CARDIAC FUNCTION ASSESSED BY SPECKLE TRACKING ECHOCARDIOGRAPHY IN OBESE ADOLESCENTS WITH HYPERLEPTINEMIA

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A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Cardio Thoracic Technology
November 2017

Thesis entitled "Cardiac function assessed by Speckle Tracking Echocardiography in obese adolescents with hyperleptinemia"

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ACKNOWLEDGEMENT

According to the cooperation between Naresuan University and the Faculty of Allied Health Sciences, the author is grateful to thank dean of Faculty of Allied Health Sciences, Professor Emeritus Taweesook Kanluan, and the President of Naresuan University, Professor Dr.Kanchana Ngourungsi, for arranging a grant.

The author would like to thank and offer my sincere gratitude to my advisor, Dr.Teonchit Nuamchit and co-advisors Dr.Teerapat Yingchoncharoen and Dr.Duangduan Siriwittayawan. I greatly appreciated the guidance, support, and encouragement that were offered throughout the length of the study. I also would like to give special thanks to Assistant Professor Dr.Sansanee Mekrungruengwong and Dr.Piyameth Dilokthornsakul for their assistance and helpful comments.

The author is sincerely grateful to the IDS Medical Systems (Thailand) company for excellent technical assistance and material support.

Unforgettably, I would like to thanks to the participants who voluntarily participated in the study, none of this would have been possible without them.

Finally, I would like to give special thanks to my mother for her endless love, understanding and support.

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Title	CARDIAC FUNCTION ASSESSED BY SPECKLE TRACKING ECHOCARDIOGRAPHY IN OBESE ADOLESCENTS WITH HYPERLEPTINEMIA
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Academic Paper	Thesis M.S. in Cardio Thoracic Technology, Naresuan University, 2017
Keywords	obesity, adolescents, leptin, speckle tracking, echocardiography, cardiac function, cardiac morphology

ABSTRACT

The purposes of this study were to use speckle tracking echocardiography to: 1) evaluate cardiac function of obese adolescents compared to non-obese adolescents, 2) assess cardiac function of obese adolescents with hyperleptinemia compared to obese adolescents with normoleptinemia and 3) analyze the relations of leptin levels and other clinical parameters on cardiac function in obese adolescents. Eighty-one participants aged 16-19 were recruited and classified as either belonging to a normal weight group (n=30) or an obese group (n=51). Demographic and anthropometric data as well as resting heart rate and blood pressure were assessed. Blood analysis, including complete blood count, lipid profile, serum uric acid and leptin levels was performed. The obese participants were then divided into two subgroups: 1) an obese with normoleptinemia group (obese NL, n=25) and 2) an obese with hyperleptinemia group (obese HL, n=26). Cardiac function parameters in all participants were measured by 2D-conventional echocardiography and 2D-speckle tracking echocardiography (STE). The echocardiographic parameters between the non-obese and obese groups and between the obese NL and obese HL groups were analyzed and compared. The results showed that there was a significant increase in the LV mass, LVMI, and RWT in the obese group compared to the non-obese group (103.20 ± 4.60 vs. 140.80 ± 5.04 gm, $p < 0.001$ for LV mass, 65.30 ± 2.27 vs. 74.35 ± 2.63 g/m^2 and 0.32 ± 0.01 vs. 0.36 ±

0.00 cm, for LVM and RWT, respectively, $p < 0.05$).The IVSDd, LVIDd and LVPWd were also higher in the obese group than in the non-obese group (0.77 ± 0.02 vs. 0.90 ± 0.02 cm, 4.43 ± 0.06 vs. 4.82 ± 0.05 cm and 0.72 ± 0.02 vs. 0.85 ± 0.01 cm, for IVSd, LVIDd and LVPWD, respectively, $p < 0.001$). However there was no significant difference in the main systolic and diastolic functional indices. 2D-STE demonstrated that the absolute average longitudinal strain in A2C was significantly lower in the obese group than in the non-obese group (19.5 ± 0.3 vs. 21.2 ± 0.4%, $p = 0.006$) as was the absolute average longitudinal strain in A4C (19.1 ± 0.3 vs. 20.6 ± 0.5%, $p = 0.01$). Absolute average longitudinal strain in APLAX was also reduced in the obese group (18.8 ± 0.4 vs. 20.7 ± 0.7%, $p = 0.01$). Absolute GLS was significantly lower in the obese group when compared with the non-obese group (19.1± 0.3 vs. 21.1 ± 0.3%, $p< 0.001$). It was shown that there was no significant difference in 2D-echocardiographic parameters between the obese HL and obese NL adolescents. However, absolute average longitudinal strain in A2C and absolute GLS were significantly lower in the obese HL group when compared with the obese NL group (18.6 ± 0.4 vs. 20.4 ± 0.5%, $p= 0.02$ for average longitudinal strain in A2C and 18.5 ± 0.3 vs. 19.8 ± 0.4%, $p= 0.03$ for absolute GLS in the obese HL group and the obese NL group, respectively). There were no significant differences between groups regarding absolute average longitudinal strain in A4C and absolute average longitudinal strain in APLAX between the obese HL group and the obese NL group. Multivariate regression analysis showed that the leptin level was associated with GLS with $\beta = 0.35$, $p = 0.02$ in the obese adolescents.

In conclusion, subclinical LV systolic dysfunction was found in obese adolescents. GLS was worsen in hyperleptinemia obese adolescents. It was also found that the leptin level was associated with a worsening of cardiac function.

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CHAPTER I

INTRODUCTION

Background

Obesity in adolescents is an essential general medical problem and is related with diminished lifespan (1-2). In the United States, the prevalence of overweight and obesity in teenagers has almost tripled in the previous two decades (3). It is reported that overweight adolescents tend to turned out to be obese as grown-ups (4).

Obesity in adolescence consequences in alterations of various systems. It is found that obesity in adolescents increases risk of hypertension, stroke, sleep apnea, type 2 diabetes mellitus, metabolic syndrome, cancers, disability, liver disease, and cardiovascular disease (5). The studies of Nordet, P., et al. showed a strong correlation amongst overweight and the increase in atherosclerosis in coronary arteries and aorta in children (6-7). By using computed tomography, Mahoney, et al. found that overweight was the strongest predictor of coronary calcium, a marker for plaque formation and also a risk factor for myocardial infarction (8). In addition, obesity is believed to be the cause of hypertension. Assessment of national survey information suggested that obese children and adolescents has a tendency of higher blood pressure (9).

In children, there are many evidence supporting the effects of obesity on cardiac morphology especially in left atrial (10-14) and left ventricular (LV) (10-13, 15-20) dimension. It has been shown that LA and LV dimensions are significantly greater in children with obesity compared to that of lean children. Several studies also indicated greater LV mass in children with obesity (10-11,15-16,18-24).

Changes in cardiac morphology might be a forerunner of cardiac dysfunction. Some studies demonstrated altered cardiac function including diastolic dysfunction (10,12,15-16,19-20,24-28) and systolic dysfunction at rest (28) and during exercise in obese children and adolescents (19). Many studies showed changes in left ventricular function, left atrial dimension, posterior wall thickness, relative wall thickness (RWT), left atrial (LA) index, left ventricular mass index (LVMI), doppler E/e' septal,

myocardial performance index (MPI) and doppler mitral EA ratio in obese subjects (10,12,15-16,19-20,24-28). Mahfouz, et al. found that aortic and pulmonary artery stiffness was increased in obese children when compared to lean children. Moreover, they had subclinical cardiac dysfunction (28). It was also found that left ventricular (LV) diameters and LV mass at rest in pre puberty boys were increased when compared with the lean subjects by using conventional and tissue Doppler echocardiography. The obese subjects had greater stroke volume, cardiac output and had lower diastolic tissue velocities than controls, indicating adaptive compensatory cardiac changes in obesity (19). However, the effects of obesity on cardiac function are controversial. Some studies indicated normal or even supernormal LV ejection fraction of obese subjects (29-31). Erick, et al. found a normal LV endocardial fractional shortening (%FS) with mildly decreased mid wall fractional shortening in severely obese participants. (29). In additional, some studies found a normal LV systolic function with reduced LV diastolic function in obese subjects (30). Moreover, supernormal systolic left ventricular function was also demonstrated (31). One of the explanations for this controversy is that cardiac dysfunction may be subclinical.

Normal ejection fraction obtained from conventional 2D-echocardiography might not fully demonstrate the contractile properties of the myocardium. Contractile abnormalities can be shown up when using more sensitive echocardiographic technique such as tissue Doppler, strain and strain rate analyses (32). Two-dimensional speckle tracking echocardiography (2D-STE) is an acceptable method for evaluating ventricular function and detecting sub-clinical dysfunction (33). However, the use of speckle tracking for assessing contractile function of obese myocardium is limited and the results are still controversial. It was shown that the obese children and adolescence had changed in longitudinal strain and strain rate (11,32) whereas some studies suggested changing in global strain and strain rate in obese children and adolescents (26). Furthermore, a study demonstrated the changes in longitudinal strain, radial strain, circumferential strain, LV twist and LV torsion in obese subjects (18).

The pathogenesis of obesity involves many mechanisms including insulin resistance, hyperinsulinemia, dyslipidemia, essential hypertension, endothelial dysfunction, inflammation, and level of a key hormone, leptin level, resulting to increased cardiovascular morbidity and mortality. One alteration is the change of leptin

hormone. Leptin is a 16 kDa hormone produced from the OB gene, play an important role in the weight regulation system. The absence of leptin results in increased appetite and diminished energy consumption and subsequently results in obesity (34). However, the obese population is characterized by hyperleptinemia, which indicates a leptin-resistant state (35). Hyperleptinemia is defined as baseline values above 20 ng/ml for boys and 24 ng/ml for girls (36). The prevalence of hyperleptinemia was 25.92% in obese Portuguese adolescents (37).

Several studies have indicated that hyperleptinemia is a powerful risk factor for cardiovascular disease related with obesity. There are supporting studies showing impacts of leptin on cardiovascular system. The role of leptin in the pathogenesis of atherosclerosis has been addressed. Leptin receptors have been found in human with atherosclerosis and several clinical studies demonstrated that plasma leptin concentration was related with atherosclerosis (38-39). Furthermore, leptin-induced endothelial dysfunction is correlated with elevated oxidative stress, reduction in anti-oxidant molecules and decreased NO bioavailability (40-41). In addition, recent studies illustrated the positive correlation between leptin and the risk for thrombosis in both In vivo study and obese individual (42-43). A clinical study revealed that obese patients had more leptin-sensitive platelets and had increased platelet aggregation comparing with their lean subjects (44).

Leptin also affects both cardiac structure and function. Previous studies showed the positive correlation between plasma leptin levels and cardiac hypertrophy. In vitro study, leptin was able to induce hypertrophy in neonatal rat ventricular myocytes (45-47) and human cardiomyocytes (48). Cardiac function assessed by echocardiography in leptin- deficiency mice was altered. Samuelsson AM, et al. investigated the influence of exogenous leptin administration in neonatal rats on parameters of cardiovascular function. Echocardiography indicated impaired left ventricular morphology and systolic function in 30-day female leptin-treated rats including increased LV internal diameter at diastole (LVIDd), LV internal diameter at systole (LVIDs), LV posterior wall at diastole (LVPWd), increased LV volume at systole, increased LV mass, decreased intraventricular septal thickness at diastole (IVSd) with decreased ejection fraction (LVEF) and fractional shortening when

compare with saline treated rats (49). These results provide information supporting the alteration of cardiac function in hyperleptinemia.

According to limited data about the effects of leptin on cardiac function in human as well as the controversial data on cardiac function of obese adolescents, the purposes of this study are 1) to evaluate cardiac function of obese adolescents by using speckle tracking echocardiography 2) to assess cardiac function of obese adolescents with hyperleptinemia comparing to that of participants with normo-leptinemia by using speckle tracking echocardiography and 3) to analyze the association of the leptin levels and other risk factors on cardiac function assessed by speckle tracking echocardiography in obese adolescents.

Objectives of Study

The purposes of this study are 1) to evaluate cardiac function of obese adolescents by using speckle tracking echocardiography 2) to assess cardiac function of obese adolescents with hyperleptinemia comparing to that of participants with normo-leptinemia by using speckle tracking echocardiography and 3) to analyze the association of the leptin levels and other risk factors on cardiac function assessed by speckle tracking echocardiography in obese adolescents.

Significance of Study

This project aims to investigate the alteration of cardiac function of obese adolescents who have hyperleptinemia and normo-leptinemia using speckle tracking echocardiography. The results of this experiment will bring more understanding of functional effect of obese with alteration in leptin level. It may be useful for prevention and proper management of obese in adolescence with specific condition.

Scope of Study

This study will be performed at Faculty of Allied Health Sciences, Naresuan University. The subjects will be recruited from students aged 16-19 year old. The recruitment of the participants will be performed by advertising in purposive selection style after the project has been approved from Naresuan University Ethical committee.

The sample size will be calculated resulting in 120 subjects in total, 45 for lean group, 45 for obese with normo-leptinemia and 30 for obese with hyperleptinemia.

