Regulation and function of vascular endothelial growth factor in hypercholesterolemia:

"Differences among normal and subjects with low and high cardiovascular risks"

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BACKGROUND

VEGF : Roles in cardiovascular medicine

I. Protective role

1. **High VEGF levels for stimulating angiogenesis in tissue repair and reperfusion after tissue ischemia (i.e. formation of collateral circulation to bypass the occluded vessels).**

2. **Low circulating VEGF levels for maintaining vascular tone thereby healthy vascular function by stimulating endothelial production of nitric oxide (NO) and prostacyclin (PGI_2) which have vasodilating, anticoacgulation and antiproliferating effects on vascular tissues.** *[Zachary, I and Morgan, RD. Heart 2011; 97: 181-9.]*

II. Pathogenic role in atherosclerosis

VEGF: has pro-inflammatory properties via the recruitment of inflammatory cells .

In experimental models, VEGF may take part in the atherosclerotic process by recruiting monocytes in bone marrow and peripheral blood to atherosclerotic vessels to help plaque formation and plaque destabilization via interaction with Flt-1 receptors (VEGFR-1) expressing on monocytes which in return secrete inflammatory cytokines and angiogenic VEGF.

VEGF REGULATION

1. Bioavailability of VEGF is regulated by sVEGFR-1 via ligand trapping.
2. Both VEGF and sVEGFR-1 are upregulated by hypoxia. sVEGFR-1 is also activated by VEGF expression.
3. Under healthy state, sVEGFR-1 regulates VEGF at low physiologic level.
4. For tissue damage or ischemia such as acute myocardial infarction (AMI), in acute phase sVEGFR-1 is down-regulated to allow elevation of VEGF. When AMI is treated, tissue damage is repaired, sVEGFR-1 is up-regulated to normalize VEGF at low physiologic level.

VEGF DYSREGULATION

Maladaptive increase in sVEGFR-1 (at baseline or at acute phase) can lead to severe heart failure in patients with

➢ **acute myocardial infarction, AMI (*Onoue K, et al. Am J Cardiol 2009; 104: 1478-1483*).**

➢ **chronic heart failure (*Ky B, et al. J Am Coll Cardiol 2011; 58: 386-394*).**

How about hypercholesterolemic subjects with CVD risk factors?

✓**CVD risk factors are known to cause endothelial dysfunction a state of impaired vascular tone and abnormal reactivity which is thought to precede the development of atherogenesis and linked to atherosclerosis clinical sequelae.**

✓**VEGF can resolve endothelial dysfunction via production of nitric oxide (NO) and prostacyclin which have vasodilating, anticoacgulation and antiproliferating effects on vascular tissues.**

✓**Degree of endothelial dysfunction is related to the global CVD risk score.** [Vita, J. A., et al.(1990) Circulation 81, 491–497]

Research Questions

❖ **How will VEGF be regulated in subjects with hypercholesterolemia in comparing to controls?**

❖ **How will VEGF be regulated in hypercholesterolemic subjects with low and high CVD risks?**

❖ **Whether VEGF exerted protective or adverse effect to vascular health in hypercholesterolemic subjects with different CVD risks?**

OBJECTIVES

1. To characterize VEGF regulation by sVEGFR-1 in hypercholesterolemic subjects with low (1 risk factor, only hypercholesterolemia), intermediate (2–4 risk factors excluding diabetes) and high risk (1–4 risk factors plus diabetes and/or medical history of peripheral and cardiovascular diseases) comparing to subjects without any modifiable risk factors (zero risk).

2. To evaluate whether VEGF exert protective or adverse effect to vascular health, based on how CVD risk score profiles changed with VEGF concentrations.

Classification of risk

Subjects were classified into four risk categories.

1. **A, the zero risk category** recruited subjects without any modifiable CVD risk factors.

2. **B, the low risk category** included subjects with hypercholesterolaemia as the only risk factor.

3. **C, the intermediate risk category** comprised subjects with hypercholesterolaemia plus 1–3 of the following risk factors: hypertension, abdominal obesity and cigarette smoking.

4. **D, the high risk category** enrolled hypercholesterolaemic subjects with additional risk factors plus diabetes and/or medical history of cardiovascular disease, cerebrovascular event and peripheral vascular disease.

MATERIALS AND METHODS

Subjects

Subjects were selected among participants of the ongoing program, the Electricity Generating Authority of Thailand (EGAT) study on cardiovascular risks .

Study design : Cross-sectional design, N=227.

This study consisted of 56 subjects without any modifiable CVD risk factors and 171 subjects with hypercholesterolaemia with or without additional risk factors.

Procedure: Blood samples were obtained for clinical chemistry analyses and VEGF, sVEGFR-1 assays while subjects attending the EGAT follow-up survey. After blood sampling, subjects underwent physical examination and medical history evaluation .

The inclusion criteria :

Subjects aged between 35–65 years with or without the following CVD risk factors :

❖hypercholesterolaemia (total cholesterol ≥ 240 mg/dl or current use of cholesterol lowering therapy),

❖hypertension (blood pressure ≥ 140/90 mmHg or currently taking antihypertensive medication),

❖diabetes (according to the World Health Organisation criteria),

❖abdominal obesity (waist circumference ≥ 90 cm for male and 80 ≥ cm for female),

❖current smoker,

❖medical history of cardiovascular disease, cerebrovascular events and peripheral vascular disease.

The exclusion criteria:

❖ subjects who had recent surgery, coronary bypass graft, post-myocardial infarction, stroke (within 12 weeks),

❖ acute infections requiring antibiotic therapy (within 2 weeks),

❖ history of neoplastic disease and connective tissue disease.

RAMA-EGAT ten-year cardiovascular risk scoring

Score	−2	0	2	3	4	5	6	8	10
Age (Y)	35-39	40-44	45-49		50-54		55-59	60-64	≥ 65
Gender		female		male					
Cholesterol mg/dL		<280			≥ 280				
Smoking		No	Yes						
Diabetes		No				Yes			
BP: >140/90		No		Yes					
Waist M ≥90 cm, F≥80 cm		below		above					

