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Original Article

Physiology Section

Effect of Metaboreflex on Cardiovascular System in Subjects of Metabolic Syndrome

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ABSTRACT

Introduction: Metaboreflex is a reflex in which muscle receptors send signals regarding metabolic (metabolites accumulation like lactic acid, potassium, adenosine) conditions of the muscles to nucleus tractus solitarius via afferent III and IV fibres to cause haemodynamic adjustments in order to regulate blood flow on the basis of the status of contracting muscle. Dysregulation in its mechanism in metabolic syndrome is demonstrated.

Aim: To study the effect of metaboreflex by both isometric and rhythmic handgrip exercise on CVS parameters {Blood Pressure (BP), Cardiac Output (CO) and Systemic Vascular Resistance (SVR)} in subjects of metabolic syndrome.

Materials and Methods: In this study, 27 subjects aged 25 to 45 years were enrolled after ethical clearance and proper consent. They were divided into: a) subjects without metabolic syndrome; and b) subjects with metabolic syndrome. Impedance cardiovasography was done to assess cardiac parameters (systolic and diastolic blood pressure, cardiac output, systemic vascular resistance). Pre-exercise parameters were assessed followed by isometric exercise and post-isometric exercise parameter measurement. Again after rest, rhythmic exercise was followed. Finally post exercise parameters were assessed. Student paired t-test for comparison between pre and post exercise parameters were done.

Results: Changes in diastolic BP following exercise were statistically significant in subjects without metabolic syndrome (p-value 0.01 and 0.001 following isometric and rhythmic exercise respectively). In subjects with metabolic syndrome also these changes were significant, but to a lesser extent (p-value 0.1 and 0.01 respectively for isometric and rhythmic exercise).

Changes in systolic BP following exercise were statistically significant in subjects without metabolic syndrome (p-value 0.001 and 0.001 following isometric and rhythmic exercise respectively). In subjects with metabolic syndrome also these changes were significant (p-value 0.01 and 0.001 respectively for isometric and rhythmic exercise).

Conclusion: Diminished pressor response is found after exercise in subjects with metabolic syndrome.

Keywords: Autonomic nervous system, Cardiac parameters, Haemodynamics, Isometric exercise, Rhythmic exercise

INTRODUCTION

Physical activity is any bodily movement produced by skeletal muscles that results in energy expenditure [1]. An emphasis has been placed on understanding the role of physical activity in the prevention and management of obesity which is responsible for the increasing prevalence of metabolic syndrome in the U.S. and worldwide due to sedentary lifestyles [2-4].

When physical activity is planned, structured, and repetitive with an objective to improve or maintain the physical fitness, then it is known as exercise [1]. Exercise can be of three types: isometric, isotonic or rhythmic and isokinetic [1]. The cardiovascular and haemodynamic changes during exercise are regulated by autonomic activity which itself is under control of several neural mechanisms [5]. These neural mechanisms are central command, exercise pressor reflex and baroreflex. Central command is a feed-forward mechanism from the cortical centres in brain. The metaboreflex or exercise pressor reflex is feedback mechanism from the exercising skeletal muscle. The baroreflex is a negative feedback mechanism that can either be arterial, originating from the carotid sinus and aortic arch, or cardiopulmonary, originating from the heart and pulmonary vasculature [5].

In healthy individuals, exercise causes the sympathetic tone to dominate over parasympathetic tone thereby augmenting the heart-rate, myocardial contractility and peripheral vasoconstriction in the vascular beds of organs and tissues not involved in exercise [6]. Some dysregulation in these mechanisms has been demonstrated in various diseases, mainly circulatory and metabolic diseases [6]. Further investigation in this field is warranted on the correction of the dysregulation of these reflexes [6].

