"A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND LIPID PROFILE IN THE POSTMENOPAUSAL WOMEN"

By

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IN

PHYSIOLOGY

Under the guidance of

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LIST OF ABBREVIATIONS USED

(In alphabetical order)

Ach- Acetylcholine

APO-Apoproteins

ANS- Autonomic Nervous System

ATP-Adenosine Tri Phosphate

BMI- Body Mass Index

BP- Blood pressure

bpm- Beats per minute

BSA- Body Surface Area

AV node- Atrioventricular node

CE-Cholesterol Ester

CETP-Cholesterol ester transfer protein

cm-Centimeter

CVD-Cardiovascular Diseases

DBP- Diastolic blood pressure

dl- Deciliter

ECG- Electrocardiogram

ERT-Estrogen Replacement Therapy

FMP-Final Menstrual Period

FSH-Follicle Stimulating Hormone

GnRh-Gonadotrophin Releasing Hormone

HDL-C-High Density Lipoprotein Cholesterol

HMG CoA-Hydroxy-methylglutaryl-CoA

HR- Heart rate

HRT-Hormonal Replacement Therapy

Ht- Height

IDL-Intermediate Density Lipoprotein

I-E -Inspiration- Expiration

Kg-Kilogram

Kg/m^2-Kilogram Per meter Square

LCAT-Lecithin Cholesterol Acyltransferase

LDL-C-Low Density Lipoprotein Cholesterol

LH-Leutenizing Hormone

mg-Milligram

mmHg-Millimeter Of Mercury

NADPH-Diydronicotinamide Adenine Dinucleotide Phosphate

PR-Pulse Rate

RR-Respiratory rate

SA node- Sinoatrial node

SBP-Systolic Blood Pressure

SD-Standard Deviation

SHBG-Sex Hormone Binding Globulin

TC-Total Cholesterol

TG-Triglyceride

VM- Valsalva maneuver

Wt- Weight

^0C-Degree Celsius

ABSTRACT

Background :

Menopause is a normal ageing phenomenon in women. The risk of cardiovascular diseases gradually increases in postmenopausal women, which may be due to lower level of estrogen. Alterations in autonomic nerve functions may occur in menopausal women and it commonly affects cardiac vagal control and usually associated with sympathetic hyperactivity. The hormonal changes associated with menopause exerts significant effect on metabolism of plasma lipids and lipoproteins .A significant number of postmenopausal women suffer from various menopause related complications including autonomic nerve dysfunctions and dyslipidemia .

Objectives of the study:

To study and compare Cardiovascular Autonomic Functions and lipid profile between postmenopausal women and premenopausal women.

Methods: 72 healthy women in the age group of 40-60 yrs who were attending the OPD of Shri B.M. Patil Medical College Bijapur were included in the study. They were divided into premenopausal (control)(n=36) and postmenopausal (study) (n=36) group depending upon attainment of menopause. The anthropometric parameters, physiological parameters, cardiovascular autonomic function tests and serum lipid profile were recorded in control and study groups.

Results: There was statistically significant difference in all the autonomic function tests except blood pressure response to standing between pre and postmenopausal women .There was decrease in parasympathetic functions and increase in sympathetic functions in postmenopausal women. There was a increase in the total autonomic

function score in postmenopausal women compared to premenopausal women .There was statistically significant difference in serum lipid profile parameters between pre and postmenopausal women. There was increase in Total Cholesterol,Triglycerides, LDL levels and decrease in HDL level in postmenopausal women .

Conclusion:

Autonomic function tests showed a decrease in parasympathetic activity and increase in sympathetic activity in postmenopausal women. Serum lipid profile showed a increase in serum total cholesterol, LDL and triglycerides and decrease in cardioprotective HDL in postmenopausal women compared to premenopausal women

Key words: Menopause, Parasympathetic function tests, Sympathetic function tests, autonomic function score, lipid profile.

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INTRODUCTION

Menopause is the permanent cessation of menstruation due to loss of ovarian function which occurs at a median age of 51.4 years. There is gradual transition from the reproductive to non-reproductive phase of life.[1] Menopausal period has an important role in the reproductive life of a woman which is associated with many physical and mental problems. As life expectancy is increasing and age at menopause remains relatively unchanged, women are spending more of their life in the post-menopausal period. Changes in menopause experience for women in different parts of the world and in different ethnic groups provide evidence for specific cultural and ethnic impacts of menopause. As such, healthcare workers need significant information on menopause in order to plan healthcare services. [2, 3]

With the increasing life expectancy, a women spends almost a third of her life in menopause.[4]Menopause is recognized by all women in all cultures as cessation of menstruation for one year. Numerous physical and psychological symptoms have been attributed to the hormonal changes of menopause. This reproductive landmark is not always the same for all women in all cultures. The prevalence of menopausal symptoms varies widely not only among individuals of the same population but also between different ethnic populations. Even there is a great diversity in nature of symptom and frequencies across countries, even in the same cultures .[5,6]

The menopause confounds many traditional cardiovascular disease risk factors (CVRF`s),including changes in body fat distribution from a gynoid to an android pattern ,reduced glucose tolerance, abnormal plasma lipids, increased sympathetic tone, endothelial dysfunction and vascular inflammation. [7]

The portion of the nervous system that regulates most of the visceral functions of the body is called as Autonomic Nervous System.[8] Its main aim is to maintain the optimal internal environment (Homeostasis) of the body. It governs various body functions which are normally carried out without conscious control .[9]

Women, during their childbearing age have reduced risk of cardiovascular diseases than men. However this risk gradually increases in postmenopausal women, which may be due to decreased level of estrogen during this period of life.[10]Moreover, alterations in autonomic function have been observed in postmenopausal women that commonly affect cardiac vagal control. [11] Alteration in autonomic function may lead to hypertension, cardiac arrythmia or sudden cardiac death.[12,13]

The risk of coronary artery disease increases in women after menopause. The hormonal changes associated with menopause such as low estrogen, increased leutenizing hormone and follicular stimulating hormone exerts significant effect on metabolism of plasma lipids and lipoproteins. The effect of hormonal changes associated with menopause on serum lipid levels play an important role in most cardiac disorders .[14]

A significant number of postmenopausal women are a part of the elderly female population in our country. They often suffer from various menopause related complications including autonomic dysfunctions and dyslipidemia. Hence, the present study was undertaken to know more about cardiovascular autonomic functions and serum lipid profile in postmenopausal women.

OBJECTIVES OF THE STUDY

To study and compare Cardiovascular Autonomic Functions and Lipid profile between postmenopausal women and premenopausal women.

REVIEW OF LITERATURE

Menopause is defined retrospectively as the time of the final menstrual period followed by 12 months of amenorrhea. Postmenopause describes the period following the final menses.

Mean age at menopause in Indian women ranges from 40.32 to 48.84yrs and in developed countries from 48.0 to 51 yrs. The age of menopause appears to be genetically determined and is unaffected by race, socioeconomic status, age at menarche or number of prior ovulations. Factors that are toxic to the ovary often result in an early menopause, as do many women exposed to chemotherapy or pelvic radiation. Women who had hysterectomy, despite retention of their ovaries, may also experience early menopause. Premature menopause is defined as menopause before the age of 40 yrs. Occurs in approximately 1% of women.[15] If the menopause fails to occur even beyond 55yrs, it is called delayed menopause. Climacteric is the physiologic period in woman's life during which there is regression of ovarian function. This covers a wide period ranging 5-10years on either side of menopause. Due to increased life expectancy, the clinical importance has increased specially in affluent society as about one-third of life span will be spent during the period of estrogen deprivation stage with long term symptomatic and metabolic complications.[15]

PHYSIOLOGICAL CHANGES DURING MENOPAUSE:

I. Changes in hypothalamo pituitary-ovarian axis: The transition from ovulatory cycles to menopause typically begins in late 40s and in early menopausal transition. Levels of FSH rise slightly and lead to an increased ovarian follicular response with overall higher estrogen levels. There is an increase in serum estrogen levels produced from an increased number of follicles in the stimulated cohort responding to rising FSH levels. Also, during this time ovarian follicles undergo an accelerated rate of loss until eventually in the late menopausal transition, the supply of follicles is depleted. These changes including the increase in FSH levels reflect the reduced quality and capability of aging follicles to secrete inhibin. As follicular depletion continues, episodes of anovulation become more common. With ovarian failure in the menopause, ovarian steroid hormone release ceases and the negative feed-back loop is opened. Subsequently, GnRH is released at maximal frequency and amplitude. As a result, circulating FSH and LH levels rise up to fourfold higher than in reproductive years.

II. Changes in sex steroid level.

Estrogen-There is significant fall in the level of serum oestradiol from 50-300pg/ml before menopause to 10-20pg/ml after menopause. Following menopause, the predominant estrogen is oestrone and to lesser extent oestradiol. Serum level of oestrone is higher than that of oestradiol. The major source of oestrone is peripheral (aromatization) conversion of androgens from adrenals. The aromatization occurs at

the level of muscle and adipose tissue. The trace amount of oestradiol is derived from peripheral conversion of oestrone and androgens.

Androgens. After menopause, stromal cells of ovary continue to produce androgens due to increase in LH. The main androgens are androstenedione and testosterone. They are produced mainly by the adrenal and partly by the ovary.

Progesterone. A trace amount of progesterone detected is probably of adrenal in origin.

III. Ovarian changes: Ovarian senescence is a process that has been shown to actually begin in utero within the embryonic ovary due to programmed oocyte atresia. From birth onwards, primordial follicles are continuously being activated, mature partially and then regress. This follicular activation continues in a constant pattern that is independent of stimulation by pituitary. But, in late reproductive life the regular follicular activation is altered. A more rapid depletion of ovarian follicles starts in the late 30s and early 40s and continues until a point at which the menopausal ovary is virtually devoid of follicles. The process of atresia of the nondominant cohort of follicles, largely independent of menstrual cyclity, is the prime event that leads to the eventual loss of ovarian activity and menopause.

IV. Changes in sex hormone binding globulin level: The principal sex steroids, estradiol and testosterone, circulate in the blood bound to a glycoprotein carrier produced in the liver, known as sex hormone binding globulin (SHBG). Production of SHBG declines after the menopause and may lead to increased levels of free or unbound estrogen and testosterone.[16]

V. Endometrial changes: There is cessation of endometrial proliferation with resulting endometrial atrophy. The endometrium becomes very thin, scanty endometrial glands, some of which are enlarged and cystic, are seen within a depleted stroma.

VI. Lower reproductive tract changes: Estrogen deficiency during postmenopausal period causes vagina to loose collagen, adipose tissue and ability to retain water. As a result, vaginal walls shrink, rugae flatten and the vagina attains a flat walled, pale-pink appearance. The surface epithelium thins to few layers of cells, markedly reducing the ratio of superficial to basal cells. In addition, vaginal pH becomes more alkaline and a pH greater than 4.5 is observed with estrogen deficiency.

VII. Breast changes: The breast undergoes change during menopause mainly because of hormonal withdrawal. At menopause, withdrawal of estrogen and progesterone leads to a relative reduction in the volume and percentage of dense tissue on mammography and these areas become replaced with adipose tissue.

VIII. Dermatological changes: Skin changes include hyperpigmentation, wrinkles and itching. These are caused in part from skin aging which results from the synergistic effects of the intrinsic aging, photoaging and hormonal aging. These changes include a reduced thickness due to reduced collagen content, a decrease in sebaceous gland secretion, loss of elasticity, diminished blood supply and epidermal changes.

IX. Central thermoregulation changes: Vasomotor symptoms, which may be described as hot flushes and night sweats, are most common during menopausal

transition. An individual hot flush generally lasts 1-5min and skin temperature rises because of peripheral vasodilatation. This change is particularly marked in the fingers and toes, where skin temperature can increase 10-15 ^{0}C. Hot flushes are associated with increase in systolic blood pressure. In addition, heart rate increases by 7 to 15 bpm at approximately the same time as peripheral vasodilatation and the metabolic rate also significantly rises. Hot flushes may also be accompanied by palpitation, anxiety, irritability and panic. The pathophysiology of vasomotor symptoms is not clearly understood. Some dysfunction of central thermoregulatory centers in the hypothalamus is likely the cause of this common symptom. Estrogen plays a vital role in the development of hot flushes. Estrogen withdrawal or rapid fluctuation in levels rather than low estrogen concentration is suspected as a cause of hot flushes. The reduction and significant fluctuation in estradiol levels leads to decline in inhibitory presynaptic alpha-2 adrenergic receptors and an increase in hypothalamic norepinephrine and serotonin release. They lower the set point in the thermoregulatory nucleus and allow heat loss mechanisms to be triggered by subtle changes in core body temperature.

X. **Sleep dysfunction and fatigue**: Sleep disruption is a common complaint of women with hot flushes. Women may awake several times during the night and may be drenched in sweat. Disturbed sleep can lead to fatigue, irritability, depressive symptoms, cognitive dysfunction and impairment in daily function.

XI. **Bone metabolism and structural changes**: During menopause, the rate of decline in bone mass increases to 2-5% per year for the first five to ten years and then to 1% per year. The subsequent risk of fractures depends on bone mass at the time of menopause. Primary osteoporosis refers to bone loss associated with aging and

menopausal estrogen deficiency. As its levels fall after menopause, estrogens regulatory effect on bone resorption is lost. As a result, bone resorption is accelerated and is usually not balanced by compensatory bone formation.

XII. Weight gain and fat distribution: Weight gain is common among women in the menopausal transition. Weight gain during this period is associated with fat deposition in the abdomen and around the hip. [16]

IXV. Autonomic changes: A fundamental etiology associated with menopause is an intricate link between estrogen metabolism and autonomic nervous system.[17] Estrogen metabolism is associated with disease protection in women prior to menopause .[18] Also, the presence of estrogen receptors in the heart, vascular smooth muscle and autonomic brain stem centers (e.g. nucleus tractus solitarius, ventrolateral medulla) suggest a possible involvement in the regulation of the cardiovascular system .[19]Research indicates that premenopausal women have higher ANS function than postmenopausal women and that estrogen influences the ANS both centrally and peripherally by suppressing sympathetic tone and elevating parasympathetic tone [20] Higher sympathetic activity has been related to a higher susceptibility to fatal arrhythmias and to the development of coronary heart disease.[21]

Various research evidences suggest that cardiovascular autonomic balance is related to baroreceptor sensitivity and heart rate variability .Overiectomy suppresses baroreflex sensitivity and HRV. It has also been demonstrated that hormone replacement therapy in postmenopausal women increases baroreflex sensitivity and HRV which in turn improves parasympathetic control of the heart .[22]

XIII. Cardiovascular changes: The risk of cardiovascular diseases increases exponentially for women as they enter menopause and estrogen levels decline. Before menopause, women have a much lower risk for cardiovascular events compared with age matched men . Reasons for protection from CVD in premenopausal women are complex, but a significant contribution can be assigned to the greater HDL-C levels in younger women which is an effect of estrogen. However, after menopause this benefit disappears over time and women begin to have a risk identical to that of a male of comparable age. Physiological levels of estrogen maintain favorable lipoprotein profiles in women specifically throughout adulthood. HDL levels are approximately 10mg/dl higher in women and this difference continues throughout the premenopausal period . Total cholesterol and LDL-C levels are lower in premenopausal women than in men. After menopause and with the subsequent decrease in estrogen, this favorable effect on lipid is lost, HDL levels decrease and total cholesterol levels increase.[16]

AUTONOMIC NERVOUS SYSTEM

Anatomical and Physiological aspects of autonomic nervous system

The term autonomic (autonomous – self governing) nervous system was introduced to describe "the system of nerves which controls the unstriated tissues, the cardiac muscles and the glandular tissue of the mammals".[23]

Originally the term was applied only to neurons with axons outside the CNS. More recently, the discovery that discrete neuronal groups in the brain stem diencephalon and the cerebral cortex are involved in the control of autonomic function has broadened the definition of the ANS to include not only peripheral

afferent and efferent pathways but also complex network of neurons within the CNS.[23]

ANS controls most visceral functions of the body. This system helps control arterial pressure, gastrointestinal motility, gastrointestinal secretion, urinary bladder emptying, sweating, body temperature and many other activities some of which are controlled almost entirely and some only partially by the autonomic nervous system. One of the salient features of the ANS is the rapidity and intensity with which it can change visceral functions.[24]

The autonomic nervous system is governed centrally by brain stem centers, Hypothalamus, Cerebellum, Frontal cortex and Limbic system. Hypothalamus is most important. In fact , Sherrington rightly called it as "Head Ganglion of the autonomic nervous system".[25]

Anatomic organization of the autonomic outflow[25]:

The autonomic nervous system like the somatic nervous system is organized on the basis of reflex arc which contains a visceral receptor, an afferent pathway, centre, an efferent pathway and effector organ.

The peripheral motor portions of the ANS are made up of preganglionic and postganglionic neurons. The cell bodies of preganglionic neurons are located in the visceral efferent intermediolateral gray column of the spinal cord or the homologous motor nuclei of the cranial nerves. These axons are mostly myelinated, relatively slowly conducting B fibers. The axons synapse on the cell bodies of the postganglionic neurons that are located in all cases outside the CNS. Each

preganglionic axon diverges on an average of 8-9 postganglionic neurons. The axons of postganglionic neurons mostly unmyelinated C fibers end on the visceral effectors.

Anatomically, the autonomic outflow is divided into 2 components: the sympathetic and parasympathetic divisions of the ANS. In the GIT, both of them communicate with the enteric nervous system and this is sometimes called a third division of the ANS.

Sympathetic division:

The axons of the sympathetic preganglionic neurons leave the spinal cord with the ventral roots of the first thoracic to the third or fourth lumbar spinal nerves. They pass via white rami communicans to the paravertebral sympathetic ganglion chain, where most of them end on the cell bodies of postganglionic neurons. The axons of some postganglionic neurons pass to the viscera in various sympathetic nerves. Others re-enter the spinal nerves via grey rami communicans and are distributed to the autonomic effectors in areas supplied by these spinal nerves. The postganglionic sympathetic nerves to the head originate in the superior, middle and stellate ganglia in the cranial extension of the sympathetic ganglion chain and travel to the effectors with the blood vessels. Some preganglionic neurons pass through the paravertebral ganglion chain and end on postganglionic neurons in the collateral ganglia close to the viscera.

Parasympathetic division:

The cranial outflow of the parasympathetic division supplies the visceral structures in the head via the Oculomotor (III), facial (VII) and Glossopharygeal (IX) and in the thorax and upper abdomen via the Vagus (X) nerves. The sacral outflow

supplies the pelvic viscera via the pelvic branches of the second to the fourth sacral spinal nerves. The preganglionic fibers in both outflows end on short postganglionic neurons located on or near the visceral structures.

Chemical divisions of the ANS:

On the basis of the chemical mediator released, the ANS can be divided into cholinergic and noradrenergic divisions. The neurons that are cholinergic are

1) All preganglionic neurons
2) Anatomically parasympathetic postganglionic neurons
3) The anatomically sympathetic postganglionic neurons which innervate sweat glands.
4) Anatomically sympathetic neurons which end on the blood vessels in skeletal muscles and produce vasodilatation when stimulated.

The remaining postganglionic neurons are noradrenergic.

Functions:

Sympathetic division:- The sympathetic division in addition to sub-serving basic functions as maintenance of blood pressure and body temperature helps the individual to cope up with the emergencies. Sympathetic stimulation leads to relaxation of accommodation and dilatation of the pupils, acceleration of heart beat, increase in blood pressure, increase in blood flow to muscles and decreased blood flow to skin and abdominal viscera, elevated plasma glucose and free fatty acid levels. On the basis of these effects, Cannon called this emergency reaction as ‘preparation for flight or fight’.

Parasympathetic division:- Generally, the functions promoted by this division are those concerned with the vegetative aspects of day to day living,for example digestion and absorption of food. Hence, the cholinergic division is called as anabolic and the sympathetic as catabolic. The various functions of sympathetic and parasympathetic divisions aim at maintaining homeostasis. Research indicates that premenopausal women have higher ANS function than postmenopausal women and that estrogen influences the ANS both centrally and peripherally by suppressing sympathetic tone and elevating parasympathetic tone .[20]

Figure 1: Organization of Autonomic Nervous System

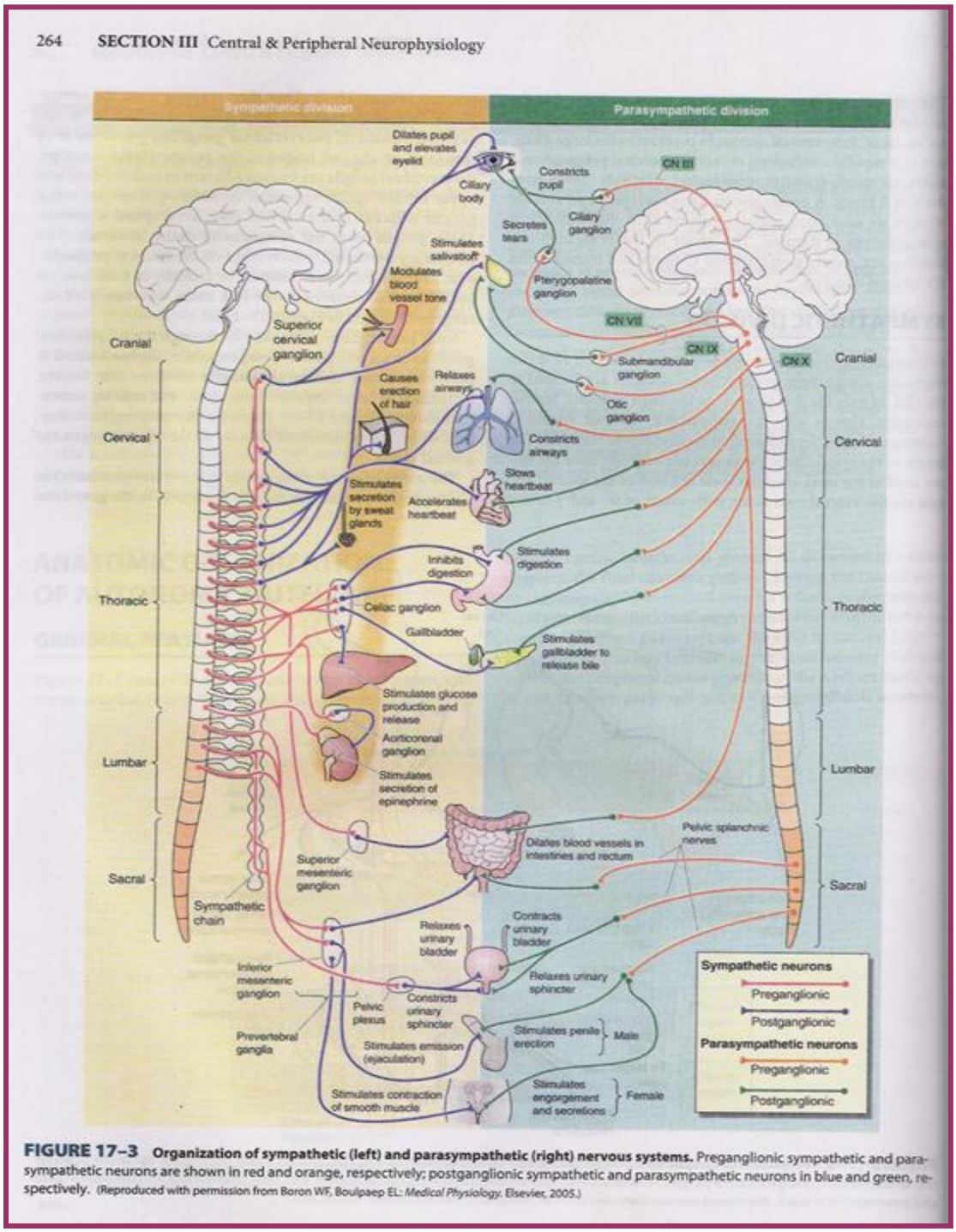


FIGURE 17–3 Organization of sympathetic (left) and parasympathetic (right) nervous systems. Preganglionic sympathetic and parasympathetic neurons are shown in red and orange, respectively; postganglionic sympathetic and parasympathetic neurons in blue and green, respectively. (Reproduced with permission from Boron WF, Boulpaep EL: *Medical Physiology*. Elsevier, 2005.)

