CORRELATION OF ALTERED LIPID PROFILE TO THE RELATIVE DERANGEMENT OF THE TWO CONTRASTING VASCULAR BIOMARKERS IN APPARENTLY HEALTHY NON-OBESE AND OVERWEIGHT/OBESE SUBJECTSPreeti Kanawjia¹, Manish Bajpai², Sunita Tiwari³**HOW TO CITE THIS ARTICLE:**

Preeti Kanawjia, Manish Bajpai, Sunita Tiwari. "Correlation of Altered Lipid Profile to the Relative Derangement of the two Contrasting Vascular Biomarkers in Apparently Healthy Non-Obese and Overweight/Obese Subjects". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 40, September 01; Page: 10215-10223, DOI: 10.14260/jemds/2014/3328

ABSTRACT: With progressive vascular dysfunction, the endothelial cells mediate increased vessel tone by the synthesis and release of the vasoconstrictor substances primarily endothelin and endothelium-derived constricting factor(s), and by reduced elaboration of vasodilator products primarily nitric oxide. With this background, our study sought correlation of altered lipid profile; a cause of progressive vascular dysfunction, to the relative derangement of the two contrasting vascular biomarkers with respect to anthropometry in apparently healthy individuals. This was a case-control pilot study comprising of 30 overweight/obese healthy subjects (BMI ≥ 25 kg/m² and/or WHR (female >0.85 ; male >1) and 30 non-obese healthy subjects (BMI <25 kg/m² and/or WHR (female <0.85 ; male <1) excluding all subjects with secondary cause of abnormal blood flow. Reactive hyperemia (RH) for indirect evaluation of nitric oxide levels was measured by impedance plethysmography (NICOMON, Larsen & Toubro) in the subject's forearm along with fasting plasma glucose, serum lipids and serum endothelin-1. A significant difference in blood flow index (BFI) at 1 min post-occlusion time (mean $\pm$ % SD) {control 1.81 ± 0.42 vs. study 1.60 ± 0.38 } ($P=0.041$) was detected but RH, plasma glucose, lipid profile and serum endothelin were comparable in both the groups with lipid profile derangement present in either group, though not significant. So the two groups were clubbed to seek further association. Out of the two contrasting biomarkers, endothelin-1 and nitric oxide, only latter was significantly altered (raised) in subjects with deranged serum VLDL ($P=0.026$) and serum triglyceride ($P=0.044$). Therefore we concluded that altered lipid profile irrespective of measured anthropometric variables, affects the release of nitric oxide and it's like, before any detectable change in serum endothelin-1 levels. This in turn suggested the use of the former to predict the 'subclinical prelude' to 'manifest' metabolic disorder in such apparently healthy subjects and thus ensuring timely premorbid level care.

KEYWORDS: Endothelin-1, reactive hyperemia, nitric oxide, impedance plethysmography.

INTRODUCTION: The endothelial cell serves several functions in the vascular milieu, one of the most important of which is the regulation of vascular tone. Poised to respond to physical and molecular factors in the vasculature, the endothelium modulates the vascular smooth muscle contractile state and, as a result is a critical determinant of vasomotion and consequently blood flow.

Under normal conditions, the endothelium maintains comparatively low basal vascular tone by the production and release of factors that relax vascular smooth muscle, including prostacyclin, endothelium-derived relaxing factor, and endothelium-derived hyperpolarizing factor. With progressive dysfunction or injury, the endothelial cell supports increased vessel tone by the synthesis

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and release of the vasoconstrictor substances like endothelin and endothelium-derived constricting factor(s), and by reduced elaboration of vasodilator products.⁽¹⁾

In recent years, it has become increasingly apparent that the earliest vascular abnormality in many chronic vascular disorders is endothelial dysfunction. This pathologic phenotype is commonly manifested as an abnormality in the endothelium-dependent vasodilator response. In extreme cases, this abnormality may lead to a paradoxical vasoconstrictor response.⁽²⁾

In contrast to this, our study aimed to unveil the correlation of lipid profile to the two contrasting vascular biomarkers that is endothelin-1 (ET-1) as vasoconstrictor versus nitric oxide (NO) as vasodilator in apparently healthy overweight/obese & non-obese subjects and not in cases of established chronic vascular disease.