VEGF and sVEGFR-1 Assay

TMB Substrate

Sandwich Assay

HRP HRP HRP

TMB Substrate

HRP

VEGF

Blue

Stop

Yellow

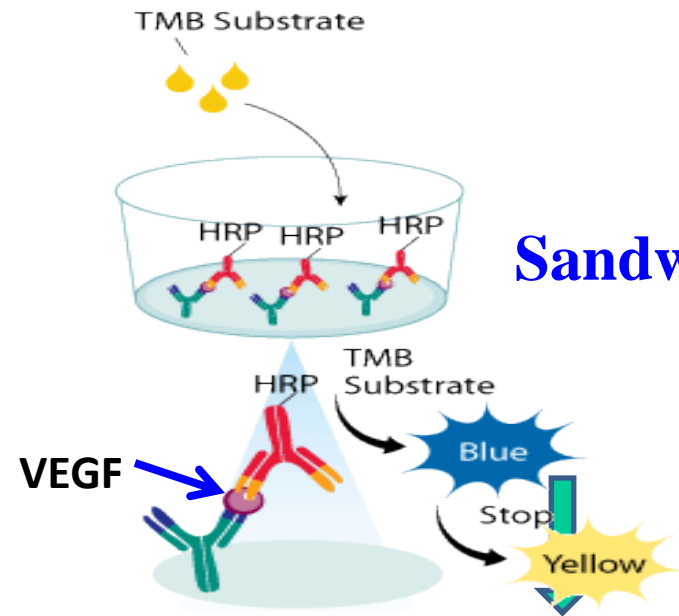**ELISA kits (Quantikine R&D Systems, Abingdon, UK).**

1. **The VEGF assay kit (DVE 00)**
2. **The sVEGFR-1assay (DVR 100)**
3. **According to the manufacturer, the minimal detectable concentrations of VEGF and sVEGFR-1 were < 9 and 5.01 pg/ml, respectively.**
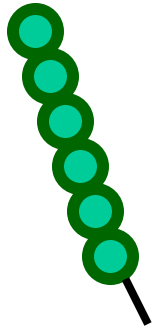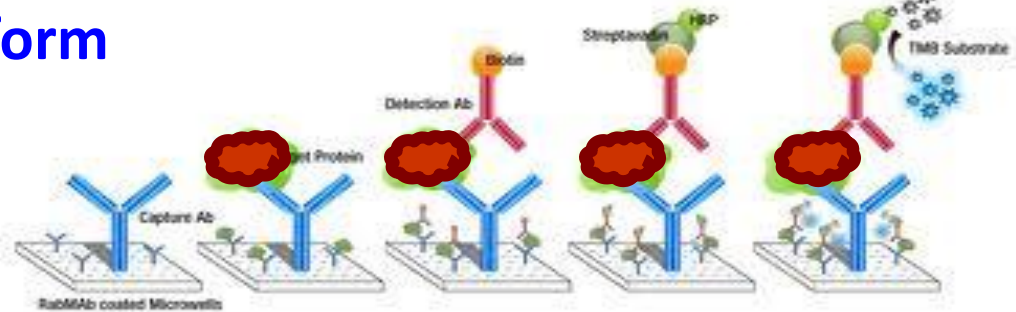
VEGF assay (kit DVE 00) measures only unbound bioactive VEGF due to steric hindrance of the bound form
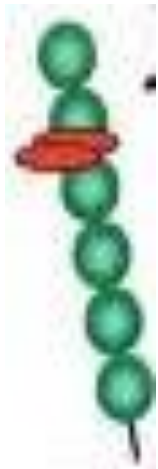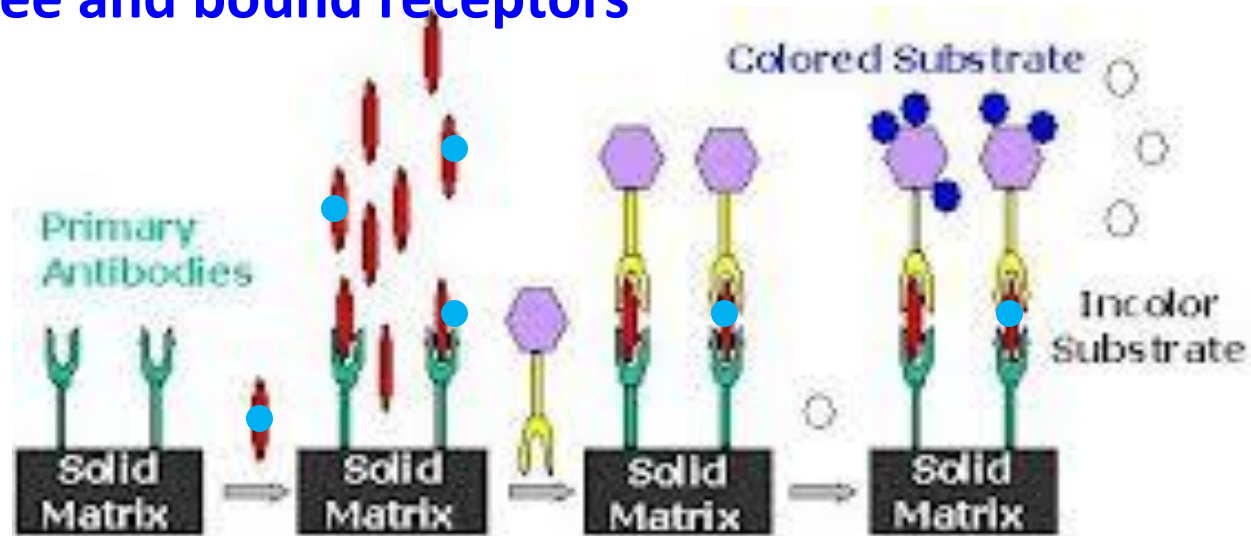
VEGF: dimer

sVEGFR-1

sVEGFR-1 assay (kit DVR 100) measures both free and bound receptors

Bound VEGF by sVEGFR-1

•**Bailey, A. P., et al.(2006) Med. Sci. Moni. 12, CR45–CR50**
•**Maynard, S. E., et al. (2003) J. Clin. Invest. 111, 649–658**

Statistical analysis

- Parametric data are expressed as mean (S.D.), nonparametric data as median (interquartile range) or actual numbers (percent).
- Difference among means was analysed by one-way analysis of variance (ANOVA), between or among medians by χ^2 analyses of contingency tables.
- Frequency data between groups were analysed by χ^2 – test or Fisher exact test as appropriate.
- Correlations for sVEGFR-1- to-VEGF ratio or sVEGFR-1 levels with VEGF levels were assessed by Spearman rank test.
- Comparisons between risk categories for any differences in associations between VEGF levels and CVD risk profiles were evaluated by χ^2 analyses of $2\times3\times3$ three-dimensional contingency tables.
- Analysis of linear regression line was performed to assess the relation between VEGF levels and the proportion of low-to-high risk scores or proportion of high-to-low risk scores.

All statistical tests were 2-tailed and $P < 0.05$ was considered statistically significant.

RESULTS

Table 1   Characteristics of the study population

Risk category

1. **A, the zero risk category :** subjects without any modifiable CVD risk factors.
2. **B, the low risk category** : subjects with hypercholesterolaemia as the only risk factor.
3. **C, the intermediate risk category :** subjects with hypercholesterolaemia plus 1–3 of the following risk factors: hypertension, abdominal obesity and cigarette smoking.
4. **D, the high risk category:** hypercholesterolaemic subjects with additional risk factors plus diabetes and/or medical history of cardiovascular disease (CVD), cerebrovascular event (CVA) and peripheral vascular disease (PVD).