AUTONOMIC INNERVATION TO THE CARDIOVASCULAR SYSTEM[23]

The heart receives parasympathetic and sympathetic innervations. The cell bodies of parasympathetic preganglionic neurons innervating the heart are located in the medulla (Nucleus Ambigus and Dorsal Motor Nucleus of the Vagus). The axons of these neurons which are part of vagus nerve join the cardiac neural plexus after entering the thorax to synapse with the neurons in the intracardiac ganglia. From these ganglia, short postganglionic parasympathetic neurons emerge to innervate myocardial tissue. Vagal innervations are particularly abundant in the nodes and the atrioventricular conducting system.

The heart draws its sympathetic innervation from the neurons in the intermediolateral columns of the spinal cord at the T_1- T_4 levels. Axons of these neurons synapse in the superior, middle and inferior (stellate) cervical ganglia which are the origin of postganglionic sympathetic neurons. Sympathetic axons innervate the sinoatrial (SA) and atrioventricular (AV) nodes, the conducting system and myocardial fibers, most prominently in the ventricles. The arteries and veins of the systemic circulation are innervated primarily by the sympathetic system.

Postganglionic parasympathetic neurons release ACh which activates muscarinic receptors in the heart, causing a decrease in heart rate as well as reductions in the conduction, excitability and contractility of myocardial cells. Postganglionic sympathetic axons release norepinephrine which primarily via β-adrenergic receptors increases the heart rate and conduction, excitability and contractility of the myocardium. Cardiac vagal neurons are activated through the baroreflex when arterial pressure increases and are inhibited during respiration. Because ACh acts quickly and

is rapidly inactivated by cholinesterase, Vagus controls the heart rate on a beat-to-beat basis.

Sympathetic outflow to the arteries, arterioles and veins of the peripheral circulation produces vasoconstriction by activating alpha adrenergic receptors. The presence of vasodilator cholinergic sympathetic fibers to skeletal muscle of humans is debatable.

Autonomic outflow to the heart and blood vessels is controlled on a moment to moment basis by a variety of reflexes, which are initiated by arterial baroreceptors , chemoreceptors and by several types of cardiac receptors. Of these reflexes, one of the best studied is the arterial baroreflex, which is a classic negative feedback mechanism that buffers fluctuations in the arterial blood pressure.

TESTS FOR CARDIOVASCULAR REFLEXES

Autonomic function tests

It is imperative that any test for autonomic function has to be simple, noninvasive, and reliable and should be able to demarcate clearly normal and abnormal. The physiological basis of the test should be clearly understood. These criteria are fulfilled by tests based on cardiovascular reflexes which measure the heart rate, systolic and or diastolic blood pressure responses to a number of simple maneuvers. The cardiovascular tests: The Valsalva maneuver[26,27,28], Heart rate response during periods of deep breathing[29], The Heart rate response to standing up[30,31] and Blood pressure response to standing[30] , the diastolic blood pressure response to sustained hand grip[32,33], have been employed in the present study to assess autonomic function. The

first three tests evaluate the cardiac parasympathetic while the latter two tests show an altered response when there is sympathetic imbalance.

Physiological basis of the study tests:

1. Valsalva Maneuver Nearly three centuries ago ,Antonio Maria Valsalva recommended forced expiration against a closed glottis for expelling pus from an infected middle ear. The procedure known thereafter as Valsalva maneuver has been widely used in the treatment of Eustachian tube obstruction. Weber (1851) found that the Valsalva maneuver caused changes in the pulse volume. Flack (1920) modified the maneuver by having subjects blow against a column of mercury than against closed glottis.[34,35] Results by earlier workers showed that more valid assessment of Valsalva response can be made when performed in the supine or sitting position. Sitting position for the Valsalva maneuver test is used in the present study.

During Valsalva maneuver in a normal individual, one can recognize four phases of change in haemodynamics.[27]

Phase I: At the onset of expiratory strain (forced expiration against closed glottis),a sudden increase in the intrathoracic pressure is transmitted to all the vessels within the thorax including aorta and its branches producing an abrupt rise in arterial systolic and diastolic pressures.

Phase II: As the increased intrathoracic pressure impedes venous return to the right atrium, there is a progressive reduction in the left ventricular stroke volume accompanied by a fall in arterial pressure. The diminished pulse pressure acting through baroreceptors reflexly stimulates increased sympathetic activity which manifests as tachycardia and peripheral vasoconstriction.

Phase III: Upon release of the strain, there is an abrupt increase in venous return as well as in the capacity of the pulmonary vascular bed. For several beats following the release of the strain, there may be a further diminution in the arterial pressure due to transient pooling of the right ventricular output in the expanded pulmonary vascular bed.

Phase IV: When the augmented venous return reaches the left ventricle, there is a progressive increase in left ventricular stroke volume which is ejected into constricted systemic vasculature, the arterial pressure rises, exceeding the control level "Over shoot". Finally, rising pulse pressure stimulates vagal activity which manifests as bradycardia.

In the Valsalva maneuver, forced expiration against resistance (expiratory strain) causes complex reflex circulatory changes mediated by both parasympathetic and sympathetic pathways. While straining, the heart rate rises. After release of strain, heart rate slows. With autonomic damage, the expected sympatho-adrenal discharge fails to occur and thus there may or may not be a change in the heart rate. The occurrence of requisite changes in the intrathoracic pressure with this procedure, integrity of the autonomic pathway and responding end organ are essential to obtain the correct response. The heart rate changes are unaffected by sympathectomy, indicating that the baroreceptors and the vagi mediate these changes. The blood pressure falls gradually during the maneuver and does not overshoot.

2. Heart rate variation (R-R interval) during deep breathing

The clinical relevance of HRV was first appreciated in 1965 when Hon and Lee noted that fetal distress was preceded by alterations in interbeat intervals before

any appreciable change occurred in heart rate itself. Twenty years ago, Sayers and others focused attention on the existence of physiological rhythms embedded in the beat-to-beat heart rate signal. During the 1970s, Ewing et al devised a number of simple bedside tests of short-term RR differences to detect autonomic neuropathy in diabetic patients. The association of higher risk of post infarction mortality with reduced HRV was first shown by Wolf et al in 1977. In 1981, Akselrod et al introduced power spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control. The clinical importance of HRV was appreciated in the late 1980s, when it was confirmed that HRV was a strong and independent predictor of mortality after an acute myocardial infarction.[36]

The heart rate varies with the phases of respiration, accelerating during inspiration and decelerating during expiration. These changes are marked in younger individuals and during deep breathing and are abolished by atropine showing the involvement of parasympathetic nerves. The impulses from stretch receptors in the lungs are relayed via afferent fibers in the vagi and inhibit the cardio-inhibitory centre in the medulla oblongata during inspiration. The tonic vagal discharge which keeps the heart rate slow decreases, thereby increasing the heart rate. There will also be withdrawal of inhibition of the cardio-inhibitory centre and excitation of vasomotor centre contributing to the acceleration of heart rate during inspiration.[25]

Respiratory sinus arrhythmia is explained by four basic mechanisms,

1. Alteration in autonomic activity: During inspiration, sympathetic discharge increases and during expiration vagal activity increases.

2.Activation of Bainbridge reflex: During inspiration, increased venous return to right atrium increases heart rate. The decrease in intrathoracic pressure during inspiration increases right atrial filling and stretches the right atrium .Thus, atrial tachycardia producing receptors are activated that produces tachycardia. Right atrial stretching also stretches SA node which causes tachycardia.

3. Irradiation from inspiratory center: Increased irradiation from inspiratory center to the vasomotor center during inspiration increases the heart rate.

4. Activation of atrial stretch reflex: Increased venous return during inspiration stimulates type B atrial stretch receptors and this increases the heart rate.[37]

In presence of autonomic imbalance or autonomic neuropathy, the change in heart rate during respiration is impaired or even absent.[38] The heart rate may be fixed in patients with severe autonomic damage involving both sympathetic and parasympathetic innervations of heart.

For practical purposes, the test can be best performed breathing deeply at six breathes per minute and measuring the difference between maximum and minimum heart rates over a period of one minute.[29]

3. Postural stress tests:

(Heart rate and blood pressure response to standing from lying down position)

Immediate heart rate and blood pressure response to standing is used as a postural stress test. Postural stress tests are useful in assessment of cardiovascular reflex responses of normal subjects who may be involved in specialized occupations.[39]

The maintenance of normal blood pressure and limitation of peripheral blood pooling is a function of cardiovascular reflexes.

Postural stress reduces intra thoracic volume and shifts to legs where it forms a pool, thereby producing fall of blood pressure and reduction in the circulating blood volume.[40] Compensation to this is brought by - 1) Reflex tachycardia 2) Arteriolar constriction – aimed at maintenance of arterial blood pressure 3) Vasoconstriction- helps to limit expansion of blood pool in legs, keeps up the preload and is helped up by the increased activity of abdomino-thoracic respiratory pump, the pumping action of calf muscles and the venous valves.[41,42]

The change of posture (lying down to standing) – displaces about 600 ml of blood from thorax to the legs.[43] This deactivates baroreceptors to produce a reflex tachycardia which is mediated mostly by vagal tone withdrawal.[38] Displacement of blood produces light fall in systolic blood pressure and a slight increase in diastolic blood pressure. The immediate heart rate increase on standing in healthy young subjects is seen by about 15^{th} beat and this later settles down to a steady state value at about 30^{th} beat which is 10-15 beats per minute higher than in supine position. In patients with autonomic neuropathy, the tachycardia response is markedly attenuated and may be accompanied by postural hypotension. If the 30:15 ratio is used, then the counting of beats should start when the subject starts to stand and not when he has finished the movement of standing up. The beats are counted when the subject starts to stand up in the present study.

D.J. Ewing, L. Hume et al in their study on heart rate response to standing analyzed their results by the use of time after standing rather than beats after standing. But they were of opinion that counting time had no advantage and was more laborious

to calculate. The maximum responses occured around 15th and the 30th beats, and the shortest and longest R-R intervals around these beats should therefore be measured.

D.J. Ewing, L. Hume, I.W.Campbell et al have studied cardiovascular reflex responses during standing up on pharmacological basis and confirmed the above mechanism.[44]

In the present study, Heart rate and Blood pressure changes on quiet standing from lying position was selected because of the simplicity of the test. To find postural fall in blood pressure, cuff was inflated above systolic blood pressure before subject stood up. Immediate heart rate response to standing (30:15 ratio) was used to study the parasympathetic pathway sensitivity and systolic blood pressure response to study the sympathetic sensitivity. Values of 1.04 or more were taken as normal for heart rate response and fall of 10 mm Hg or less for systolic blood pressure response to standing (D.J.Ewing and B.F.Clarke).[29]

Blood pressure response to sustained handgrip

Sustained handgrip is a type of isometric exercise. With the start of an isometric muscle contraction, heart rate is increased. This is largely due to a reduction in vagal tone, although increased discharge in cardiac sympathetic fibers also plays a role. Shortly thereafter, the systolic and diastolic blood pressure also rises sharply. This is brought about by increased peripheral resistance. The increase in peripheral resistance results from vasoconstriction in the inactive muscles produced by sympathetic outflow. The rise in blood pressure will be abnormally small if there is extensive peripheral sympathetic abnormality.[29] Dwain L. Eckberg and B. Gunnar

Wallin in their study have found that there was an increase in heart rate and arterial pressure with the onset of isometric exercise (Brief handgrip – 30% of maximum). Their results suggested that exercise modifies, in small but significant ways, early sympathetic and vagal responses to abrupt changes of arterial baroreceptor input in humans.[45]

Many workers have studied the effect of different types of isometric exercise on blood pressure and heart rate. Most of the workers were of the opinion that irrespective of the muscle mass involved in isometric exercise there is a pressure response of the same magnitude but the pressure depends upon maximal voluntary contraction.

LIPIDS AND LIPOPROTEINS:

Lipids are a heterogeneous group of compounds related, either actually or potentially to the fatty acids. They have the common property of being 1) Relatively insoluble in water 2) Soluble in nonpolar solvents such as ether, chloroform and benzene.

I. Bloor's modified classification of lipids.

A. Simple lipids: Esters of fatty acids with various alcohols. Eg. Fats, waxes

B. Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

1) Phospholipids-Eg. glycerophospholipids
2) Glycolipids

3) Sulfolipids

4) Amino lipids

C. Derived lipids: These include fatty acids, glycerol, steroids other alcohols, fatty aldehydes, and ketone bodies.[46]

II. Lipids can also be classified as

1) Cellular lipids.

2) Brown fat.

3) Plasma lipids.

1) Cellular lipids:

a) Structural lipids-Inherent part of the membranes and other parts of cell.

b) Neutral fat-Stored in fat depots as adipose tissue.

2) Brown fat: It makes up a small percentage of total body fat. It is abundant in infants but is present in adults as well, is located between the scapulae, at the nape of the neck along the great vessels in the thorax and abdomen.[25]

3) Plasma lipids: Average concentration of plasma lipids are as follows.

Free fatty acids-15mg/dl

Cholesterol-180mg/dl

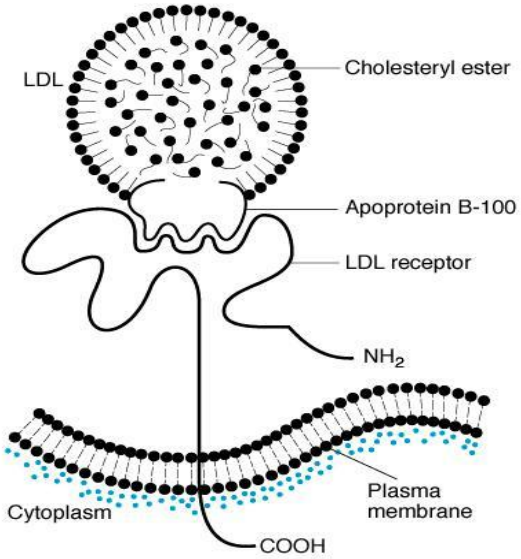
Triglyceride-160mg/dl

Phospholipid-160mg/dl[8]

The major plasma lipids are relatively insoluble in aqueous solution and do not circulate in the free form. Free fatty acids are bound to albumin, whereas cholesterol, triglycerides and phospholipids are transported in the form of lipoprotein complexes.

Lipoprotein complexes- In general the lipoprotein consists of hydrophobic core of triglyceride and cholesterol esters surrounded by phospholipids and protein.

Fig 2: Structure of lipoprotein:



The protein constituents of lipoproteins are called apoproteins. The major apoproteins are APO-A, APO-B, APO-C and APO-E with their subgroups. There are six families of lipoproteins graded in size and lipid content and relative densities.[25]

Composition of lipoproteins in human plasma:[46]

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylo-microns					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Transport of lipids:

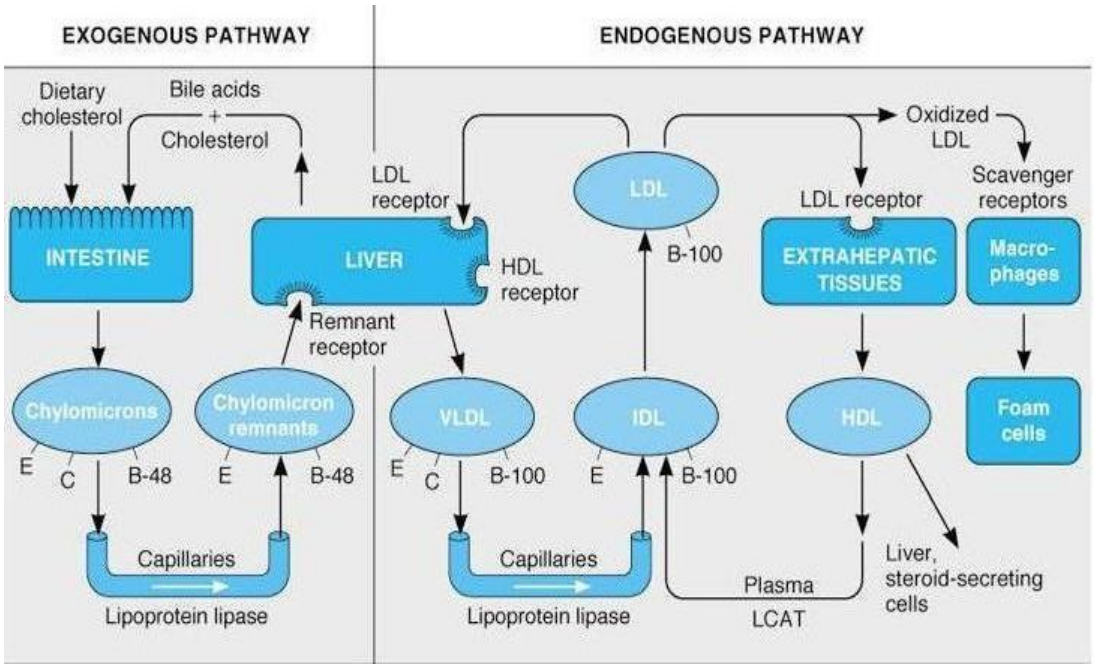
1) **Exogenous pathway.**
2) **Endogenous pathway.**
3) **Reverse cholesterol transport.**

1) Exogenous pathway: This pathway transports lipids from the intestine to the liver. The chylomicrons and their remnants constitute transport system for ingested exogenous lipids. Chylomicrons are formed in the intestinal mucosa during the absorption of the products of fat digestion. They are very large lipoprotein complexes that enter the circulation via lymphatic ducts. The chylomicrons are cleared from the circulation by the action of lipoprotein lipase. This enzyme catalyzes the breakdown of the triglyceride in the chylomicrons to FFA and glycerol, which then enter adipose cells and are re-esterified. Alternatively, the FFA remain in the circulation bound to albumin. Chylomicrons which are depleted of their triglyceride remain in the circulation as cholesterol rich lipoproteins called chylomicron remnants which are 30-80nm in diameter. The remnants are carried to the liver, where they bind to

chylomicron remnant and LDL receptors. They are immediately internalized by receptor mediated endocytosis and are degraded in lysosomes.

2) Endogenous pathway: This pathway transports lipids to and from the tissues. An endogenous system made up of VLDL, IDL, LDL and HDL. This system also transports triglyceride and cholesterol throughout the body. VLDL is formed in the liver and transports TG formed from fatty acids and carbohydrate in the liver to extra hepatic tissues**.** After their TG is largely removed by the action of lipoprotein lipase they become IDL. The IDL give up phospholipids and, through the action of the plasma enzyme lecithin-cholesterol acyltransferase (LCAT) pick up cholesteryl esters formed from cholesterol in HDL. Some IDL are taken up by the liver. The remaining IDL then lose more TG and protein probably in the sinusoids of the liver, and become LDL. During this conversion, they lose APO-E but APO-B100 remains. LDL provides cholesterol to the tissues. The cholesterol is an essential constituent in cell membranes and is used by gland cells to make steroid hormones. In the extra hepatic tissues and liver, LDL is also taken up by a lower affinity system in the macrophages and some other cells. The macrophages preferentially take up LDL that has been modified by oxidation. The receptors on macrophages and related cells are called the Scavenger Receptor. When the macrophages become overloaded with oxidized LDL, they become the "foam cells" that are seen in early atherosclerotic lesion.[25]

Fig 3: Transport of lipids [25]

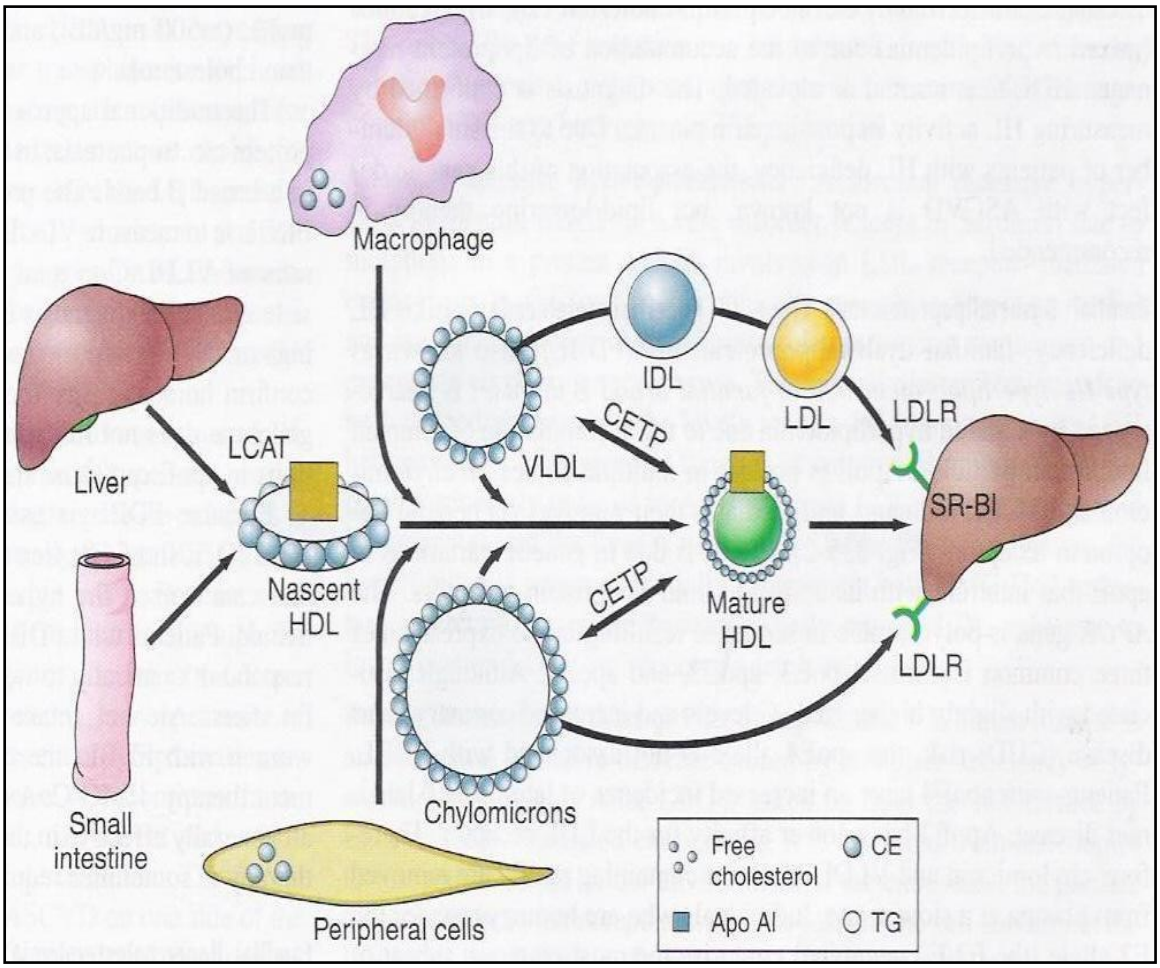


3) Reverse cholesterol transport. Cholesterol in peripheral cells is transported from the plasma membranes of peripheral cells to the liver by an HDL-mediated process termed reverse cholesterol transport.

