweight.(3) It is evident that Some East Asian countries classify lower values to define obesity. There are evidences which depict obesity causes increase in the likelihood of various diseases like type 2 diabetes mellitus, obstructive sleep apnea, cardiovascular disease, osteoarthritis and certain types of cancer.(4) Many obesity-related disorders have close association with vascular dysfunctions. A combination of excessive food intake, lack of physical activity, and genetic susceptibility may cause Obesity.(5) It is also seen that a few cases are caused primarily by genes, mental illness, disorders or medications.

Obesity was formally recognized as a global epidemic by the WHO in the year 1997. In 2008 a survey carried out by WHO estimates 500 million adults (>10%) were obese, with more women than men. Obesity was once considered a problem of high-income countries, the rates have seen steady rise worldwide and affects both the developed and developing nations.(6)

Various studies have shown the importance of angiogenesis in obesity, expansion of the capillary bed in regional fat depots in adipose tissues. Experiments done on obese mouse with angiogenic agents shows protection against high fat diet-induced insulin resistance.(7) Endothelial cells and adipocytes are main abundant cells in adipose tissue which secretes many angiogenic factors among them vascular endothelial growth factor(8) (VEGF) is a most important angiogenic factors.

Vascular endothelial growth factor (VEGF) is avascular permeability factor (VPF) produced by various cells in the body that stimulates both angiogenesis and vasculogenesis. When blood circulation is inadequate such as in hypoxic conditions this signal protein restores the adequate oxygen supply to tissues. The normal function of VEGF is to create new blood vessels after injury and new vessels (collateral circulation) to bypass blocked vessels.

There are seven members in VEGF family: VEGF-A, B, C, D, E, F and PlGF. All 7 members have a similar VEGF homology domain. Among them VEGF-A is a most important factor, it is dimeric with molecular weight of 34 to 42 kDa, disulfide-bound glycoprotein. The main function is seen in angiogenesis – increases the migration of endothelial cells, mitosis of endothelial cells, matrix metalloproteinase activity, αvβ3 activity, creation of blood vessel lumen and creates fenestrations. Other functions are Chemotactic for macrophages and granulocytes and Vasodilation (indirectly by NO release).

Materials and Method

Source of data: This study has been carried out with the approval of ethical and research committee at BMC & RI. It was carried out on obese and lean subjects

attending the outpatient & inpatient Departments of General Medicine and OBG in Victoria hospital and Hospital attached to Bangalore Medical College and Research Institute, Bangalore. All subjects involved in the study have participated voluntarily.All overweight/obese subjects and normal subjects aged 18-44 years diagnosed by BMI (overweight between 25-29kg/m^2, obese >30 kg/m^2 and normal between 18.5-24.9 kg/m^2) and WC (men>102cm, women>88cm). Patients with diabetes mellitus, kidney and liver disorder and thyroid abnormalities which are known to influence serum levels of VEGF, were excluded from the study. Consent was obtained to carry out the study from the subjects involved.

Under aseptic condition, 5 ml blood samples were collected from median cubital vein with all necessary precautions. It was allowed to clot for 25 minutes in a clean dry test tube and was subjected to centrifugation for 5 minutes to separate the serum. The serum samples were stored at -70ºC till they were analyzed. Estimation of Serum VEGF Levels was done using ELISA. The human VEGF kit is a solid phase sandwich Enzyme Linked Immuno Sorbant Assay [ELISA].[9] A polyclonal antibody specific for human VEGF has been coated onto the wells of the microtiter strips provided. Samples including standards of known human VEGF content, control specimen and unknown are pipetted into these wells.

Statistical Methods: Results are presented as Mean ± SD (Min-Max) and in order to find whether there is significance between study parameters, a 2 tailed and independent 't' test was used. Also, to ascertain correlation between the parameters of the study, Pearson's correlation analysis was used.

Statistical software: The analysis was carried out using Statistical software (SPSS 15.0). Also, generous use of Microsoft word and Excel in order to generate graphs, tables etc. was included.

Significant figures
+ Suggestive significance (p value: 0.05<p<0.10)
* Moderately significant (p value: 0.01<p≤ 0.05)
** Strongly significant (p value: p≤0.01)

Results

Among 30 obese subjects, the age group of 15-25Y had 10 (comprising of 33% of total), 15 in the age group of 26-35Y (comprising of 50%) and 5 in the age group of 36-45Y (making the remaining 17%). Among 30 lean subjects, 11 were in the age group of 15-25Y with 37%, 16 were in the age group of 26-35Y with 53% and 3 were in the age group of 36-45Y with 10%.