AIM & OBJECTIVES:

- To assess variation in cardiovascular response through reactive hyperaemia (RH) where $RH = (\text{peak blood flow} - \text{resting blood flow}) / \text{resting blood flow}$.
- To study the circulating levels of fasting lipids and endothelin-1 in apparently/clinically healthy subjects.
- Comparing the lipid profile to endothelin-1 and reactive hyperaemia in apparently/clinically healthy subjects.

MATERIAL & METHODS: The study was conducted in Cardiovascular Physiology Laboratory, Department of Physiology, K.G.M.U., Lucknow after an approval from the Institutional Ethics Committee. Study group (aged 18-45 years) comprised of 30 overweight/obese subjects {BMI >25 and/or WHR >0.85 in females and >1 in males.} and 30 non-obese subjects {BMI <25 and/ or WHR < 0.85 in females and <1 in males}^(3,4) as controls.

The cases chosen for the study were mostly those enrolled in the 'yoga camp for obese' held at K.G.M.U, Lucknow. The controls were mostly recruited from Physiology department and college staff with no previous or current history of any significant disease. Each patient was subjected to a questionnaire regarding weight gain and a thorough clinical examination to rule out any disease. Exclusion of all subjects with identifiable secondary cause of abnormal blood flow was done.

A written informed consent was taken from all subjects screened and underwent the study. The test was planned on overnight fasting subjects. For Impedance Cardio Vasography (ICVG) testing (Model- NICOMON, Larsen & Toubro) ⁽⁵⁾, the subject was made to lie supine comfortably with both the arms by the side in the level of his/her heart, the blood pressure was measured from the left arm, electrocardiograph (ECG) electrodes were applied over the chest and sensing electrodes V1 and V2; 25 cm. apart; were applied around the forearm and the current electrodes I1 and I2 were applied at the neck and wrist respectively.

Blood flow index (BFI) at rest was measured via ICVG followed by measurement of the blood pressure of the subject. Subsequently cuff pressure was raised to 50 mm Hg above systolic pressure, to completely occlude the arterial blood flow in the left forearm for 3 minutes. Subsequently pressure was released and brought down to 0 mm Hg. Following this, blood flow index (BFI) was again recorded at 1st, 2nd, 3rd, 5th, 7th and 9th minute to see post occlusive reactive hyperaemia (RH). Post ICVG, 3ml venous blood of the subject was drawn for biochemical analysis (fasting glucose, lipid

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profile and serum endothelin-1) (Endothelin-1 ELISA Kit manufacturer-Enzo Life Sciences International, Inc, North America; Sensitivity 0.40 pg/mL; Range 0.78 - 100 pg/mL).

Statistical Analysis: For each variable, the mean and standard deviation were calculated. For repeated testing of the endothelial data, survival analysis (Montel- Cox Log Rank) has been performed. Kaplan-Meier test has been conducted to see the difference in duration of reactive hyperaemia in the two groups. Owing to small and non-uniform sample size with variable outcome, nonparametric test (Mann-Whitney "U") has been used to study any statistical difference between the two groups with regards to a dependent variable. The confidence interval of the study was kept at 95%, hence a "p" value less than 0.05 indicated a statistically significant difference. The data was analyzed using Statistical Package for Social Sciences (SPSS) Version 15.0.

Method for Cardiovascular physiology study ^(6,7): The study involved the use of NICOMON, an Impedance Cardio -Vasograph (ICVG) device developed at the Electronics Division, Bhabha Atomic Research Centre, Mumbai (Jindal et al 1985) and commercially manufactured by Larsen & Toubro Limited.

Normal Range:

1. BFI = maximum amplitude of NdZ/dt waveform (Normal range = 1.50 ± 0.31).⁽⁸⁾
2. Mean Arterial Pressure (MAP) (Normal average value = 95 mm Hg).⁽⁹⁾

Derangement criteria for Blood pressure:

Systolic BP $\geq$ 140 mm Hg and/or

Diastolic BP $\geq$ 90 mm Hg ⁽¹⁰⁾ following, the given.