The nascent HDL particles are synthesized in the intestine and liver. The newly formed discoidal HDL particles contain APO-A1 and phospholipids but rapidly acquire unesterified cholesterol and additional phospholipids from peripheral tissues via transport by the membrane protein ATP-binding cassette protein A1. Once incorporated into the HDL particle, cholesterol is esterified by lecithin cholesterol acyltransferase, a plasma enzyme associated with HDL. As HDL acquires more cholesterol esters it becomes spherical and additional apolipoproteins and lipids are transferred to the particles from the surfaces of chylomicrons and VLDL during lipolysis.

HDL cholesterol is transported to hepatocytes by both an indirect and direct pathway. In indirect pathway, HDL cholesteryl esters are transferred to APO-B containing lipoproteins in exchange for TG by the cholesteryl ester transfer protein (CETP). The cholesteryl esters are then removed from circulation by LDL receptor-mediated endocytosis. In direct pathway, HDL cholesterol is taken up directly by hepatocytes via the scavenger receptor class B1.

Fig 4: REVERSE CHOLESTEROL TRANSPORT:



Classification of LDL, TC and HDL-C as given by National Cholesterol Education Programme (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults.[47]

Classification of LDL, Total, and HDL cholesterol(mg/dl)	
LDL cholesterol	
<100	Optimal
100-129	Near or above normal
130-159	Borderline High
160-189	High
>190	Very high
Total cholesterol	
<200	Desirable
200-239	Borderline high
>240	High
HDL-cholesterol	
<40	Low
>60	High

Cholesterol metabolism:

Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. A little more than half the cholesterol of the body arises by synthesis and remainder is provided by the average diet. The liver and intestine account for approximately 10% each of total synthesis in humans.

Cholesterol synthesis: All tissues containing nucleated cells are capable of cholesterol synthesis which occurs in the endoplasmic reticulum and cytosol. It is synthesized from acetyl CoA obtained from catabolism of glucose or fatty acid.

Biosynthesis of cholesterol may be divided into 5 steps.

I. Synthesis of Mevalonate from Acetyl CoA: Two molecules of Acetyl CoA condense to form Acetoacetyl-CoA catalyzed by thiolase. Acetoacetyl-CoA condenses with further molecule of acetyl CoA catalyzed by HMG- CoA synthase to form HMG- CoA which is reduced to Mevalonate by NADPH catalyzed by reductase.

II. Formation of isoprenoid units: Mevalonate is phosphorylated sequentially by ATP by three kinases and after decarboxylation the active isoprenoid unit, isopentanyl diphosphate is formed.

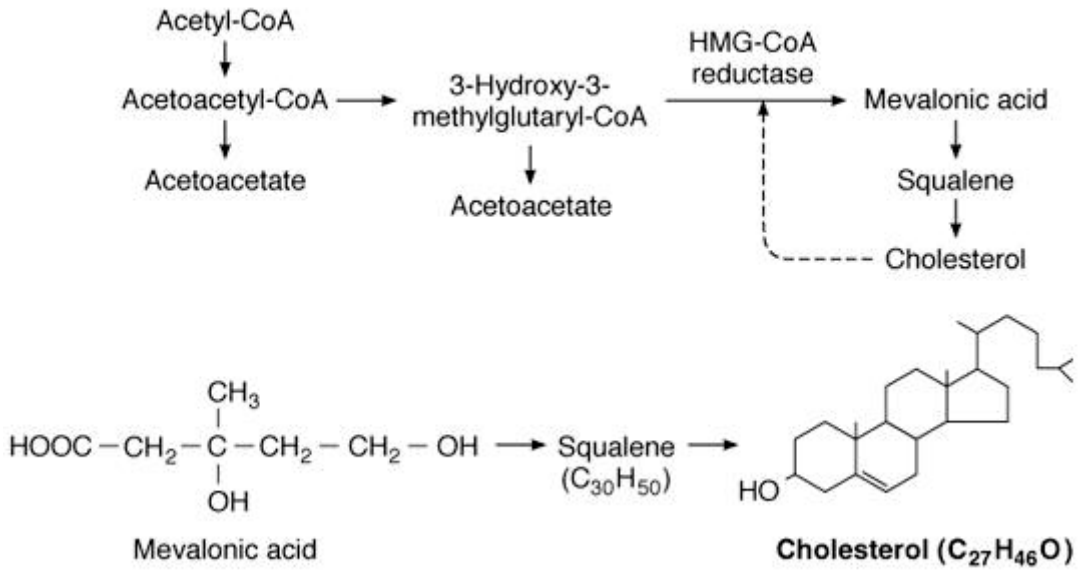
III. Form of squalene:Isopentanyl diphosphate is isomerized by a shift of the double bond to form dimethylallyl diphosphate, and then condenses with another molecule of isopentanyl diphosphate to form the ten carbon intermediate geranyl diphosphate. A further condensation with isopentanyl diphosphate forms fernasyl

diphosphate. Two molecules of fernasyl diphosphate condenses at diphosphate end to form Squalene[6 isoprenoid units].

IV. Formation of lanosterol: Squalene can fold into a structure that closely resembles steroid nucleus. Before ring closure occurs, it is converted into squalene 2,3-epoxide by a mixed function oxidase called squalene epoxidase. The squalene 2,3-epoxide is converted to oxidosqualene by lanosterol cyclase. It occurs in endoplasmic reticulum.

V. Formation of Cholesterol: This takes place in the membranes of the endoplasmic reticulum. It involves changes in the steroid nucleus and side chain. The lanosterol is converted to desmosterol, which is then converted to cholesterol by reducing the double bond of side chain. [46]

SYNTHESIS OF CHOLESTEROL:



Absorption, transport and excretion of cholesterol:

Cholesterol is readily absorbed from the small intestine if bile, fatty acids, and pancreatic juice are present. With cholesterol synthesized in the intestine, the absorbed cholesterol is incorporated into chylomicrons that enter the circulation via lymphatics. 95% percent of the chylomicron cholesterol is delivered to the liver in Chylomicron remnants, and most of the cholesterol secreted by the liver in VLDL is retained during the formation of IDL and ultimately LDL which is taken up by the LDL receptor in liver and extra hepatic tissues.

About 1gm of cholesterol is eliminated from the body per day. Approximately half is excreted in the feces after conversion to bile acids. The remainder is excreted as cholesterol.[25]

Biomedical importance:

1**)** It is an essential structural component of membranes and of the outer layer of lipoproteins.

2) It is precursor of all other steroids in the body like corticosteroids, sex hormones, bile acids and vit D.

3) It is a major constituent of gallstones.

4) It has a chief role in pathologic process of atherosclerosis of vital arteries, causing cerebrovascular, coronary and peripheral vascular diseases.[46]

Factors affecting cholesterol level:

1. Age: As age advances, cholesterol level in blood also increases.
2. Sex: Males have higher blood cholesterol level than females.

3. Menopause: Menopausal women have higher level of cholesterol than premenopausal women
4. Diet: Vegetarian diet contains mainly polyunsaturated fatty acid which lowers blood cholesterol levels. Non vegetarian diet contains high proportion of saturated fatty acids which in turn will cause increase in blood cholesterol.
5. Obesity: It is associated with increased cholesterol level.
6. Smoking: It increases total cholesterol level.
7. Hormones: The plasma LDL-C is decreased by thyroid hormones and estrogens, both of which increase the number of LDL receptors in the liver. HDL-C is increased by estrogen.
8. Plasma cholesterol is elevated by biliary obstruction and in untreated diabetes mellitus.
9. Drugs lowering plasma cholesterol
    a) Resins like colestipol
    b) Vitamin niacin
    c) Statins like lovastatin[25]

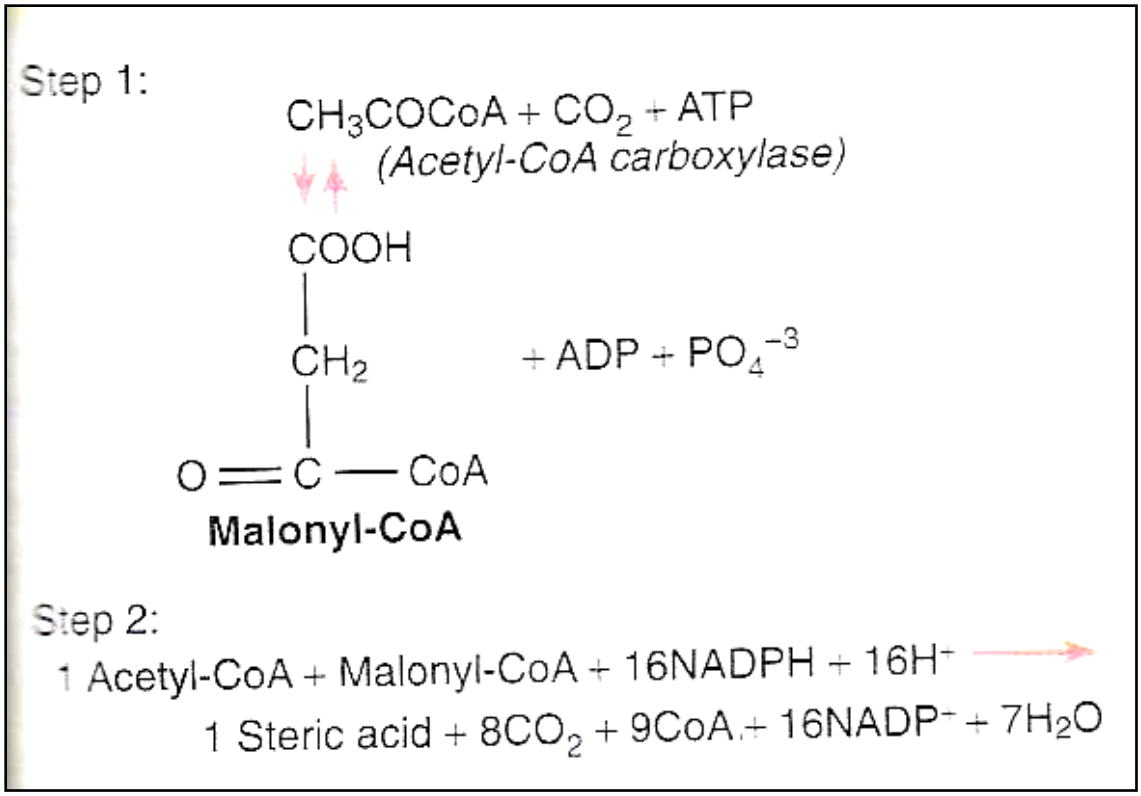
Triglyceride metabolism:

In human beings, most TG synthesis occurs in the liver but minute quantities are also synthesized in the adipose tissue. TG synthesized in liver are transported mainly in VLDL to the adipose tissue where they are stored.

I. Synthesis of acetyl co A into fatty acids:

The first step in the synthesis of TG is conversion of carbohydrates into acetyl – CoA during the normal degradation of glucose by glycolytic system.

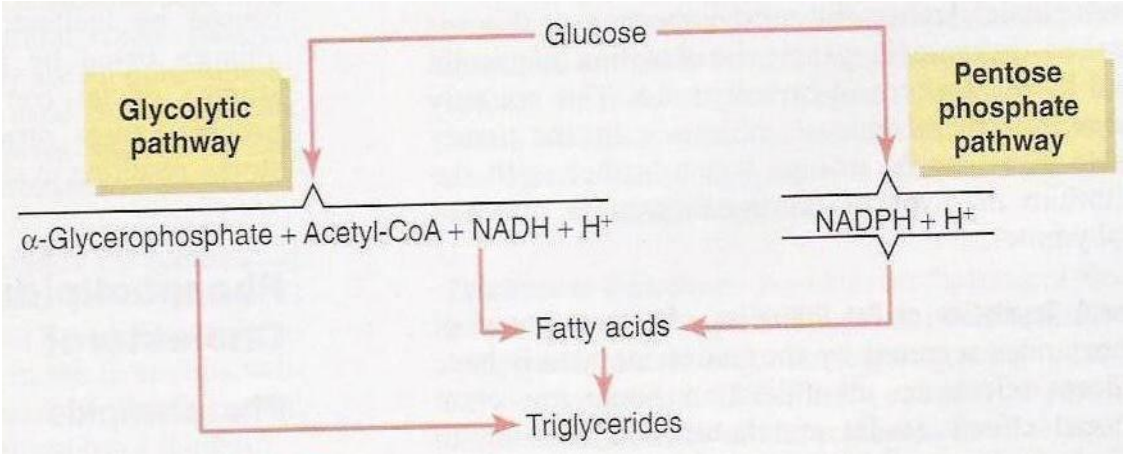
Steps in synthesis of fatty acid:



II. Combination of fatty acids with glycerophosphate to form TG

Fatty acid+glycerol ------Triglyceride

Synthesis of triglyceride:



Synthesis of TG from proteins: Many amino acids can be converted into acetyl CoA and then acetyl CoA can be converted into TG.

Dietary TG: The most abundant fats of the diet are neutral fats (triglycerides). It is a major constituent in food of animal origin but, much less so in food of plant origin. Most of the TG of the diet is split by lipase into free fatty acid and 2-monoglyceride in the intestine. The free fatty acid and monoglycerides are absorbed by intestinal epithelial cells which are taken by cell's smooth endoplasmic reticulum. Here they are mainly used to form new TG that are subsequently released in the form of chylomicrons through base of the epithelial cell to flow upward through the thoracic lymph duct and empty into the circulating blood. [48]

Following studies have been done on Autonomic functions tests in postmenopausal women

In a study conducted by Shailaja S on 70 subjects, it was found that systolic blood pressure in premenopausal women was comparatively less than postmenopausal women.They also found that diastolic blood pressure in premenopausal women was less compared to postmenopausal women. In their study they also found heart rate in premenopausal women to be lower than postmenopausal women. Using heart rate variability test they found increased sympathovagal balance in postmenopausal women compared to premenopausal women .[49]

Shikha G conducted a study on 90 adult women.They were divided into three groups, group I premenopausal, Group 1A In proliferative phase of menstrual cycle Group 1B In secretory phase of menstrual cycle group II - Postmenopausal women who had not yet been put on HRT and group III - Postmenopausal women who were on oral HRT.

They found that postmenopausal women had alteration in their autonomic status with higher sympathetic and lower vagal tone compared to premenopausal women. In women on HRT, the sympathovagal balance was shifted towards parasympathetic dominance. Across the menstrual cycle, higher parasympathetic activity was seen in the secretory phase while no change was observed in the sympathetic activity in the two phases. [19]

A study was conducted by Latifa N on 60 subjects in Dhaka, it was found that systolic blood pressure and diastolic blood pressure in postmenopausal women were high compared to premenopausal women. They also found that there was more fall in systolic blood pressure in standing position in postmenopausal women compared to premenopausal women.The diastolic blood pressure response to handgrip test was high in postmenopausal women compared to premenopausal women.[50]

Naher LD studied on 30 apparently healthy postmenopausal women aged 45 to 60 years(study) (Group B) and 30 healthy premenopausal women with age ranged from 20 to 30 years(control)(Group A).They carried out parasympathetic function tests like heart rate response to valsalva maneuver, heart rate response to deep breathing , heart rate response to standing. They concluded the parasympathetic cardiac autonomic nerve function was lower after menopause which was associated with estrogen deficiency.[22]

A study was done on 27 subjects in Brazil by Neves FC on Autonomic modulation of heart rate of young and postmenopausal women undergoing estrogen therapy. They found that heart rate in supine position and sitting position in premenopausal women to be higher than postmenopausal women.[10]

Lathadevi GV and co workers conducted a study on 60 normal women volunteers which included perimenopausal and postmenopausal. The parasympathetic function tests included the standing to lying ratio (S/L ratio), the 30:15 ratio and the valsalva ratio. The sympathetic function tests like the blood pressure response to the isometric handgrip (IHG) and the blood pressure response to standing (BPS) were done. The parasympathetic function tests were reduced to below the normal values. The sympathetic activity was increased in both the groups, indicating an increased risk of cardiovascular disease.[51]

Following studies have been done on lipid profile in postmenopausal women

A study was conducted by Igweh JC on 126 subjects in Nigeria (74 premenopausal and 52 postmenopausal) . Serum total cholesterol and their subfractions- high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG) were estimated using enzymatic and established mathematical methods. There was no significant difference in the total serum cholesterol and triglyceride between the two groups. There was however, a significant reduction of HDL in the postmenopausal group ($P<0.005$) and a significant increase in the level of LDL in the postmenopausal group ($P<0.005$).[52]

Bitoska I and others conducted a study on 70 subjects in Macedonia which included 46 postmenopausal women and 24 healthy premenopausal women. They found significant difference in total cholesterol levels (0,0034; $p<0,05$), as well as in LDL-C ($p= 0,0021; p<0,05$)) and ApoB ($p=0,027; p<0,05$) among healthy premenopausal and postmenopausal women and there was no statistically significant difference in TG level ($p=0,067; p>0,0$)), HDL-c ($p=0,623; p>0,05$) and apoA1($p=0,196; p>0,05$).

Similar results with no significant difference in the triglyceride level were obtained as far as the Apo B/A1 ratio is concerned (p= 0,069; p>0, 05). [53]

Arunima C and co workers carried out a study on heart rate variablity tests and lipid profile in 60 postmenopausal women. The heart rate variability tests showed significant decrease in values of Valsalva ratio, deep breath test, and 30:15 R-R interval ratio in subjects having abnormal autonomic functions. Results of lipid profile showed significant increase in values of total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), cholesterol/high density lipoprotein (HDL) ratio and insignificant decrease in HDL values in subjects having abnormal autonomic functions.[1]

The above reviews reveal that there is alteration in Cardiovascular Autonomic functions, and serum lipid profile in the postmenopausal women.

MATERIALS AND METHODS

Source of Data

The study was conducted in apparently healthy premenopausal and postmenopausal women attending BLDEU`s Shri B. M .Patil Medical College, Hospital and Research Centre Bijapur.

Method of collection of Data

Subjects: Apparently healthy women of 40-60 years of age attending BLDEU'S Shri B.M.Patil Medical College hospital and research centre Bijapur were randomly selected for the study. They were divided into premenopausal (control) and postmenopausal (study) group depending upon attainment of menopause.

Duration of the study: November 2010 to April 2012.

Sample size: Mean number of menopausal symptoms was 6.70 and standard deviation was 5.76.[7] The calculated sample size when allowable error L= ± 2 was

$$n = \frac{4\sigma^2}{L^2}$$

$$= \frac{4(5.76)^2}{(\pm 2)^2} \cong 36$$

Hence a total of 72 subjects including 36 study and 36 controls were taken for the study.

As all the distributions will merge into normal distribution, sample size i.e., 30 is enough because inference that can be drawn based on 30 observations will more or less remain the same inspite of any increase in the sample size.[54]

INCLUSION CRITERIA

Only healthy subjects were included in the study. The apparent health status of each subject was determined through thorough clinical examination and history taking.

EXCLUSION CRITERIA

The following subjects were excluded from the study.

1. Subjects with history of alcohol intake.
2. Subjects with history of tobacco consumption in any form.
3. Subjects with history or clinical signs of structural cardiovascular diseases.
4. Subjects receiving drugs known to affect Cardiovascular Autonomic Function and serum lipid profile.
5. Subjects with history of Diabetes Mellitus and Hypertension.

The following parameters were recorded in the subjects:

1. Recording of Physical Anthropometry of subjects

a) Height (in centimeters). This was measured with subject standing without foot wears nearest to 0.1 centimeter.
b) Weight (in kilograms): The subjects were weighed by standardized machine with the minimum clothing nearest to 0.1 kilogram.

c) Body Surface Area (square meters): This was calculated by using Dubois nomogram.

d) Body Mass Index (kilogram/meter^2): This was calculated for each subject from weight in kgs and height in meters by using Quetlet index.

Recording of Physiological Parameters:

In each subject following physiological parameters were recorded.

a) Respiratory rate (cycles/minute)

b) Heart rate (Beats/minute)

c) Systolic and Diastolic blood pressure (mm of Hg) by using mercury sphygmomanometer.

Recording of Autonomic Function Parameters

The Autonomic Nervous Function Parameters were selected as recommended by American Diabetic Association and performed as per methods described by Sir Roger Bannister.[42]

A) The Parasympathetic activity was assessed by:

1) Heart Rate response to Valsalva Maneuver

2) Heart rate response to deep breathing

3) Immediate heart rate response to standing

1. Heart rate response to Valsalva maneuver:

The subject was asked to sit comfortably. A nose clip was applied and subject was asked to blow through the mouth piece attached to the mercury manometer for 15 seconds maintaining a pressure of 40 mm Hg. A small air leakage in the mouth piece was done to ensure that the subject does not to blow with his cheeks (open glottis method). Throughout the maneuver ECG was recorded continuously and for 30 seconds after release of strain.