Hypothesis of Study

Obese adolescents have worsened cardiac function compared to non-obese adolescents when assessed by speckle tracking echocardiography.

Obese adolescents with hyperleptinemia demonstrate worsened cardiac function assessed by speckle tracking echocardiography compared to obese adolescents without hyperleptinemia.

Leptin level is associated with cardiac dysfunction in obese adolescents.

CHAPTER II

REVIEW LITERATURE

Adolescents

1. Definition and classification

Adolescent is defined as "the developmental period of transitional between childhood and adulthood, from ages 10 to19" (50). Adolescence begins at 10 to 13 year old and ends around 18 and 22 year old. Developmentalists divided adolescence in two stages early and late stage. It is termed that "early adolescence refers to middle school or junior high school years and includes most pubertal change while late adolescence refers to the latter half of the second decade of life" (50).

2. Biological development in adolescents

A Pubertal and physicals changes during adolescence is a result of hormones. Hormonal changes of testosterone in male, estrogen in female including follicle-stimulating hormone (FSH), luteinizing hormone (LH), gonadotropin-releasing hormone (GnRh) and growth hormone are involved (51). FSH and LH which were secreted by pituitary gland, are regulated the levels of sex hormones. FSH activates follicle development in females and sperm production in males while LH regulates estrogen secretion and ovum development in females and testosterone secretion in males (52). Include in GnRh which were secreted in the hypothalamus. These hormones are stimulated by a negative feedback. If the level of sex hormones increases too high, the hypothalamus and pituitary gland were reduced sex hormones secretion. If the level of sex hormones falls too low, then production of the hormones increases (52).

Moreover the hypothalamus and the pituitary gland also secreted hormones that regulate growth, skeleton maturation and produce growth effects which called growth hormone. In addition, cortisol can also influenced growth during adolescence (53).

Obesity

1. Definition and classification

Obesity defined as "the excessive body fat associated with an increased risk of disease and premature death, which is the result of the positive energy balance and weight gain" (54). Nowadays, it is known that the excessive of fat, especially visceral fat and intra-abdominal, associated with risk of cardiovascular disease (CVD) (54). Body mass index (BMI) is a simple index of 'weight-for height'. The calculation of BMI is

Body Mass Index (BMI) = Weight in kg / Height (meters^2) or

Body Mass Index (BMI) = Weight in pounds x 703 /Height (inches^2).

The World Health Organization (WHO) states that for adults, "the healthy range for BMI is between 18.5 and 24.9. Overweight is defined as a body mass index of 25 to 29.9, and obesity is defined as a body mass index of 30 or higher"(55). However, there was a debate issue on developing different BMI cut-off points for different ethnic groups According to Asia-Pacific classification of obesity, in Asians, the cut-offs for overweight and obesity in Asians is 23.0 kg/m^2 and 25.0 kg/m^2 respectively (56). (Table 1)

Table 1 Classification of obesity in adults Asians according to BMI

Obesity class		BMI (kg/m^2)	Associated risk
Underweight		< 18.5	Low (but risk of other health problems increased)
Normal		18.5 – 22.9	Normal
Overweight		≥ 23	
At risk		23 – 24.9	Increased
Obese	I	25 – 29.9	Moderate
Obese	II	≥ 30	Severe

BMI still has several limitations; BMI does not give the information about fat distribution and cannot use to evaluate the body fat content. The simple method to estimate adiposity is waist circumference (WC). Waist circumference relates with

intra-abdominal fat content than BMI alone. Waist circumference is measured at the end of expiration, in the midline between the lower costal margin and the iliac crest. Increased waist circumference associated with increased risk in CVD (54).

2. Epidemiology

The prevalence of obesity is increasing in worldwide. Increasing prevalence of obesity is associated with the increasing in type 2 diabetes (type 2 DM), mortality rate and cardiovascular disease. Throughout the world, there are 22 million overweight children in aged <5 years. In the United States, the prevalence of overweight and obese adolescents has increased three-times in the last two decades (3). In Thailand, the prevalence of obese adolescents has increased from 22.2% in 2009 to 36.5% in 2010. Thailand National Statistical Office reported that in 2012, there are 10.9 % of obese children in aged < 5 years (57). It is reported that overweight adolescents tend to become obese as adults (4).

3. Pathophysiology and etiology of obesity

Obesity occurs when energy intake is more than energy expenditure for a longtime leading to increasing in adipose tissue and decreasing in lean body mass. There are many factors involving in obesity including genetic, hormone, biochemistry, and environment (58). Obesity in children is related to genetics factor rather than an environment factor. Fat distribution is a significant key in pathophysiology of obesity, for example, excessive abdominal fat is related with a higher risk of diabetes and CVD (58). The mechanism of obesity has been illustrated. This mechanism involves in skeletal muscle and adipose tissue metabolism, genetic and heritable factors, lifestyle and environmental factors, and level of a key hormone, leptin level (58).

3.1 Impaired skeletal muscle and adipose tissue metabolism

30-40 % of body weight is from the skeletal muscles, which is a major storage source of carbohydrate and have an essential role in glucose, amino acid, and lipid metabolism. Impairment of muscle oxidative metabolism leads to more storage of body energy. The important defect involving skeletal muscle metabolisms in obesity is decreased in glucose utilization and storage, leading to insulin resistance, and impaired fatty acid oxidation (58).

In addition, increasing in fat deposition and fat distribution plays a significant role in major systems and biochemical pathways. There are many studies indicate the role of non-esterified fatty acids involving in the development of insulin resistance. Recently, new adipokines involving in skeletal metabolism has been discovered. The role of leptin, adiponectin, resistin, visfatin, and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF- α) are being studied widely. They can provide a helpful information about the pathophysiology of obesity and its consequences, and they also give potential therapeutic targets (59)

3.2 Genetics

Heritable factors play a significant role of weight gain. Genetic and heritable factors have been contributed in 45–75% to develop obesity from family, adoptee, and twin studies. For example, alterations in the leptin receptor (LEPR) gene (missense or non-sense mutations) have been reported in about 3% of individuals with early onset obesity, especially in blood relation couples (60-61).

Although the genetic factors have a lower influence to weight gain, but several genetic variations can produced a clinically significant weight gain.

3.3 Lifestyle and environmental factors influencing obesity

Environmental and behavioral changes have highly influential in increasing rate of obesity. Data from migration studies have shown that the obesity risk increases dramatically when they are exposed to 'Western' lifestyles. The data from the UK National Food Survey (NFS) show that the average daily food intake in Britain has not changed significantly, but levels of physical activity have fallen (62).

Over the last 50 years, the availability of labor-saving devices, better transportation, changes in work patterns, lack of outdoor activities and sedentary lifestyles such as watching television and playing computer games have all been implicated in reducing physical activity levels. Some data supported that time spent with television and the use of cars, are linked to an increase in obesity risk, particularly in children (63).

3.4 Leptin

Leptin was produced within adipocytes and its serum levels correlate with the fat mass. Leptin receptors and signaling pathways are located in the hypothalamus. Administration of leptin in animals is results in a profound reduce in appetites and lose weight. The leptin-dificient (ob/ob) mouse, which is completely deficient in leptin and very obese. Because leptin can trigger the pro-opiomelanocortin (POMC) pathway and inhibit the neuropeptide-Y pathway resulting in reduced food consumption and increased thermogenesis (64)

4. Associations and complications of obesity

4.1 Metabolic syndrome

Metabolic syndrome is characterized by a group of medical disorders that often occurs together in one individual, which relates to the higher risk of cardiovascular disease and diabetes. In 1998, the American Diabetes Association (ADA) stated that "glucose intolerance, central obesity, dyslipidemia (elevated triglycerides (TG), reduced high-density lipoprotein (HDL), and elevated small dense low-density lipoprotein (LDL)), hypertension, increased prothrombotic and antifibrinolytic factors and a predisposition for atherosclerosis vascular disease" as parts of the metabolic syndrome (65).

Obesity is strongly and independently linked to all the other components of metabolic syndrome including insulin resistance, hyperinsulinemia, dyslipidemia (elevated TG, reduced HDL, and elevated LDL), alteration in adipocyte activity, type 2 DM, fatty liver, essential hypertension, endothelial dysfunction, renal insufficiency, polycystic ovary syndrome, inflammation, hypercoagulability and atherosclerosis resulting to increased cardiovascular morbidity and mortality. Most of the above definitions recognize central obesity [categorized by waist circumference (WC) or waist-to-hip ratio (WHR)] as the essential component of metabolic syndrome rather than generalized obesity, as the intra-abdominal fat content has a more stronger association with insulin resistance and hyperinsulinemia. The relationship between insulin resistance and cardiovascular disease was described by Reaven (Figure) (66).

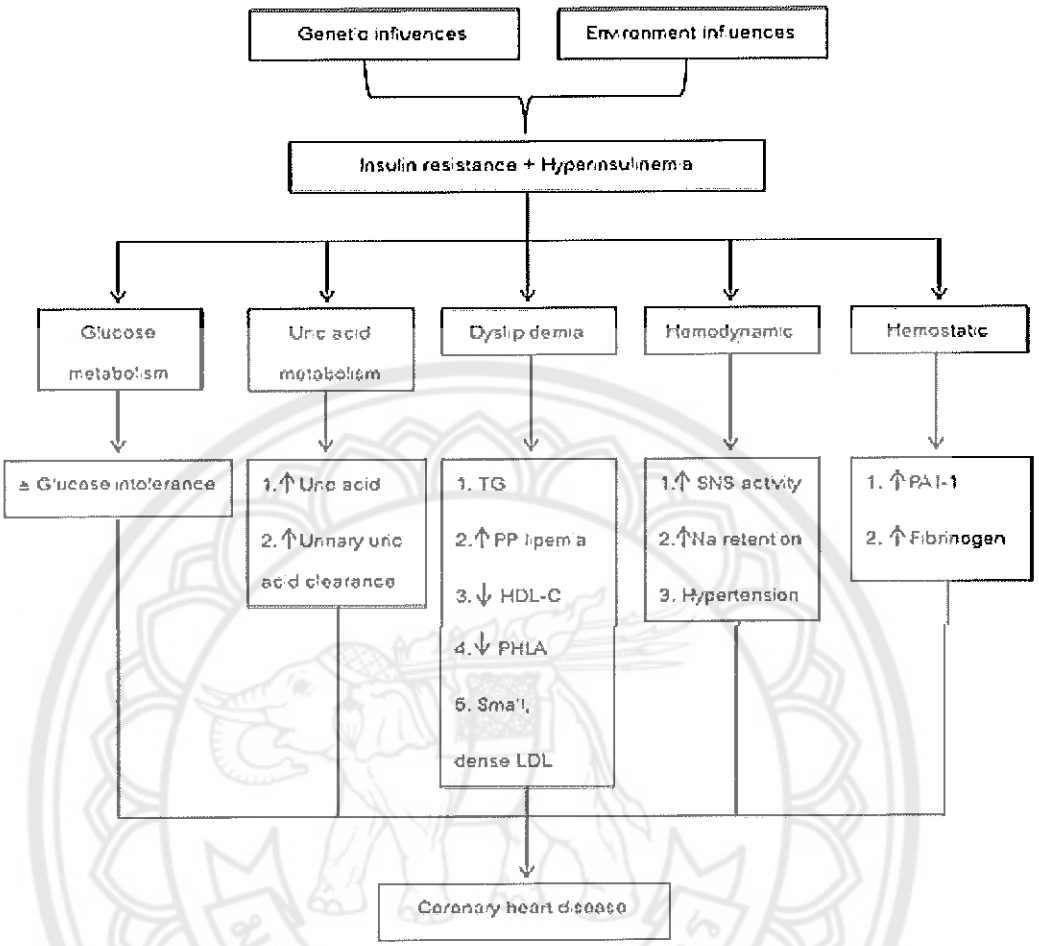


Figure 1 Relationship between insulin resistance and CVD

Note: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; PHLA, post-heparin lipolytic activity; PP, post prandial; SNS, sympathetic nervous system; TG, triglyceride

Source: Modified from Reaven, et al. (66)

Epidemiological data show that the increase in prevalence of obesity is strongly associated with increases in the prevalence of type 2 DM. The pathophysiological links between obesity, type 2 diabetes, and metabolic syndrome are well established.

Obesity is strongly linked to dyslipidemia, which is almost always included in the criteria for diagnosis the metabolic syndrome. The "lipid overflow–ectopic fat model" or the "hypertriglyceridic waist" model proposes that alterations in lipid metabolism play a vital role in the pathogenesis of metabolic syndrome (67).

4.2 Respiratory disease and obstructive sleep apnea

As the prevalence of obesity increases, clinicians are seeing increasing numbers of patients with obesity-related lung disease. Breathlessness is common respiratory symptom in obesity, this may be brought on by deconditioning or increased work of breathing due to the excessive weight being carried, rather than lung disease itself. It is also important to consider that the breathlessness may arise from cardiovascular disease. However, there are a number of respiratory conditions that are linked to obesity.