Table 1: Age distribution among obese and lean subjects

Age (in years)	Obese subjects		Lean subjects	
	No	%	No	%
15 - 25	10	33	11	37
26 – 35	15	50	16	53
36 – 45	5	17	3	10
Total	30	100	30	100
Mean ± SD	28.77 ± 8.47		27.53 ± 7.21	

Obese and lean subjects are age matched. From table I and Figure 2, the mean age of the obese subjects is 28.7 years with an SD of 8.4 years and in lean subjects is 27.53 with an SD of 7.2.

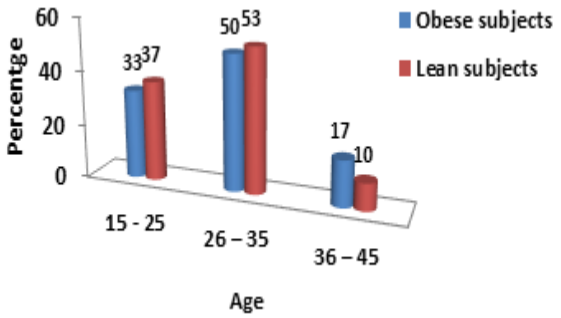

Fig. 1: Comparison of obese and lean subjects according to age group

Table 2: Gender distribution among obese and lean subjects

Gender	Obese subjects		Lean subjects	
	No	%	No	%
Male	10	33	12	40%
Female	20	67	18	60%
Total	30	100	30	100

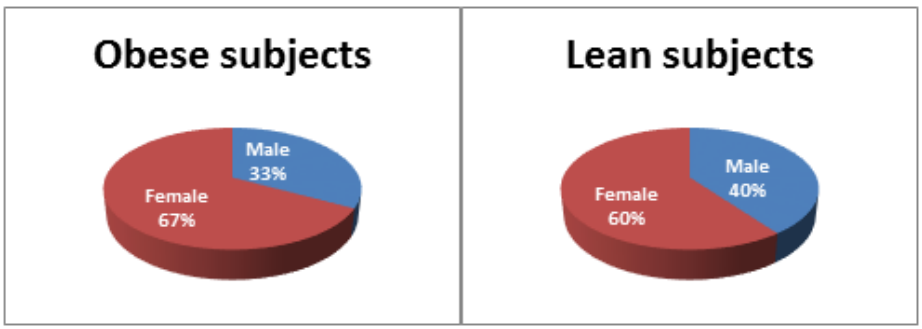

Fig. 2: Comparison of obese and lean subjects according to sex

Distribution of BMI in obese and lean subjects

Among 30 obese subjects studied the BMI were <25.5 with 7%, 25.5-30 with 20% and ≥ 30 with 73%. Among 30 lean subjects < 25.5 with 83%, 25.5-30 with 13% and ≥30 with 3%.

Table 3: Distribution of BMI in obese and lean subjects

BMI (Kg /m^2)	Obese subjects		Lean subjects	
	No	%	No	%
<25.5	2	7	25	83
25.5 – 30	6	20	4	13
≥ 30	22	73	1	3
Total	30	100	30	100
Mean ± SD	32.3 ± 4.6		22.5 ± 3.3	

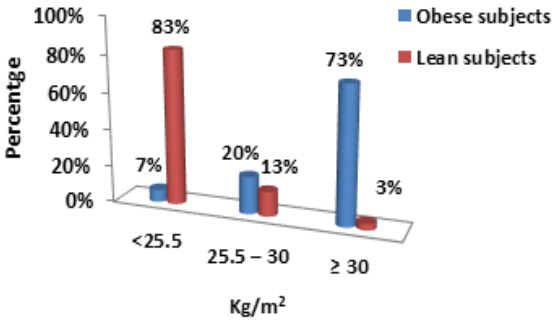

Fig. 3: Distribution of BMI in obese and lean subjects

Comparison of BMI and WC between study subjects

Mean ± SD of BMI in obese and lean subjects are 32.3±4.6and 22.5±3.3 respectively with p value <0.005.

Mean ± SD of WC in cases and controls are 104.08±9.51and 80.58±7.50 respectively with p value <0.005.

Table 4: Comparison of waist circumference and BMI in patients studied

	Obese subjects	Lean subjects	p – value
WC (cm)	108.08 ± 8.86	78.59 ± 6.80	<0.005
BMI (Kg /m^2)	32.3 ± 4.6	22.5 ± 3.3	<0.005

Fig. 4: Comparison of Waist circumference and BMI in patients

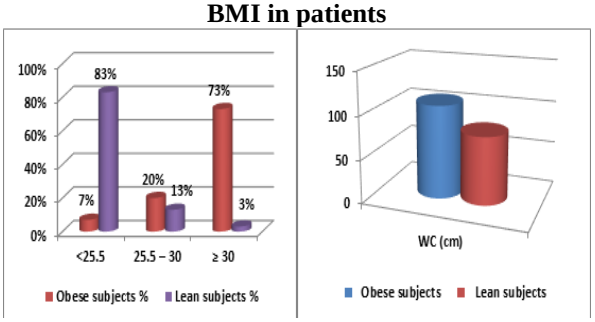

Table 5: Serum VEGF comparison in the two groups studied

Parameter	Obese subjects	Lean subjects	P value
VEGF (ng/ml)	99.6±5.1	51.9± 7.2	<0.005

Normal serum VEGF levels is 31-86 ng/ml.