Parameter	A	B	C	D	P value
n	56	43	74	54	
Gender (male/female)	28/28	32/11	52/22	48/6	<0.001
Age (years)	49 (4.4)	49.6 (4)	51.6 (4.7)	51.9 (4.4)	<0.001
Medical history, CVD				20	
CVA				9	
PVD				14	
Diabetes mellitus				33	
Hypertension			38	32	
Current smoker			14	17	
Total cholesterol (mg/dl)	203.6 (27)	268.2(25.3)	263.6 (30.7)	227 (55.2)	<0.001
Glucose (mg/dl)	93.5 (9.5)	96 (7.1)	98.3 (9.3)	146.7 (83.7)	<0.001
Systolic BP (mmHg)	114.7 (10)	114 (9.8)	127.6 (15.9)	132.3 (16.1)	<0.001
Diastolic BP (mmHg)	74 (8.8)	73.1 (7.2)	83 (11.1)	85 (10.8)	<0.001
Waist circumference(cm)	81.5 (8.1)	81.1 (5.3)	90.2 (9.5)	93 (8.3)	<0.001
EGAT score	5 (4-8)	6 (5-9)	10 (9-14)	13 (11-17)	<0.001
VEGF (pg/ml)	**12.7 (9-18.1)**	**11 (5.2-18)**	**13.9 (7.9-21.1)**	**13.7 (6.8-18.3)**	**0.66**
sVEGFR-1 (pg/ml)	**51.1 (45.5-70)**	**57.2 (50.3-66)**	**56.5 (50.3-65.2)**	**53.4 (51.3-70)**	**0.61**

Hypotheses

VEGF in dimeric form
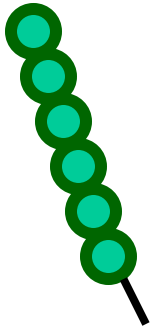
sVEGFR-1

Bound VEGF by sVEGFR-1

H1: Normal VEGF regulation in subjects with CVD risk factors in comparing to controls.

H2: Compensatory response in VEGF regulation as such to normalize VEGF levels in subjects with CVD risk factors.

VEGF Regulation

Based on one-to-one ligand-receptor binding

Dimeric VEGF, mole wt 45 kD

+

sVEGFR-1, mole wt 100 kD

R-to-V mass ratio = 100/45 = 2.22
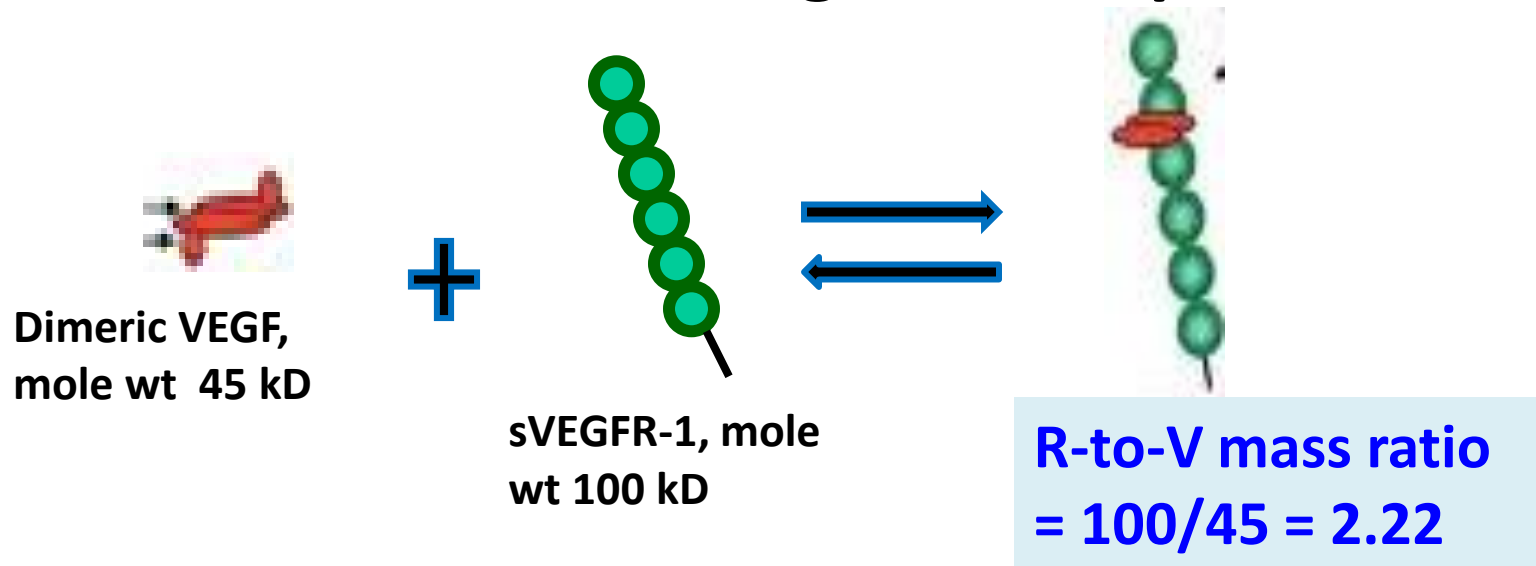
➢High VEGF levels, R-to-V mass ratio << 2.22, sVEGFR-1 insufficient, or sVEGFR-1 down-regulation or VEGF up-regulation.

➢Low VEGF levels , R-to-V mass ratio >> 2.22, sVEGFR-1 in excess or sVEGFR-1 up-regulation.

[Kendall, et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10705–10709]

A 25th % 2.87 75th % 7.99

L I H

Scatter plot between free VEGF and sVEGFR-1-to-VEGF mass ratio for zero risk control.

R-to-V mass ratio = 100/45 = 2.22 for one-to-one ligand-receptor binding.