Classification of blood pressure for adults 18 years & older:

Category	Systolic Pressure(mm Hg)	Diastolic Pressure (mmHg)
Optimal	<120	<80
Normal	<130	<85
High Normal	130-139	85-89
Hypertension		
Stage 1 (mild)	140-159	90-99
Stage 2 (moderate)	160-179	100-109
Stage 3 (severe)	180-209	110-119

Source: The sixth Report of Joint National Committee on Prevention, Detection, Evaluation and treatment of High blood pressure. JAMA

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Derangement criteria for Fasting Plasma glucose:

Fasting Plasma glucose >110 mg/dl ⁽¹¹⁾ following, the given classification:

Classification adapted from American Diabetic Association, Clinical Practice Guidelines 2000	
Category	Fasting plasma glucose (mg/dl)
Normal glucose tolerance	<110 mg/dl
Impaired glucose tolerance	110-125 mg/dl
Diabetes Mellitus	>126 mg/dl

Derangement criteria for Fasting lipid profile ^(12, 13):

Category	(mg/dl)
Total Pl. Cholesterol	>200 mg/dL
S. HDL	<40 mg/dL
S. LDL	>130 mg/dL
S. VLDL	>30 mg/dL
S. TG	>150 mg/dL following the classification given below

ATP III Classification of Total, LDL and HDL Cholesterol (mg/dl)			
Category	Total Pl. Chl (mg/dL)	LDL-Chl (mg/dL)	HDL-Chl (mg/dL)
Desirable	< 200	< 130	≥ 60
Borderline	200-239	130-159	40-59
Undesirable	≥ 240	≥ 160	< 40

ATP III Classification of Triglycerides
Normal triglycerides: <150 mg/dL
Borderline-high triglycerides: 150-199 mg/dL
High triglycerides: 200-499 mg/dL
Very high triglycerides: >500 mg/dL

ATP III Classification of VLDL
Desirable VLDL: ≤ 30mg/dL
Undesirable VLDL: > 30 mg/dL

OBSERVATION & RESULTS: On comparison of biochemical variables in the two groups of overweight/obese versus non obese, no significant difference in the fasting lipid profile and serum endothelin-1 levels was seen though deranged lipid profile was present in both the study and control groups. (Table 2) On comparison of RH recordings in the two groups, no significant difference in its onset, time of attaining peak value (2nd minute post-occlusively) and duration were seen except for a significant difference in BFI at 1 min post-occlusion time (mean ± % SD) {control 1.81 ± 0.42 vs. study

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1.60 ± 0.38} (P=0.041) (table 1). As no significant difference in RH, serum endothelin-1 and lipid profile was observed hence for subsequent analysis, to seek an association between various cardiovascular risk factors, both the groups were clubbed i.e. they were combined into one group. In the clubbed group, only those subjects who had deranged serum Very Low Density Lipoprotein (VLDL) and serum Triglyceride (TG) had significantly raised RH response (P = 0.026, P = 0.044 respectively) (table 2) and a normal serum endothelin-1 level (Table 3).

DISCUSSION: We sorted two groups of healthy overweight/obese and non-obese subjects. On analysing their serum lipid profile, a non-universal association of its derangement to anthropometric variables was seen i.e. both the groups comprised of subjects with deranged as well as normal lipid profile. Considering impedance plethysmographic technique of measuring RH response as an indirect and non-invasive test for assessing the nitric oxide (NO) activity of the vascular endothelium ⁽¹⁴⁾ RH response was measured.

So before going further, it needs to be stressed that NO scripted anywhere in the paper should be equated to RH unless mentioned otherwise. It is also to be noted that during the ICVG procedure we stuck to the '3 minute brachial artery occlusion time' protocol for eliciting RH response as researches reveal the importance of time factor in promoting release of specific vascular biomarkers and in our case it was assumed to be NO.⁽¹⁴⁾ A normal response was one when the basal/resting blood flow index peaked at 2nd minute post occlusion time and then gradually tapered down to the pre-occlusive values at 9th minute post occlusion time.

Except for a significant difference in BFI at 1st minute post-occlusion time in the obese group, no other significant difference in RH was sought, (table 1) thus suggesting that subjects with isolated adverse anthropometric measurements (BMI, WHR) have an unfavourable influence on the vascular hemodynamics.