4.2.1 Lung function

Cross-sectional and longitudinal studies have demonstrated that increases in body weight are linked to a reduction in lung function. As BMI increases, the forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), total lung capacity, functional residual capacity, and expiratory reserve volume decrease, although these changes may not be evident until the BMI is >40 kg/m^2. Truncal obesity leads to direct mechanical effects by impeding movement of the diaphragm and chest wall and this may be reflected in a restrictive spirometry pattern (FEV1/FVC ratio >0.7). Respiratory muscle strength may also be reduced in obesity (reduced chest wall compliance and operating lung volumes may lead to reduced muscle efficiency). Ventilation/perfusion abnormalities and arterial hypoxemia at the lung bases also arise as a result of obesity.

4.2.2 Asthma

There is a clear link between obesity and a clinical diagnosis of asthma, although there is conflicting evidence as to whether airways hyper-responsiveness or allergy is increased by obesity. The incidence of asthma is increased by 50% in obese subjects. In patients with pre-existing asthma, increased BMI is linked with worse asthma control. The relationships between asthma and obesity are complex.

Although the mechanisms behind the links between asthma and obesity are poorly understood, it is clear that there is a link and some studies have shown that weight loss results in better asthma control.

4.2.3 Obstructive sleep apnea (OSA)

OSA is a condition in which there is intermittent and repeated upper airway collapse during sleep. Pharyngeal muscles fail to maintain upper airway patency, resulting in intermittent upper airway obstruction. This leads to hypoxemia, hypercapnia, and high blood pressure. Patients (or their bed partner) may report snoring, apneic spells and choking at night. They may feel unrefreshed after sleep and report excessive daytime sleepiness. OSA is associated with increased mortality from accidents (particularly road traffic accidents) and cardiovascular disorders associated with this condition. Obesity is a major risk factor for OSA: up to 70% of patients with OSA are obese, and approximately 40% of obese patients have OSA. Reduced operating lung volumes, increased airway collapsibility and increased fat deposition in the soft tissues of the neck contribute to the development of OSA.

4.3 Thrombo-embolic disease

Obesity more than doubles the risk of pulmonary embolism and deep venous thrombosis. This may be in part due to the more sedentary lifestyle that many obese subjects follow, but also due to venous stasis that arises from increased intra-abdominal pressure and the hypercoagulable state associated with obesity (increased levels of factor VIII and Von- Willebrand factor are seen in obese subjects)

4.4 Renal disease

Obesity is closely linked to chronic kidney disease directly and indirectly (Figure 2). In addition to its indirect effects on the renovascular system through its association with vascular risk factors such as hypertension and dyslipidemia, obesity has been shown to directly cause several functional alterations in renal physiology that may eventually lead to renal glomerulosclerosis (68).

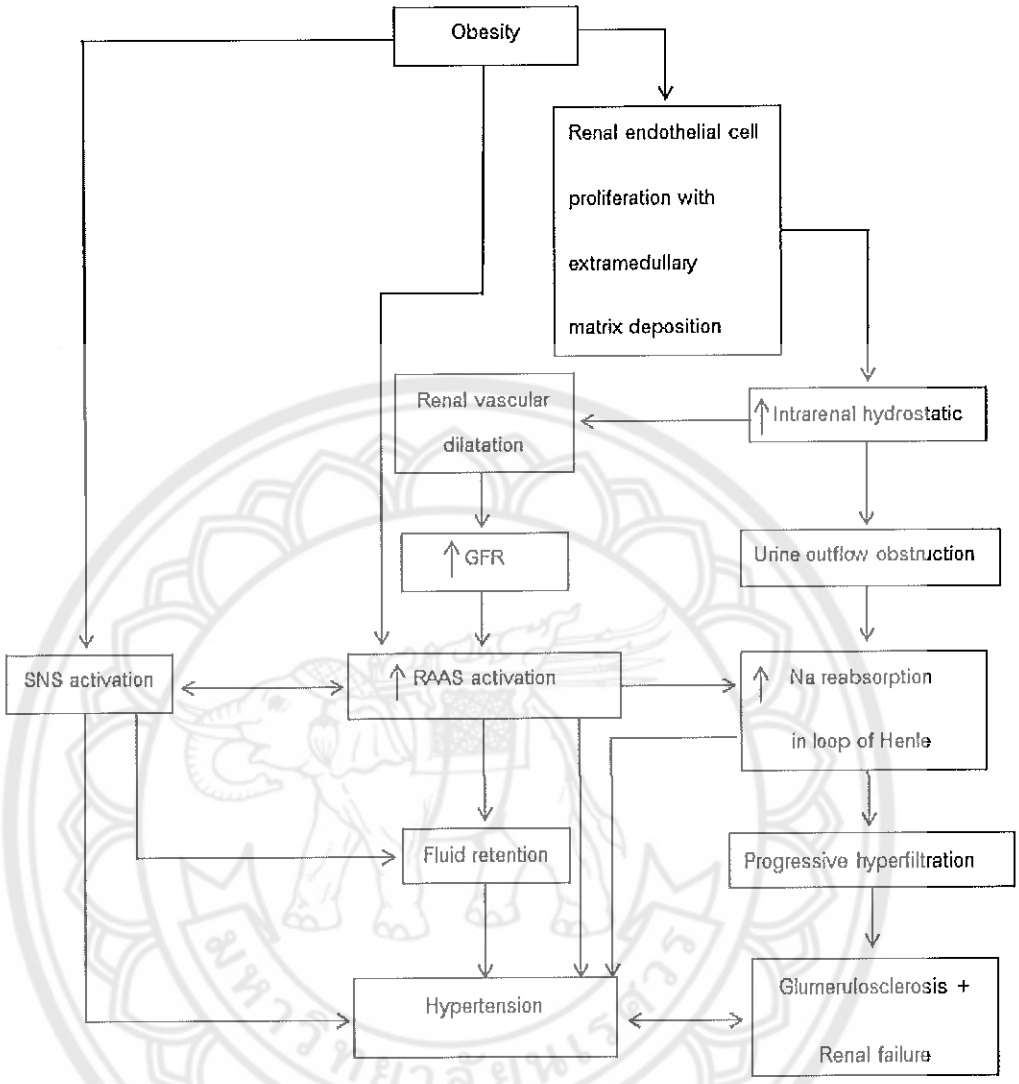


Figure 2 Mechanisms responsible for progressive renal failure in obesity and hypertension

Note: GFR= glomerular filtration rate, Na= sodium, RAAS= renin–angiotensin–aldosterone system and SNS= sympathetic nervous system

Source: Modified from Ejerblad, et al. (69)

In a large epidemiological study demonstrated that overweight (BMI $\geq$25 kg/m^2) was correlated with a significant three times increases in risk for renal failure while obesity (BMI $\geq$30 kg/m^2) and morbid obesity (BMI $\geq$35 kg/m^2) at any time during their lifetime was related to three to four times increases in risk for

diabetic nephropathy, but two to three fold risk elevations were shown for all major subtypes of chronic renal failure (68).

4.5 Gastrointestinal diseases

The prevalence of obesity and overweight elevated risk of gastrointestinal diseases (58). For example: Fatty liver, steatohepatitis, gastro-esophageal reflux disease, gallstones, cholecystitis and altered bowel habits.

4.6 Cancer and obesity

There is abundant evidence from studies showed that obesity is correlated with increased risk of cancer and death. Several studies have shown strong associations between BMI and various cancers but the exact mechanisms underlying this are still unclear.

4.7 Musculoskeletal

Overweight and obese subjects are at an increased prevalence of musculoskeletal disorders such as chronic joint pain and osteoarthritis (OA) leading to problems with posture, activities of daily living, and mobility. Normally affected in the weight-bearing joints areas such as knees, ankles hips, feet, and lower back but there is also increased incidence of problems in shoulders, hands, upper back, and neck.

4.8 Cerebrovascular disease

Obesity increases the risk of cerebrovascular disease independently as well through its association with hypertension, dyslipidemia, insulin resistance, and diabetes. The study from National Health and Nutrition Examination Survey (NHANES) IV demonstrated the significantly higher risk of cerebrovascular disease in middle-aged women when compared with the NHANES III population. The only difference between the two groups was higher BMI and waist circumference (70).

4.9 Psychosocial effects

Obese patients have many physical limitations and much psychosocial effects to overcome (58). For example depressive illness and anxiety, low self-esteem, daytime sleepiness, fatigue, body dysmorphic disorder and social stigmatization.

4.10 Miscellaneous

Obesity increases the risk of developing varicose veins and venous ulcers. Obesity is associated with increased intra-abdominal pressure and poor blood

flow in leg veins leading to enlargement of the veins and valve incompetence. Deep venous thrombosis also can damage the valves permanently. This, along with chronic lymphedema, increases the risk of recurrent cellulitis, leg ulcers, and venous eczema.

5. Effects of obesity on cardiac function

Obese adolescents were associated with a higher prevalence of disease and death such as type 2 DM, metabolic syndrome, liver disease, disability, cancers, hypertension, sleep apnea, stroke and CVD. Moreover, cardiovascular risk factors were increased in overweight children (BMI between 85th to 95th percentile), and in obesity (BMI > 95th percentile), indicating that even lowest levels of fatness lead to cardiovascular risk (5). The effects of obesity on cardiac function are shown below.

5.1 Cardiac dimensions

Obesity is an anabolic event. The obese child is defined as "an increase in lean body mass, acceleration of linear growth, enhanced skeletal maturation and advanced sexual development (71)". This somatic growth resulting in increased blood volume left ventricular (LV) hypertrophy and dilated cardiac chamber.

In obese adult autopsy study, indicated an increased in heart weight, biventricular enlargement and eccentric hypertrophy with independently of coronary artery disease and hypertension. Moreover, LV mass, chamber dimensions and wall thickness were directly associated with severity and duration of obesity. For example, Alpert, et al. discovered an increase in mean left ventricular end diastolic dimension (LVEDD) from 4.8 cm to 6.5 cm in 5 years duration in obesity (72). In addition, studies from magnetic resonance imaging (MRI) studies have confirmed these results (73).

Similarly findings were found in obese children and adolescents. Several studies revealed a larger and thicker cardiac chamber in obese participants compared to non-obese participants (12,74). In the study of Alpert MA. the result revealed a 15-20% higher in cardiac mass (correlated to height or body surface area (BSA)) in mild-moderate obesity compared to normal-weight children (75).

Several cross-sectional studies showed the correlations between body fat content and LV mass, wall thickness and chamber dimensions in obese adults (76), children and adolescents (15,36,77). Kono, et al. (77) demonstrated a directly

association (r=0.6) between body fat content and LV mass in 6 years old boys. Rowland and Dunbar found a positive correlation between BMI and LVEDD in early stage female adolescents (BMI range of 14-63 $kg \cdot m^2$) (78).

5.2 Hemodynamics and ventricular function at rest

Because the increased in heart size in the obese, it is not surprising that these individuals present higher values of resting stroke volume (SV) and cardiac output (CO) than lean participants. As shown in studies in children and adults, for example, Alexander and Alpert demonstrated an higher oxygen uptake, blood volume, SV and CO in morbid obesity and they found that oxygen uptake, blood volume, SV and CO are positively correlated with severity of obesity (75). Moreover, A reduce in these hemodynamic parameters is seen following weight loss (79).

Rowland and Dunbar (78) showed a strongly positive correlation between BMI and resting CO and SV in obese female adolescents. Giordano, et al. (80) found a greater resting CO in obese compared to their lean children (7.3 ± 1.9 vs. 5.7 ± 1.2 $L \cdot min^{-1}$, respectively ($p < 0.05$)). Consistent in the study of Pflieger, et al. and Chinali, et al. that reported a positive correlation between severity of obesity and both SV and CO by Doppler echocardiography in 6-15 years old participants and 14-20 years old participants, respectively (BMI range 16 to 57$kg \cdot m^2$) using Doppler echocardiography (80-81).

Although these variables of increased heart size and hemodynamics, obese patients often present evidence of reduced cardiac function, which is directly correlated with the severity and duration of fatness. These findings were first described in studies of longstanding morbid obesity adults, which. their hearts were reduced in left ventricular ejection fraction (LVEF), chamber dilatation, and increased in left ventricular end-diastolic pressures (LVEDP) with independently from hypertension and coronary artery disease (75).

The obesity induced cardiomyopathy occurs in approximately 10% of morbid obesity adult (BMI >40 $kg \cdot m^2$) and in more than 10 years of obesity (75). Recent echocardiographic studies demonstrates, however, that subclinical evidence of alteration in cardiac function, especially diastolic function, is often observed even in mild-to-moderate obesity adults (82). In the study of Wong, et al. the study showed that LVEF was no differ in all groups, however an inverse correlations between BMI

and myocardial systolic peak velocity (as measured by tissue Doppler imaging) were found in middle-aged men and women participants (r=-0.59). Moreover, similarly trends were found between the diastolic function parameters and BMI including isovolumetric relaxation time (IVRT) and tissue Doppler early diastolic tissue velocity (e') were decreased whereas the ratio of mitral inflow velocity (E/e') were increased with increasing BMI, these findings demonstrating the cardiac diastolic dysfunction and elevated left ventricular filling pressures in obese participants (83).