In metaboreflex, during exercise, metabolites like lactate, potassium, phosphate, adenosine, bradykinin and arachidonic acid products start accumulating in the skeletal muscle [7,8]. These metabolites are sensed by the receptors present within the muscles, which relay this information to the dorsal horn of spinal cord via Group III and IV muscle afferents [7,8]. Finally the information reaches to the nucleus tractus solitarius and other cardiovascular controlling areas in the brain stem that regulate the blood flow in contracting skeletal muscles by several haemodynamic adjustments [7,8].

In metabolic syndrome, drug therapies directed towards the individual risk factors (i.e., hypertension, obesity, dyslipidemia) might be required [9]. In order to know the effect of individual components of metabolic syndrome on EPR, many studies were conducted [10-13]. Trombetta IC et al., reported that weight loss improves neurovascular and muscle metaboreflex control in obesity [10]. Negrão CE et al., found that muscle metaboreflex control is diminished in normotensive obese women [11]. Sausen MT et al., showed enhanced metaboreflex sensitivity in hypertensive humans [12]. Delaney EP et al., concluded exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans [13]. These studies did not take metabolic syndrome as criteria to divide

subjects, instead either obesity or hypertension was used as criteria. Obesity and hypertension are usually a part of cascade of diseases known as metabolic syndrome. The present study was performed on subjects with and without metabolic syndrome to find the effect of metaboreflex on central haemodynamics and cardiac parameters by performing isometric and rhythmic exercise. The aim of this study was to evaluate the effect of metaboreflex by both isometric and rhythmic handgrip forearm exercise on haemodynamic parameters (blood pressure, cardiac output, and systemic vascular resistance) in subjects with metabolic syndrome.

MATERIALS AND METHODS

The experimental longitudinal study was conducted in Department of Physiology in Impedance CardioVasoGraphy Laboratory, KGMU, Lucknow, Uttar Pradesh, India. An approval from the Institutional Ethical Committee of KGMU, Lucknow was taken. Twenty seven subjects (both male and female) aged 25 to 45 years were enrolled and divided into two groups:

a. Subjects without metabolic syndrome=15;
b. Subjects with metabolic syndrome (according to NCEP: ATP III 2001 criteria for metabolic syndrome) = 12.

NCEP ATP III Criteria

If three or more of the following are present, subject is classified into metabolic syndrome group.

1. Central obesity: waist circumference > 102 cm (M), >88 cm (F).
2. Hypertriglyceridemia: Triglyceride level ≥ 150 mg/dL, or specific medication.
3. Low HDL Cholesterol: <40 mg/dL and <50 mg/dL for men and women, respectively, or specific medication.
4. Hypertension: blood pressure ≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or specific medication.
5. Fasting plasma glucose level ≥ 100 mg/dL or specific medication or previously diagnosed type 2 diabetes.

Inclusion and Exclusion Criteria

Subjects aged 25 to 45 years with or without metabolic syndrome were included in the study. Any subject with systemic disease, cardiovascular and respiratory abnormalities, or history of smoking, heavy exercise within 12 hours, hypertension, drug intake interfering with exercise response like beta blockers, vasodilators, ACE inhibitors, Channel Antagonist (CA), digitalis, antiarrhythmic agents and diuretics, hyperkalemia and hypokalemia was excluded from the study.

Informed consent was taken from each subject for anthropometry, isometric and rhythmic handgrip exercise, impedance cardio-vasography and blood sampling for mentioned biochemical analysis before and after exercise.

Anthropometry (waist circumference) and biochemical parameters [Fasting Blood Sugar (FBS), serum HDL, and serum triglycerides] were measured to classify the subjects into subjects with and without metabolic syndrome.

Waist circumference was measured using measuring tape. It was measured halfway between lowest rib and top of hip bone. Subject was asked to breathe out normally before measuring [14]. Blood sample (3 ml before exercise) was drawn from antecubital vein. Biochemical parameters {serum High Density Lipoprotien (HDL) and serum triglycerides} were analysed on vitros 250 dry chemistry full autoanalyser.