High — 18.58 pg/ml, 75th %

Intermediate — 12.99 pg/ml, 50th %

Low

Free VEGF, pg/ml

sVEGFR-1-to-VEGF mass ratio

A

sVEGFR-1-to-VEGF mass ratio

VEGF, pg/ml	n	Low <2.87	Intermediate 2.87-7.99	High >7.99
High > 18.58	13	7 (**53.8%**)	6 (**46.2%**)	
Intermediate 12.99-18.58	15	2 (13.3%)	12 (**80%**)	1 (6.7%)
Low <12.99	28		16 (**57.1%**)	12 (**42.9%**)

25th % **2.87**   75th % **7.99**

L   **I**   **H**

High   **18.58 pg/ml, 75th %**

Intermediate   **12.99 pg/ml, 50th %**

Low

Free VEGF, pg/ml

sVEGFR-1-to-VEGF mass ratio

Regulation of high VEGF levels

sVEGFR-1-to-VEGF mass ratio

Group	VEGF, pg/ml median (range)	n	Low (< 2.87)	Intermediate (2.87-7.99)	High (>7.99)	P value
A	23.1 (18.6-53.8)	13	7 (53.8%)	6 (46.2%)		_
B	23.5 (19.6-46.2)	9	9 (100%)			0.046
C	23.3 (19.6-137.2)	22	18 (81.8%)	4 (18.1%)		0.081
D	30.2 (18.8-292.4)*	13	13 (100%)			0.015

Regulation of intermediate VEGF levels

sVEGFR-1-to-VEGF mass ratio

Group	VEGF, pg/ml median (range)	n	Low (< 2.87)	Intermediate (2.87-7.99)	High (>7.99)	P value
A	15.6 (13-18.3)	15	2 (13.3%)	12 (80%)	1 (6.7%)	_
B	15.1 (13-18.5)	10	2 (20%)	8 (80%)		1
C	16 (13.4-18.6)	17	3 (17.7%)	14 (82.4%)		0.82
D	15.4 (13.6-18.3)	15	2 (13.3%)	13 (86.7%)		1

Regulation of low VEGF levels

sVEGFR-1-to-VEGF mass ratio

Group	VEGF, pg/ml median (range)	n	Low (< 2.87)	Intermediate (2.87-7.99)	High (>7.99)	P value
A	9 (1.4-12.5)	28		16 (57.1%)	12 (42.9%)	_
B	5.5 (0-12.1)	24	1 (4.2%)	9 (37.5%)	14 (58.3%)	0.3
C	7.6 (0-12.5)	35		21 (60%)	14 (40%)	0.84
D	6.6 (0-12.9)	26		8 (30.8%)	18 (69.2%)	0.052

➢ Is high VEGF level good or bad to vascular health in subjects with CVD risk factors?

➢ Is low VEGF level good or bad to vascular health in subjects with CVD risk factors?

EGAT score profile

Group (n)	VEGF levels	≤ 5	6-10	≥ 11
A (56)	High	6 (10.7%)	7 (12.5%)	
	Intermediate	10 (17.9%)	4 (7.1%)	1 (1.8%)
	Low	21 (37.5%)	6 (10.7%)	1 (1.8%)
B (43)	High	6 (14%)	3 (7%)	
	Intermediate	6 (14%)	4 (9.3%)	
	Low	9 (21%)	10 (23.3%)	5 (11.6%)

➢ EGAT score ≤ 5, correspond to ≤ 1% 10-year risk, represent non-significant health risk.

➢ EGAT score 6-10, correspond to 2%-4% 10-year risk, represent no serious health risk which can be nullified by changing life style.

EGAT score profile

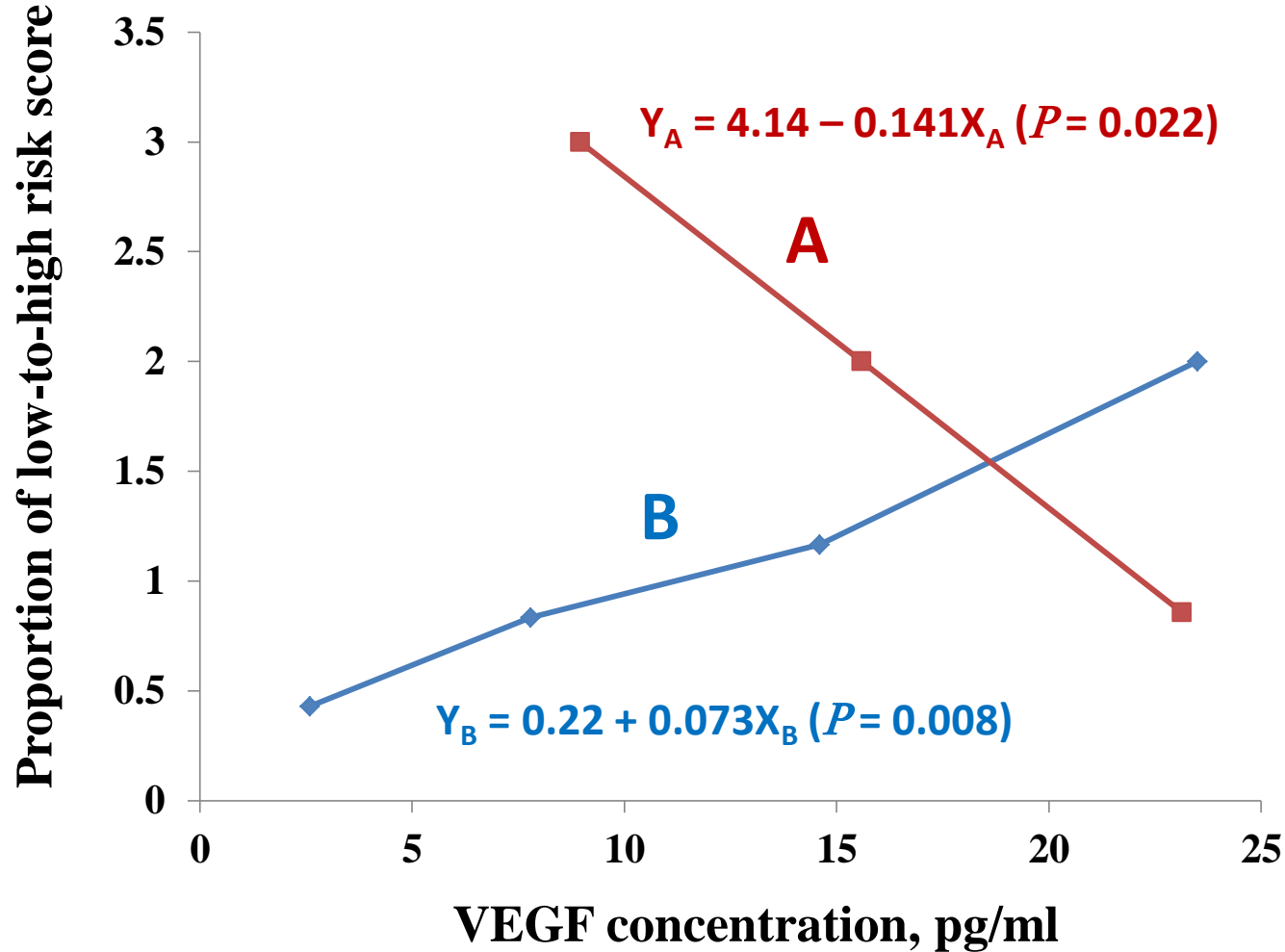| Group (n)	VEGF levels	≤ 5	6-10	≥ 11
C (74)	High	4 (5.4%)	10 (13.5%)	8 (10.8%)
	Intermediate	3 (4%)	7 (9.5%)	7 (9.5%)
	Low	3 (4%)	15 (20.3%)	17 (23%)
D(54)	High	2 (3.7%)	5 (9.3%)	6 (11.1%)
	Intermediate		4 (7.4%)	11 (20.4%)
	Low	2 (3.7%)	5 (9.3%)	19 (35.2%)

➢EGAT score ≥ 11, correspond to ≤ 4% 10-year risk, represent serious health risk requiring medical advices and therapy for preventive measures.