Heart rate response to Valsalva maneuver was expressed as:

$$\text{Valsalva ratio} = \frac{\text{Longest R-R interval after the maneuver}}{\text{Shortest R-R interval during the maneuver}}$$

2. Immediate heart rate response to standing (30:15 ratio):

The subject rested in supine position for 5 minutes after which he was asked to stand up unaided within 5 seconds and to remain standing for 1 minute. Continuous ECG recording was done starting from 1^{st} beat after standing up to the 60^{th} beat. The shortest R-R interval at or around the 15^{th} beat and the longest R-R interval at or around the 30^{th} beat after starting to stand were measured with a ruler. The heart rate response was expressed by the 30:15 ratio. The result was expressed as:

$$\text{Max/min} = \frac{\text{Longest R-R interval around } 30^{th} \text{ beat}}{\text{Shortest R-R interval around } 15^{th} \text{ beat}}$$

3. Heart rate response during deep breathing:

The subject was seated quietly for 1 minute and after a verbal command subject was instructed to breathe deeply and continuously at a rate of 6 breaths/min (5seconds inspiration and 5 seconds expiration) as trained before. ECG was recorded continuously for one minute. The heart rate ratio during deep breathing was expressed as:

$$\text{E/I ratio} = \frac{\text{Mean of longest R-R intervals during each expiration.}}{\text{Mean of shortest R-R intervals during each inspiration}}$$

B. **The sympathetic activity was assessed by:**

1. Blood pressure response to standing

2. Blood pressure response to sustained handgrip exercise.

1. Blood pressure response to standing

The subject rested comfortably in supine position for 15 minutes. Then the subject was asked to stand up unaided and remain standing. Systolic blood pressure (SBP) was recorded in resting supine position and again immediately when she stands up. And the difference in SBP was noted.

2. Blood pressure response to sustained Hand grip exercise

The subject was asked to sit comfortably in chair. Initially the subject was asked to exert maximal hand grip strength on hand grip dynamometer with dominant hand. First the maximum voluntary contraction (MVC) was determined and then the subject was asked to exert 30% of MVC for 5 minutes (at least for 3 min) with dominant

hand. Diastolic blood pressure was measured in the non-dominant hand at rest and at one minute intervals during hand grip. The maximum rise in diastolic BP during 30% of MVC over the resting diastolic blood pressure was noted.

Grading (Ewing and Clarke) [29] and autonomic function score of the results:

	Normal	Borderline	Abnormal
Score	**0**	**1**	**2**
1. H.R response to Valsalva maneuver	>1.21	1.11-1.20	<1.10
2. H.R variation during deep breathing	>15bts/min	11-14bts/min	<10bts/min
3. H.R response to standing	>1.04	1.01-1.03	<1.0
4. BP response to standing	<10mmHg	11-29mmHg	>30mmHg
5. BP response to sustained hand grip	>16mmHg	11-15mmHg	<10mmHg

Criteria for grading autonomic function as whole[55]

Scores $\leq$ 3 -Normal autonomic function

> 3 & < 8 -Borderline dysfunction

$\geq$ 8 to 10 -Abnormal function

Lipid profile parameters:

Collection of sample: After overnight fasting, 2ml of venous blood sample was collected from each subject, clear unhemolyzed serum was obtained by

centrifuging blood at 3000rpm for 15min, and lipid profile was done by semi-automated analyzer using enzymatic method.

Lipids analyzed were:

1. Triglyceride
2. Total-cholesterol
3. HDL- cholesterol
4. LDL- cholesterol
5. HDL/LDL

a) Estimation of serum total cholesterol: The total cholesterol was estimated by the enzymatic method (cholesterol Oxidase – Peroxidase) with Endpoint Colorimetry.

Principle: The estimation of cholesterol involves the following enzymatic reactions:

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{CO} \text{Choleste-3-one + } H_2O_2$$

$$H_2O_2 \text{ + Phenol + 4-AAP} \xrightarrow{POD} \text{Quinoneimine dye + } H_2O$$

CE = Cholesterol esterase, CO = Cholesterol Oxidase , POD = Peroxidase , 4AAP = 4- Aminoantipyrine

Absorbance of quinoneimine was measured at 505 nm is proportional to cholesterol concentration in the specimen. [56]

Reagents:

Reagent 1 Cholesterol Mono reagent*:*

Goods Buffer (pH 6. 7) ……. 50 mmol / L

Cholesterol Oxidase ……. . . ≥ 50 U / L

Cholesterol Esterase……≥ 100 U/ L

Peroxidase……. ≥ 3 KU /L

Reagent 2 Cholesterol Standard*:*

Cholesterol standard …. 200 mg /dL

Sample: Unhemolyzed sample of serum was used.

Assay Programme :

Mode	End Point
Wavelength	505nm (490 – 530)
Temperature	37^0C
Optical path length	1 cm
Blanking	Reagent blank
Sample volume	10µL
Working reagent volume	1000µL
Incubation time (mins)	10 at 37^0C
Concentration of Standard	200 mg / dL
Maximum absorbance limit	2. 0
Linearity	750 mg / dL
Stability of color	1 hour
Units	mg / dL

Procedure:

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Serum	--------	----------	10µL
Standard	--------	10 µL	--------
Cholesterol Reagent	1000µL	1000µL	1000µL

Contents were mixed well and incubated at at 37^0C for 10 minutes. As per assay parameters the analyzer was programmed. The absorbance of absorbance of standard and sample against reagent, blank at 505 nm within 60 minutes was measured.[57]

Calculation:

$$\text{Cholesterol (mg /dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 200$$

Cholesterol concentration (mmol / L) = concentration (mg /dL) x 0. 0259

b) ESTIMATION OF SERUM HDL-CHOLESTEROL

The serum chylomicrons, VLDL, LDL, and TG were precipitated by using precipitating reagent, leaving only HDL in the solution. The precipitated lipoproteins were sedimented by centrifugation and the clear HDL containing supernatant was recovered for HDL- cholesterol estimation.[56]

Reagents:

Reagent 3 precipitating Reagent:

Polyethylene glycol 6000……. . … . . 200mM / L

Reagent 4 HDL-cholesterol Standards*:* HDL-cholesterol standard 50 mg / dL

Sample: Unhemolyzed sample of serum was used.

Assay Programme :

Mode	End Point
Wavelength	505nm (490 – 530)
Temperature	37^0C
Optical path length	1 cm
Blanking	Reagent blank
Sample volume	100µL
Working reagent volume	1000µL
Incubation time (mins)	10 at 37^0C
Concentration of Standard	50 mg / Dl
Maximum absorbance limit	2. 0
Linearity	120mg / dL
Stability of color	1 hour
Units	mg / dL

Procedure:

Step-1: HDL-cholesterol separation

Pipette into tubes marked	Test
Serum	200µL
Reagent 3	200µL

Contents were mixed well and incubated at 37^0C for 10 minutes. Later it was centrifuged for 15min at 2000rpm and clear supernatant was separated. For HDL-cholesterol estimation, supernatant was used.

Step-2: HDL-cholesterol estimation

Pipette into tubes marked	Blank	Standard	Test
Supernatant from step-1	--------	----------	100µL
Standard	--------	100µL	--------
Cholesterol Reagent 1	1000µL	1000µL	1000µL

Contents were mixed well and incubated at 37^0C for 10 minutes. As per assay parameters the analyzer was programmed .The absorbance of standard and sample against reagent blank at 505 nm was measure within 60 minutes. [57]

Calculation:

$$\text{HDL-Cholesterol (mg /dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 50$$

(2=Dilution factor, as sample is diluted 1:1 in step 1)

c) Estimation of serum Triglycerides*:* Plasma triglycerides were estimated by Enzymatic (Glycerol-3-phosphate Oxidase) method with Endpoint colorimetry .

Princple: The estimation of Triglycerides involved the following enzymatic reactions-

$$\text{Triglycerides} + H_20 \xrightarrow{LPL} \text{Glycerol + FFA}$$

$$\text{Glycerol + ATP} \xrightarrow{GK} \text{Glycerol-3-Phosphate + ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{GPO} \text{DHAP} + H_2O_2$$

$$2H_2O_2 + \text{4- AAP} \xrightarrow{POD} \text{Quinoneimine dye} + 4H_2O$$

LPL = Lipoprotein Lipase ; FFA = Free Fatty Acids ; GK = Glycerol Kinase ,GPO = Glycerol – 3 – Phosphate Oxidase ; POD = Peroxidase , ATP = Adenosine Triphosphate ; AAP = 4- Aminoantipyrine ; ADP = Adenosine Diphosphate; DHAP = Dihydroxyacetone phosphate.

Absorbance of quinoneimine measured at 505 nm was proportional to Triglycerides concentration in the specimen.[56]

Reagents:

Reagent 1 Triglycerides Mono Reagent:

Pipes Buffer ……. . … . . 50 mmol / L

4-Chlorophenol……. . . 05 mmol / L

Magnesium ion……05 mmol / L

ATP……. . . 1. 0 mmol / L

Lipase……. . . . . .≥ 5000 U / L

Peroxidase…… ≥ 1000 U / L

Glycerol Kinase ……. . . . . ≥ 400 U / L

4- Aminoantipyrine……. . 0. 4 mmol U / L

Glycerol – 3 – Phosphate Oxidase…. . . ≥ 4000 u / l

Detergents, Preservative & stabilizer

Reagent 1 Triglycerides Standards*:* Triglycerides standard 200 mg / dL

Sample: Unhemolized sample of serum was used.

Assay Programme :

Mode	End Point
Wavelength	505nm (490 – 550)
Temperature	37^0C
Optical path length	1 cm
Blanking	Reagent blank
Sample volume	10µL
Working reagent volume	1000µL
Incubation time (mins)	10 at 37^0C
Concentration of Standard	200 mg / dL
Maximum absorbance limit	2. 0
Linearity	1000 mg / dL
Stability of color	1 hour
Units	mg / dL

Procedure :

Pipette into tubes marked	Blank	Standard	Test
Serum	--------	----------	10µL
Standard	--------	10µL	--------
Triglycerides Reagent	1000µL	1000µL	1000µL

Contents were mixed well and incubated at 37^0C for 10 minutes. As per assay parameters, the analyzer was programmed. The absorbance of standard and sample against reagent blank was measured at 505 nm within 60 minutes. [57]

Calculation:

$$\text{Triglycerides (mg /dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 200$$

Triglycerides concentration (mmol / L) = concentration (mg /dL) x 0. 0114

d) ESTIMATION OF LDL-CHOLESTEROL: - By Friedwald's formula

LDL-C=Total cholesterol –TG/5- HDL-cholesterol

Figure 5: Showing Computerized 4-channel Physiopac.

Figure 6: Showing instruments used: Hand grip Dynamometer, Stethoscope, Sphygmomanometer

Fig7: Recording of Blood pressure response to Sustained Hand Grip

Fig 8: Recording of blood pressure response to standing

Fig 9: Recording of ECG at Rest

Fig 10: Recording of Heart rate response to Valsalva maneuver

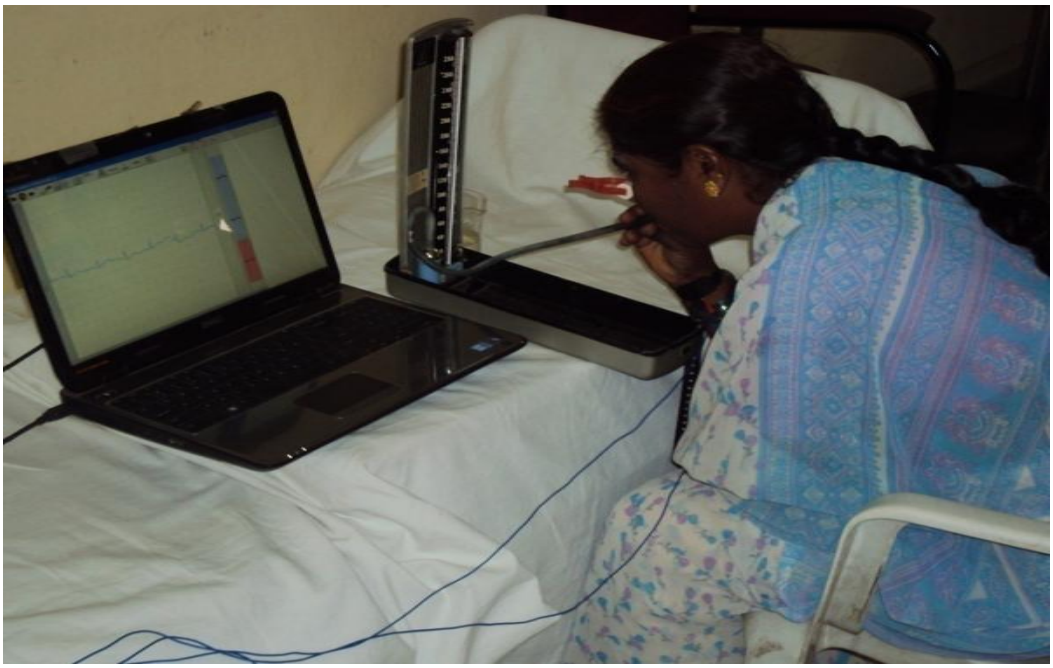


Figure 11: ECG showing Heart rate response to standing

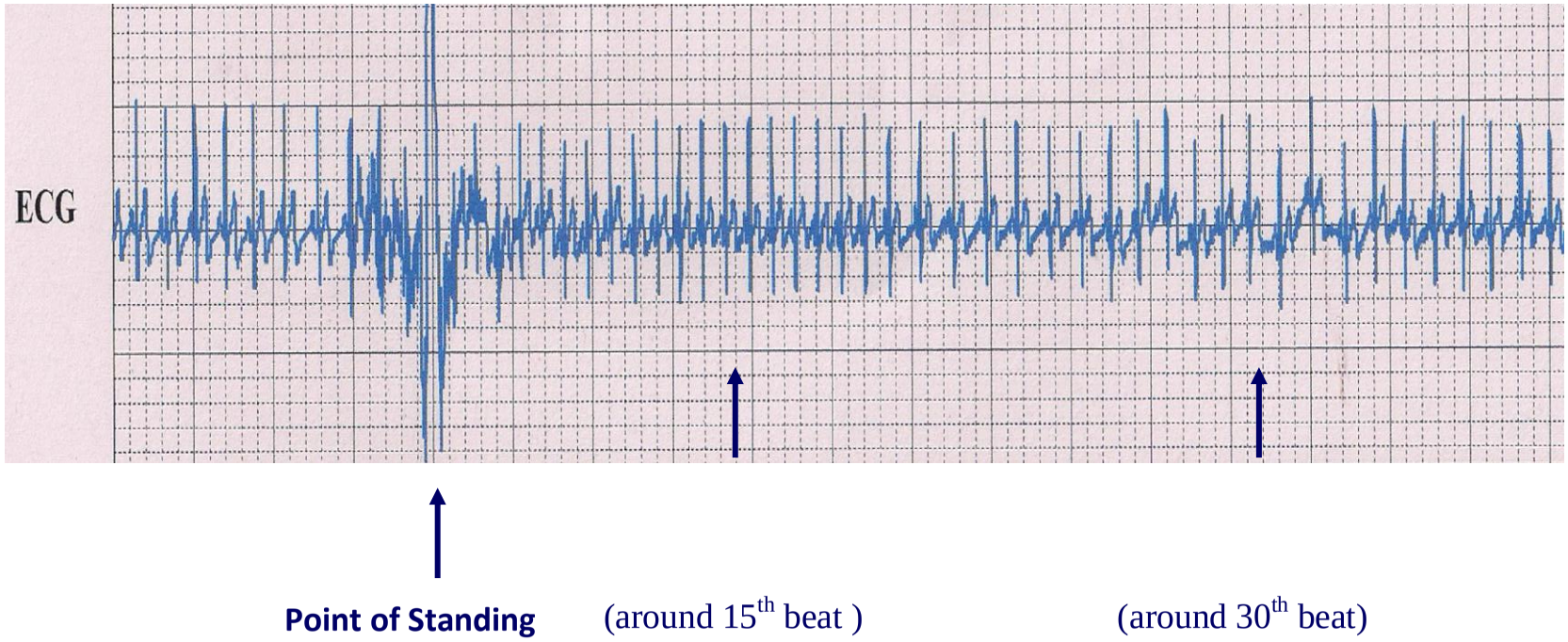


Figure 12: ECG showing Heart rate response to Valsalva maneuver

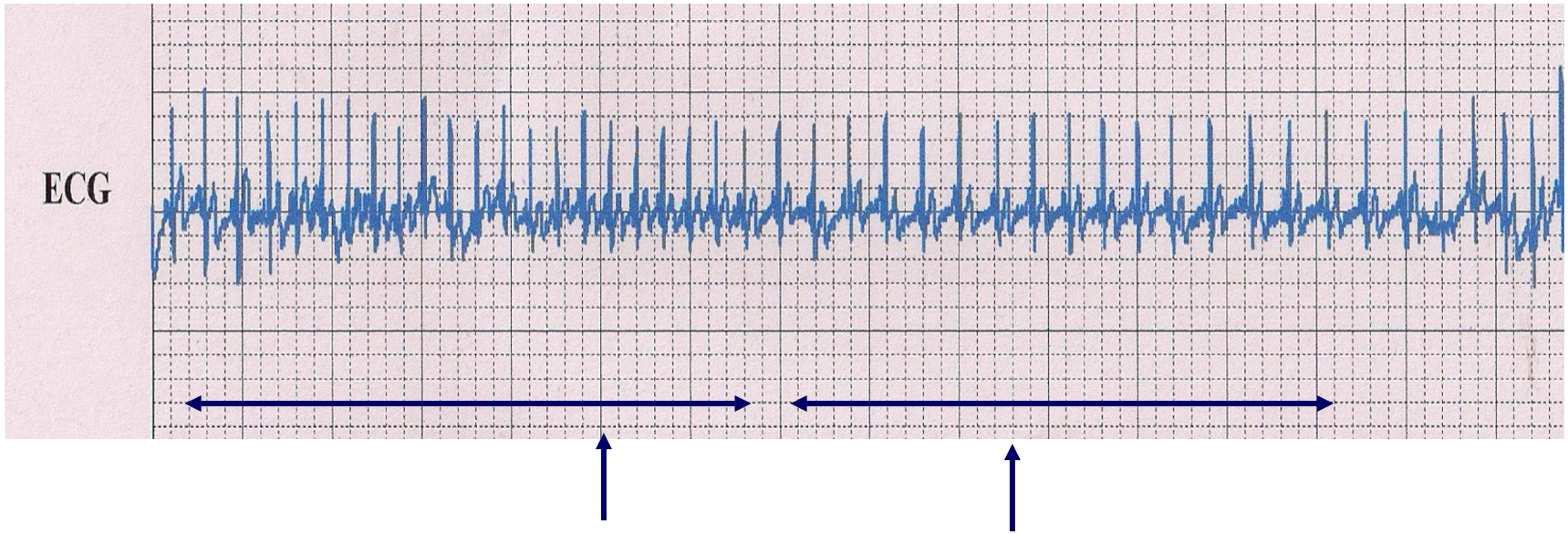
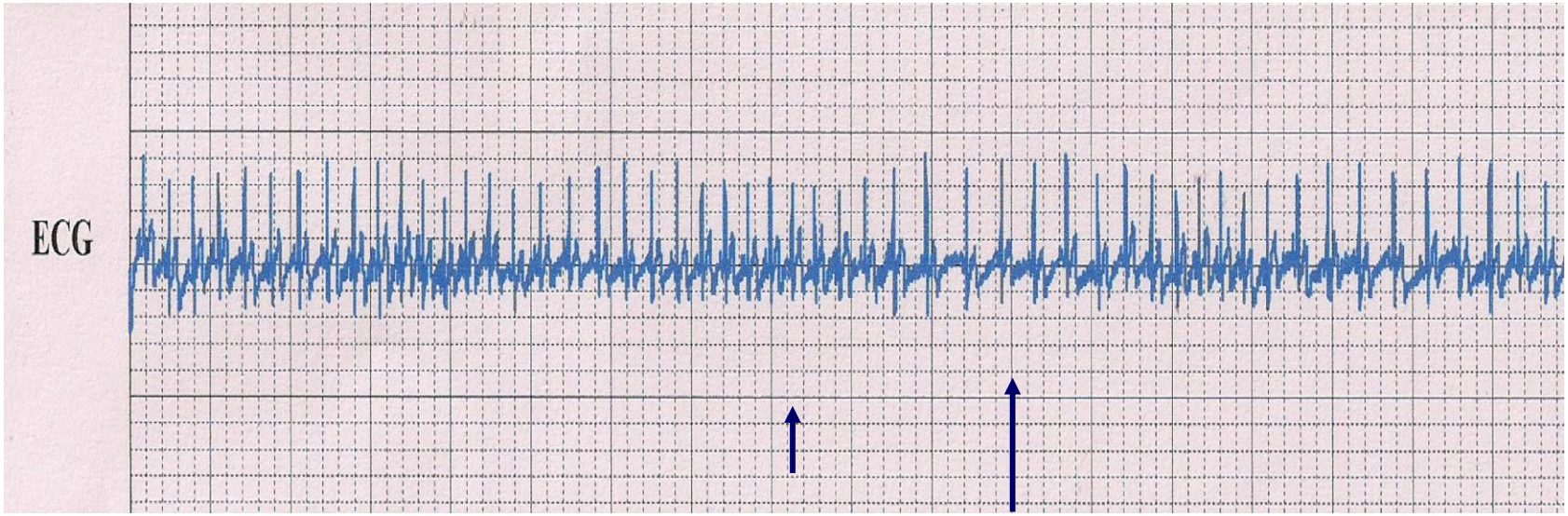


Figure 13: ECG showing Heart rate variation during deep breathing



Statistical Analysis

All values are presented as Mean ± Standard Deviation (Mean ± SD). Comparison of mean values of parameters between premenopausal and postmenopausal group was done by students unpaired t test.

p value >0.05 was taken as not significant.[54]

1. p value <0.05 was taken as significant.
2. p value <0.01 was taken as highly significant.
3. p value <0.001 was taken as very highly significant.

RESULTS

The study of cardiovascular autonomic functions and lipid profile was done in postmenopausal and premenopausal women of B.L.D.E.U'S Shri.B.M.Patil Medical College and Research centre, Bijapur. Total number of subjects in each group was as follows.

I] Control (premenopausal women), n=36

II] Study (postmenopausal women), n=36

Various anthropometric, physiological, cardiovascular autonomic function tests and lipid profile parameters were evaluated in pre and postmenopausal women. The observations of all the parameters were statistically analyzed by using unpaired student t-test and compared in pre and postmenopausal women. The results are shown in tables 1-5 for all the variables used.