Serum ET-1 was also analysed for obtaining more information about the health of vascular endothelium and that too was in normal range.

To seek further association, the two healthy groups were clubbed. The clubbed group was then divided into two sub-groups of normal versus deranged lipid profile and both reactive hyperaemia and serum endothelin-1 levels were studied with respect to them.

As per researches, chronic vascular diseases affect the nitric oxide activity much before any change in serum endothelin -1 levels⁽²⁾ but in our study the apparently healthy overweight/obese and non-obese subjects though indicated the same chronology of biomarker derangement but were inverse quantitatively i.e. derangement in lipids; serum VLDL (P = 0.026) and serum triglyceride (P = 0.044) was associated with 'raised' RH response (table 2) with no observable change in serum endothelin-1 levels (Table 3).

This reflected the difference which the apparently healthy subjects with deranged lipid profile bore with respect to unhealthy subjects having established chronic vascular disease.

Explaining the cause of this rise seemed nearly impossible, if we had to say that RH response was solely due to nitric oxide activity. Instead this study gave indications of some other parallel phenomenon/ bio chemicals/ vasodilators which were keeping the endothelium healthy in spite of an unfavourable lipid profile.

On-going researches in the last few years have stressed the role of one such endothelium-derived vasodilator known as Endothelium-Derived Hyperpolarizing Factor (EDHF). After many

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pathologic conditions, dilation produced by endothelium-derived nitric oxide can be significantly attenuated.

The primary reasons for the attenuated dilations include an excessive production of reactive oxygen species, which inactivate nitric oxide, and/or dysfunction in endothelial nitric oxide synthase (eNOS) generation of nitric oxide. In contrast, Endothelium-Derived Hyperpolarizing Factor (EDHF) seems to be resistant to reactive oxygen species.

In fact, EDHF has been reported to be up regulated after a variety of pathologic conditions when nitric oxide-mediated dilations have been attenuated.⁽¹⁵⁾ Thus the up-regulation could be a possible answer to the raised RH response, only if it be equated to collective NO and EDHF activity.

CONCLUSION: To summarize, deranged lipids irrespective of body mass index and waist hip ratio clandestinely grows up as an alteration in levels of contrasting biomarkers, not always in a conventional way of positive feedback phenomenon but also as an 'escape phenomenon' elucidated by a raised reactive hyperaemic response suggesting the probable role of yet undeciphered phenomena and vascular biomarkers. Thus it is very likely that this is how subclinical biochemical abnormality in an apparently healthy individual may take a course, before manifesting as metabolic syndrome.

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Parameter/ Variable	Control Group (n=30)		Study Group (n=30)		P
	No./ Mean	%/ SD	No./ Mean	%/ SD	
Female gender	12	40	12	40	1
Age	29.20	9.01	33.00	10.38	0.136
Fasting glucose	103.77	14.64	100.38	11.46	0.322
S.T.Chl.(mg/dl)	151.30	39.15	152.92	30.06	0.858
S.HDL.(mg/dl)	42.84	9.17	38.85	10.23	0.117
S.LDL.(mg/dl)	85.84	32.71	87.86	27.93	0.797
S.VLDL.(mg/dl)	22.62	11.23	28.24	16.16	0.123
S.TG.(mg/dl)	108.41	50.72	138.02	83.14	0.101
LDL/HDL	2.01	0.57	2.32	0.67	0.052
BMI(kg/m ²)	22.55	2.96	29.59	2.87	0.000
Waist Hip ratio	0.86	0.06	0.95	0.09	0.000
Calories intake	1307.83	381.08	1626.50	488.97	0.007
Resting blood flow index	1.76	0.36	1.61	0.38	0.137
PO 1st min BFI	1.81	0.42	1.60	0.38	0.041
PO 2nd min BFI	2.15	0.53	1.92	0.47	0.076
PO 3rd min BFI	2.04	0.44	1.87	0.45	0.141
PO 5th min BFI	2.00	0.46	1.81	0.42	0.090
PO 7th min BFI	1.99	0.40	1.82	0.39	0.104
PO 9th min BFI	1.91	0.38	1.76	0.34	0.109
S.endothelin1(pg/ml)	19.03	7.25	21.52	5.01	0.128
Reactive hyperemia [(Peak PO blood flow – Resting blood flow)/ Resting blood flow]	0.294	0.201	0.274	0.264	0.735