Similar trend of subclinical cardiac dysfunction were found in the pediatric populations. For example, Gutin, et al. revealed a negative relation (r = -0.37) between the percent body fat and left ventricular fraction al shortening (%FS) in 7 to 13 year old children (84). Mensah, et al. discovered a strongly negative correlation between midwall %FS and central adiposity in black adolescents (85). Chinali, et al. discovered a decreased LVEF in 14-20 year old obese participants compared to lean participants (15). In the study of Rowland and Dunbar, the result showed a negative correlation between %FS and BMI (r=-0.47) in females adolescents however, no participants showed a %FS below the normal range (28 percent) (78). Naylor, et al. discovered an increased in tissue Doppler E/e' in obese children compared to lean children (8.16 ± 0.26 versus 6.86 ± 0.20 $cm \cdot sec^{-1}$, respectively), proposing mildly increased left ventricular filling pressure from diastolic dysfunction (86). Echocardiographic results in the study of Mehta, et al. showed that there were no differences in %FS, LVEF and the ratio of E/e' between the study groups (obese group, overweight group and lean group), proposing no systolic dysfunction and no elevate in left ventricular filling pressures in the obese. However, some diastolic dysfunction were discovered, especially a lower septal e' (indicating an impaired cardiac relaxation) in overweight and obese children compared to theirs lean subjects (87).

Several factors have been included to account in systolic and diastolic dysfunction, which correlated to both severity and duration of fatness. Myocardial dysfunction in the obesity may be the result from chronic volume overwork, which called "high output" congestive heart failure and described by myocardial dysfunction and chamber dilatation which is result from chronic increases in CO. In addition, metabolic disorders in obesity may lead to cardiac dysfunction. Insulin resistance can

altered myocardial substrate utilization, resulting an increase in myocardial fatty acid oxidation and oxygen consumption and leading to a decrease in cardiac efficiency (88). Moreover, leptin secreted by adipocyte has been proposed to cause an alteration in myocardial function in rats (89).

The alteration in cardiac autonomic in obesity might play an important role in myocardial dysfunction but the mechanisms of these changes have not been clear (90). A summary of the mechanisms of obesity induced cardiovascular complications (Figure 3).

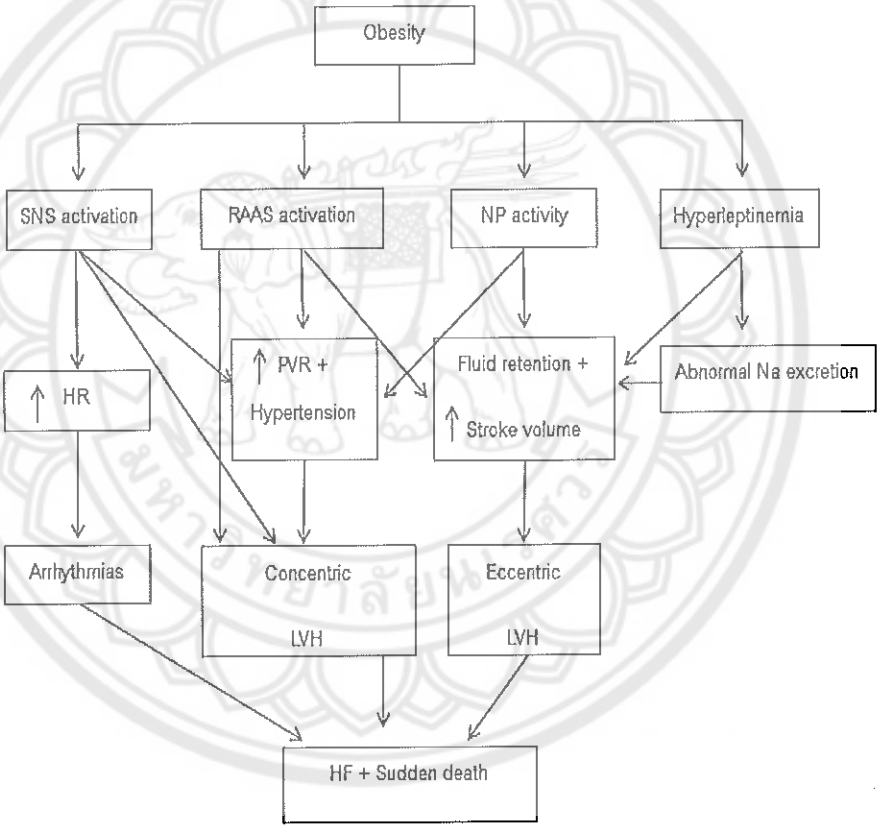


Figure 3 A summary of the mechanisms of obesity induced cardiovascular complications

Note: BP= blood pressure, HF= heart failure, HR= heart rate, LVH= left ventricular hypertrophy, Na= sodium, NP= natriuretic peptide, PVR= peripheral vascular resistance, RAAS= renin-angiotensin–aldosterone system and SNS= sympathetic nervous system

Source: Modified from Liatis, et al. (90)

Leptin

Leptin is a 16 kDa hormone made from the OB gene. Leptin plays a critical role in maintaining energy homeostasis. It has been identified as an important protein involved in the weight regulation system and the absence of leptin leads to increased appetite and decreased energy expenditure resulting to obesity. However, obesity as a result of a leptin deficiency has been rarely observed in children (34). A high plasma leptin level is correlated with total body fat mass in obese populations and animal models, and is considered to be a result of leptin resistance.

In addition, leptin is produced by the placental tissue, digestive epithelial, vascular smooth muscle and heart. Many studies have shown that leptin has direct effects on the cardiovascular system. Therefore, leptin is considered to be an important link between obesity and cardiovascular diseases. Much evidence has shown that leptin plays an important role in obesity-related cardiovascular diseases. The relationship between leptin and cardiovascular diseases are shown below.

1. Leptin and cardiovascular diseases

1.1 Hypertension

Clinical and animal studies have indicated a strong relationship between obesity and hypertension (91). One factor linking excess fat mass to hypertension might be the sympathetic cardiovascular actions of leptin.

Overactivity of the sympathetic nervous system (SNS), a common feature in the obese population, increases arterial pressure by causing peripheral vasoconstriction and by increase sodium reabsorption in renal tubular. Many Experiments show that an infusion of leptin causes sympathetic activation in several organs, such as brown adipose tissue, the kidney and the adrenal gland. The administration of leptin also increases plasma concentrations of norepinephrine and epinephrine. This sympathetic activation in response to leptin increases blood pressure.

Clinical evidence suggests that leptin is linked to mean blood pressure in lean populations with essential hypertension. In transgenic mice with hyperleptinemia, the high level of leptin resulted in higher arterial pressure and also increased lumbar and renal sympathetic nerve activity (92). Furthermore, leptin deficient obese people are overweight, but have hypotension. There are observations

in leptin deficient (ob/ob) mice supporting that arterial pressures were lower and the administration of leptin resulted in an increase in systolic blood pressure. These experiments show that in obese populations, leptin-induced sympathetic over activity plays a pathological role in the development of hypertension.

In addition there are other mechanisms might contribute to the development of obesity-related hypertension. As an example, leptin increased the amount of reactive oxygen species (ROS) in endothelial cells and stimulated the production of several pro-inflammatory cytokines, such as tumors necrosis factor (TNF)-α and interleukin (IL)-6, which promotes the hypertension and atherosclerosis. Additionally, leptin also increased some vasoconstrictor, such as endothelin-1 (ET-1) and angiotensin II (AngII). However, some studies have shown that leptin has a direct vasodilatory effect by inducing nitric oxide (NO) production in endothelial cells and smooth muscle cells (93-94).

1.2 Atherosclerosis

Many studies suggest a role for leptin as a contributor of atherosclerosis. In in vivo experiments, leptin deficient ob/ob mice were resistant to atherosclerosis, although leptin directly increased atherosclerosis in apolipoprotein mice. Furthermore, leptin receptors have been detected in human atherosclerosis, and clinical studies have shown that plasma leptin concentrations were associated with atherosclerosis in different patients (38-39). These results support that leptin signaling is involved in the promotion of atherosclerosis. The pro-atherogenic effects of leptin include the development of hypertension, oxidative stress, endothelial dysfunction, inflammation, platelet aggregation, endothelial cell migration and proliferation, and vascular smooth muscle cells (VSMC) proliferation, migration and calcification.

1.3 Endothelial dysfunction

Leptin-induced endothelial dysfunction is associated with increased oxidative stress and decreased NO bioavailability. Various studies have shown that leptin-treated mice have elevated levels of oxidative stress markers and a reduction in anti-oxidant molecules (41). Although leptin has a direct vasodilatory effect by inducing NO production, the acute effects of leptin can be quite different from long-term elevation. Korda, et al. (41) reported that the long-term (12 h) exposure of endothelial cells to leptin decreased bioavailable NO, despite a two-fold increase in

endothelial NO synthase (eNOS) expression in the endothelium. In obese mice, leptin increased eNOS expression and decreased intracellular L-arginine, resulting in eNOS-uncoupling and greater O^{-}_{2} production. Overproduction of O_{-2} fast-reacted with NO to form cytotoxic ($ONOO^{-}$), which resulted in a ($NO/ONOO^{-}$) imbalance, leading to impaired endothelial function (41)

1.4 Immune and inflammatory response

Leptin is involved in the regulation of immune function and cytokine secretion, by which promotes endothelial dysfunction and atherogenesis. Several immune cells, including T- lymphocytes, macrophages and monocytes, are involved in and play a key role in atherogenesis. Pro-inflammatory cytokines, such as IL-2, IL-6, IL-12 and TNF-α, are mediated the immunological cascade that leads atherosclerosis. Many studies have shown that leptin and pro-inflammatory cytokines show same modulation and a shared association with atherosclerosis (95). At the same time, leptin also regulates immune functions in humans and rodents. Leptin stimulates central T-cell production and a peripheral shift in favor of T helper (Th) 1 adaptive immune responses (proinflammatory). Then, further augmenting the inflammatory pathway that lead to atherosclerosis, leptin also promotes intimal monocyte recruitment, induces foam cell formation and induces the secretion of atherogenic cytokines (96). In other words, leptin itself can be considered a pro-inflammatory cytokine.

1.5 Vascular cell proliferation

Vascular cell proliferation is associated with inflammation, oxidative stress and thrombosis, leading to atherosclerosis. Leptin has been identified as a main factor that induces vascular cell proliferation. In vivo and in vitro studies have shown that leptin promotes growth and matrix remodeling through vascular smooth muscle cells (VSMC) proliferation and endothelial cell matrix metalloproteinase (MMP) expression (97). ROS might be a second messenger in leptin-induced smooth muscle cell proliferation. Ling, et al. showed that leptin induced human aortic smooth muscle cell (HASMC) proliferation and MMP-2 expression through the protein kinase C dependent activation of NADPH oxidase with subsequent activation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) and nuclear factor kappaB (NF-kB) pathways (98).

Angiogenesis, which is involved in endothelial cell proliferation and remodeling, plays an important role in the growth of atherosclerotic plaques. Recently evidence has shown that leptin stimulates angiogenesis by inducing endothelial cell and matrix proliferation through stimulation of endothelial expression of vascular endothelial growth factor receptors (VEGFR), MMP and tissue inhibitors of metalloproteinase in atherosclerosis

In addition, a recent study indicated that hyperleptinemia increased caveolin-1 protein expression in vascular endothelial cells, which can contribute to the development of atherosclerosis and can impair leptin signaling (99).

1.6 Thrombus formation

The formation of a thrombus is a cause of an acute coronary event, particularly in obese individuals. Recent studies have given evidence for a direct link between leptin and the risk for thrombotic complications in obese individuals (42-43). An in vivo study has shown that lower levels of thrombus formation were observed in ob/ob mice, but was reversed with leptin supplementation (42). Similarly, wild-type mice that were treated with a leptin-neutralizing antibody before inducing carotid artery injury with ferric chloride showed prolonged times to thrombotic occlusion (43).

Leptin promotes thrombosis by increasing platelet aggregation (44). Leptin receptor (Ob-Rb) was found on platelets, and leptin enhanced platelet aggregation in the presence of this receptor (42). A clinical study showed that platelets from obese patients were leptin-sensitive and had enhanced adenosine diphosphate-induced aggregation compared with the platelets from lean patients (44). A more interesting observation is that high concentrations of leptin corresponding to levels in obese individuals increased platelet aggregation, whereas lower concentrations did not. This result suggests that the prothrombotic effect of leptin might be limited to obese hyperleptinemia individuals.

In addition, the pathophysiology of thrombosis shows that tissue factor (TF) plays a pivotal role in triggering the formation of intracoronary thrombi. A recent study has shown that leptin promoted expression of TF and cellular adhesion molecules (CAM) on human coronary endothelial cells (HCAEC), which was modulated by eNOS-production of oxygen free radicals through the activation of

nuclear factor (NF)-kB (100).Taken together, these results provide support to the view that leptin acts as a unique link between obesity and cardiovascular events.