Then the individuals were made to lie supine in bed and made comfortable and given rest for five minutes. Pre exercise cardiac and haemodynamic parameters of the subjects were measured. Blood Pressure {Systolic (SBP) and Diastolic (DBP)} was measured using mercury sphygmomanometer and stethoscope. Cardiac parameters {Cardiac Output (CO), Cardiac Index (CI)] and haemodynamic parameters- SVR, Total Peripheral Resistance (TPR), Systemic Vascular Resistance Index (SVRI)} were measured using impedance cardiovasography (from Larsen and Tourbo) in Department of Physiology. It is a non invasive measurement of impedance requiring use of surface electrodes [15]. The calibrated network, through a relay, passed a sinusoidal current of constant amplitude to the body segment with the help of current electrodes amplitude [15]. Voltage sensing electrodes were applied at desired locations on the body segment along the current path [15]. The amplitude of sensed sinusoidal signal is directly proportional to the instantaneous Z (Impedance) of the body segment between the sensing electrodes [15]. Z is processed yielding basal impedance Zo, $\Delta Z(t)$ and dZ/dt waveform which are read through an interface [15]. Small change in the impedance of the body segment caused by physiological processes like blood circulation is obtained as waveforms [15]. Measurement of these physiological processes from these impedance signals is known as Impedance Cardiovasography (ICVG). It has most often acceptable accuracy, precision and responsiveness in a wide range of circulatory situation [16].

Subjects' Maximum Voluntary Contraction (MVC) was recorded by handgrip dynamometer. It was followed by isometric handgrip exercise (forearm exercise) at 30% of MVC (for 2 minutes), followed by Post Exercise Cuff Occlusion (PECO [17]) 20 mmHg above systolic blood pressure to isolate metaboreflex. Post-isometric exercise cardiac and haemodynamic parameters were assessed. Again rest for 10 minutes was given. Then rhythmic handgrip exercise (forearm exercise) at 30% of MVC [18] at a rate of 30 times per minute (for two minutes), followed by PECO 20 mmHg above systolic blood pressure was done, followed by post rhythmic exercise measurement of cardiac and haemodynamic parameters of the subjects. Vasoconstriction mediated pressor response (changes in DBP and SVR) and flow mediated pressor response (changes in SBP and CO) due to metaboreflex activation were evaluated.

Sample Size Calculation

The sample was calculated by using the following formula [19].

$n=Z_{1-\alpha/2}^2 \times SD^2/d^2$, where

$Z_{1-\alpha/2}$=Power of the study

SD: Standard deviation of the study variable

d: Absolute error

In a study by Milia R et al., an exaggerated increase in systemic vascular resistance from baseline during the metaboreflex was found in the Metabolically Healthy but Obese (MHO) patients 0.52±177.6 [20]. Assuming 80% power, 5% significance level with 95% confidence interval as well as absolute error being 40, the total sample size calculated was 38.

STATISTICAL ANALYSIS

Student paired t-tests for comparison between pre and post exercise parameters were done.

RESULTS

[Table/Fig-1] shows the normal ranges and the equation for the calculation of the assessment parameters. There was no significant difference in DBP at pre-exercise, post isometric and post rhythmic exercise between subjects with metabolic syndrome and subjects without metabolic syndrome. The increase in the DBP from pre-exercise to post isometric exercise was significant ($p<0.05$) in subjects without metabolic syndrome. However, the increase in DBP from pre-exercise to post rhythmic exercise was observed to be statistically significant ($p<0.05$) in both the groups [Table/Fig-2]. There was no significant difference in SBP at pre-exercise, post isometric exercise and post rhythmic exercise between subjects with