I. Anthropometric Parameters:

The Mean Value and Standard Deviation, Level of Significance of each parameter was calculated for each group and presented in Table-1

Table 1: Anthropometric Parameters (Mean ± SD) of Control and Study Groups:

Parameters	Control Group	Study Group	Level of significance
Age (Years)	41.5 ± 1.82	50.25 ± 5.97	0.0005***
Height (cms)	157.94 ± 5.97	153.88± 5.23	0.003**
Weight (Kg)	62.22±8.87	62.72 ± 9.21	0.81
BMI (kg/m^2)	24.82 ±2.98	26.50± 3.85	0.04*
BSA (Sq m)	1.61±0.13	1.62±0.12	0.84

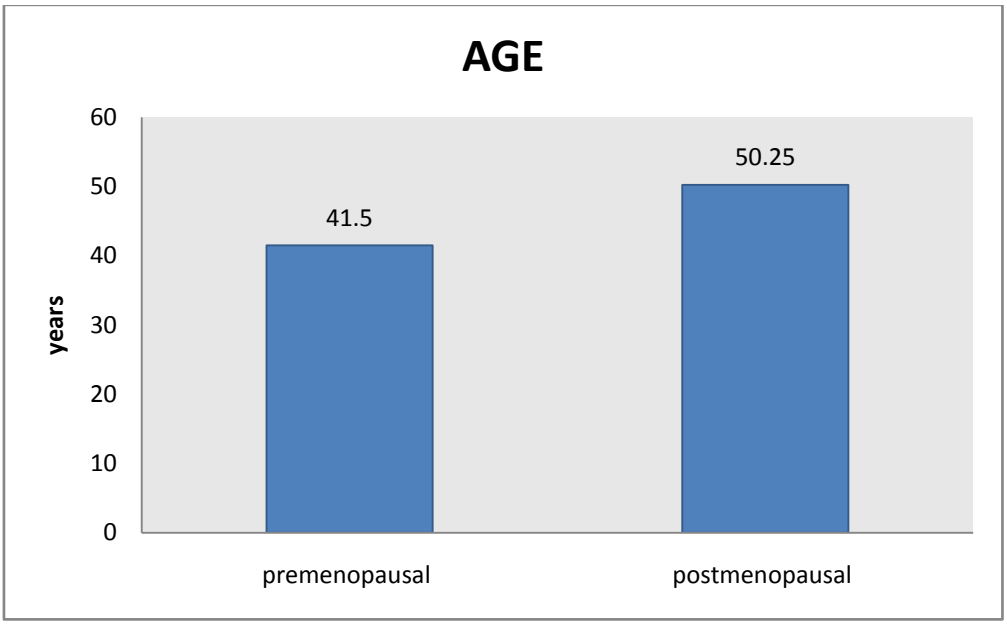
*p <0.05: Significant, ** p <0.01: Highly significant, *** p <0.001: Very highly significant

AGE:

The mean± SD of age of premenopausal women and postmenopausal women were found to be 41.5 ± 1.82 years and 50.25 ± 5.97 years respectively.

- The mean age was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of age in between the pre and postmenopausal women. ($p<0.05$)

Graph 1: Mean values of Age in pre and postmenopausal women

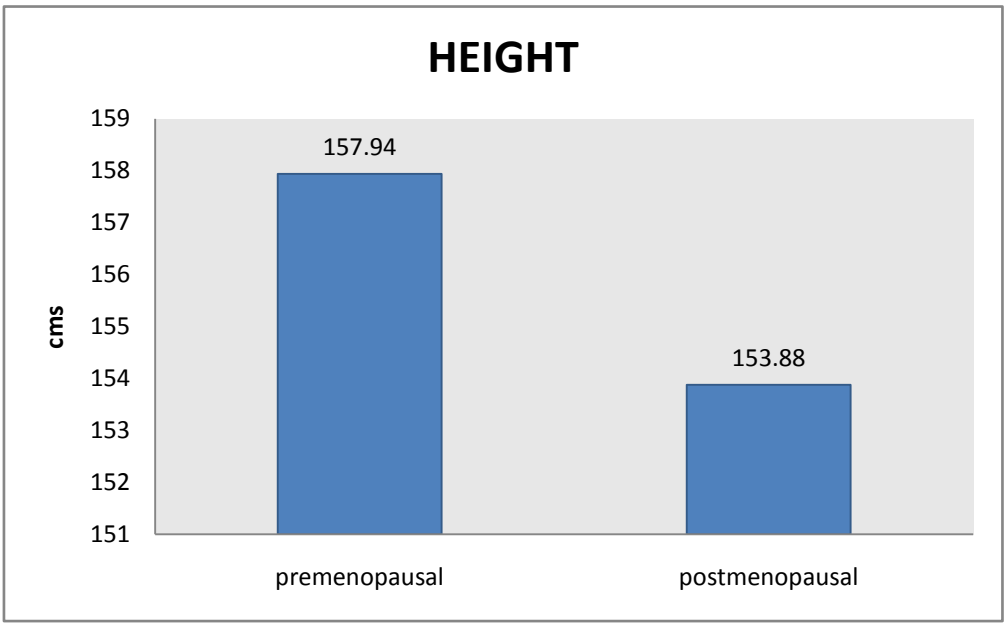


HEIGHT:

The mean± SD height of pre and postmenopausal women were found to be 157.94 ± 5.97cms and 153.88± 5.23cms respectively.

- The mean height was less in postmenopausal women as compared to premenopausal women
- There was significant statistical difference in the mean values of height in between the premenopausal and postmenopausal women. ($p<0.05$)

Graph 2: Mean values of Height in pre and postmenopausal women

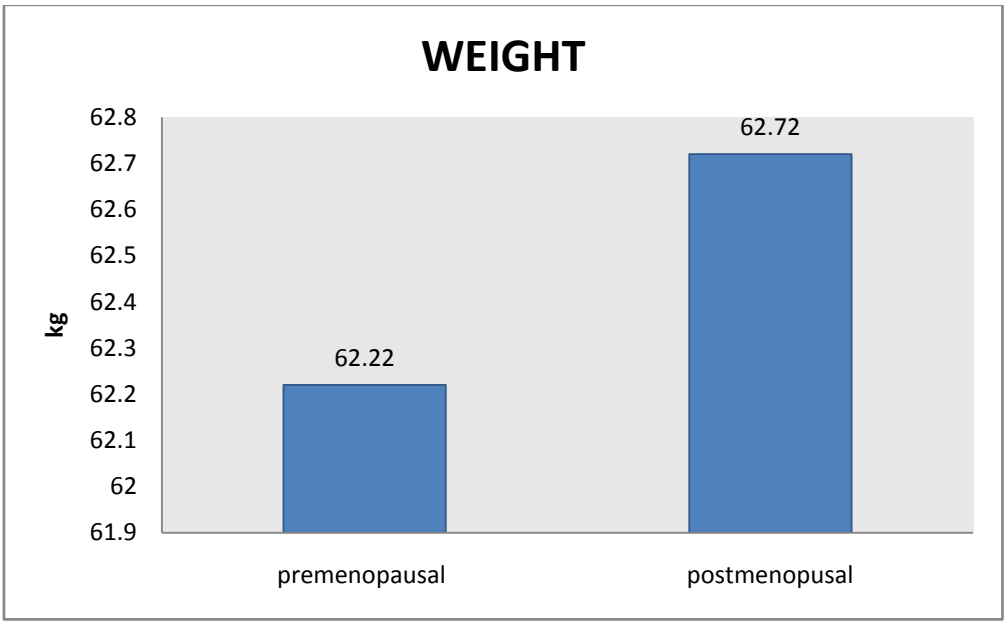


WEIGHT:

The mean± SD of weight of premenopausal women and postmenopausal women were found to be 62.22±8.87kgs and 62.72 ± 9.21 kgs respectively.

- The mean weight was more in postmenopausal women as compared to premenopausal women.
- There was no significant statistical difference in the mean values of weight in between the premenopausal women and postmenopausal women. (p>0.05)

Graph 3: Mean values of weight in pre and postmenopausal women

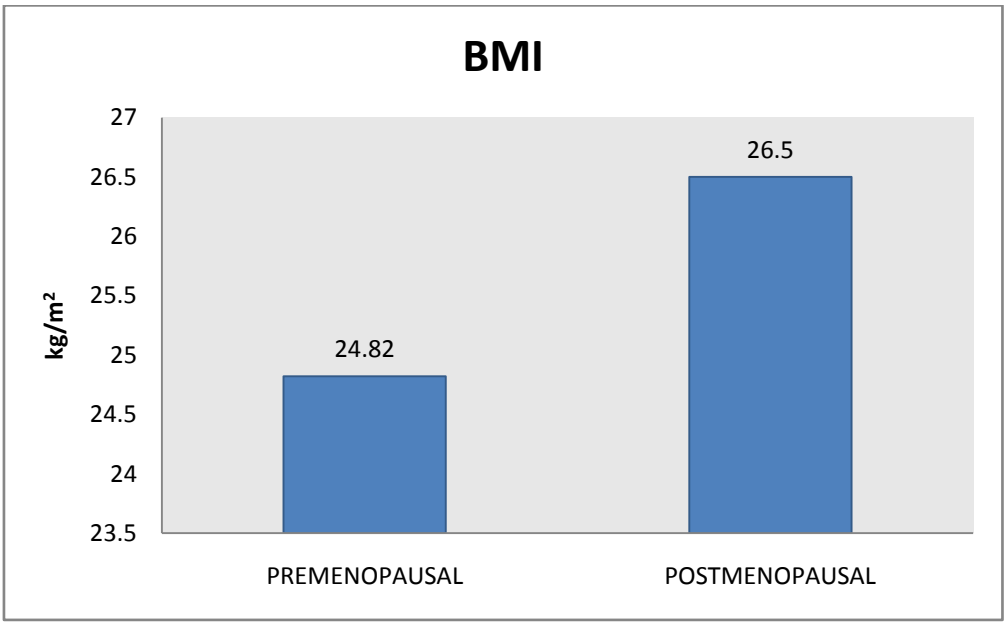


BMI:

The mean± SD of BMI of premenopausal women and postmenopausal women were found to be 24.82 ±2.98 kg/m^2 and 26.50± 3.85 kg/m^2 respectively.

- The mean BMI was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of BMI in between the premenopausal women and postmenopausal women ($p<0.05$)

Graph 4: Mean values of BMI in pre and postmenopausal women

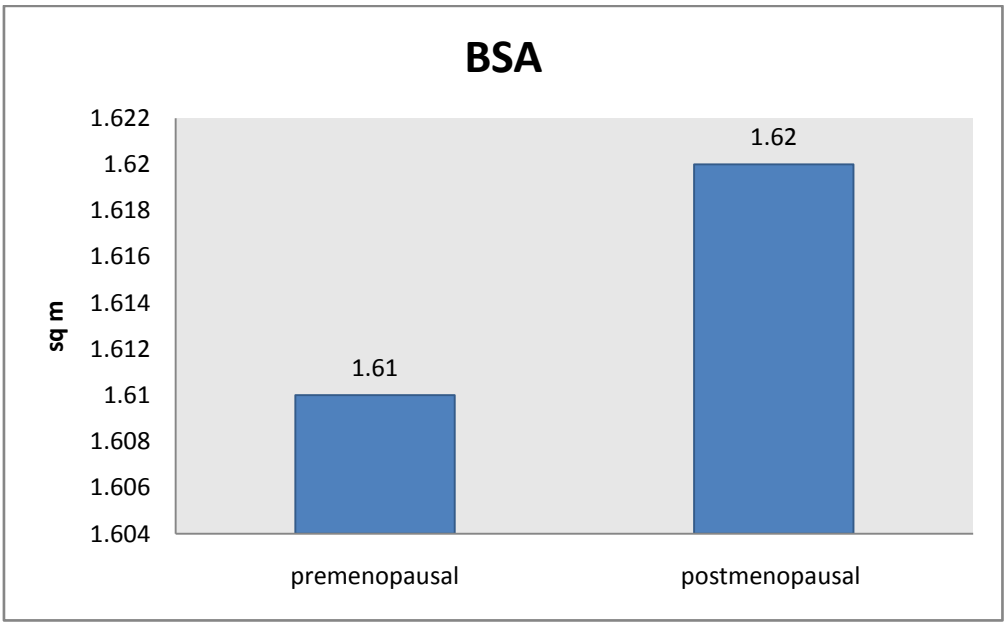


BSA:

The mean± SD of BSA of premenopausal women and postmenopausal women were found to be 1.61±0.13 sq m and 1.62±0.12 sq m respectively.

- The mean BSA was more in postmenopausal women as compared to premenopausal women.
- There was no significant statistical difference in the mean values of BSA in between the premenopausal women and postmenopausal women. (p>0.05)

Graph 5: Mean values of BSA in pre and postmenopausal women



II. Physiological Parameters in control and study groups

The Mean Value , Standard Deviation and Level of Significance of each parameter was calculated for each group and presented in Table-2

Table 2: Physiological Parameters in control and study groups

Parameters	**Control Group**	**Study Group**	**Level of significance**
Resting PR (bpm)	76.38±6.58	77.83±7.20	0.37
Resting RR (cycles/min)	16.5±2.15	14.58±2.64	0.001***
Resting SBP (mm of Hg)	121.5±7.74	128.05±10.06	0.002**
Resting DBP (mm of Hg)	77.27±6.93	81.16±7.83	0.02*

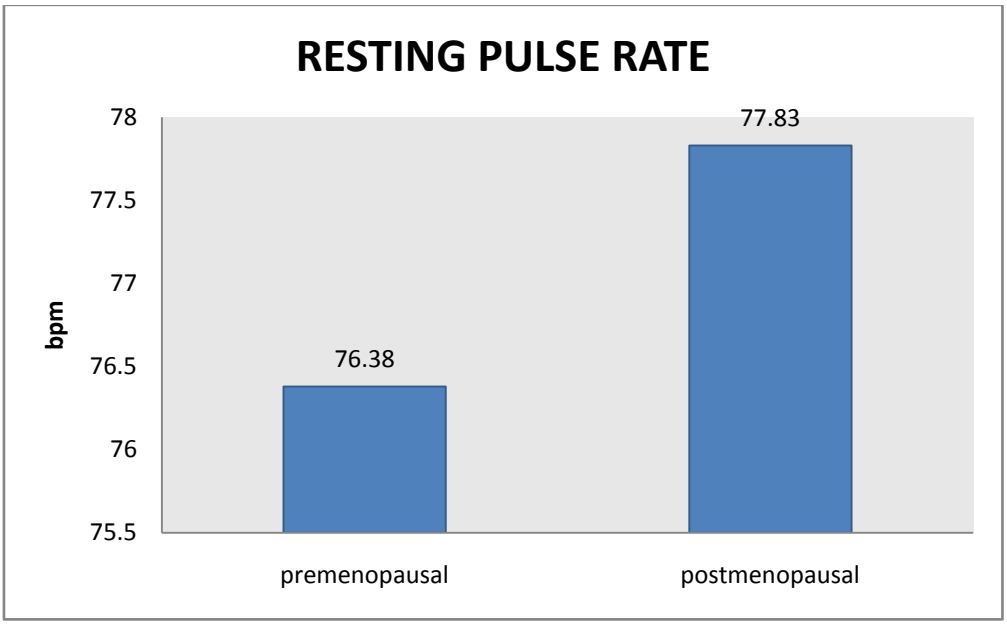
*p <0.05: Significant, ** p <0.01: Highly significant, *** p <0.001: Very highly significant

Resting Pulse Rate:

The mean± SD of resting pulse rate of premenopausal women and postmenopausal women were found to be 76.38±6.58 bpm and 77.83±7.20bpm respectively.

- The mean resting pulse rate was more in postmenopausal women as compared to premenopausal women.
- There was no significant statistical difference in the mean values of resting pulse rate in between the premenopausal women and postmenopausal women($p>0.05$)

Graph 6: Mean values of resting Pulse Rate in pre and postmenopausal women

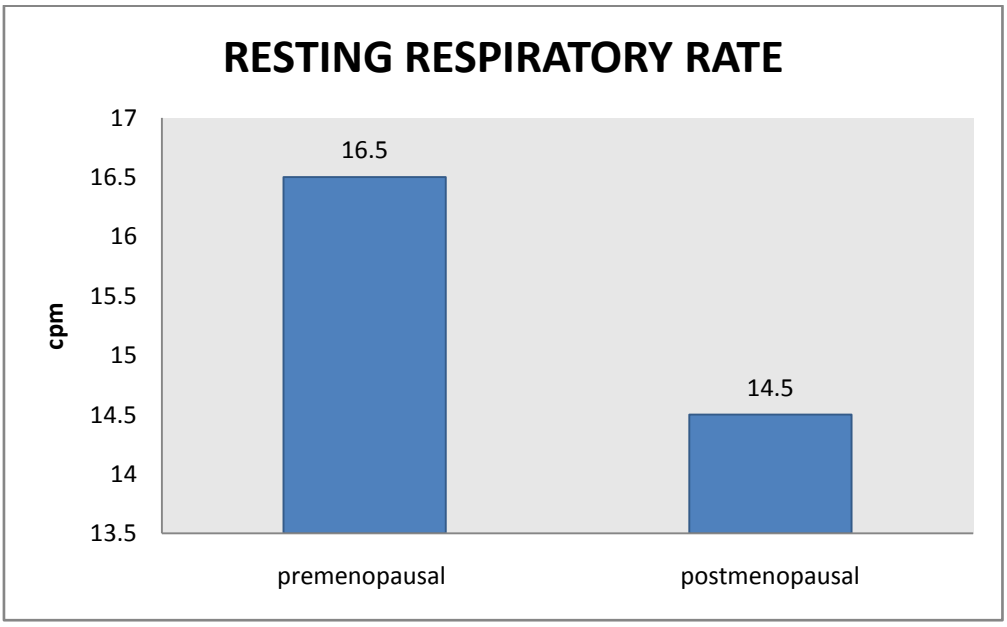


Resting Respiratory Rate:

The mean± SD of resting respiratory rate of premenopausal women and postmenopausal women were found to be 16.5±2.15 cycles per min and 14.58±2.64 cycles per min respectively.

- The mean resting respiratory rate is less in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of resting respiratory rate in between the premenopausal women and postmenopausal women ($p<0.05$).

Graph 7: Mean values of resting Respiratory rate in pre and postmenopausal women

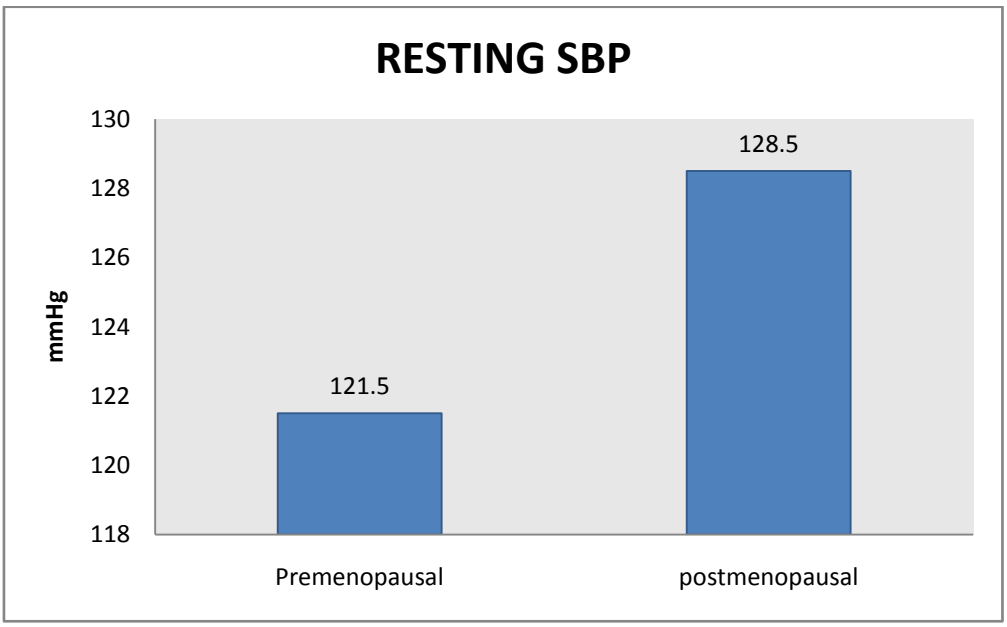


Resting Systolic Blood Pressure :

The mean± SD of resting Systolic Blood pressure of premenopausal women and postmenopausal women were found to be 121.5±7.74 mmHg and 128.05±10.06 mmHg respectively.

- The mean resting Systolic Blood pressure was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of resting Systolic Blood pressure in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph 8: Mean values of resting Systolic Blood Pressure in pre and postmenopausal women

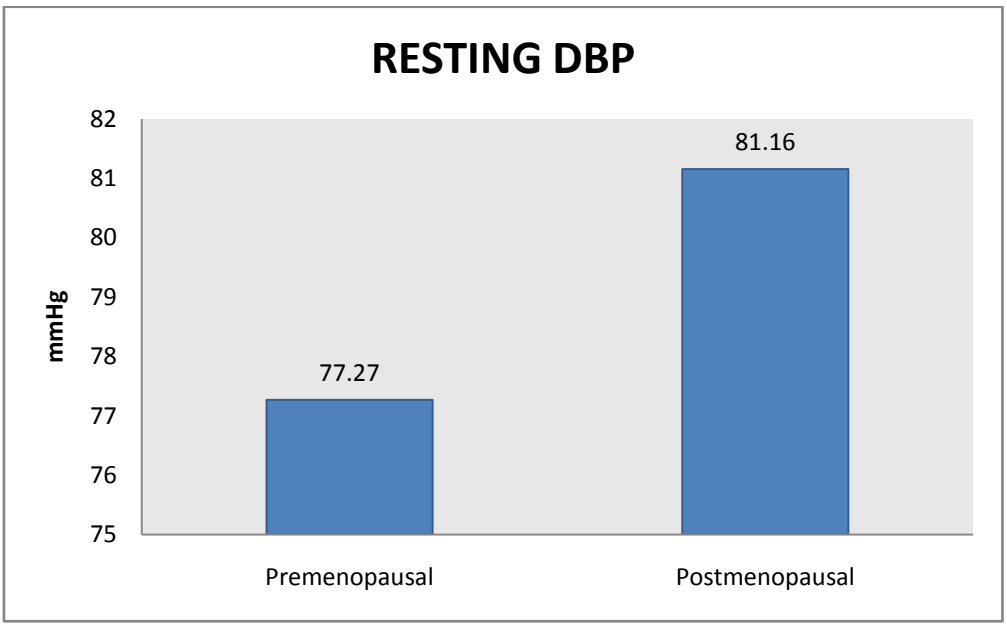


Resting Diastolic Blood Pressure :

The mean±SD of resting diastolic blood pressure of premenopausal women and postmenopausal women was found to be 77.27±6.93 mmHg and 81.16±7.83 mmHg respectively

- The mean resting diastolic blood pressure was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of resting diastolic blood pressure in between the premenopausal women and postmenopausal women ($p<0.05$).

Graph 9: Mean values of resting Diastolic Blood Pressure in pre and postmenopausal women



III. AUTONOMIC FUNCTION PARAMETERS IN SUBJECTS OF CONTROL AND STUDY GROUP

The Mean Value, Standard Deviation and Level of Significance of various Autonomic function parameters like Heart rate response to Valsalva maneuver (Valsalva Ratio), Heart rate variation during deep breathing (I-E), Immediate heart rate response to standing, Blood pressure response to standing and Blood pressure response to sustained hand grip were measured for each group and presented in Table-3 .