➢EGAT score 6-10, correspond to 2%-4% 10-year risk, represent no serious health risk which can be nullified by changing life style.

Groups A, B
Plotting median VEGF concentrations VS proportion of subjects with EGAT score ≤ 5 to proportion with score >5, *proportion of low-to-high risk score.*

$$Y_A = 4.14 - 0.141X_A \ (P = 0.022)$$

A

Proportion of low-to-high risk score

VEGF concentration, pg/ml
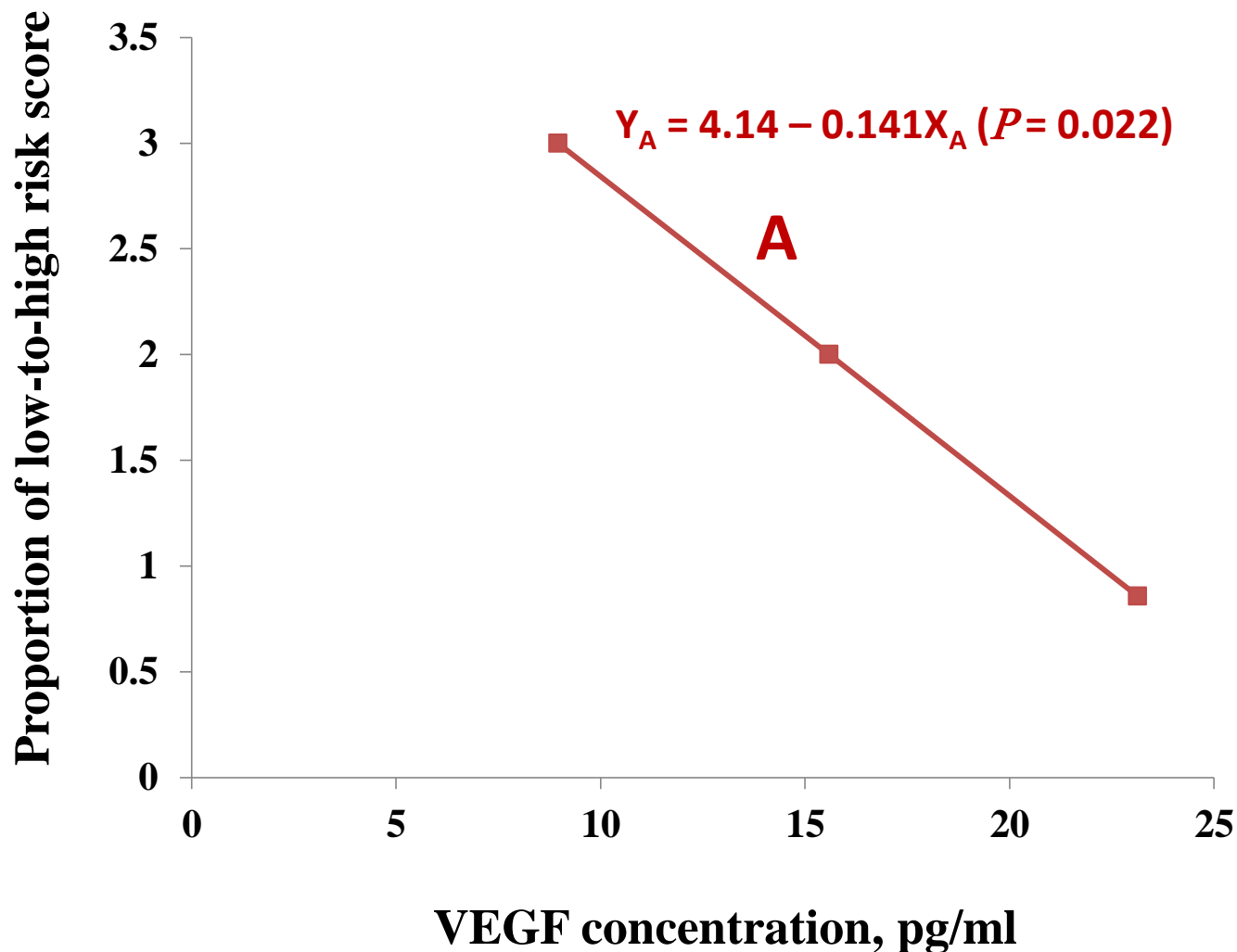
High value of low-to-high risk score ratio, suggestive of good vascular health, are associated with low VEGF levels.

$Y_A = 4.14 - 0.141X_A$ ($P = 0.022$)

$Y_B = 0.22 + 0.073X_B$ ($P = 0.008$)