Parameters	Equation	Normal Range
Systolic (SBP)		90 - 140 mmHg
Diastolic (DBP)		60 - 90 mmHg
Cardiac Output (CO)	PR x SV/1000	4.0 - 8.0 l/min
Cardiac Index (CI)	CO/BSA	2.5 - 4.0 l/min/m^2
Systemic Vascular Resistance (SVR)	80 x (MAP - RAP)/ CO	800 - 1200 dynes sec/cm^5
Systemic Vascular Resistance Index (SVRI)	80 x (MAP - RAP)/CI	1970 - 2390 dynes sec/cm^5/m^2

[Table/Fig-1]: Normal values and ranges.

metabolic syndrome and subjects without metabolic syndrome. The increase in the SBP from pre-exercise to post isometric exercise and post rhythmic exercise was observed to be statistically significant ($p<0.05$) in both the groups [Table/Fig-2].

DISCUSSION

Metabolic syndrome is a cluster of abnormalities with basic characteristics being insulin resistance and visceral obesity. The major concerns of obesity and metabolic syndrome are the co- morbidities, such as type 2 diabetes, cardiovascular disease, stroke, and other life threatening conditions [21].

Sympathetic Nervous System (SNS) activity is associated with energy balance and alteration in SNS activity is associated with metabolic syndrome [22]. There is important contribution of the muscle metaboreflex to blood pressure changes during exercise. Previous research show exaggerated blood pressure responses to whole-body exercise in adults with metabolic syndrome [23-25].

This study was planned with an aim to study if metabolic syndrome is having any effect on metaboreflex, to see if it contributes to cardiovascular risks [26].

The mean age of the first group (subjects without metabolic syndrome) and second group (subjects with metabolic syndrome) was 32.73±12.07 and 33.17±18.22 years respectively. The difference was statistically not significant ($p>0.05$). There were six females and nine males in first group (subjects without metabolic syndrome) and six males and six females in the second group (subjects with metabolic syndrome. So, there was no significant ($p>0.05$) difference in the gender between subjects with metabolic syndrome and subjects without metabolic syndrome. All these subjects performed handgrip isometric and rhythmic exercises as per the protocol [26,27].

There was no significant difference in any cardiac parameter in pre-exercise, post-isometric exercise and post rhythmic exercise values between subjects without metabolic syndrome and subjects with metabolic syndrome as found via unpaired t-test.

Vasoconstriction (SVR) Mediated Pressor Response

Changes in DBP following exercise were statistically significant in subjects without metabolic syndrome. SVR and SVRI showed statistically significant increase following rhythmic exercise. In subjects with metabolic syndrome also, these changes were significant, but to a lesser extent. SVR and SVRI showed a decrease following rhythmic exercise which was statistically significant. Thus, it was found that rhythmic exercise is more associated with vasoconstriction response compared to isometric exercise and vasoconstriction mediated pressor response is decreased in subjects with metabolic syndrome during exercise.

Negrão CE et al., reported that in normotensive obese women, muscle metaboreflex control was diminished, a finding similar to that of our study [11]. However, Negrão CE et al., observed only vasoconstriction mediated pressor response following static exercise. It was suggested that the obese individuals have more fat content in their skeletal muscle that could have desensitized the metaboreceptors, thereby reducing the metaboreflex-mediated muscle sympathetic nerve activity.

The reduction in response in subjects with metabolic syndrome cannot be explained by reduced exercise force performed during handgrip exercise. The force used for exercise was adjusted to the percentage of the maximum voluntary contraction force in both groups. Also, in the study by Negrão CE et al., the maximal voluntary force was similar between the two groups [11].

The findings of this study could also be due to insulin resistance [28] in subjects with metabolic syndrome, which reduces glycolysis in skeletal muscle, which, in turn, attenuates muscle acidosis during exercise in these individuals. Thus, the metaboreceptors underwent less stimulation during exercise in these subjects. This might have led to decreased vasoconstriction mediated pressor response.