1.7 Cardiac hypertrophy and remodeling

1.7.1 Cardiac hypertrophy

Recently evidence has shown the direct effect of leptin on cardiomyocyte hypertrophy. Clinical data show that increased plasma leptin levels are positively correlated with cardiac hypertrophy (101). In vitro studies have shown that leptin induces hypertrophy in a concentration-dependent manner in cultured neonatal rat ventricular myocytes (45-47). Evidence shows that the activation of MAPK mediates the hypertrophic effect of leptin in both rat and human cardiomyocytes (48). The mechanism of leptin-induced hypertrophy is, however, unclear.

It has been well documented that ROS mediate cardiac hypertrophy of well-established hypertrophic agents, such as Angiotensin II (AngII), norepinephrine and ET-1 (102). Previous study showed that leptin-induced neonatal rat cardiomyocyte hypertrophy occurred through a mechanism involving ET-1 and ROS generation, whereas the blockade of the endothelin receptor type A (ETA receptor) by Atrasentan (ABT-627) could not completely abolish leptin-induced ROS production and hypertrophy(46). In another study, the hypertrophic effect induced by ET-1 or Ang II was inhibited by antibodies to either leptin or leptin receptors. However, antagonists of neither ET-1 nor Ang II affected the hypertrophic effect of leptin (45).Thus, there appears to be a marked difference between the mechanisms underlying the direct hypertrophic effect of leptin versus its endogenous role in mediating the hypertrophy produced by ET1. Peroxisome proliferator-activated receptor a (PPARa) is a key regulator of fatty acid metabolism in the heart. In vivo and in vitro studies have found that PPARa overexpression induced cardiomyocyte hypertrophy (103-104). Recent study showed that leptin upregulated PPARa expression and activity; treatment with the PPARa antagonist, GW6471, significantly inhibited leptin-induced hypertrophy and ROS production in cultured neonatal rat cardiomyocytes. These data suggest that the PPARa pathway mediates leptin-induced cardiac hypertrophy (104). It is well known that hypertrophic cardiomyocytes are usually associated with morphological changes in cell shape and remodeling of the actin cytoskeleton. The Rho-ROCK pathway, a pathway involving a downstream

target protein of small GTP-binding protein, is one of the major pathways that affects cell morphology and regulates transcription factors, leading to cellular hypertrophy. Zeidan, et al. (47) showed that the Rho-ROCK pathway and the G/F-actin ratio were important components of leptin-induced hypertrophic signaling in cardiomyocytes. Furthermore, inhibition of Rho and ROCK completely abrogated the activation of ERK1/2 and p38 MAPK in response to leptin, suggesting that the Rho A/ROCK pathway is an upstream mediator of leptin-induced p38 and ERK1/2 phosphorylation (105). Subsequently, researchers in this group focused on the role of lipid rafts/caveolae in the response of cardiomyocytes to leptin. The data showed that the p38 MAPK translocation to the nucleus induced by leptin was dependent on intact caveolae, activity of the RhoA pathway and actin dynamics. Although the phosphorylation of ERK1/2 was similar to p38 MAPK, it was associated with only minimal and insignificant nuclear translocation (106).

In contrast, some studies have shown leptin repletion to be anti-hypertrophic in ob/ob hearts. A 4–6 week leptin infusion reduced weight and reversed LV hypertrophy in ob/ob mice (107). These paradoxical results suggest that more or less leptin can contribute to the development of hypertrophy, although a leptin deficiency is rarely seen in obese patients.

1.7.2 Extracellular matrix remodeling

The myocardial extracellular matrix (ECM) commonly consists of structure (collagen, elastic fibers) and adhesive (fibronectin, laminin) elements. Turnover of collagen is regulated by the family of MMP (108). Alterations in the composition and structure of the ECM make an important contribution to changes in cardiac size, structure and function in heart failure (108). Leptin signaling might contribute to the regulation of the cardiac ECM in obesity, because increased fibrosis has been described in the hearts of ob/ob mice (109), Zucker (fa/fa) rats and diet-induced obesity (DIO) mice (110). However, to date, reports of the effect of leptin on ECM remodeling are few. It has been shown that leptin selectively regulates different forms of collagen. For example, leptin has been shown to increase procollagen type III and type IV mRNA and decrease procollagen type I levels without changing total collagen synthesis in primary human pediatric ventricular myocytes (111). In neonatal rat fibroblasts, leptin increased intracellular and secreted procollagen type I levels, but

decreased procollagen type III levels (112). Furthermore, leptin enhanced MMP-2 expression and activity in neonatal rat fibroblasts (112) and primary human pediatric ventricular myocytes (111). Further studies are needed to confirm leptin's effects on cardiac remodeling in obesity.

1.8 Heart metabolism

The normal adult heart uses both glucose and fatty acids (FA) for adenosine triphosphate production. The energy substrate switches between FA and glucose according to nutritional state, physical activity and diurnal rhythms. An imbalance in metabolism substrate utilization leads to cardiac function detriment. Recently, studies carried out in genetic obesity models have observed that myocardial metabolism in obesity is characterized by an increase in FA utilization and decrease in glucose utilization (64). These changes are associated with increased myocardial oxygen consumption and decreased cardiac efficiency (113).

Accumulating evidence has shown the potential for leptin to contribute to this imbalance of metabolism substrate utilization. In isolated working rat hearts, a leptin infusion increases FA oxidation and triacylglycerol lipolysis, although this effect was independent of changes in the 5'-AMP-activated protein kinase (AMPK)–acetyl-CoA carboxylase–malonyl CoA axis (64). Sharma, et al. (114) showed that leptin stimulated fatty acid oxidation by a STAT3-NO-p38 MAPK dependent mechanism, which was upstream of the mitochondria. In an in vitro study, the treatment of murine cardiomyocytes with leptin has been shown to cause increases in FA uptake, which corresponded with increased cell surface CD36 levels, as well as elevated fatty acid transport protein 1 and CD36 protein content (115). These effects of leptin are usually considered to be unfavorable. Notably, in this in vitro study, the FA oxidation was increased after a short-term (1 h) leptin treatment, whereas it was decreased with a long-term (24 h) treatment, leading to intracellular lipid accumulation that can result in lipotoxic damage in heart failure (115). This metabolic change was dependent on the AMPK–acetyl-CoA carboxylase–malonyl CoA axis. This finding is supported by a rodent model study (115). The hearts of Zucker rats showed an increased uptake of FA without an upregulation in cardiac FA oxidation, eventually leading to an accumulation of myocardial triglycerides and lipotoxicity (116). In Zucker rats, the enzymes involved in FA oxidation, such as acyl-CoA oxidase and

carnitine palmitoyltransferase1, are at attenuated levels. Hydrolysis of the increased triglycerides to fatty acyl-CoA activated the iNOS expression, which leads to the cardiomyocyte apoptosis.

In addition, the hydrolysis effect indirectly increases ceramide synthesis, which is involved in the development of cardiomyocyte hypertrophy (117). The observation of lipid accumulation in human hearts provides strong evidence for the pathophysiological significance of this lipotoxicity phenomenon.

Furthermore, there are some discrepancies in the effect of leptin on glucose utilization. In HL-1 cardiomyocytes and an isolated working heart, leptin had no effect on glucose utilization. In contrast to these findings, a study of Langendorff-perfused rat hearts showed that leptin stimulated glucose uptake (118). Additionally, in Zucker rats, a reduction in glucose metabolism has been described even before the onset of hyperglycemia (119). These differences show that the various metabolic effects of leptin need to be further investigated.

In conclusion, clinical and experimental studies have established the direct effects of leptin on obesity-related cardiovascular diseases. The involved mechanisms that mediated these effects, such as sympathetic nerve activity, endothelial dysfunction, oxidative stress, hypertrophy and remodeling, as well as metabolism shift, have been elucidated in this review. However, some paradoxical results and several intriguing questions are not yet fully understood. For instance, whether leptin has a hypertrophic or anti-hypertrophic effect on the myocardium; whether leptin peripheral resistance exits or not; and how leptin regulates glucose utilization. These issues could be resolved by appropriate future investigation in this area.

Assessment of left ventricular systolic function by echocardiography

1. Two-dimensional echocardiography

The echocardiographic assessment of cardiac mechanics can be divided into methods that quantify global function and those that assess regional contractility.

1.1 LV volume and LV ejection fraction

The most common functional surrogate is LV ejection fraction (LVEF), the percentage of chamber volume ejected in systole, which is well measured

by echocardiography. The first step in calculating LVEF is correct visualization of endocardial borders. Although in most cases this can be done by standard approach, it is sometimes necessary to use ultrasound contrast for LV opacification; this is especially true in the morbidly obese, in the presence of apical masses, and during stress echocardiography (120).

To obtain LV volumes and EF, one can use formula that have been validated for M-mode measurements and single and biplane 2-dimensional (2D) assessments, but these are based on geometric assumptions and so work best in symmetric ventricles (120-122). For maximal accuracy, particularly with ventricular aneurysms or other asymmetric abnormalities, 3-dimensional (3D) echocardiography should be used, which has an accuracy rivaling magnetic resonance imaging (MRI) (123-124).

Although EF is universally used, it is limited by its sensitivity to preload and afterload, resulting in the false reassurance of a high EF in severe mitral regurgitation and the low reversibly EF with severe aortic stenosis. Because of this, much effort is given to developing less load-dependent methods to measure true contractility, the most accurate of which requires continuous acquisition of ventricular pressure and volume data during sudden preload change. From these data, several indexes can be calculated, with end-systolic elastance (125) and preload recruitable stroke work being the most popular such as the LV power (a product of peak systolic flow and pressure), indexed to the square of end-diastolic volume, accurately estimates end-systolic elastance (126-127) and the myocardial performance index.

1.2 Assessment of regional myocardial contraction

Regional function is commonly assessed by dividing the LV into 17 segments and assigning a qualitative grade to each ranging from 1 (normal) to 5 (aneurysmal) (128). This method had changed little from the initial descriptions of wall motion abnormalities by echocardiography (129) and is observer-dependent. However, novel insights into myocardial structure and new echocardiographic modalities may dramatically change the way we assess regional function.

2. Tissue Doppler imaging (DTI)

By eliminating the wall filter and using low-gain amplification, it is possible to display myocardial tissue velocity either as a PW Doppler signal at a

specific place in the myocardium, a color map, or M-mode. Imaged from the apex, it is possible to visualize long-axis motion towards the apex in systole, reversing itself in diastole, with complex biphasic movements during isovolumic periods. Newer machines can simultaneously capture multiple colors DTI planes arranged around a common axis. Analysis of such simultaneously acquired DTI data over several LV regions has been used to assess LV synchrony (130-132) and as an additional tool during stress echocardiography (133). Doppler tissue imaging assessment of contractility has a number of limitations: 1) like all Doppler methods, it can only measure the component of motion parallel to the ultrasound beam; 2) velocity may reflect gross translation (as in right ventricular volume overload) (134), rather than actual local contraction; and 3) even akinetic segments show motion due to tethering of adjacent normally contracting segments.

3. Strain and strain rate measurements

Myocytes lie in planes parallel to the long axis of the heart. Within these planes, myocyte orientation varies. It is circumferential at the mid-wall, but rotates clockwise (as viewed from the outside) to form a -60° left-handed helix in the epicardium and counterclockwise to a +60° right-handed helix in endocardium (135). With these issues in mind, it is obvious that new imaging modalities should differential between various segments and layers of the heart to fully assess contractility.

Strain is a mathematically complex construct, reflecting local tissue deformation. Deformation can occur by linear compression or expansion along the x-, y-, or z-axes.

Strain (ε) is a change of dimension divided by the initial dimension, $\Delta L/L0$, where L0 is the end-diastolic dimension of a myocardial segment, so Δ is negative for long-axis strain (myocardium shortens) and circumferential strain and positive for radial strain (wall thickening). It is expressed as a percentage.

Strain Rate (ε') represents the myocardial deformation rate. It is expressed as $seconds^{-1}$

3.1 DTI-derived strain measurements

DTI-derived strain and strain rate have been recently proposed as new parameters of regional LV function. Doppler tissue imaging– obtained strain and strain

rate have the time resolution capability far superior to any other non-invasive method. Doppler tissue imaging– derived strain accurately measures longitudinal deformation of the heart (136) and is sensitive to early stages of ischemia (137). Strain rate imaging is also useful in the assessment of myocardial viability after infarction (138). Normal age-stratified values for DTI, myocardial displacement (the integral of DTI velocity, reflecting total movement of the myocardium during systole), strain, and strain rate have been published (139). In addition, strain rate should be more sensitive to pathology than strain (because prolonged contraction may yield normal strain despite low strain rate), but in practice, strain rate data are noisier, whereas the integration process makes strain data more reproducible. Finally, strain and systolic strain rate show some preload dependency due to their sensitivity on initial diastolic dimension through the Starling mechanism.