Increasing VEGF levels are associated with lower risk scores in B, suggestive of increasing VEGF to restore vascular health. Highest attainable proportion is ~ 2 not 3 for as for A.
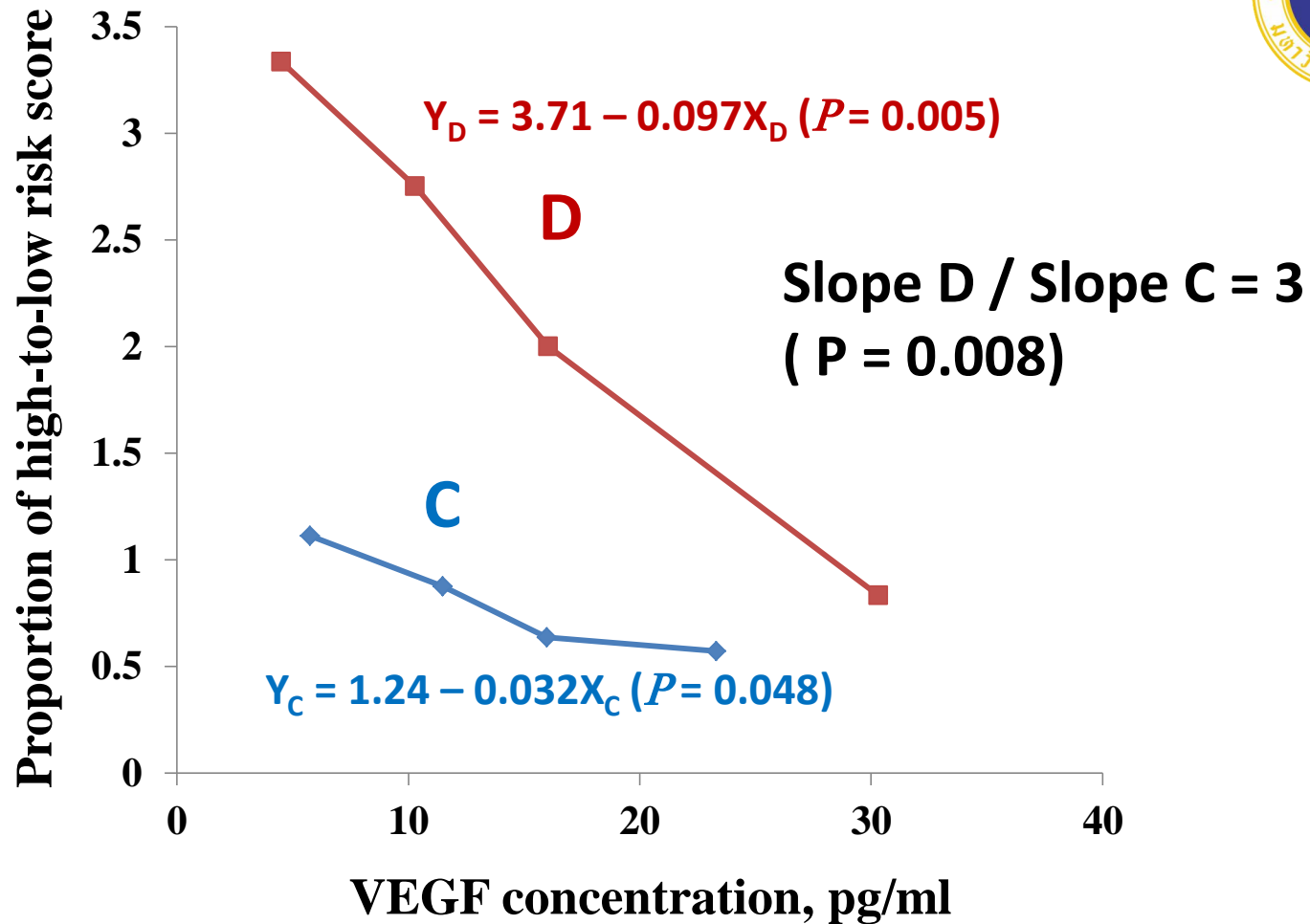
Groups C, D

Plotting median VEGF concentrations VS proportion of subjects with EGAT score ≥ 11 to proportion with score < 11, *proportion of high-to-low risk scores.*

$Y_D = 3.71 - 0.097X_D$ ($P = 0.005$)

D

Slope D / Slope C = 3
(P = 0.008)

C

$Y_C = 1.24 - 0.032X_C$ ($P = 0.048$)

Increasing VEGF levels , lowering the proportion of high to low risk scores, the better vascular health can be expected

Conclusions

1. **High VEGF levels confer protective effect to cardiovascular health in subjects with CVD risk factors and that sVEGFR-1 is down-regulated to increase VEGF levels so as to sustain high VEGF levels represents an adaptive or reparative response.**

2. **Despite this protective response, high VEGF levels can restore the vascular function in CVD risk groups only partially.**

Conclusions

3. Low VEGF concentrations are adverse to cardiovascular health of subjects at risks but are associated with the healthy vascular functional state for subjects without any modifiable risk factors.

4. The tendency of over-expressed sVEGFR-1 to subdue VEGF levels observed in the high risk subjects (D) may implicate a health threat alarm for the deficiency in vascular repair factor.

Have a heart loving habit.

Thank You!

Fed up with her diet is going, Chariene takes a more serious aim at her target weight.

❖Vessel growth is required for nutrient delivery to the growing plaque.

❖Neovessels growing from adventitia into the plaque are immature and leaky which directly contribute to the instability of the plaque *through intraplaque hemorrhage*.

❖Intraplaque hemorrhage is an important process in the progression of asymtomatic plaques into high risk unstable lesions leading to plaque rupture, a principal cause of luminal thrombosis in acute coronary syndromes occurring in 75% of patients dying of an acute myocardial infarction.

[Holm, PW, et al Ann Med 2009; 41: 257 Kolodogie, FD,et al. N Eng J Med, 2003; 349: 2316]

sVEGFR-1, a negative regulator of VEGF bioavailability via ligand trapping

3. In the setting of acute myocardial infarction (AMI).

3.1 In acute phase (24 h): low level of sVEGFR-1 and high level of free VEGF are observed in comparing to control subjects.

3.2 Two weeks after treatment ± Atorvastatin: increased in sVEGFR-1 level and decreased in free VEGF level are noted along with improved in left ventricular ejection fraction (LVEF) .

3.3 Six months after treatment +Atorvastatin treatment: further increase in sVEGFR-1 and decrease in free VEGF with further improved in LVEF. *[Atorvastatin exert its effects via lipid lowering and anti-inflammation]*

[Kodama Y, et al. J Amer Coll Cardiol 2006; 48: 43-50]

Example 1: Normalizing VEGF levels by sVEGFR-1 during exercise training.

In physiologic context (e.g. exercise), sVEGFR-1 is up-regulated in responding to the rise in VEGF during exercise and is responsible for normalizing the VEGF level to baseline.

Bailey, A. P., Shparago, M. and Gu, J. W. (2006) Exercise increases soluble endothelial growth factor receptor-1 (sFlt-1) in circulation of healthy volunteers. Med. Sci. Moni. 12, CR45–CR50

Example 2: Rise and fall of VEGF modulated by sVEGFR-1 before and after treatment of AMI

2. **In the setting of acute myocardial infarction (AMI).**

    2.1 In acute phase (24 h): low level of sVEGFR-1 and high level of free VEGF are observed in comparing to control subjects.

    2.2 Two weeks after treatment ± Atorvastatin: increased in sVEGFR-1 level and decreased in free VEGF level are noted along with improved in left ventricular ejection fraction (LVEF) .

    2.3 Six months after treatment +Atorvastatin treatment: further increase in sVEGFR-1 and decrease in free VEGF with further improved in LVEF. *[Atorvastatin exert its effects via lipid lowering and anti-inflammation]*

[Kodama Y, et al. J Amer Coll Cardiol 2006; 48: 43-50]

VEGF : Roles in cardiovascular medicine

I. Protective role

3. **Resolve endothelial dysfunction ,** *a state of vascular function impairment ,i.e. caused by the presence of CVD risk factors ,* **via production of nitric oxide (NO) and prostacyclin .**

[Zachary, I and Morgan, RD. Heart 2011; 97: 181-9.]

Basic information: Subjects with CVD risk factors.

1. CVD risk factors are known to cause endothelial dysfunction via the induction of oxidative stress to deplete endothelium-derived relaxing factors, such as NO, prostacyclin.