Table 3:Autonomic function parameters of subjects in Control and Study Groups.

Autonomic function parameters	**Control Group**	**Study Group**	**Level of significance**
Valsalva Ratio	1.71±0.43	1.20±0.14	0.001***
HR variation to deep breathing (Maximum-Minimum)	31.61± 5.96	14.98± 6.30	0.0005***
Immediate HR response to standing (30:15)	1.23±0.19	1.05±0.06	0.0004***
BP response to Standing (Fall in SBP)	7.77± 2.73	8.83 ±2.76	0.10
BP response to sustained Hand grip (Increase in DBP)	18.08± 3.54	22.55± 5.37	0.0001***

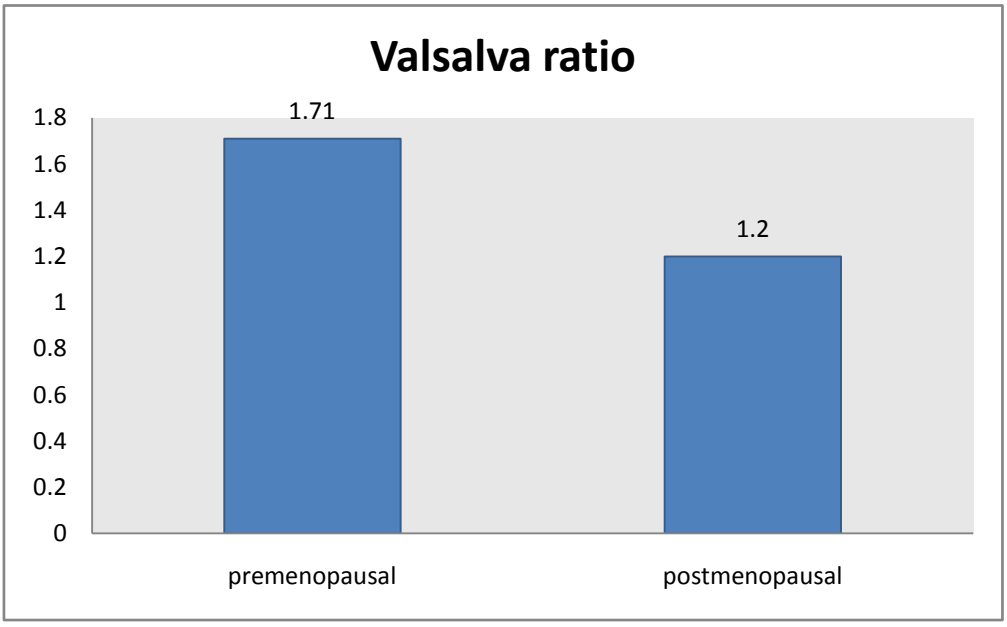
*p <0.05: Significant, ** p <0.01: Highly significant, *** p <0.001: Very highly significant

Valsalva Ratio:

The mean± SD of valsalva ratio of premenopausal women and postmenopausal women were found to be 1.71±0.43 and 1.20±0.14 respectively.

- The mean valsalva ratio was less in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of valsalva ratio in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph 10: Mean values of Valsalva Ratio in pre and postmenopausal women

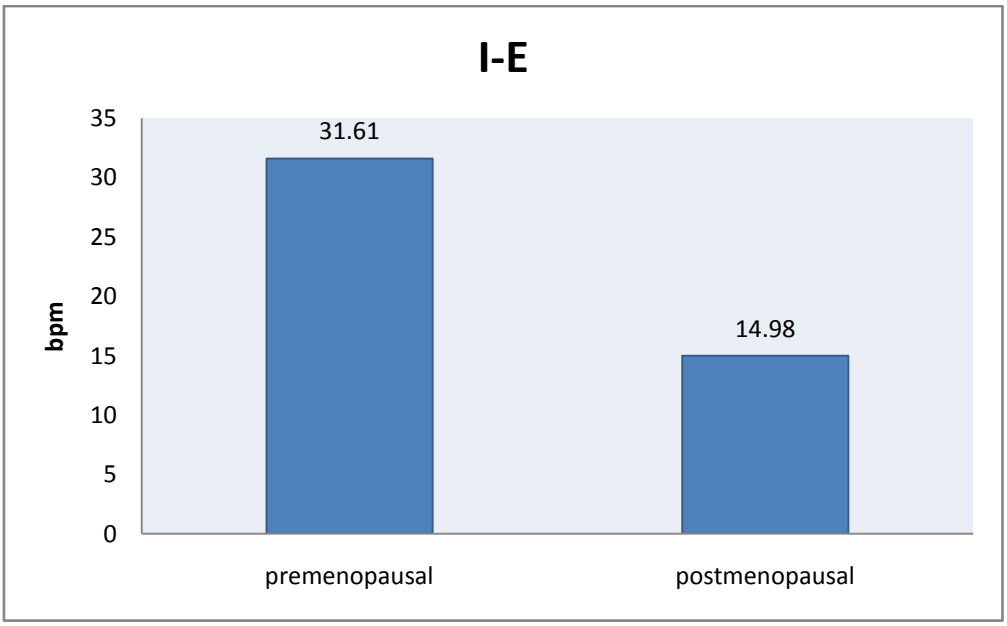


HR variation to deep breathing (Maximum-Minimum):

The mean± SD of HR variation to deep breathing of premenopausal women and postmenopausal women were found to be 31.61± 5.96bpm and 14.98± 6.30bpm respectively

- The mean HR variation to deep breathing was less in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of HR variation to deep breathing in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph11: Mean values of HR variation to deep breathing in pre and postmenopausal women

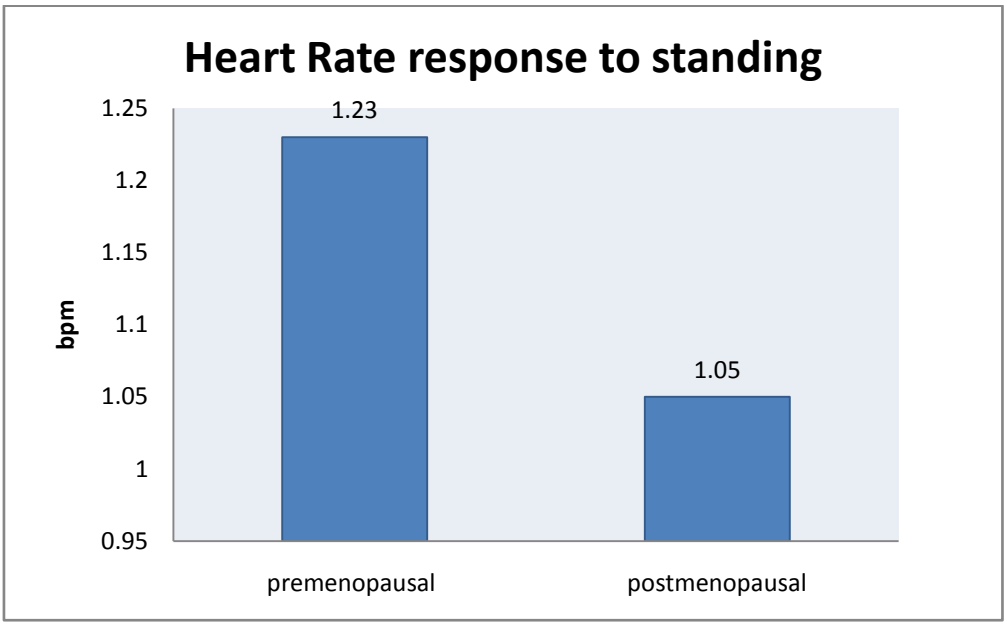


Immediate HR response to standing (30:15) :

The mean±SD of Immediate HR response to standing of premenopausal women and postmenopausal women were found to be 1.23±0.19 and 1.05±0.06 respectively.

- The mean Immediate HR response to standing was less in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of Immediate HR response to standing in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph 12: Mean values of Immediate HR response to standing (30:15) in pre and postmenopausal women

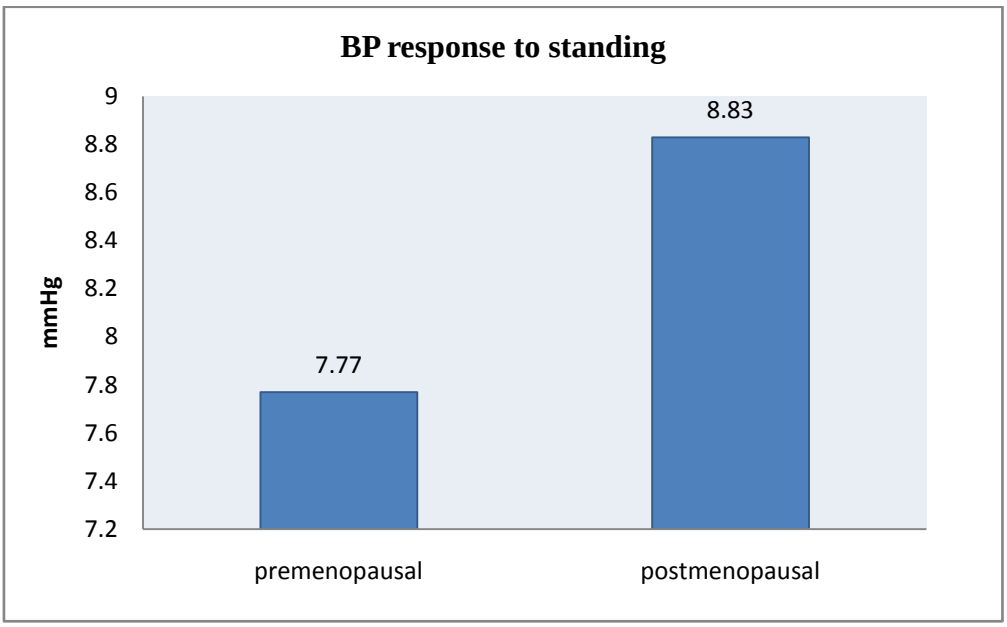


BP response to Standing (Fall in SBP) :

The mean± SD of BP response to Standing (Fall in SBP) of premenopausal women and postmenopausal women were found to be 7.77± 2.73 mmHg and 8.83 ±2.76 mmHg respectively.

- The mean BP response to Standing (Fall in SBP) was more in postmenopausal women as compared to premenopausal women.
- There was no significant statistical difference in the mean values of BP response to Standing (Fall in SBP) in between premenopausal women and postmenopausal women (p>0.05)

Graph13: Mean values of BP response to Standing in pre and postmenopausal women

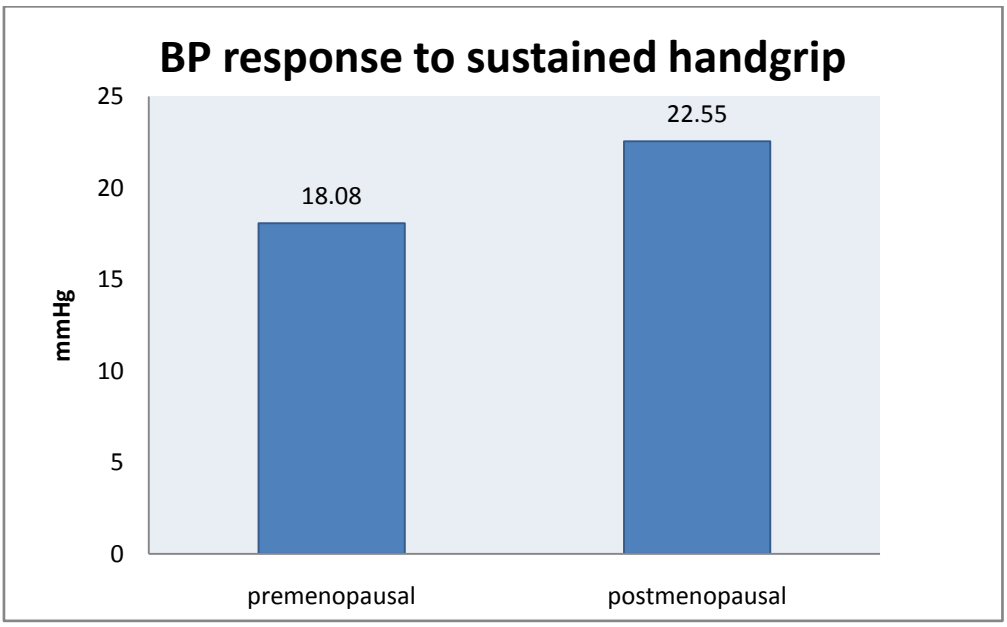


BP response to sustained Hand grip (Increase in DBP):

The mean± SD of BP response to sustained Hand grip (Increase in DBP) of premenopausal women and postmenopausal women were found to be 18.08± 3.54 mmHg and 22.55± 5.37 mmHg respectively.

- The mean BP response to sustained Hand grip (Increase in DBP) was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of BP response to sustained Hand grip (Increase in DBP) in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph 14: Mean values of BP response to sustained Hand grip in pre and postmenopausal women



AUTONOMIC FUNCTION SCORE

Total scores were done according to D.J. Ewing and B.F.Clarke.[29] Number of subjects expressed as percentage of the total number of the subjects in the groups and compared.Higher the score, more is the dysfunction.Total Autonomic function scores were calculated for each group and presented in Table-4

Table 4: Total Autonomic function score in control and study group

Group	0	1	2	3	4	5	6	7	8
Premenopausal	63.8%	27.77%	8.33%	0%	0%	0%	0%	0%	0%
Postmenopausal	11.11%	11.11%	27.77%	16.66%	25%	5.55%	2.77%	0	0

Graph 15: Total autonomic function score in pre and postmenopausal women

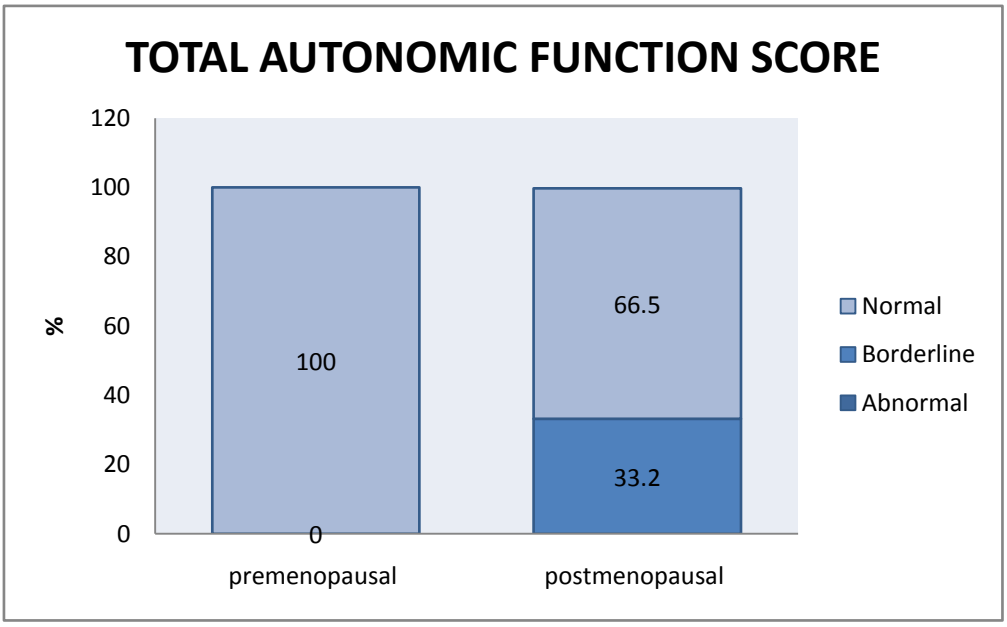


Table 5: Lipid profile parameters of subjects in Control and Study Groups.

Lipid profile parameters	Control Group	Study Group	Level of significance
Triglyceride(mg/dl)	88.33±21.99	109.58±39.49	0.006**
Total-cholesterol(mg/dl)	163.5±20.18	180.13 ±28.97	0.006**
HDL- cholesterol(mg/dl)	39.63±5.98	36.75±5.69	0.03*
LDL- cholesterol(mg/dl)	106.52±18.16	117.72 ±26.81	0.04*
HDL/LDL	0.36±0.09	0.34 ±0.10	0.36

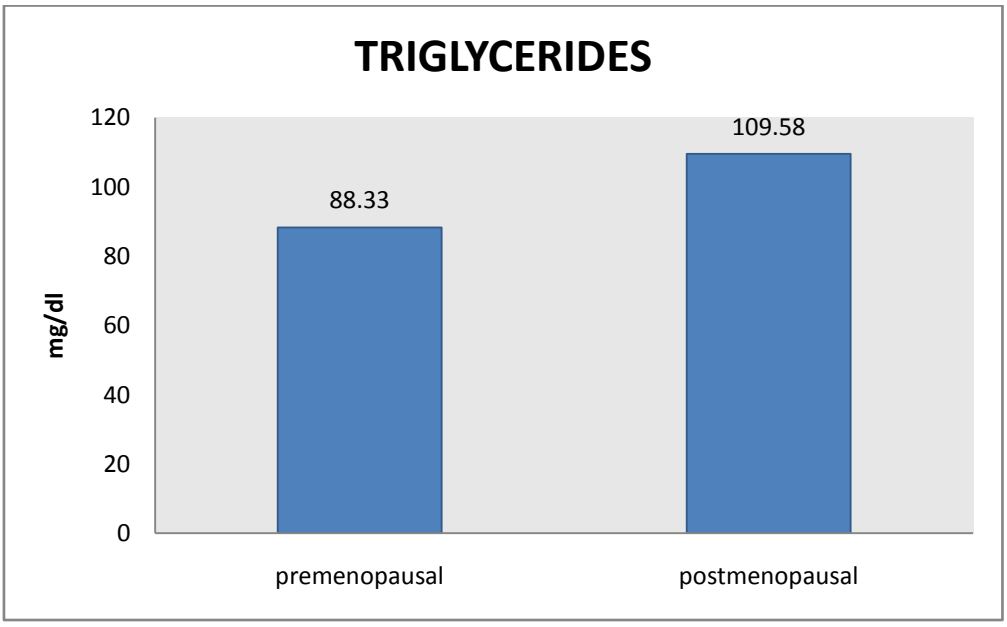
*p <0.05: Significant, ** p <0.01: Highly significant, *** p <0.001: Very highly significant

Triglyceride :

The mean± SD of Triglyceride of premenopausal women and postmenopausal women were found to be 88.33±21.99mg/dl and 109.58±39.49mg/dl respectively.

- The mean Triglyceride was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of Triglyceride in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph 16:Mean values of Triglyceride (TG) in pre and postmenopausal women

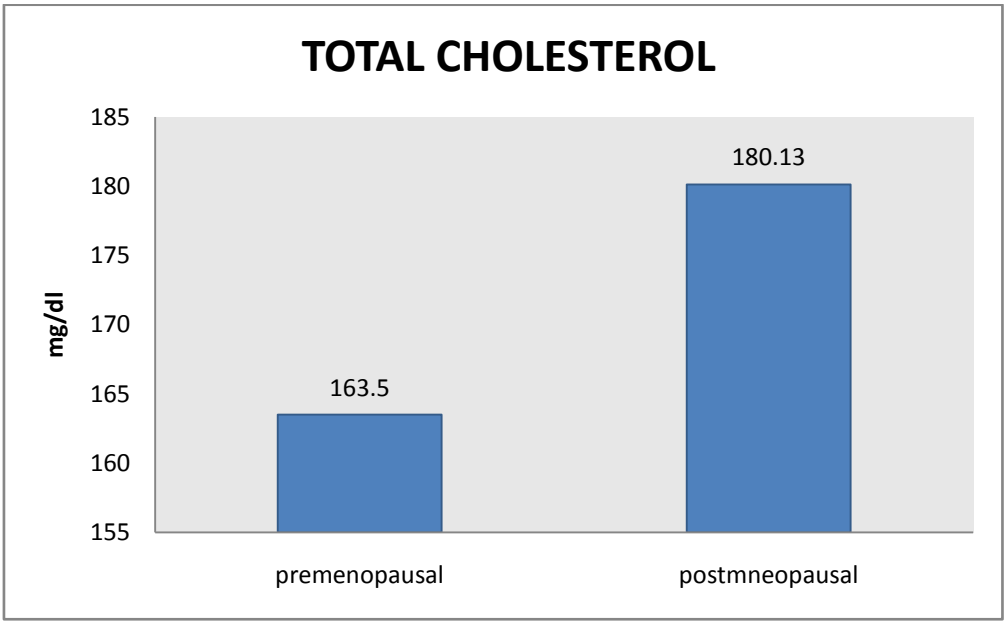


Total-Cholesterol:

The mean± SD of Total-cholesterol of premenopausal women and postmenopausal women were found to be 163.5±20.18mg/dl and 180.13 ±28.97mg/dl respectively.

- The mean Total-cholesterol was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of Total-cholesterol in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph17: Mean values of Total-cholesterol (TC) in pre and postmenopausal women

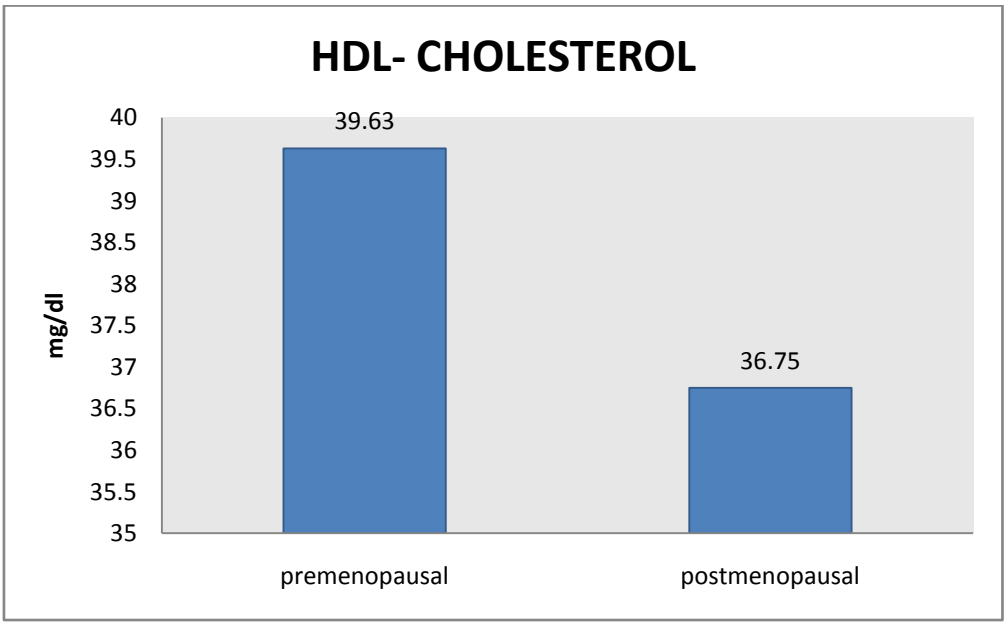


HDL- cholesterol:

The mean± SD of HDL- cholesterol of premenopausal women and postmenopausal women were found to be 39.63±5.98mg/dl and 36.75±5.69 mg/dl respectively.