The mean serum VEGF levels in obese subjects was 99.6 ng/mL with a standard deviation of 5.1ng/mL and p value <0.005. The mean serum VEGF levels in lean subjects was 51.9ng/mL with standard deviation of 7.2ng/mL and p value <0.005.

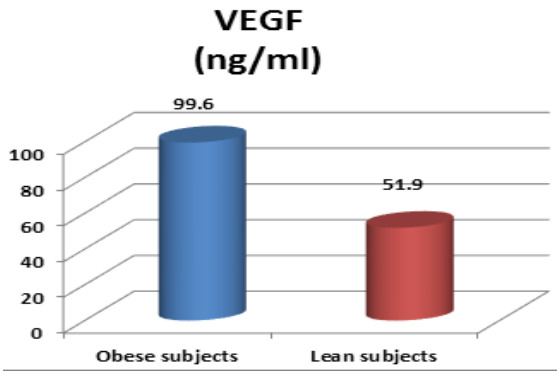

Fig. 5: Comparison of serum VEGF levels between obese and lean subjects

Table 6: Pearson correlation of VEGF with BMI and WC in obese subjects studied

Parameter Pair	Obese subjects	
	r score	p value
VEGF (ng/ml) vs BMI (Kg/m^2)	+0.9408	<0.005
VEGF (ng/ml) vs WC(cm)	+0.8328	<0.005

A positive correlation is observed between Serum VEGF with both BMI and WC, it is statistically significant.

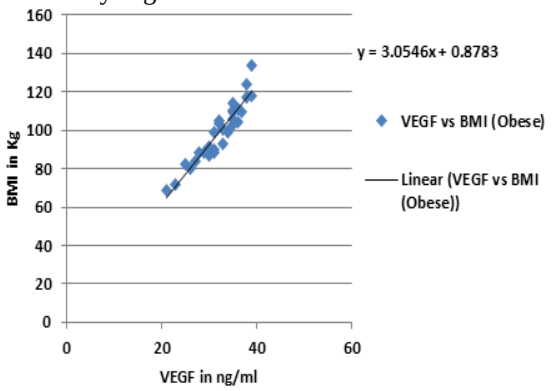

Fig. 6: Plot I: Scattor plot I: Between serum VEGF and BMI

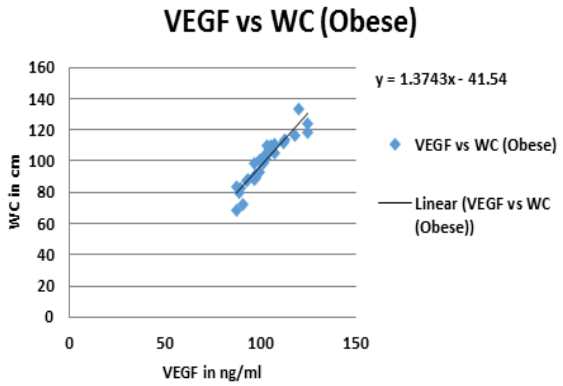

Fig. 7: Scatterplot II: Between serum VEGF and WC

Discussion

For proper expansion and homeostasis of Adipose tissue (AT), adequate oxygen, nutrients, hormones and growth factors are supplied by macro and micro vasculature. There are various angiogenic growth factors secreted in our body. More importantly factors such as leptin, adiponectin, angiopoeitin-2 and VEGF –A are secreted by adipocyte, implies an auto regulatory function for angiogenesis in AT. Among all the angiogenic growth factors VEGF-A plays a major role in adipose tissue. The action of VEGF-A is mediated by two tyrosine kinase receptors, VEGF receptors 1 (R1) and 2 (R2). Most of the cellular responses of VEGF-A mediated by VEGF R2, the function of VEGF R1 is less defined. The regulation of serum levels of VEGF-A in AT by exercise, hypoxia, insulin, a subset of cytokines and referral growth factors. However, they do not respond to local hypoxia. There are several studies showing the association of obesity with increased levels of angiogenic growth factor connected with pathogenesis of obesity or more likely in improvement of insulin resistance and obesity related problems.

During embryogenesis and pathological conditions such as tumorigenesis, the VEGF family of proteins is important for the development of blood vessels. VEGF is a heparin binding glycoprotein with angiogenic, mitogenic and vascular permeability factor specific for endothelial cells.