2. Endothelial dysfunction , a state of impaired vascular tone and abnormal reactivity characterized by pro-inflammatory, pro-thrombotic and pro-proliferative, is thought to precede the development of atherogenesis and linked to atherosclerosis clinical sequelae.

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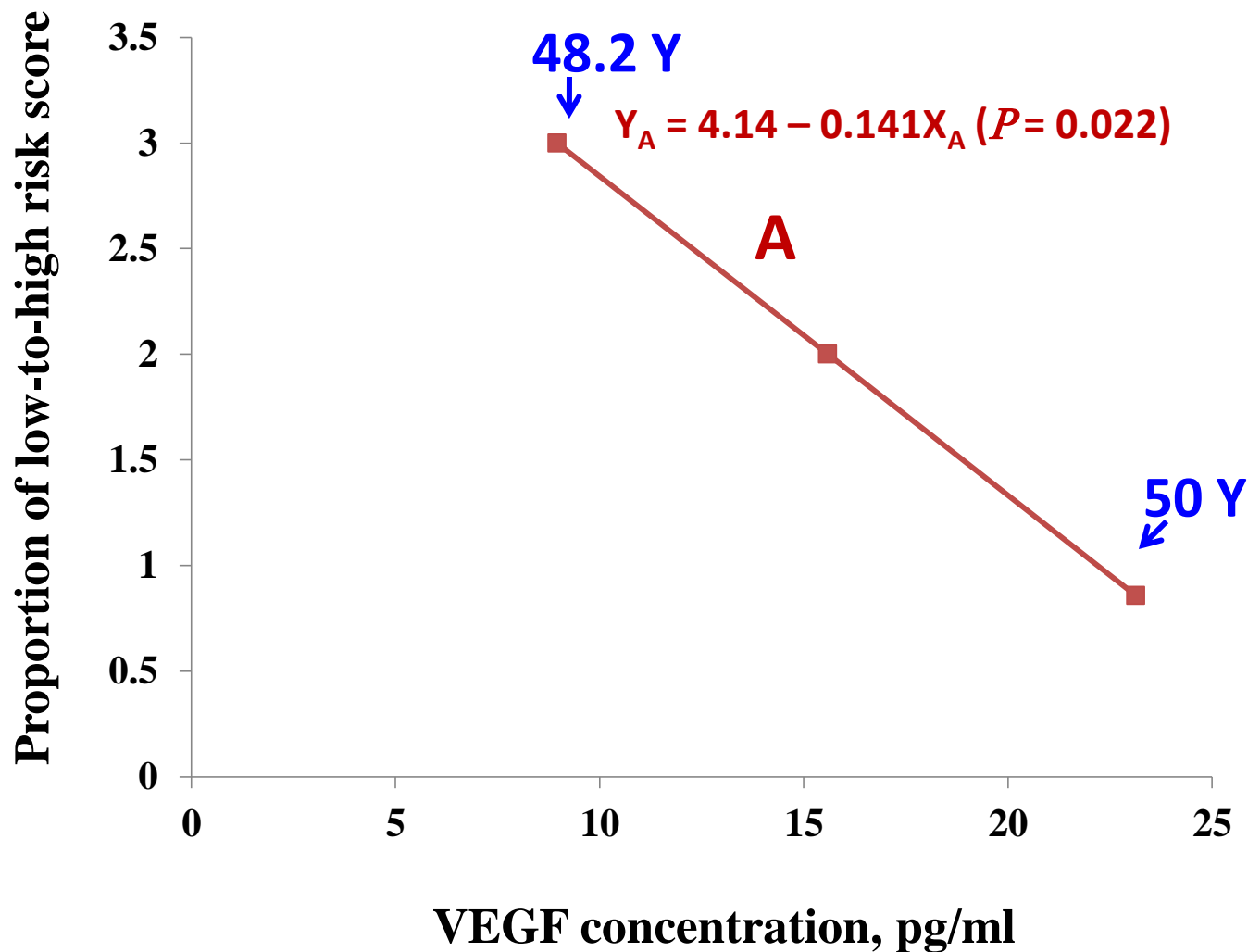# Basic information: Subjects with CVD risk factors.

3. **VEGF can resolve endothelial dysfunction** [*i.e. restore vascular tone and normalize vascular reactivity*] **via production of nitric oxide (NO) and prostacyclin .**
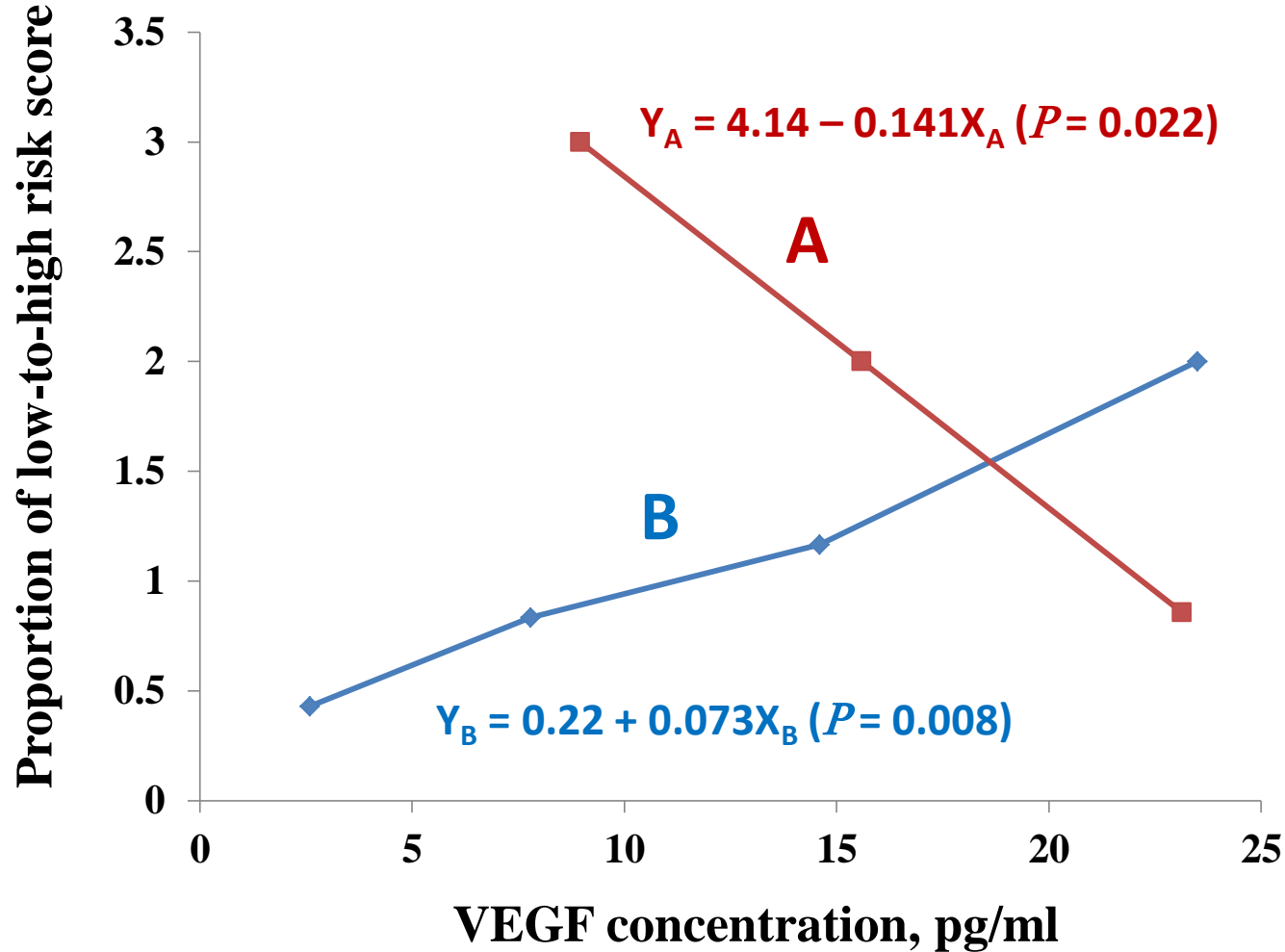
4. Degree of endothelial dysfunction is related to the global CVD risk score.