- The mean HDL-cholesterol was less in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of HDL- cholesterol in between the premenopausal women and postmenopausal women. ($p<0.05$)

Graph18: Mean values of HDL- Cholesterol in pre and postmenopausal women

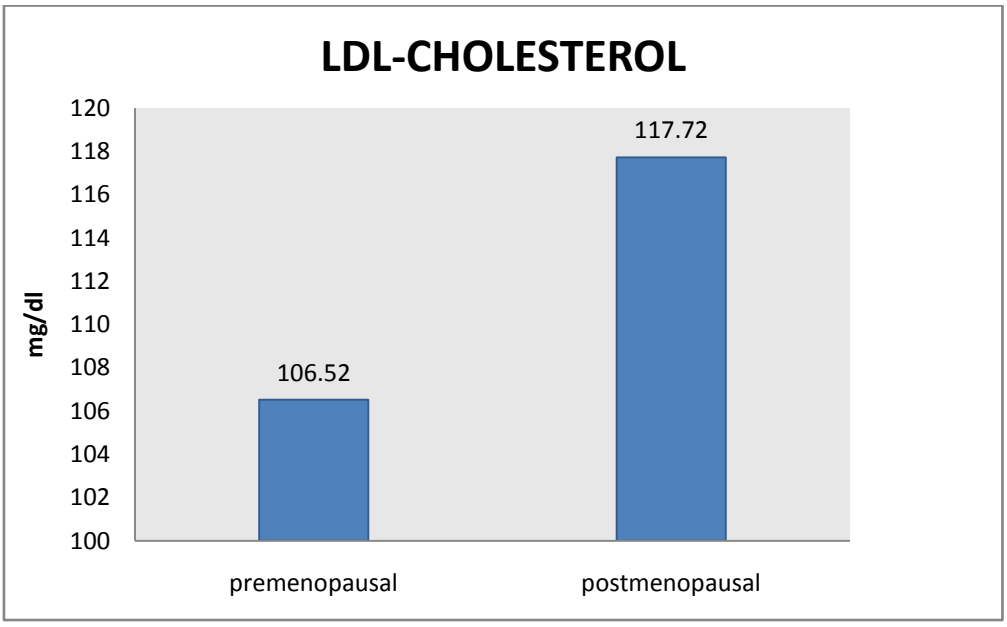


LDL- cholesterol:

The mean± SD of LDL- cholesterol of premenopausal women and postmenopausal women were found to be 106.52±18.16mg/dl and 117.72 ±26.81 mg/dl respectively

- The mean LDL- cholesterol was more in postmenopausal women as compared to premenopausal women.
- There was significant statistical difference in the mean values of LDL- cholesterol in between the premenopausal women and postmenopausal women. ($p<0.05$).

Graph19: Mean values of LDL- Cholesterol in pre and postmenopausal women

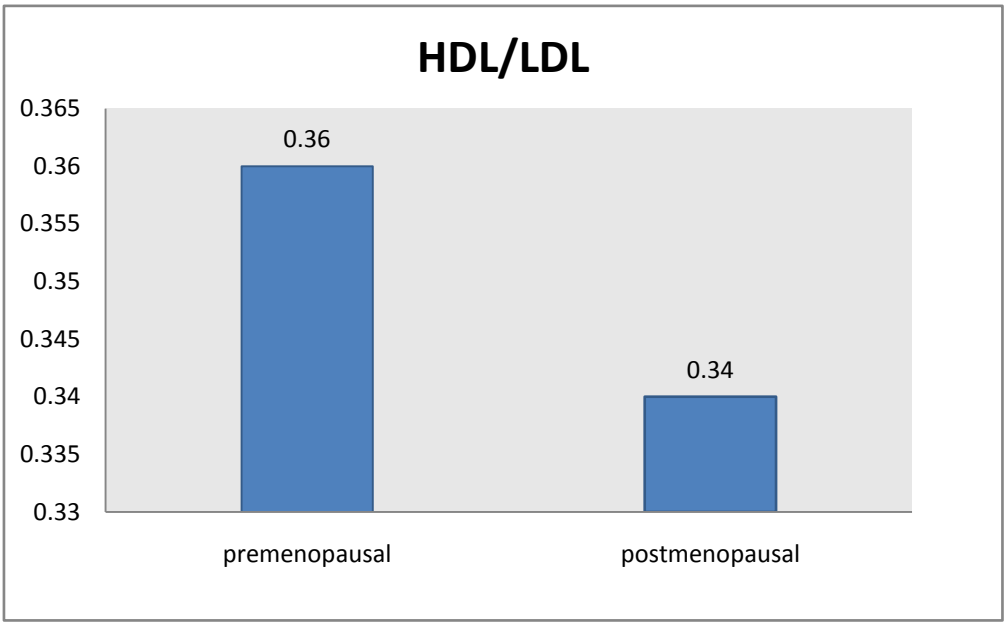


HDL/LDL

The mean± SD of HDL/LDL of premenopausal women and postmenopausal women were found to be 0.36±0.09 and 0.34 ±0.10 respectively.

- The mean HDL/LDL was more in premenopausal women as compared to postmenopausal women.
- There was no significant statistical difference in the mean values of HDL/LDL in between the premenopausal women and postmenopausal women. (p>0.05)

Graph 20: Mean values of HDL/LDL in pre and postmenopausal women



DISCUSSION

We conducted a cross-sectional study to evaluate cardiovascular autonomic functions and lipid profile in apparently healthy women in age range of 40-60 years attending BLDEU'S Shri B M Patil Medical College Hospital and Research Centre Bijapur. The subjects were divided into study group (postmenopausal women) (n=36) and control group (premenopausal women)(n=36) depending upon attainment of menopause. We studied anthropometric parameters, physiological parameters, cardiovascular autonomic functions and lipid profile in study and control groups. We performed the Parasympathetic Function tests (Valsalva maneuver, Heart rate variation during deep breathing, and Heart rate response to standing) and Sympathetic function tests (Blood pressure response to standing and Blood pressure response to sustained hand grip). Lipid profile parameters like TG, TC, HDL-C, LDL-C, and HDL/LDL were estimated by semi auto analyzer using enzymatic method.

I. Anthropometric Parameters:

The anthropometric parameters were compared between premenopausal women and postmenopausal women.

In the present study (Table no 1), significant statistical difference was seen in the mean values of all anthropometric parameters between pre ($p<0.05$) and postmenopausal women except weight and BSA($p>0.05$). BMI was significantly high in postmenopausal women compared to premenopausal women.

Our findings are in accordance with earlier studies

A study was done by Koskova I on 146 healthy Czech women between 20-65yrs age group. They classified subjects according to four reproductive phases: fully reproductive women, pre-menopausal women, menopausal women and postmenopausal women. BMI increased significantly in all the groups except for the premenopausal group and was greatest in the menopausal group. [58]

In a study done by Zafar hussain and co workers on three groups G-I(premenopausal women),G-II(early postmenopausal women),G-III(late postmenopausal women)showed that there was no significant difference in weight between premenopausal and early postmenopausal women. Weight was more in late postmenopausal women compared to premenopausal and early postmenopausal women and it was statistically significant. [59]

Quinglong wang and co-workers analyzed total and regional body composition changes in 373 (age:49-69 yrs) early postmenopausal women. Weight was neither related to age($r=0.06$,$p>0.05$) nor to years since menopause($r=-0.05$, $P>0.05$), BMI was related to age($r=0.15$, $p<0.01$) not with years since menopause($r=-0.01$, $P>0.05$).[60]

PHYSIOLOGICAL PARAMETERS

The physiological parameters were compared between premenopausal and postmenopausal women.

In the present study (Table no 2), we found resting systolic blood pressure ($p=0.002$), resting diastolic blood pressure ($p=0.02$) were significantly higher and Resting respiratory rate significantly lower ($p=0.001$) in study group compared to control group but were within physiological limit. We found no statistical difference in resting pulse rate between premenopausal and postmenopausal women. These changes may be attributed to menopause which potentiate age related increase in blood pressure.

In a study done by Renata C et al on randomly selected 908 female residents of a Prague district, aged 45–54 years, they found Age-adjusted and BMI-adjusted systolic blood pressure and diastolic blood pressure did not differ among the groups regardless of the definition of menopause. There was also no difference in the prevalence of hypertension and in the age-adjusted and BMI-adjusted odds ratio for hypertension. They concluded that the rise in blood pressure after the menopause appeared to be due to increased BMI rather than to ovarian failure per se.[61]

Karen A. Matthews and others conducted study on Changes in Cardiovascular Risk Factors during the Perimenopause, Postmenopause and Carotid Artery Atherosclerosis in Healthy Women .They found that rise in Systolic blood pressure at follow-Up of Premenopausal to First Year of menopause to be 2.7 (0.6), Postmenopausal First to Fifth Year to be 6.6 (0.8) and Premenopausal to Fifth Year Postmenopausal 9.3 (0.8) mmHg.[62]

In a study done by JA Staessen and others on Conventional and ambulatory blood pressure and menopause in a prospective population study, they found higher values of SBP and DBP in postmenopausal women compared to perimenopausal women .[63]

AUTONOMIC FUNCTION PARAMETERS

A.PARASYMPATHETIC FUNCTION TESTS:

I. Heart rate response to Valsalva maneuver :

A normal response to Valsalva maneuver is characterized by a decrease in the pulse pressure and tachycardia during strain and blood pressure overshoot and bradycardia following the strain[64]. The Valsalva maneuver tests the integrity of both parasympathetic and sympathetic divisions of autonomic nervous system. The hemodynamic changes during the maneuver are mediated via baroreceptors. With parasympathetic affection, the baroreceptor mediated reflex bradycardia response to elevated blood pressure will be reduced.

In the present study, the mean value of Valsalva ratio (Table no.3) in study group was significantly less (0.001) compared to control group. Valsalva ratio was less than the normal range in study group (normal value > 1.21, according to Ewing and Clarke grading).[29]

Our findings are in accordance with earlier studies done by Arunima C [1], Naher LAD et al [22] .

Lathadevi G V and others in their study found decreased valsalva ratio in postmenopausal women compared to perimenopausal .[51]

II. Heart rate response to deep breathing:

Heart rate response to deep breathing (sinus arrhythmia) is a normal phenomenon and is primarily due to fluctuation of parasympathetic output to the heart.

In the present study, the mean value Heart rate response to deep breathing (Table no.3) was significantly less (p=0.0005) in postmenopausal women compared to premenopausal women . Heart rate response to deep breathing was less than the normal range in study group (normal value > 16bpm , according to Ewing and Clarke grading).[29]

Our study is in accordance with Arunima C et al [1], Naher LAD et al [22].

III. Heart rate response to Standing (30: 15 ratio):

Heart rate response to standing in normal subjects consists of tachycardia maximum around 15th beat followed by relative bradycardia around 30th beat after standing.[22] These hemodynamic responses are mediated by baroreceptors.

In the present study the mean value of Heart rate response to standing (Table no.3) in study group was significantly lower compared to control group (p=0.0004). Heart rate response to standing is within the normal range in study group (normal value > 1.04, according to Ewing and Clarke grading).[29]

Our findings are in accordance with Arunima C et al [1],G.V. Lathadevi et al [51], Naher LAD et al [22].

Shikha gautam and co workers studied heart rate response to standing in premenopausal women in proliferative phase, premenopausal women in secretory phase, postmenopausal women and postmenopausal women on hormone replacement therapy (HRT). They found significantly lower values in postmenopausal women compared to premenopausal women in secretory phase, proliferative phase and postmenopausal women on HRT.[19]

B. SYMPATHETIC FUNCTION TESTS

I. Blood pressure response to standing

With change of posture from supine to standing, the autonomic nervous system acts to produce a rise in heart rate & vasoconstriction in order to maintain blood pressure.[65] Vasoconstriction is mediated through sympathetic innervations to blood vessels during standing.

In our study ,we found greater fall in systolic blood pressure on standing (Table 3) in study group compared to control group but this difference was not statistically significant (p=0.10)

In study done by G.V. Lathadevi and Warun K they found fall in blood pressure response to standing to be higher in postmenopausal women compared to perimenopausal.[51]

In a study done by Shikha gautam and others on women in premenopausal in proliferative phase, premenopausal women in secretory phase, postmenopausal

women and postmenopausal women on HRT, they found no significant changes in systolic BP in postural challenge test and in diastolic BP in sustained handgrip test .[19]

II. Blood pressure response to sustained hand grip

D.J Ewing et al (1973) first showed that during sustained hand grip, there was a sharp rise in diastolic blood pressure (DBP) due to increase in peripheral vascular resistance.[32]

We observed mean values of diastolic blood pressure in response to sustained handgrip(Table 3) to be significantly higher in postmenopausal women compared to premenopausal women.

Our study is in accordance with G.V. Lathadevi et al .[51]

Shailaja S et al found sympathetic hyperactivity in postmenopausal women by measuring heart rate variability .[49]

AUTONOMIC FUNCTION SCORE IN CONTROL AND STUDY GROUP .

More is the score, more is the dysfunction**.**

Criteria for grading autonomic function as whole[55]

Scores ≤ 3 Normal autonomic function

Scores > 3 and < 8 Borderline dysfunction

Scores ≥ 8 to 10 abnormal function

For grading of individual cardiovascular autonomic function, results were classified into normal, borderline, and abnormal according to Ewing and Clarke's classification[29].

	Normal 0	Borderline 1	Abnormal 2
Tests reflecting Parasympathetic function			
1. Heart rate response to Valsalva maneuver (Valsalva ratio)	>1.21	1.11-1.20	<1.10
2. Heart rate variation (R-R interval) during deep breathing (maximum-minimum heart rate)	>15 bpm	11-14 bpm	<10 bpm
3. Immediate heart rate response to standing (30:15 ratio)	>1.04	1.01-1.03	<1.00
Tests reflecting Sympathetic function			
1. Blood pressure response to standing (fall in systolic blood pressure)	<10 mm Hg	11-29 mm Hg	>30 mm Hg
2.Blood pressure response to sustained hand grip (increase in diastolic blood pressure)	>16 mm Hg	11-15 mm Hg	<10 mm Hg

An overall score ≤ 3 was considered to indicate normal autonomic function. Scores >3 and ≤ 8 were considered borderline and scores ≥ 8 were judged abnormal.[43]

Total autonomic function score (Table no 4) showed increase in the number of subjects with borderline autonomic function in study group compared to control group.

Though the grading and function scores are accepted no reference in the literature is available following these criteria. So, we have tried to present the autonomic function in the form of score which may help to grade the subjects easily rather than expressing the results as pure values of different tests.

We found significantly lower mean values of three parasympathetic function tests and significant higher values of one sympathetic function test in study group compared to control group. This suggested that there was increased sympathetic activity and decreased parasympathetic activity in postmenopausal women (study) compared to premenopausal women (control).

This can be attributed to decreased sex hormones in postmenopausal women.[19] The possible explanation could be the decline in level of estrogen from premenopausal to postmenopausal status which favors the shifting of autonomic balance towards the sympathetic dominance. Earlier studies based on the autonomic changes before and after oophorectomy in premenopausal women have shown that estrogen has a role in increasing vagal and reducing sympathetic action. Similar observations were also made by studying the effect of conjugated estrogen replacement therapy in reversing the vagal deficit in postmenopausal women. Findings of our study suggest that even in physiological doses, estrogen contributes to the alterations in sympathovagal balance. Sympathetic hyperactivity has been linked to the development of atherosclerosis, cardiovascular hypertrophy, cardiac arrhythmia and sudden death. Our findings suggest that by enhancing sympathetic

dominance, decreased level of estrogen in postmenopausal women produces an unfavorable alteration in cardiac autonomic function. There are several mechanisms through which reproductive hormonal status may influence cardiovascular autonomic reactivity. These include altering receptor sensitivity, density or neurotransmitter availability. The role of estrogen on cardiac autonomic modulation action can be explained by the effect of estrogen on enhancing the cholinergic muscaranic activity in central nervous system and such modulation at central and peripheral levels tends to suppress sympathetic but elevate parasympathetic tone.[49]

Ageing is also associated with an increased dependency on sympathetic control of cardiac responses and reduced vagal responsiveness. Though age is an important confounding factor it adds to the decline in estrogen level which is associated with increased sympathovagal balance in postmenopausal women .[49]

III. LIPID PROFILE PARAMETERS:

1. TRIGLYCERIDE (TG):

In the present study, we found serum TG value(Table 5) to be significantly higher in postmenopausal women compared to premenopausal women ($p=0.006$).

Our findings are in accordance with Arunima C and co workers who found significantly higher values of triglycerides in postmenopausal women compared to premenopausal women ($p<0.001$).[1]

Our study is in accordance with Zafar hussain et al who studied on three groups G-I(premenopausal women),G-II(early postmenopausal women),G-III(late

postmenopausal women).They found significant difference in TG between early postmenopausal and late postmenopausal women p<0. 05.[59]

In a study done by Fukami K showed that from 4 years before to 1 year after menopause the serum TG remained virtually unchanged during the perimenopausal and postmenopausal periods.[66]

Nese Ozbey and others in their study observed TG was more in younger postmenopausal women (age<50yrs) compared to older postmenopausal women (age>50yrs). This difference was not statistically significant.[67]

Our study is also comparable with Gwen J et al .[68]

2. TOTAL CHOLESTEROL (TC):

In present study, we found Total Cholesterol values(Table 5) to be significantly high in postmenopausal women compared to premenopausal (p=0.006).

Our study is in accordance with Lejla M et al [69],C A O Usoro et al[14] , Arunima C.[1]

In a study done by Nese Ozbey on 405 obese postmenopausal women the TC was low in younger postmenopausal women(age<50yrs) compared to older postmenopausal women(age<50yrs). This difference was not statistically significant.[67]

In a study by Zafar hussain et al on three groups, G-I(premenopausal women),G-II(early postmenopausal women),G-III(late postmenopausal women). They found significant increase in total cholesterol in late postmenopausal women

and early postmenopausal women compared to premenopausal women .They showed that there was no significant difference in TC between early postmenopausal and late postmenopausal women.[59]

In a study conducted by Van BE and co workers on three menopausal cohorts based on years relative to menopause: 2 years before (perimenopausal), 2 years after (early menopausal), 6 years after (late menopausal) showed that from 2 years before to 6 years after menopause serum TC concentration increased on average by at least 1.1mmol/liter. Thereafter, only minor increase was observed. [70]

3. HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C):

In the present, study we found High Density Lipoprotein cholesterol (Table 5) values to be significantly lower in postmenopausal women compared to premenopausal women .

Our study is in accordance with Arunima C and others who found significantly lower values of HDL in postmenopausal women compared to premenopausal women.[1]

Comparable results were obtained by Zafar hussain et al who studied on three groups G-I(premenopausal women),G-II(early postmenopausal women),G-III(late postmenopausal women) .They found that there was significant difference in HDL-C between premenopausal women , early postmenopausal and late postmenopausal women $p<0.05$. [59]

In a study by Chee J K et al they found HDL-C was higher in postmenopausal women compared to premenopausal women and was statistically significant. [70]

Similar results have been observed by Gwen J et al (P=0.456)[68] and C.A.O.Usoro et al [14].

4. LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C):

In the present study, we found low density lipoprotein cholesterol (LDL –C)values (Table 5) were significantly high in postmenopausal women compared to premenopausal women.

Our study is in accordance with Arunima C[1], C.A.O.Usoro et al [14]and Chee J K et al[71].

In a study by Zafar hussain et al on three groups, G-I(premenopausal women),G-II(early postmenopausal women),G-III(late postmenopausal women) showed that there was no significant difference in LDL-C between premenopausal women ,early postmenopausal and late postmenopausal women $p>0.05$. [59]

In a study by Gwen J and co workers showed that LDL-C was high in older post menopausal women(Absence of menses for more than 5years)compared to younger post menopausal women (absence of menses for less than 5 years).[68]

In a study on age matched physically active and less active postmenopausal women by Edith T and others they found LDL-C was low in physically active and high in less active postmenopausal women. [72]

5. HDL/LDL:

In the present study, we found lower HDL/LDL(Table 5) in postmenopausal women compared to premenopausal women .This difference was not statistically significant .

In a study by Edith T and others the HDL/LDL was higher in active women compared to less active postmenopausal women. [72]

Bhagya V and co workers found HDL/LDL ratio decreased with increasing age from reproductive to postmenopausal period.[73]

Gwen J et al found no significant difference in HDL/LDL between younger postmenopausal women and older postmenopausal women (P=0.99).[68]

We found significantly higher values of Triglycerides, Total Cholesterol, LDL-C and lower value of HDL-C in postmenopausal women compared to premenopausal women. A non significant decrease in HDL/LDL ratio in postmenopausal women was seen compared to premenopausal women.

These observations suggest unfavorable alteration in lipid profile in postmenopausal women. The possible underlying mechanism for changing lipid profile could be decreased estrogen level in postmenopausal women. In premenopausal women the normal levels of estrogen maintains the lipids and lipoproteins in the normal range which may be attributed to its actions such as enhancement of LDL catabolism, presumably due to increased expression of LDL receptors. Estrogen also increases the HDL levels which may be attributable to increased synthesis with decreased clearance of HDL particles through diminished hepatic lipase activity.[74] The confounding factors such as age, weight, BMI , physical inactivity may add to the altered lipid profile seen in postmenopausal status .

CONCLUSION

We conducted a cross-sectional study to evaluate cardiovascular autonomic functions and lipid profile in apparently healthy women in age range of 40-60 years attending BLDEU'S Shri B M Patil Medical College Hospital and Research Centre, Bijapur. The subjects were divided into study group (postmenopausal women) and control group(premenopausal women) depending upon attainment of menopause. We performed the Parasympathetic Function tests (Valsalva maneuver, Heart rate variation during deep breathing, and Heart rate response to standing) and Sympathetic function tests (Blood pressure response to standing and Blood pressure response to sustained hand grip). Lipid profile parameters like TG, TC, HDL-C, LDL-C and HDL/LDL were analyzed.

We conclude from our study that

1) Parasympathetic function tests showed decreased parasympathetic activity in postmenopausal women compared to premenopausal women.
2) Sympathetic function tests showed a higher sympathetic activity in postmenopausal women compared to premenopausal women.
3) Higher values of autonomic function score were observed in postmenopausal women compared to premenopausal women indicating altered cardiovascular autonomic functions in postmenopausal women.
4) The lipid profile parameters like Triglycerides ,Total Cholesterol ,LDL –C were increased and HDL–C was decreased in postmenopausal women compared to premenopausal women indicating a atherogenic lipid profile in postmenopausal women.