3.2 Speckle tracking echocardiography (STE)

To avoid this angle dependence of all Doppler techniques, STE has recently been introduced. Speckle-tracking echocardiography is a new noninvasive ultrasound imaging technique that allows for an objective and quantitative evaluation of global and regional myocardial function independently from the angle of insonation and from cardiac translational movements (140-141). Speckle-tracking echocardiography is based on an analysis of the spatial dislocation of speckles (defined as spots generated by the interaction between the ultrasound beam and myocardial fibers) on routine 2-dimensional sonograms.

By tracking the displacement of speckles during the cardiac cycle, speckle-tracking echocardiography allows semi-automated elaboration of myocardial deformation in 3 spatial directions: longitudinal, radial, and circumferential. In addition, speckle-tracking echocardiography offers an evaluation of the occurrence, direction, and velocity of left ventricle (LV) rotation (142). The semi-automated nature of speckle-tracking echocardiography guarantees good intraobserver and interobserver reproducibility (143). Longitudinal strain represents myocardial deformation directed from the base to the apex. During systole, ventricular myocardial fibers shorten with a translational movement from the base to the apex; the consequent reduction of the distance between single kernels is represented by negative trend curves (A) (144). Through longitudinal strain analyses in 4-chamber, 2-chamber, and

apical long-axis views, both regional (relative to each of the 17 LV segments) and global strain values (global longitudinal strain) can be obtained. Global longitudinal strain recently has been validated as a quantitative index for global LV function (145).

Radial strain represents radially directed myocardial deformation, ie, toward the center of the LV cavity, and thus indicates the LV thickening and thinning motion during the cardiac cycle. Consequently, during systole, given the progressive radial propulsion of single kernels, radial strain values are represented by positive curves (B). Radial strain values are obtained by speckle-tracking echocardiographic analysis of both basal and apical LV short-axis views (146).

Circumferential strain represents LV myocardial fiber shortening along the circular perimeter observed on a short-axis view (C) (146). Consequently, during systole, for circumferential speckle-to-speckle distance reduction, circumferential strain measurements are represented by negative curves. As for longitudinal strain, it is possible to obtain a global circumferential strain value.

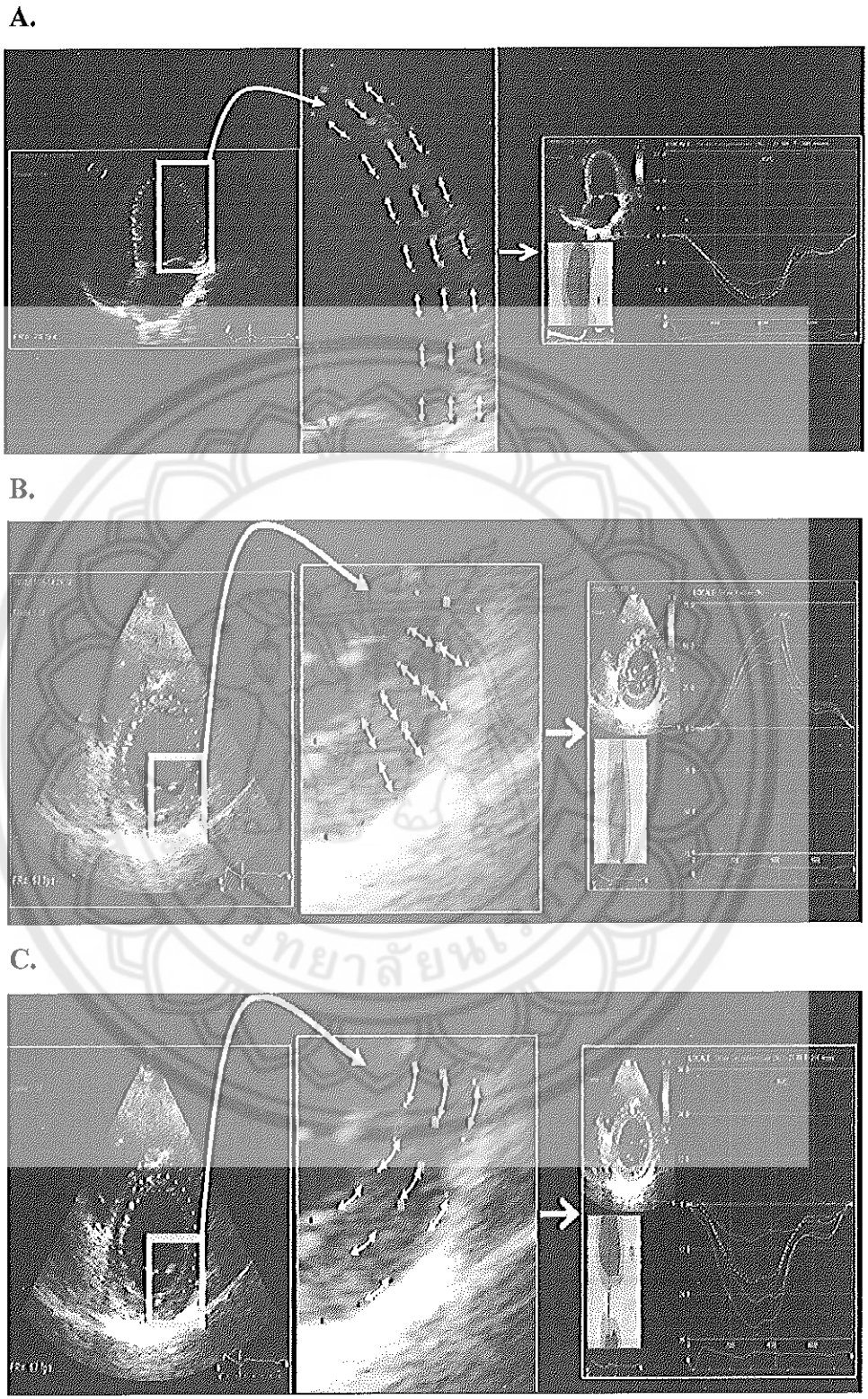


Figure 4 Speckle-tracking echocardiographic analysis of myocardial deformation showing measurements of longitudinal strain (A), radial strain (B) and circumferential strain (C) (147)

3.2.1 Obtaining Strain Parameters

Images for speckle-tracking echocardiographic analysis, currently performed offline, are obtained and recorded by using conventional 2-dimensional gray scale echocardiography. Care must be taken to obtain true apical and short-axis images using standard anatomic landmarks in each view and to avoid foreshortening of the analyzed myocardial structure, thus allowing a more reliable delineation of the endocardial border. The optimal frame rate for the 2-dimensional image acquisition is set between 60 and 110 frames per second (142,148). It is recommended to begin with speckle-tracking echocardiographic analysis of an apical long-axis chamber view to select the frame corresponding to the aortic valve closure, which is a useful reference for the subsequent analysis. Particular attention should be paid to making the LV cross section as circular as possible. Recordings are processed using specific acoustic-tracking software usually available on dedicated workstations, allowing for an offline semi-automated analysis of speckle-based strain. The endocardial surface of the myocardial segment analyzed is manually traced in apical and/or short axis views by a point-and-click approach. An epicardial surface tracing is then automatically generated by the system, thus creating a region of interest. After manual adjustment of the region of interest width and shape, the software automatically divides the region of interest into 6 segments, and the resulting tracking quality for each segment is automatically scored as either acceptable or unacceptable, with the possibility of further manual correction. Segments for which no adequate image quality can be obtained are rejected by the software and excluded from the analysis. Last, once the region of interest is optimized, the software generates strain curves for each selected myocardial segment. From these curves, the operator can obtain regional and global (by averaging values observed in all segments) peak and time-to-peak values.

If the longitudinal strain analysis is performed in all 3 apical views, the software automatically generates a topographic representation of all 17 analyzed segments (bull's eye; Figure 4).

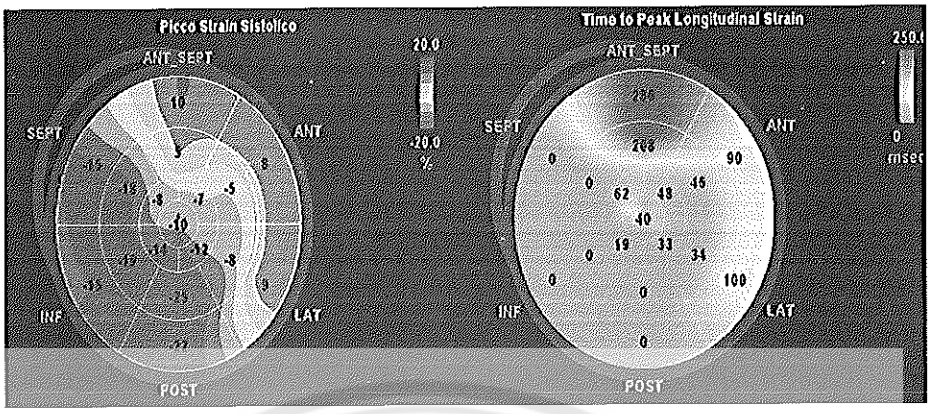


Figure 5 Topographic bull's-eye representations of different strain measures Representation of longitudinal strain (left) and time-to-peak longitudinal strain (right) in a patient with severe stenosis of the left anterior descendent artery (147)

3.2.2 Clinical Applications

In general, speckle-tracking echocardiography may allow an evaluation of myocardial systolic and diastolic dynamics across a broad range of physiologic and pathologic conditions beyond traditional echocardiographic techniques. For example, not only has a good correlation between longitudinal strain and the left ventricular ejection fraction (LVEF) been shown in several studies (145, 149), but in addition, longitudinal strain provides a quantitative myocardial deformation analysis of each LV segment, also allowing for early systolic dysfunction detection in patients with a preserved LVEF(150).

1. Hypertension

Arterial hypertension is an ideal model for assessing the changes in different varieties of deformation occurring hand in hand with the development of LV concentric geometry (concentric remodeling and concentric LV hypertrophy). Speckle-tracking echocardiography has furthered the understanding that the interaction of the different deformations is much more complex under these circumstances. In particular, it seems that longitudinal and radial strain are impaired when circumferential strain is still normal and LV torsion, also maintained in the normal range, acts as mechanistic compensation to preserve a normal ejection fraction (EF).

2. Diabetes

In asymptomatic diabetic patients with a preserved LVEF, it has been shown that speckle-tracking echocardiography has the potential for detecting subclinical LV systolic dysfunction, which is unmasked by the alteration of longitudinal strain. Speckle-tracking echocardiography might provide useful information about the development of subclinical myocardial dysfunction in the diabetic setting before the overt appearance of diabetic cardiomyopathy (151-152).

3. Coronary Artery Disease

Choi, et al. (153) reported that a lower longitudinal strain value in asymptomatic patients without wall motion abnormalities is a strong predictor of stable ischemic cardiomyopathy. Studies in patients with acute myocardial infarction found that longitudinal strain is related to peak levels of cardiac troponin T33 and the LV infarct size (154). Moreover, when measured immediately after reperfusion therapy, longitudinal strain is an excellent predictor of LV remodeling and adverse events (155). In addition, A radial peak strain cutoff value of 17.2% predicts LV functional recovery after revascularization with accuracy similar to that of a cutoff value of 43% hyperenhancement on MRI (156). A cutoff value of –4.5% for regional longitudinal strain discriminates between segments with a viable myocardium and those with transmural scar tissue on contrast-enhanced MRI, with sensitivity of 81.2% and specificity of 81.6% (157). However, data from studies on large populations are still lacking.

4. Valvular Heart Disease

Speckle-tracking echocardiographic analysis in patients with valvular heart disease has been mainly performed for the evaluation of LV function with stress (exercise or pharmacologic) testing (158). Lancellotti, et al. (33) have shown that in asymptomatic patients with degenerative mitral regurgitation undergoing valvular surgery, limited exercise-induced longitudinal LV contractile recruitment, as assessed by speckle-tracking echocardiography with global longitudinal strain, predicts postoperative LV dysfunction. In patients with aortic stenosis or aortic regurgitation immediately after aortic valve replacement, there is a substantial increase in radial and circumferential strain (159).

5. Cardiomyopathies

In patients with non-obstructive hypertrophic cardiomyopathy and a preserved EF, speckle-tracking echocardiography has shown the capability to identify early major abnormalities of all strain components of myocardial deformation (longitudinal, circumferential, and radial strain) (148). Another potential clinical application of speckle-tracking echocardiography is for differentiation of hypertrophic cardiomyopathy from athlete's LV hypertrophy (160-162) based on the lower longitudinal strain values in patients with hypertrophic cardiomyopathy who have a normal LVEF (163). Others interesting findings have recently been shown for other cardiomyopathies (164-165).

CHAPTER III

RESEARCH METHODOLOGY

The participants were recruited from students aged 16-19 year old. They were separated into 2 groups relying upon their body mass index (BMI) i.e., non-obese group and obese group. After that obese participants will be divided into 2 groups depending on their blood leptin level i.e., obese with normo-leptinemia (obese-NL) group and obese with hyperleptinemia (obese-HL) group. After that, demographic details and anthropometric data were obtained. Cardiac functions were assessed in all groups by using 2D-conventional echocardiography and 2D-STE data. The data were analyzed using commercial software program such as SPSS

Population and sample

The recruitment of participants was performed by advertising in purposive selection style after the project has been approved from Naresuan University Ethical committee in 20 January, 2016 (IRB No. 796/58).