The following are the functions of VEGF in adipose tissue:

1. Recent evidence confirms the role of VEGF in the function and energy metabolism of both white and brown adipose tissue. Studies carried out by Elias I, Franckhauser S et al showed the association of VEGF with increased thermogenesis and energy expenditure enhanced mainly in brown adipose tissue (BAT), ‘’BAT like’’ phenotype in white adipose tissue (WAT) and this is proven by increased levels of proteinPGC-1α and UCP1 in BAT.
2. Study done by Sung HK et al shows adipose VEGF regulates metabolic homeostasis through angiogenesis. They reveal the metabolic role of adipose VEGF by studying on mice with deletion or overexpression of VEGF in the adipose tissue. In VEGF deletion mice showed reduced adipose vascular density and increased hypoxia, apoptosis, inflammation and metabolic defects. In contrast, induction of VEGF expression in mice leads to increased adipose vasculature and reduced hypoxia, indicating that metabolic misbalance is reversible by increase in adipose vessel density.
3. The anti-inflammatory action of VEGF shows through chemo attractant to M2 macrophages thus maintaining an anti-inflammatory milieu. Thus VEGF- overexpression in adipose tissue acts through various mechanisms helps to decrease obesity and through anti-inflammatory action responsible for the reduction of insulin resistance.

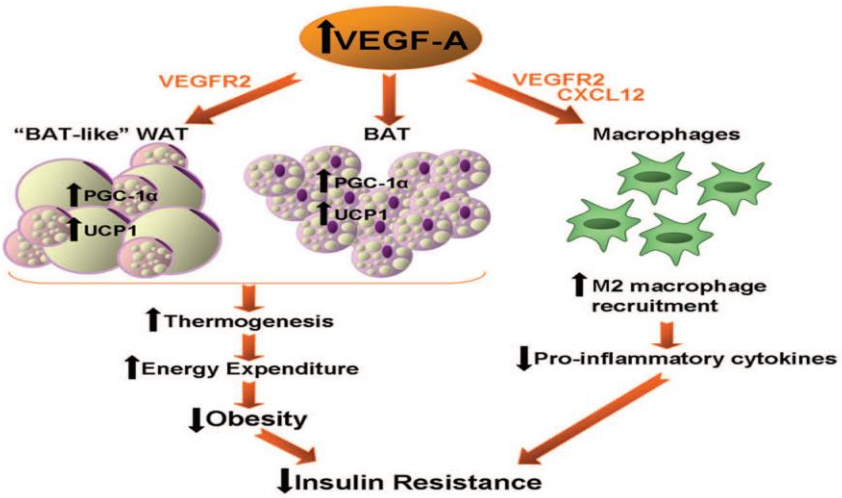

Fig. 8: Action VEGF-A in Adipose tissue

This new proven evidence highlights the importance of VEGF and other proangiogenic factors on metabolism of adipose tissue and so many new angiogenic factors are trying for treatments of obesity, insulin resistance and obesity related disorders.

Conclusion

From the current study we consider that elevated serum levels of VEGF have a good and beneficial role in obese subjects compared to lean subjects. By various mechanisms VEGF increases thermogenesis and energy expenditure in AT, so it reduces the incidence of obesity and pro-inflammatory cytokine levels which is responsible for the amelioration of insulin sensitivity.

Reference

1. Kosti RI, Panagiotakos DB. The obesity in children and adolescents in the world. Cent Eur J Public Health 2006;14:151-9.
2. Elias I, Franckhauser S, Ferré T, Vilà L, Tafuro S, Muñoz S, et al. Adipose tissue overexpression of vascular endothelial growth factor protects against diet-induced obesity and insulin resistance. Diabetes. 2012;61:1801–13.
3. "Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies". The Lancet. 363(9403):157–63.2004.
4. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX et al. "Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss". Arterioscler. Thromb. Vasc. Biol. (Review). 26(5):968–76.
5. Yadav K, Krishnan A (September 2008). "Changing patterns of diet, physical activity and obesity among urban, rural and slum populations in north India". Obes Rev. 9(5):400–8.
6. Sara N. Bleich, David Cutler, Christopher Murray, and Alyce Adams et al. "Why is the developed world obese?". Annual Review of Public Health Vol. 29:273-295.
7. Sung H K, Don Ko, Son JE, Park JG, Bae Y, Choi S et al. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. Cell Metab. 2013;17:61-72.
8. Zhang QX, Magovern CJ, Mack CA, Budenbender KT, Ko W, Rosengart TK. Vascular endothelial growth factor is the major angiogenic factor in omentum: mechanism of the omentum-mediated angiogenesis. J Surg Res. 1997;67:147–154.
9. Schmidt, SD; Mazzella, MJ; Nixon, RA; Mathews et al. 'Enzyme-Linked Immunosorbent Assay". Methods in Molecular Biology. 849:507–27.