SUMMARY

The cross-sectional study was carried out in 72 apparently healthy subjects in age range of 40-60 years of BLDEU's Shri B M Patil Medical College to assess the effect of menopause on cardiovascular autonomic functions and lipid profile . The subjects were divided into control (premenopausal women) and study groups (postmenopausal women) depending upon attainment of menopause. Anthropometric parameters, Physiological parameters, cardiovascular autonomic functions and lipid profile were evaluated.

Evaluation of status of cardiovascular autonomic nervous system was done with the help of five non-invasive tests. Parasympathetic activity was assessed by Heart rate response to deep breathing, Heart rate response to Valsalva maneuver and Heart rate response to standing. Sympathetic function was assessed by Blood pressure response to standing and Blood pressure response to sustained hand grip.

Lipid profile parameters TG, TC, HDL-C, LDL-C and HDL/LDL were analyzed by semi-automated analyzer using enzymatic method. Statistical analysis was done using Student t test. p value of <0.05 was considered as significant.

It was observed that

1) Parasympathetic function tests showed decrease in parasympathetic activiy and Sympathetic function tests showed higher sympathetic activity in postmenopausal women compared to premenopausal women.
2) Autonomic function scores were higher in postmenopausal women compared to premenopausal women.

3) The lipid profile parameters like Triglycerides, Total cholesterol ,LDL–C were increased and HDL–C was decreased in postmenopausal women compared to premenopausal women, indicating a atherogenic lipid profile in postmenopausal women.
4) Altered cardiovascular autonomic functions and altered lipid profile in postmenopausal women may be attributed to decreased estrogen level after menopause.

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Annexure 1

B.L.D.E.U'S SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586103
INSTITUTIONAL ETHICAL COMMITTEE

Ethical Committee BIJAPUR-586103 OUT WARD 123/16 Date 19/10/16 Shri B. M. PATIL Medical College

DR.M.S.BIRADAR
CHAIRMAN .I.E.C.
BLDEU'S SHRI: B.M.PATIL MEDICAL COLLEGE
BIJAPUR-586103

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 19-10-2010 at 10-30 am to scrutinize the Synopsis/Research projects of post graduate student/undergraduate student/Faculty members of this college from ethical clearance point of view. After scrutiny the following original/corrected & revised version Synopsis of the Thesis/Research project has been accorded Ethical Clearance.

Title A Study of Cardiovascular autonomic functions and lipid profile in the postmenopausal women.

Name of P.G. /U.G.Student /Faculty member Dr. Anita Deshpande
Dept of Physiology.

Name of Guide Dr. Anand. R. Dhareadkar Prof & HOD Physiology

DR.M.S.BIRADAR
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE

Following documents were placed before E.C.for securitization:
1) Copy of Synopsis/Research project
2) Copy of informed consent form
3) Any other relevant document's

Inward No. 444 Date 19.12.11
Department of Physiology
Circulation / for
n, reply

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

BLDEU/REG/GEN/ 2011-12/ 1321 19th December, 2011

To,
The Principal & Dean Faculty of Medicine
Shri.B.M.Patil Medical College,
Bijapur-586103

Sub: Change in PG guide to Dr. Anita Deshpande.....reg.

Dear Sir/Madam,

On the approval of Hon'ble Vice-Chancellor, Dr. Sumangala Patil, Associate Professor, Department of Physiology, will be PG guide to Dr.Anita Deshpande, PG in Physiology.

This is for information and further reference.

Thanking you,

Yours Sincerely,

REGISTRAR
REGISTRAR.
BLDE University, Bijapur.

Copy to:

- Prof & Head, Department of Physiology, to inform concern.
- COE for information.

Smt. Bangaramma Sajjan Campus, Sholapur Road, Bijapur – 586103, Karnataka, India.
University: Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldeuniversity.org, E-mail: office@bldeuniversity.org
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldea.org, E-mail: bmpmc1@yahoo.co.in

ANNEXURE- 2

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT	:	"A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND LIPID PROFILE IN THE POST MENOPAUSAL WOMEN"
PRINCIPAL INVESTIGATOR/ P.G. GUIDE'S NAME	:	DR.SUMANGALA PATIL MD PROFESSOR. DEPARTMENT OF PHYSIOLOGY.

1: <u>PURPOSE OF RESEARCH</u>:I have been informed that this study will assess the Cardiovascular Autonomic Functions, and serum lipid profile in premenopausal and postmenopausal women. This study will be useful academically as well as for clinically to find out impairments in the Cardiovascular Autonomic Functions and serum lipid profile in postmenopausal women.

2: <u>PROCEDURE</u>: I understand that, the procedure of the study will involve recording of various physiological and physical parameters. The procedure will not interfere with any of my physiological parameters.

3: RISK AND DISCOMFORTS:

I understand determination of Cardiovascular Autonomic functions and serum lipid profile changes will not cause any discomfort to me and do not involve any risk to my health.

4: BENEFITS:

I understand that my participation in the study may not have a direct benefit to me but this may have a potential beneficial effect in the field of Cardiovascular Autonomic function, and lipids in future.

5: CONFIDENTIALITY:

I understand that medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but will be stored in investigators research file and identified only by a code number. The code key connecting name two numbers will be kept in a separate secured location.

If the data are used for publication in the medical literature and for teaching purposes no names will be used and other identities such as photographs, audio and video tapes will be used only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

6: REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Concerned researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.

7: REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate.

8: INJURY STATEMENT:

I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided.

I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____(subject's/relevant guardian)

The purpose of the research, the procedure required and the possible risk and benefits to the best of my ability.

Investigator/ PG (Guide) Date

I confirm that _____ (Name of the P.G. Guide /Chief researcher) has explained to me the purpose of research, the study procedure that I will undergo, and the possible risk and discomforts as well as benefits that I may experience. Alternative to my participation in the study have also been to give my consent from. Therefore I agree to give consent to participate as a subject and this research project.

Participant / Guardian Date:

Witness to signature Date:

Modified from Portney L.G. Watkins M.P., in Foundation of Clinical Research, Second Edition, New Jersey, Prentice Hall Health 2000. (APPENDIX – E).

ANNEXURE 3

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

DEPARTMENT OF PHYSIOLOGY

"**A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND LIPID PROFILE IN THE POSTMENOPAUSAL WOMEN"**

PROFORMA

Name: **Age:**

Occupation: **Religion/Caste:**

Address:

GENERAL HEALTH CONDITION:

Past history:

Ischemic Heart Disease **Hypertension** **Surgery**

Tuberculosis **Syphilis** **Leprosy**

Amyloidosis **Any Significant Chronic Illness**

Family History:

Diabetes mellitus **Obesity** **Hypertension**

IHD **Any other illness.**

Personal history:

Appetite **Diet (Veg/Nonveg)** **Sleep**

Nourishment **Bowel habits** **Bladder habits**

Habits: Chewing pan/gutkha **H/o Alcohol intake,** **H/o Smoking.**

Menstrual history:

Menopause(attained/not):

Menstrual Cycle:

Last Menstrual period:

Symptoms of Autonomic Neuropathy:

- **Giddiness(Dizziness on standing)**
- **Pain in abdomen, Diarrhoea**
- **Flatulence, Nausea, Vomiting, Constipation**
- **Bladder Disturbances**
- **Sweating Disturbances**
- **Chest pain**
- **Cough, breathlessness**

Symptoms of menopause:

- **Easy Fatiguability**
- **Anxiety, nervousness**
- **Weight gain**
- **Tingling, Numbness**
- **Visual disturbances**
- **Lack of energy**
- **Cold hand and feet**
- **Irritability**
- **Palpitation of heart**

GENERAL EXAMINATION

Built	**Temperature**	**Pallor**
Cyanosis	**Edema**	**Abnormal Pigmentation**
JVP	**Lymphadenopathy**	

ANTHROPOMETRIC MEASUREMENTS

1. **Height (cms):**
2. **Weight (kgs):**
3. **Body Surface Area (sq m):**
4. **Body Mass Index (kg/m^2):**

PHYSIOLOGICAL PARAMETERS

1. **Heart Rate(beats/min):**
2. **Blood Pressure (mm of Hg):**
3. **Respiratory Rate (cycles/min):**

AUTONOMIC FUNCTION PARAMETERS

PARASYMPATHETIC TESTS

1. **Heart rate response to Valsalva Maneuvere:**

   (R-R interval, longest to shortest ratio)

2. **Heart Rate variation to Deep breathing:**

   (Beats/min)

3. **Heart rate response to standing:**

   (R-R interval, 30:15 ratio)

SYMPATHETIC TESTS

1. **Blood Pressure response to standing:**

   (mm of Hg)

2. **Blood Pressure response to sustained handgrip:**

   (mm of Hg)

GRADINGS (According to Ewing and Clarke)

	Normal	Borderline	Abnormal
SCORE	**0**	**1**	**2**
1. H.R to Valsalva Maneuver.	**>1.21**	**1.11-1.20**	**<1.10**
2. H.R to Deep breathing	**>15bts/min**	**11-14beats/min**	**<10beats/min**
3. H.R to standing	**>1.04**	**1.01-1.03**	**<1.0**
4. B.P to standing	**<10mm of Hg**	**11-29mm of Hg**	**>30mm of Hg**
5. B.P to sustained handgrip	**>16mm of Hg**	**11-15mm of Hg**	**<10mm of Hg**

BIOCHEMICAL PARAMETERS

1.Serum Cholesterol→ mg/dl
2.Serum Triglycerides → mg/dl
3.Serum High Density Lipoproteins→ mg/dl
4.Serum Low Density Lipoproteins→ mg/dl
5.HDL/LDL Ratio →

Key To Master Chart :

Ht - Height

Wt - Weight

BMI - Body mass index

BSA - Body Surface Area

PR - Pulse Rate

RR - Respiratory Rate

SBP - Systolic Blood Pressure

DBP - Diastolic Blood Pressure

HR - Heart Rate

I - E - Inspiration – Expiration

HRR - Heart rate response

BPR - Blood Pressure Response

HG - Hand Grip.

S.TG - Serum Triglycerides

S.TC- Serum Total Cholesterol

HDL- High Density Lipoprotein

LDL – Low Density Lipoprotein

HDL/LDL-Ratio of High Density Lipoprotein To Low Density Lipoprotein

ANNEXURE - 4a

Master Chart - Control Group

Sl. No	Age	Ht	Wt	BMI	BSA	Resting PR	Resting RR	Resting SBP	Resting DBP	Resting HR	valsalva ratio mean	Valsalva ratio function score	I-E	I-E F Score	HRR to stand	HRR to stand function score	BPR to standing	Bpr tostand score	BPR to HG	bpr to hg score	Total score	S.TG	S. TC	HDL	LDL	HDL/LDL
1	41	145	42	19.97	1.3	76	20	112	72	88	1.34	0	16.6	0	1.1	0	4	0	18	0	0	45	152	44	99	0.44
2	40	152	60	25.96	1.56	66	18	110	72	72	1.43	0	29.9	0	1.2	0	8	0	18	0	0	90	170	40	112	0.35
3	42	154	45	18.97	1.4	80	18	124	80	82	1.65	0	30.3	0	1.1	0	8	0	20	0	0	74	144	38	91.2	0.27
4	41	160	58	22.65	1.58	78	16	120	72	88	1.23	0	33.7	0	1.4	0	12	1	16	0	1	90	178	45	115	0.39
5	40	159	52	20.56	1.5	76	16	130	80	77	1.43	0	35.2	0	1.3	0	10	0	20	0	0	56	143	37	94.8	0.37
6	40	163	82	30.86	1.84	80	16	130	80	88	2.26	0	28.1	0	1.1	0	14	1	16	0	1	80	158	32	110	0.29
7	41	157	66	26.77	1.66	84	20	122	90	94	1.77	0	42.6	0	1.4	0	12	1	13	1	2	106	184	37	125.8	0.29
8	40	155	58	24.14	1.54	80	14	120	90	88	2.35	0	32.5	0	1	1	4	0	24	0	1	71	184	50	119.8	0.41
9	41	145	62	26.98	1.52	86	20	120	78	65	2.13	0	34.7	0	1	1	4	0	22	0	1	80	172	46	110	0.41
10	43	156	63	25.88	1.62	78	16	140	96	88	1.38	0	25	0	1.9	0	8	0	14	2	2	106	135	41	72.8	0.56
11	45	162	67	25.52	1.72	72	18	130	70	82	1.73	0	28.9	0	1.2	0	8	0	14	1	1	86	180	40	122.8	0.32

12	41	158	60	24.03	1.6	84	14	120	74	94	2.33	0	31.2	0	1.1	0	6	0	26	0	0	67	187	41	132.6	0.3
13	40	160	50	19.53	1.5	76	16	120	70	94	1.21	0	37.1	0	1.4	0	10	0	22	0	0	110	115	25	68	0.36
14	40	162	64	24.38	1.64	80	16	130	70	94	1.18	1	29.4	0	1.2	0	10	0	20	0	1	74	160	48	97.2	0.49
15	44	158	61	24.43	1.6	86	18	110	70	82	1.81	0	28.2	0	1.4	0	10	0	16	0	0	99	156	31	105.2	0.29
16	41	160	70	27.34	1.72	86	14	114	74	72	1.4	0	35.5	0	1.1	0	8	0	20	0	0	90	172	50	104	0.48
17	42	162	64	24.38	1.68	80	18	120	70	82	1.87	0	32	0	1.4	0	8	0	16	0	0	66	135	28	93.8	0.29
18	47	158	68	27.23	1.68	80	12	130	86	72	1.49	0	34.3	0	1.7	0	12	1	12	1	2	88	166	38	110.4	0.34
19	40	165	66	24.24	1.72	84	16	120	84	82	2.1	0	31.7	0	1.1	0	6	0	16	0	0	167	185	38	113.6	0.33
20	40	160	64	25	1.66	70	18	116	76	88	2.63	0	27.3	0	1.2	0	4	0	20	0	0	74	155	42	98.2	0.48
21	41	152	66	28.56	1.62	82	16	116	76	72	1.54	0	25.7	0	1.2	0	10	0	18	0	0	102	190	34	135.6	0.25
22	40	168	76	26.92	1.82	76	16	110	70	82	1.55	0	34.3	0	1.1	0	4	0	22	0	0	112	140	36	81.6	0.28
23	44	166	64	23.22	1.7	84	16	120	76	82	1.18	1	36.3	0	1.2	0	8	0	14	0	1	60	138	36	90	0.4
24	40	155	65	27.05	1.64	68	18	110	70	88	2.08	0	33.8	0	1.2	0	4	0	20	0	0	90	170	48	104	0.46
25	43	170	70	24.22	1.8	70	20	124	78	94	1.65	0	46.2	0	1.1	0	8	0	20	0	0	106	160	42	96.8	0.43
26	40	151	39	17.1	1.3	62	16	116	90	82	1.33	0	40.1	0	1.3	0	12	1	10	0	1	86	158	40	100.8	0.39
27	40	160	65	25.39	1.66	70	16	124	76	72	1.32	0	33.8	0	1.2	0	8	0	18	0	0	102	190	38	131.6	0.28

28	40	162	68	25.91	1.72	82	20	122	70	82	2.36	0	22.6	0	1.6	0	6	0	16	0	0	76	170	50	104.8	0.47
29	42	148	60	27.39	1.54	76	18	122	82	88	2.46	0	19.4	0	1.3	0	6	0	18	0	0	98	164	34	110.4	0.3
30	45	160	58	22.65	1.58	66	18	126	78	88	2.36	0	30.2	0	1.2	0	8	0	14	0	0	82	148	36	107.6	0.27
31	40	152	60	25.95	1.52	74	14	120	72	88	2.13	0	40.6	0	1.1	0	6	0	24	0	0	96	158	40	98.8	0.4
32	44	148	58	26.47	1.44	66	14	140	84	77	1.26	0	32.7	0	1.1	0	6	0	18	0	0	112	188	36	129.6	0.23
33	43	160	78	30.46	1.84	78	16	130	80	82	1.2	1	33.1	0	1.3	0	4	0	20	0	1	114	204	36	145.2	0.18
34	42	158	62	24.83	1.6	76	12	126	80	72	1.66	0	30.4	0	1.4	0	8	0	16	0	0	66	154	45	95.8	0.46
35	40	162	69	26.29	1.72	70	14	120	76	65	1.38	0	26.3	0	1.1	0	6	0	20	0	0	92	136	44	73.6	0.59
36	41	163	60	22.58	1.62	68	16	110	70	82	1.47	0	28.3	0	1	1	10	0	20	0	1	67	187	41	132.6	0.3

ANNEXURE - 4 b

Master Chart - Study Group

Sl. No	Age	Ht	Wt	BMI	BSA	Resting PR	Resting RR	Resting SBP	Resting DBP	Resting HR	valsalva ratio mean	Valsalva ratio function score	I-E	I-E F Score	HRR to stand	HRR to stand function score	BPR to standing	bpr tostand score	BPR to HG	bpr to hg score	Total score	S.TG	S. TC	HDL	LDL	HDL/LDL
1	42	148	54	24.65	1.44	72	16	110	70	82	1.7	0	9	2	1.05	0	12	1	22	0	3	85	133	39	77	0.5
2	43	160	50	19.53	1.52	70	18	120	68	82	1.55	0	8.6	2	1.07	0	14	1	20	0	3	48	151	51	90	0.6
3	45	148	58	26.47	1.54	66	12	130	78	102	1.3	0	18	0	1	2	12	1	36	0	3	80	166	35	115	0.3
4	51	148	63	28.76	1.56	74	14	130	80	82	1.08	2	16.6	0	1	2	10	0	24	0	4	118	194	43	127	0.3
5	60	148	67	30.58	1.64	80	12	138	88	88	1.38	0	14.8	1	1.02	1	8	0	28	0	2	70	225	39	172	0.2
6	47	163	67	25.21	1.72	64	14	120	78	88	1.19	1	12.1	1	1.06	0	10	0	12	0	2	152	209	32	147	0.2
7	60	154	46	19.39	1.44	74	12	110	60	82	1.35	0	10.8	1	1	2	10	0	22	0	3	157	186	30	125	0.2
8	58	156	50	20.54	1.5	70	16	130	82	82	1.32	0	11.9	1	1.02	1	8	0	18	0	2	102	186	37	129	0.3
9	45	156	67	27.53	1.7	72	14	140	90	88	1.25	0	15.4	0	1.17	0	8	0	16	0	0	87	155	33	105	0.3
10	48	155	70	29.13	1.7	80	12	138	80	94	1.25	0	23.1	0	1.07	0	10	0	22	0	0	60	210	32	136	0.2
11	43	160	70	27.34	1.76	88	10	110	70	102	1.26	0	6	2	1	2	10	0	20	0	4	103	158	33	104	0.3

12	44	160	72	28.12	1.78	76	14	128	80	94	1.3	0	5.5	2	1	2	8	0	18	0	4	92	194	36	140	0.3
13	53	150	68	30.22	1.64	84	16	140	80	95	1.15	1	25.2	0	1.05	0	16	1	26	0	2	72	230	34	182	0.2
14	42	158	54	21.63	1.48	86	18	130	86	112	1.08	2	19.5	0	1.08	0	6	0	28	0	2	82	150	35	99	0.4
15	60	160	55	21.48	1.56	66	18	136	90	77	1.21	0	10.7	1	1.07	0	12	1	32	0	2	91	153	31	104	0.3
16	58	148	60	27.39	1.54	68	16	136	82	72	1.17	1	6.9	2	1.03	1	8	0	28	0	4	86	170	36	117	0.3
17	42	163	87	32.74	1.98	86	14	140	90	95	1.21	0	20.8	0	1.08	0	8	0	32	0	0	102	252	34	98	0.3
18	45	158	66	26.43	1.7	88	12	134	90	102	1.09	2	24.5	0	1.2	0	10	0	26	0	2	76	202	40	147	0.3
19	58	150	43	19.11	1.32	78	12	110	90	88	1.08	2	17.8	0	0.97	2	10	0	24	0	4	142	190	32	130	0.2
20	55	152	68	29.43	1.68	68	20	142	74	77	1	2	9.8	2	1	2	6	0	16	0	6	82	160	36	108	0.3
21	48	151	61	26.75	1.56	76	14	110	70	82	1.23	0	17.2	0	1.02	1	10	0	18	0	1	194	195	30	126	0.2
22	48	148	60	27.39	1.56	80	16	132	80	88	1.25	0	18.6	0	1.03	1	6	0	22	0	1	120	180	34	122	0.3
23	60	147	73	33.78	1.68	82	12	130	80	88	1.14	1	23.3	0	1.22	0	12	1	20	0	2	101	120	35	65	0.5
24	55	145	70	33.29	1.64	70	18	130	78	82	1.2	1	12.3	1	1.03	1	6	0	20	0	3	96	140	38	83	0.5
25	53	154	58	24.45	1.58	82	12	118	76	88	1.1	2	5.2	2	1.08	0	8	0	28	0	4	180	192	32	124	0.3
26	53	149	49	22.07	1.42	80	18	140	90	88	1.34	0	7.2	2	1	2	10	0	30	0	4	213	143	34	66	0.5
27	47	157	63	25.55	1.64	84	14	120	94	94	1.04	2	12.7	1	0.91	2	2	0	20	0	5	166	183	50	96	0.5
28	55	151	63	27.63	1.62	86	16	124	78	94	1.02	2	18.3	0	1	2	8	0	18	0	4	156	182	40	111	0.4

29	43	163	66	24.84	1.76	90	12	130	78	102	1.24	0	9	2	1.16	0	8	0	18	0	2	110	172	36	114	0.6
30	46	152	50	21.64	1.5	90	15	120	76	112	1.24	0	22.3	0	1.08	0	6	0	20	0	0	80	186	40	130	0.4
31	53	157	70	28.39	1.74	80	20	140	88	82	1.1	2	27.5	0	1.08	0	8	0	24	0	2	94	172	38	114	0.4
32	45	148	60	27.39	1.58	80	10	130	90	88	1.16	1	25.1	0	1.16	0	8	0	26	0	1	88	210	36	156	0.2
33	47	154	68	28.67	1.68	76	12	124	76	82	1.05	2	15.1	0	1	2	8	0	18	0	4	82	150	34	100	0.3
34	53	160	73	28.51	1.82	78	14	130	88	88	1.19	1	14.2	1	1.16	0	12	1	18	0	3	110	218	34	162	0.3
35	53	152	69	29.86	1.68	76	16	140	90	82	1.18	1	8.3	2	1	2	6	0	26	0	5	101	183	56	107	0.5
36	51	157	70	28.39	1.72	82	16	120	84	88	1.12	1	16	0	1.12	0	4	0	16	0	1	167	185	38	114	0.3