The sample size has been calculated resulting in 90 subjects in total, 30 for lean group, 30 for obese with normo-leptinemia and 30 for obese with hyperleptinemia.

To determine the sample size, we used the normal approximation to the hypergeometric distribution. The sample size formulas for small (hypergeometric) populations are shown below.

Data from Student Affairs Division, Naresuan University showed that there are approximately 3000 students each year and there are approximately 300 obese students from 3000 students each year and it representing 10 % from total students (P = 0.1). The numbers of obese students in first year (age between 16-19 yearsold) are about 300 students (N = 300).

$$n = \frac{NZ^2PQ}{Ne^2 + Z^2PQ}$$

When n is the required sample size

N is the population size

e is the degrees of freedom of the error component (e = 0.05)

Z is the value that specifies the level of confidence

(from 95% confidence interval, Z = 1.96)

P and Q are the population proportions (P = 0.1), (Q = 0.9)

From 300 population size, the sample size is 90 subjects.

The inclusion criteria is

Healthy male or female adolescences aged 16-19 years old

The exclusion criteria are

1. Adolescents with structural cardiac abnormality.
2. Adolescents with underlying diseases including hypertension, sleep apnea (screening by Modified Berlin questionnaires), anemia and diabetes.
3. Participants who refused echocardiography assessment.
4. Participants with poor echocardiography window.
5. Participants who exercised regularly

The participants will be divided into 2 groups depending on their BMI. The obese group was subsequently divided into 2 groups depending on their blood leptin level.

Research variables

1. Demographic data

Age, gender, underlying disease, medicines, family history of illness including sleep apnea disorder and exercise behavior

2. Anthropometric data

Body weight, height, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR), waist-circumference(WC) and % Body fat

3. Blood analysis data

White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell

distribution Width (RDW), platelet count, neutrophil, lymphocyte, monocyte, eosinophil, basophil, total cholesterol, triglycerides, High density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid and leptin level

4. 2D-conventional echocardiographic parameters

Interventricular septal end diastolic dimension (IVSDd), interventricular septal end systolic dimension (IVSs), left ventricular internal diameter end systole (LVIDs), left ventricular internal diameter end diastole (LVIDd), left ventricular posterior wall end diastole (LVPWd), Left ventricular posterior wall end systole (LVPWs), left ventricular mass (LV mass), left ventricular mass index (LVMI), relative wall thickness (RWT), left ventricular end-diastolic volume by Teicholz's method (LVEDV(Teicholz)), left ventricular end-systolic volume by Teichholz's method (LVESV(Teicholz)), left ventricular ejection fraction by Teichholz's method (LVEF (Teicholz)), fractional shortening (%FS), aortic root diameter (Ao Diam), left atrium diameter (LA Diam), left atrium/aortic ratio (LA/Ao ratio), left ventricular ejection fraction in apical 2 chamber view (LVEF A2C), left ventricular ejection fraction in apical 4 chamber view (LVEF A4C), left ventricular ejection fraction by modified Simpson's method (LVEF biplane), left ventricular end-diastolic volume by modified Simpson's method (LVEDV biplane), left ventricular end-systolic volume by modified Simpson's method (LVESV biplane), maximum velocity of the aortic valve jet (AV Vmax), maximum pressure gradient across the aortic valve (AV maxPG), peak early-diastolic mitral valve inflow velocity (MV E velocity), peak late-diastolic mitral valve inflow velocity (MV A velocity), MV E/A ratio, deceleration time (MV DecT), peak early-diastolic mitral septal annular velocity (e' sep), peak early-diastolic mitral lateral annular velocity (e' lat), peak early-diastolic mitral valve inflow velocity/peak early-diastolic mitral septal annular velocity ratio (E/e'sep), peak early-diastolic mitral valve inflow velocity/peak early-diastolic mitral lateral annular velocity ratio (E/e'lat), maximum pressure gradient across the pulmonic valve (PV maxPG), maximum velocity of the pulmonic jet (PV Vmax), right ventricular systolic pressure (RVSP), maximum velocity of regurgitant flow of the tricuspid valve (TR Vmax) and maximum pressure gradient of regurgitant flow of the tricuspid valve (TR maxPG).

5. Two-dimensional speckle-tracking echocardiographic (2D-STE) parameters

Absolute average longitudinal strain in apical long axis view (absolute avg. long. strain in APLAX), absolute average longitudinal strain in apical 2 chamber view (absolute avg. long. strain in A2C), absolute average longitudinal strain in apical 4 chamber view (Absolute avg. long. strain in A4C) and absolute global- longitudinal strain (absolute GLS) were obtained.

Research Instrument

1. Body fat monitor scale (Omron Karada scan HBF-375)
2. Digital blood pressure monitors (Omron HEM-7230)
3. Measuring tape
4. Data collection form
5. Echocardiogram (GE Vivid S6 with off line software for speckle tracking analysis)

Data Collection

1. Preparation process

1.1 Review of related literatures and studies.

1.2 Design of a data collection form

1.3 2D-STE training

2. Collecting demographic data and anthropometric data

2.1 Recruitment and selection of participants into the study

2.2 Interviewing for demographic data: including age, gender, underlying disease, medicines, family history of illness including sleep apnea disorder and exercise behavior

2.3 Anthropometric measurement: The BMI was calculated as weight in kilograms divided by height in meters squared. Body fat monitor scale (Omron Karada scan HBF-375) was used to measure weight and fat content. Waist circumference was measured in a standing position, midway between the lower lateral costal margin and the iliac crest, in exhale.

2.4 Blood pressure and heart rate measurement: Blood pressure and heart rate were measured in a sitting position after 5 minutes of rest, repeat the same measurement three times and measurements were averaged.

3. Echocardiographic measurement

3.1 Intra- and inter-observer variability

Inter and intra-observer variability was based on sample review from 10 studies completed and recorded on CD. Ten samples of echocardiograms were performed by a researcher (T.Imerbtham) and were chosen for intra- and inter-observer variability. For intra-observer variability, 10 echocardiograms from cardiac sonographer (T. Imerbtham) were randomly selected to be reread by the same cardiac sonographer for 3 times in different time periods (at 1st and 2nd and 3rd week). For inter-observer variability, a total of 10 echocardiograms from the files from the cardiac sonographer, were selected and re-measured by expert cardiologist (T. Yingchonchareon). All re-measurements were made with the expert cardiologist blinded to the original results. Inter and intra-observer variability parameters in 2D-conventional echocardiography were LVEDV (biplane), LVESV (biplane), LVEF (biplane), %FS, LVEF (Teicholz), LA/Ao ratio, IVSDd, LVIDd, LVPWd, LV mass, LVMI, RWT, MV E velocity, MV A velocity, MV E/A Ratio, MV DecT, e' septal, e' lateral, E/e' sep, E/e' lat, RVSP, AV Vmax and PV Vmax. Inter and intra observer variability parameters in 2D-speckle tracking echocardiography were LV basal anterior wall (BA), LV mid-anterior wall (MA), LV apical anterior wall (AA), LV basal anteroeptal wall (BAS), LV mid-anteroseptal wall (MAS), LV apical anteroseptal wall (AAS), LV basal inferoseptal wall (BS), LV mid-inferoseptal wall (MS), LV apical inferoseptal wall (AS), LV basal inferior wall (BI), LV mid inferior wall (MI), LV apical inferior wall (AI), LV basal posterior wall (BP), LV mid-posterior wall (MP), LV apical posterior wall (AP), LV basal lateral wall (BL), LV mid-lateral wall (ML) and LV Apicolateral wall (AL). Intra- and inter-observer reliability were analyzed with the Intraclass Correlation Coefficients (ICCs) with 95% confidence interval using the SPSS (SPSS IncChicago, USA).

3.2 Two-Dimensional Echocardiography

2D- echocardiographic measurements were measured in the faculty of Allied Health Sciences, Naresuan University, using a Vivid S6 commercial ultrasound

scanner (GE Vingmed Ultrasound AS, Horten, Norway) with an active matrix single-crystal phased-array transducer (GEM5S-D; GE Vingmed Ultrasound AS). All participants were measured by the same observer in the left side-lying position, after 10 minutes resting. Grayscale images were recorded at a mean frame rate of ≥50 frames/s. Images were acquired in the standard tomographic views of the LV (parasternal long- and short-axis, apical four-chamber (A4C), apical long axis (APLAX) and two-chamber views (A2C)). All measurements were repeated three times and measurements were averaged. 2D- echocardiographic measurements were measured along with the criteria of the American Society of Echocardiography (166). The following parameters of cardiac function were measured:

Left ventricular systolic function: ejection fraction by Teichholz's method (LVEF (Teicholz)) and fractional shortening (%FS) were measured in the parasternal long axis views, using M mode; left ventricular end diastolic (LVEDV (biplane)) and end systolic volumes (LVESV (biplane)) and ejection fraction (LVEF (biplane)) were measured from A2C and A4C views, using a modified Simpson's biplane method.

Left ventricular diastolic function: Diastolic functions were measured by integrating Doppler measurements of the mitral valve and tissue Doppler of the septal and lateral mitral annulus velocity. Pulsed wave (PW) Doppler measurements were measured in the apical four chamber view: the Doppler beam was adjusted as perpendicularly as possible to the mitral annulus and a 5 mm PW Doppler sampling volume was aligned between the tips of the mitral valve in diastolic phase. The following parameters were calculated: mitral inflow velocities, peak early diastolic velocity (MV E velocity), peak late diastolic velocity (MV A velocity), MV E/A Ratio, E deceleration time (MV DecT), e' septal, e' lateral, E/e' septal and E/e' lateral were measured.

Left ventricular dimensions and geometry: interventricular septal end diastole and end systole (IVSDd and IVSDs), left ventricular internal diameter end diastole and end systole (LVIDd, LVIDs), left ventricular posterior wall end diastole and end systole (LVPWd and LVPWs)) were measured in the parasternal long axis views, using M mode. LV mass was determined and indexed to body surface area and height $^{2.7}$ (LVMI) (167). The relative wall thickness (RWT) was calculated (2 ×

LVPWd)/LVEDD. LV hypertrophy (LVH) was determined as LV mass indexed to body surface area >95g/m ^{2} in women and >115g/m ^{2} in men. Normal geometry was defined as RWT ≤ 0.42 and no LVH. Abnormal geometry was classified as concentric remodeling (RWT > 0.42 and no LVH); concentric hypertrophy (RWT > 0.42 and LVH); and eccentric hypertrophy (RWT <0.42 and LVH).

Left atrium to aorta ratio (LA/Ao ratio) were obtained in the parasternal long axis views or parasternal short axis view (aortic valve level), using M mode. Right ventricle systolic pressure (RVSP) was estimated from the tricuspid regurgitant jet velocity and using inferior vena cava (IVC) size and collapsibility for estimate of right atrial pressure (RAP). Aortic valve max velocity (AV Vmax) was estimated from the highest velocity in the aortic valve from apical 5 chamber view or apical long axis view. Pulmonic valve max velocity (PV Vmax) was estimated from the highest velocity in the pulmonic valve from parasternal short axis (pulmonic valve level) or RV outflow tract view. (168).

3.3 Analysis of myocardial deformation

Two-dimensional (2D) strain data were recorded in digital format for subsequent examination. 2D strain data were analyzed offline with the workstation (Echopac,PC 2008, GE, Horten, Norway). Sampling volumes were placed using the Echopac software under the lateral and septal mitral valve annulus and LV apex. The myocardium motion was measured from a user manual tracing along the endocardial to myocardial border. After manual tracing of the endocardial border in the end-systolic phase of a 2D-echocardiographic image and choosing the proper wall thickness, the software was automatically tracking and divided LV into six segments. LV longitudinal systolic deformation was represented as shortening and systolic function of LV, display in negative values which obtained from apical, mid and basal segments in the LV septum and LV lateral wall from apical views (APLAX, A4C and A2C). Data were derived when the values of all six LV segments were acceptable by the software or when a value of any one LV segment with poor tracking (fewer than two segments) was dismisses by the software however that segment will be determined to be acceptable. All strain parameters were repeated three times and measurements were averaged. Strain parameters and the global longitudinal strain

(GLS) were used to assess the left ventricular systolic function. Strain analysis was performed by the cardiac sonographer blinded to the leptin level.

4. Laboratory and blood test

All subjects were tested for standard blood analyses including complete blood count (CBC), lipid profile, uric acid and leptin level. In all subjects, blood sample were measured after an overnight fasting (>12h).

4.1 Complete blood count (CBC)

CBC was analyzed using the auto analyzer.

4.2 Uric acid and lipid profile

Total cholesterol, HDL and LDL level were analyzed using a commercially available Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor kit (CHOD-PAP-Method) according to the manufacturer's instructions. Triglyceride levels were analyzed using a commercially available Enzymatic Colorimetric Test for Triglycerides with Lipid Clearing Factor kit (GPO-PAP-Method) according to the manufacturer's instructions. And uric acid levels were analyzed using a commercial available Enzymatic Colorimetric Test for Uric Acid with Lipid Clearing Factor kit (PAP-Method) kit according to the manufacturer's instructions.