Flow (CO) Mediated Pressor Response

Changes in SBP following exercise were statistically significant in subjects without metabolic syndrome. CI showed a statistically significant increase following isometric and rhythmic exercise. In subjects with metabolic syndrome also these changes were significant. CO and CI showed statistically significant increase following rhythmic exercise. Thus, the present study found a similar flow mediated pressor response with rhythmic exercise and with isometric exercise and a diminished flow mediated pressor response in subjects with metabolic syndrome following isometric exercise.

Subjects Without Metabolic Syndrome (n=15)							
Exercise		CVS Parameters					
		SBP	CO	CI	DBP	SVR	SVRI
Isometric Exercise	Pre	121.60±9.75	5.10±1.33	2.97±0.59	74.93±9.19	1403.20±338.15	2336.67±409.62
	Post	126.80±11.48	5.34±1.44	3.13±0.67	79.33±9.49	1405.47±342.74	2341.53±468.82
	p-value	<0.001*	0.10	0.03*	0.01*	0.76	0.10
Rhythmic Exercise	Post	126.93±13.37	5.28±1.00	3.12±0.40	82.00±10.30	1418.53±279.91	2409.53±385.86
	p-value	<0.001*	0.06	0.002*	<0.001*	<0.001*	<0.001*
Subjects With Metabolic Syndrome (n=12)							
Exercise		CVS Parameters					
		SBP	CO	CI	DBP	SVR	SVRI
Isometric Exercise	Pre	116.83±8.59	4.63±1.20	2.76±0.55	77.17±7.69	1552.83±382.98	2552.33±572.48
	Post	124.17±10.14	4.80±1.25	2.86±0.62	77.33±8.06	1539.08±399.97	2525.58±565.39
	p-value	0.01*	0.09	0.11	0.10	0.23	0.11
Rhythmic Exercise	Post	126.17±12.34	5.12±1.37	3.05±0.56	81.67±7.07	1409.33±403.08	2300.50±548.60
	p-value	<0.001*	0.02*	0.02*	0.01*	<0.001*	<0.001*

[Table/Fig-2]: Pre and postexercise changes in the values.

LIMITATION

Sample size for the study could be higher. No biochemical parameter was considered to confirm the findings.

CONCLUSION

It can be concluded that, rhythmic exercise is more associated with vasoconstriction response compared to isometric exercise. It was also found that vasoconstriction mediated pressor response is decreased in subjects with metabolic syndrome during exercise. Also, similar flow mediated pressor response was found both with rhythmic exercise and with isometric exercise. There is also a diminished flow mediated pressor response in subjects with metabolic syndrome following isometric exercise.