**Table 1: Comparative Assessment of Two Groups–
Normal weight vs. overweight/Obese**

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*P: probability

S.T.Chl.: Serum Total Cholesterol

S.HDL: Serum High Density Lipoprotein

S.LDL: Serum Low Density Lipoprotein

S.VLDL: Serum Very Low Density Lipoprotein Cholesterol

S.TG: Serum Triglyceride

BMI: Body mass index

PO BFI 1st, 2nd, 3rd, 5th, 7th, 9th min: Post Occlusion blood flow index at 1st, 2nd, 3rd, 5th, 7th, 9th minute.

Sl. No.	Variable/ Parameter	Mean±SD	(Proportions) Significance of difference ("p")*
S. T.Chl (mg/dL)			
1.	Normal (<200 mg/dl) (n=56)	0.28±0.24	0.349 (Mann-Whitney U test)
2.	Deranged (≥200 mg/dl) (n=4)	0.33±0.13	
S. HDL (mg/dL)			
1.	Normal (>40 mg/dl) (n=28)	0.23±0.19	0.105 (Mann-Whitney U test)
2.	Deranged (≤40 mg/dl) (n=32)	0.33±0.26	
S. LDL (mg/dL)			
1.	Normal (<130 mg/dl) (n=56)	0.27±0.23	0.306 (Mann-Whitney U test)
2.	Deranged (≥130 mg/dl) (n=4)	0.43±0.29	
S. VLDL (mg/dL)			
1.	Normal (≤30 mg/dl) (n=45)	0.26±0.24	0.026 (Mann-Whitney U test)
2.	Deranged (>30 mg/dl) (n=15)	0.36±0.21	
S. TG (mg/dL)			
1.	Normal (≤150 mg/dl) (n=46)	0.26±0.23	0.044 (Mann-Whitney U test)
2.	Deranged (>150 mg/dl) (n=14)	0.36±0.22	
Table 2: Association of Reactive Hyperemia to lipid profile			

Table 2: Association of Reactive Hyperemia to lipid profile

*Non-parametric tests have been used owing to dissimilarity in group sizes, small sample size and high variability in outcome variable.

S.T.Chl.: Serum Total cholesterol.

S.HDL: Serum High Density Lipoprotein.

S.LDL: Serum Low Density Lipoprotein.

S.VLDL: Serum Very Low Density Lipoprotein.

S.TG: Serum Triglyceride.

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Sl. No.	Variable/ Parameter	Significance of difference ("p")*
S. T. Chl (mg/dL)		
1.	Normal (<200 mg/dl) (n=56)	0.53 (Mann-Whitney U test)
2.	Deranged (≥200 mg/dl) (n=4)	
S. HDL (mg/dL)		
1.	Normal (>40 mg/dl) (n=28)	0.90 (Mann-Whitney U test)
2.	Deranged (≤40 mg/dl) (n=32)	
S. LDL (mg/dL)		
1.	Normal (<130 mg/dl) (n=56)	0.59 (Mann-Whitney U test)
2.	Deranged (≥130 mg/dl) (n=4)	
S. VLDL (mg/dL)		
1.	Normal (≤30 mg/dl) (n=45)	0.65 (Mann-Whitney U test)
2.	Deranged (>30 mg/dl) (n=15)	
S. TG (mg/dL)		
1.	Normal (≤150 mg/dl) (n=46)	0.55 (Mann-Whitney U test)
2.	Deranged (>150 mg/dl) (n=14)	
Table 3: Association of S.Endothelin-1** to lipid profile		

*Non-parametric tests have been used owing to dissimilarity in group sizes, small sample size and high variability in outcome variable

**Cut-off value for Serum Endothelin-1 was arrived by plotting a ROC curve between Controls and Cases where normal value $\leq$ 20pg/ml (n=25).

S.T.Chl.: Serum Total cholesterol.

S.HDL: Serum High Density Lipoprotein.

S.LDL: Serum Low Density Lipoprotein.

S.VLDL: Serum Very Low Density Lipoprotein.

S.TG: Serum Triglyceride.

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