Conclusions

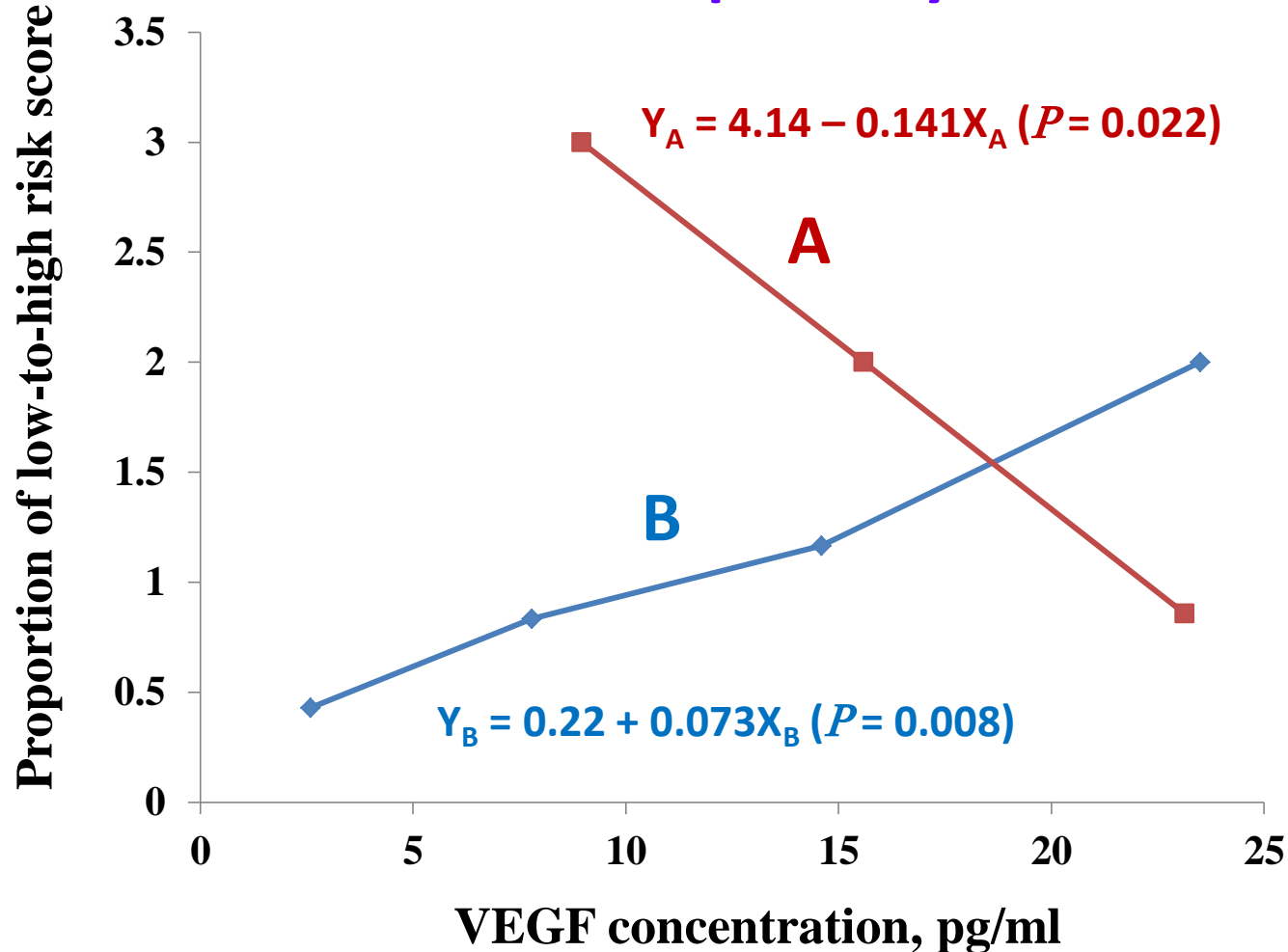4. **The tendency of sVEGFR-1 to subdue VEGF levels observed in the high risk subjects may implicate a health threat alarm particularly when VEGF suppression remains persistent.**

5. **VEGF/sVEGFR-1 as biomarkers for the long-term prognosis of peripheral and cardiovascular events in hyperchloesterolemic subjects with additional CVD risk factors is worth further investigation.**

$Y_A = 4.14 - 0.141X_A$ ($P = 0.022$)

48.2 Y

50 Y

A

Proportion of low-to-high risk score

VEGF concentration, pg/ml

$Y_A = 4.14 - 0.141X_A$ ($P = 0.022$)

A

B

$Y_B = 0.22 + 0.073X_B$ ($P = 0.008$)

Proportion of low-to-high risk score

VEGF concentration, pg/ml

Can VEGF fully restore the vascular function ?

Therefore VEGF can only restore vascular function partially.

$Y_A = 4.14 - 0.141X_A$ ($P = 0.022$)

A

B

$Y_B = 0.22 + 0.073X_B$ ($P = 0.008$)

Proportion of low-to-high risk score

VEGF concentration, pg/ml

In group B, to achieve a proportion of 3 as shown in group A requires a median concentration of 38.1 pg/ml. This is unattainable by group B for its median concentration at high VEGF level = 23.5 pg/ml.