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REFERENCES

[1] Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126–31.
[2] Saris WH, Blair SN, van Baak MA, Eaton SB, Davies PS, Di Pietro L, et al. How much physical activity is enough to prevent unhealthy weight gain? Obes Rev. 2003;4:101–14.
[3] Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc. 2009;41:459–71.
[4] Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med. 2003;163:427–36.
[5] Paul JF. Neural control of the circulation during exercise in health and disease. Front Physiol. 2013;4:224.
[6] Nobrega ACL, O'Leary D, Silva BM, Marongiu E, Piepoli MF, Crisafulli A. Neural regulation of cardiovascular response to exercise: role of central commands and peripheral afferents. BioMed Res Int. 2014;2104:478965.
[7] Smith SA, Mitchell JH, Garry MG. The mammalian exercise pressor reflex in health and disease. Exp Physiol. 2006;91(1):89–102.
[8] Megan NM, Masaki M, Mitchell JH, Scott AS. Cardiovascular regulation by skeletal muscle reflexes in health and disease. Am J Physiol Heart Circ Physiol. 2011;301(4):H1191–H204.
[9] Scott MG. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. J Am Coll Cardiol. 2006;47(6):1093-100.
[10] Trombetta IC, Batalha LT, Rondon MU, Laterza MC, Kuniyoshi FH, Gowdak MM, et al. Weight loss improves neurovascular and muscle metaboreflex control in obesity. Am J Physiol Heart Circ Physiol. 2003;285(3):H974-82.
[11] Negrão CE, Trombetta IC, Batalha LT, Ribeiro MM, Rondon MU, Tinucci T, et al. Muscle metaboreflex control is diminished in normotensive obese women. Am J Physiol Heart Circ Physiol. 2001;281(2):H469-75.
[12] Sausen MT, Delaney EP, Stillabower ME, Farquhar WB. Enhanced metaboreflex sensitivity in hypertensive humans. Eur J Appl Physiol. 2009;105:351–56.
[13] Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, Farquhar WB. Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. Am J Physiol Heart Circ Physiol. 2010;299:1318–27.
[14] Ross R, Berentzen T, Bradshaw AJ, Janssen I, Kahn HS, Katzmarzyk PT, et al. Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? Obes Rev. 2008;9(4):312-25.
[15] Karamchandani S, Dixit M, Jain R, Bhowmick M. Application of neural networks in the interpretation of impedance cardiovasograms for the diagnoses of peripheral vascular diseases. Conf Proc IEEE Eng Med Biol Soc. 2005;7:7537-40.
[16] Squara P, Denjean D, Estagnasie P, Brusset A, Dib JC, Dubois C. Noninvasive cardiac output monitoring (NICOM): a clinical validation. Intensive Care Med. 2007;33(7):1191-94.
[17] Limberg J, Morgan B, Schrage W. Mechanical and metabolic reflex activation of the sympathetic nervous system in younger adults with metabolic syndrome. Auton Neurosci. 2014;183:100-05.
[18] Meldrum D, Cahalane E, Conroy R, Fitzgerald D, Hardiman O. Maximum voluntary isometric contraction: reference values and clinical application. Amyotroph Lateral Scler. 2007;8(1):47-55.
[19] Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med. 2013;35(2):121–26.
[20] Milia R, Velluzzi F, Roberto S, Palazzolo G, Sanna I, Sainas G, et al. Differences in haemodynamic response to metaboreflex activation between obese patients with metabolic syndrome and healthy subjects with obese phenotype. Am J Physiol Heart Circ Physiol. 2015;309(5):H779-89.
[21] Alicia AT, Markus PS. Relevance of sympathetic nervous system activation in obesity and metabolic syndrome. J Diabetes Res. 2015;2015: 341583.
[22] Kevin P, Jeb S. Sympathetic nervous system behavior in human obesity. Neurosci Biobehav Rev. 2009;33(2):116–24.
[23] Gaudreault V, Després JP, Rhéaume C, Alméras N, Bergeron J, Tremblay A, et al. Exercise-induced hypertension in men with metabolic syndrome: anthropometric, metabolic, and haemodynamic features. Metab Syndr Relat Disord. 2013;11(1):07-14.
[24] Miyai N, Shiozaki M, Yabu M, Utsumi M, Morioka I, Miyashita K, et al. Increased mean arterial pressure response to dynamic exercise in normotensive subjects with multiple metabolic risk factors. Hypertens Res. 2013;36(6):534-39.
[25] Tsioufis C, Kasiakogias A, Tsiachris D, Kordalis A, Thomopoulos C, Giakoumis M, et al. Metabolic syndrome and exaggerated blood pressure response to exercise in newly diagnosed hypertensive patients. Eur J Prev Cardiol. 2012;19(3):467-73.
[26] Limberg J, Morgan B, Schrage W. Mechanical and metabolic reflex activation of the sympathetic nervous system in younger adults with metabolic syndrome. Auton Neurosci. 2014;183:100-05.
[27] Vøllestad NK, Hallén J, Sejersted OM. Effect of exercise intensity on potassium balance in muscle and blood of man. J Physiol. 1994;475(2):359-68.
[28] Bjorntorp P. Endocrine abnormalities in obesity. Diabetes Rev. 1997;5:52–68.

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