4.3 Leptin

The Leptin levels were measured using a commercially available enzyme linked Immune sorbent assay kit (ELISA) according to the manufacturer's instructions. According to this protocol:

1. Dilute the concentrated Wash Buffer 10 fold by adding the entire contents of both bottles of buffer to 900 mL de-ionized or glass distilled water.

2. Remove required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8 °C. Assemble strips in an empty in an empty plate holder and add 300 µL of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. Do not let wells dry before proceeding to the next step.

3. Add 75 µL Assay Buffer into all wells

4. Add in duplicated 25 µL Assay Buffer to blank wells.

5. Add in duplicate 25 µL Human Leptin Standards in order of ascending concentration to the appropriate wells. Add sequentially 25 µL of samples in duplicate to the remaining wells.

6. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed about 400 to 500 rpm.

7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

8. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

9. Add 100 µL Detection Antibody to each well. Cover plate with sealer and incubate at room temperature for 30 minutes on the microtiter plate shaker.

10. Remove sealer and decant solution from the plate. Tap as before to remove residual solutions in the wells.

11. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

12. Remove sealer, decant solution from the plate, and tap plate to remove the residual fluid.

13. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

14. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for 5-20 minutes. Blue color should be formed in wells of leptin standards with intensity proportional to increasing concentrations of leptin.

15. Remove sealer and add 100 µL of Stop Solution and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a

plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference in absorbance units.

16. Plot a standard curves.

Data Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software or other commercial programed. Data was presented as mean ±SD. The data was tested for normal distribution by using Kolmogrov-Smirnov test. The unpaired T-test or Mann-Whitney U test was used to compare continuous variables in study groups depending on the distribution of the data. The relationships between the absolute GLS and the independent variables in all obese participants were examined with Univariate linear regression and Multivariate linear regression analysis.

CHAPTER IV

RESULTS

Intra-observer and inter-observer variability

1. Intra-observer variability

1.1 2D-conventional echocardiography

Intra-observer variability for all conventional echocardiographic parameters was assessed and then statistically calculated for intra class correlation coefficient (ICC). The data demonstrated good intra-observer reliability with ICC ranging from 0.883 to 0.996 (for MV E velocity and LVEDV (biplane), respectively). The detail of the data was shown in Table 2.

Table 2 Intra-observer variability for 2D-conventional echocardiography

Parameters	Intraclass correlation coefficient (ICC)	95% Confidence Interval	
		Lower Bound	Upper Bound
LVEDV (biplane)	0.996	0.987	0.999
LVESV (biplane)	0.986	0.961	0.996
LVEF (biplane)	0.988	0.967	0.997
%FS	0.975	0.877	0.988
LVEF (Teicholz)	0.952	0.861	0.957
LA/Ao ratio	0.987	0.962	0.996
IVSDd	0.938	0.819	0.983
LVIDd	0.953	0.864	0.987
LVPWd	0.974	0.928	0.993
LV mass	0.994	0.983	0.998
LVMI	0.911	0.746	0.976
RWT	0.984	0.955	0.996
MV E velocity	0.883	0.659	0.968

Table 2 (cont.)

Parameters	Intraclass correlation coefficient (ICC)	95% Confidence Interval	
		Lower Bound	Upper Bound
MV A velocity	0.974	0.927	0.993
MV E/A Ratio	0.986	0.906	0.991
MV DecT	0.991	0.974	0.998
e' septal	0.960	0.886	0.989
e' lateral	0.935	0.810	0.982
E/e' sep	0.988	0.956	0.997
E/e' lat	0.973	0.923	0.993
RVSP	0.985	0.957	0.996
AV Vmax	0.974	0.926	0.993
PV Vmax	0.993	0.980	0.998

1.2 2D-Speckle tracking echocardiography

Two-dimensional (2D) strain data were stored in digital format and then were analyzed offline. Since the circumferential and radial strain measurement showed high intra observer variability, only longitudinal strain was performed. The ICC was calculated from the measurement of 18 LV segments (six basal, six mid LV, and six apical). The intra observer reliability was excellent. The test showed that ICC for longitudinal strain ranged from 0.920 to 0.995 (for LV BL wall and LV MP wall, respectively). The summary of the test was shown in Table 3.

Table 3 Intra-observer variability for longitudinal strain echocardiography

Longitudinal echocardiographic strain measurement	Intraclass correlation coefficient (ICC)	95% Confidence Interval	
		Lower Bound	Upper Bound
LV basal anterior wall (BA)	0.927	0.766	0.981
LV mid-anterior wall (MA)	0.989	0.967	0.997
LV apical anterior wall (AA)	0.966	0.904	0.991
LV basal anteroeptal wall (BAS)	0.951	0.859	0.987
LV mid-anteroseptal wall (MAS)	0.986	0.962	0.996
LV apical anteroseptal wall (AAS)	0.942	0.831	0.984
LV basal inferoseptal wall (BS)	0.950	0.856	0.987
LV mid-inferoseptal wall (MS)	0.933	0.805	0.983
LV apical inferoseptal wall (AS)	0.974	0.927	0.993
LV basal inferior wall (BI)	0.986	0.960	0.996
LV mid inferior wall (MI)	0.959	0.884	0.989
LV apical inferior wall (AI)	0.960	0.880	0.990
LV basal posterior wall (BP)	0.971	0.916	0.992
LV mid-posterior wall (MP)	0.995	0.986	0.999
LV apical posterior wall (AP)	0.965	0.899	0.990
LV basal lateral wall (BL)	0.920	0.771	0.978
LV mid-lateral wall (ML)	0.943	0.833	0.985
LV Apicolateral wall (AL)	0.964	0.963	0.997

2. Inter-observer variability

To assess inter-observer variability, a total of 10 echocardiography recorded by the researcher were selected and re-measured by expert cardiologist who was blinded to the original results.

2.1 2D-conventional echocardiography

The evaluation of inter-observer variability for conventional echocardiography was performed and also shown by the ICC. The lowest ICC was

found in MV E velocity (0.801) and the highest ICC was from LVEDV (biplane) measurement (0.997). The echocardiographic parameters obtained by 2 observers were minimally different. The data was shown in Table 4

Table 4 Inter-observer variability for 2D-conventional echocardiography

Conventional echocardiographic parameters	Inter-rater reliability (ICC)	N = 10	
		Observer 1	Observer 2
LVEDV (biplane)	0.997	90.1±12.96	89.60±12.55
LVESV (biplane)	0.986	28.88±4.31	28.49±4.32
LVEF (biplane)	0.984	66.38±4.14	66.20±4.38
%FS	0.946	37.09±3.12	37.75±3.21
LVEF (Teicholz)	0.930	69.72±3.52	70.17±3.30
LA/Ao ratio	0.983	1.19±0.18	1.21±0.16
IVSDd	0.933	0.80±0.12	0.79±0.14
LVIDd	0.937	4.25±0.29	4.30±0.22
LVPWd	0.962	0.74±0.12	0.73±0.15
LV mass	0.995	106.20±13.29	106.90±13.36
LVMI	0.818	61.90±3.18	61.50±3.24
RWT	0.975	0.34±0.07	0.35±0.07
MV E velocity	0.801	1.00±0.15	0.95±0.08
MV A velocity	0.975	0.41±0.06	0.41±0.06
MV E/A Ratio	0.976	1.96±0.29	1.98±0.23
MV DecT	0.988	184.38±26.36	181.15±28.23
e' septal	0.936	0.16±0.02	0.17±0.03
e' lateral	0.852	0.19±0.03	0.18±0.02
E/e' sep	0.986	5.69±0.49	5.65±0.41
E/e' lat	0.963	4.76±0.32	4.79±0.35
RVSP	0.964	20.30±4.44	20.56±3.79
AV Vmax	0.986	1.08±0.14	1.10±0.14
PV Vmax	0.993	0.96±0.23	0.96±0.26

2.2 2D-Speckle tracking echocardiography

The test of Inter-observer variability for longitudinal strain echocardiography showed a good correlation between observer 1(the researcher) and observer 2 (the expert cardiologist). The ICC was between 0.858 to 0.989 (for LV BL wall and LV MA wall and LV MP wall, respectively). The result of all parameters was shown in Table 5.

Table 5 Inter-observer variability for longitudinal strain echocardiography

Longitudinal echocardiographic strain measurement	Inter-rater reliability (ICC)	N = 10	
		Observer 1	Observer 2
LV basal anterior wall (BA)	0.898	-16.93±2.30	-17.15±2.08
LV mid-anterior wall (MA)	0.989	-19.86±6.59	-20.01±6.47
LV apical anterior wall (AA)	0.986	-16.14±3.53	-15.41±3.21
LV basal anteroeptal wall (BAS)	0.921	-14.76±1.82	-14.39±2.16
LV mid-anteroseptal wall (MAS)	0.975	-15.81±4.62	-15.89±5.59
LV apical anteroseptal wall (AAS)	0.978	-15.60±3.50	-15.34±3.12
LV basal inferoseptal wall (BS)	0.923	-16.19±1.34	-16.48±1.40
LV mid-inferoseptal wall (MS)	0.958	-19.89±4.11	-19.72±3.26
LV apical inferoseptal wall (AS)	0.949	-21.29±4.82	-21.86±3.88
LV basal inferior wall (BI)	0.982	-15.88±4.67	-16.29±5.44
LV mid inferior wall (MI)	0.981	-18.34±3.58	-18.00±4.18
LV apical inferior wall (AI)	0.950	-18.79±4.04	-18.31±4.39
LV basal posterior wall (BP)	0.962	-14.35±6.92	-14.09±7.27
LV mid-posterior wall (MP)	0.989	-17.05±6.17	-17.72±6.59
LV apical posterior wall (AP)	0.939	-16.67±0.93	-16.32±0.89
LV basal lateral wall (BL)	0.858	-15.87±5.20	-16.63±4.78
LV mid-lateral wall (ML)	0.931	-16.55±2.84	-16.91±3.70
LV Apicolateral wall (AL)	0.971	-14.11±5.31	-14.02±4.80

Cardiac function of the non-obese and obese adolescents

The 91 participants aged 16-19 years were enrolled and divided into 2 groups depending on their BMI, obese group (BMI ≥ 25kg/m^2) and non-obese group (BMI < 25kg/m^2) according to Asia-Pacific classification of obesity (56).Among these, 4 participants were excluded due to poor-echocardiographic image quality and 6 participants were withdrawn from the study, therefore, there were 81 participants in total, 30 for non-obese group and 51 for obese group.

1. Basic characteristics

The mean age of the studied population was 17.8 ± 0.1 and 18.4 ± 0.1 years in the obese group and non-obese group, respectively. There were no differences between groups regarding to mean age, sex and body weight. Body fat-related parameters, i.e., BMI, waist circumference and body fat percentage varied significantly between obese and non-obese adolescents. Mean SBP of the studied population was 123.5 ± 1.4 and 113.4 ± 2.5 mmHg in the obese group and non-obese group, respectively. Mean SBP was significantly increased in obese group when compared with non-obese group ($p < 0.001$). Mean DBP of the participants was 74.7 ± 1.1 and 67.7 ±1.4 mmHg in the obese group and non-obese group, respectively. Mean DBP was significantly higher in obese group when compared with non-obese group ($p < 0.001$). However, mean pulse pressure of two groups was not different significantly (48.7 ± 1.4 and 45.70± 2.1 mmHg in the obese group and non-obese group, respectively, $p = 0.22$). Mean heart rate of obese and non-obese group did not vary significantly (77.4 ± 1.7 and 80.9 ± 1.6 beats/min for obese group and non-obese group, respectively, $p = 0.17$).

Family history for hyperlipidemia, hypertension, and cardiovascular diseases did not differ significantly between non-obese group and obese group. Only DM was significantly higher in obese group when compared with non-obese group ($p = 0.02$). The detail was shown in Table 6.

Table 6 Baseline characteristics of the non-obese and obese group

Parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
Age (years)	18.4 ± 0.1	17.8 ± 0.1*	0.03
Sex (%)	F = 73	F = 61	0.25
Weight (kg)	55.3 ± 1.5	79.6 ± 1.8**	<0.001
Height (cm)	163.4 ± 1.3	165.3 ± 1.0	0.27
BMI (km/m^2)	20.6 ± 0.3	29.0 ± 0.5**	<0.001
Waist circumference (cm)	72.8 ± 1.1	92.6 ± 1.2**	<0.001
Body fat (%)	22.8 ± 1.3	28.9 ± 0.8**	<0.001
SBP (mmHg)	113.4 ± 2.5	123.5 ± 1.4**	<0.001
DBP (mmHg)	67.7 ±1.4	74.7 ± 1.1**	<0.001
PP (mmHg)	45.7± 2.1	48.7 ± 1.4	0.22
HR (beats/min)	77.4 ± 1.7	80.9 ± 1.6	0.17
Family history (%)			
Hyperlipidemia	3.3	1.9	0.70
DM	30	9.8*	0.02
Hypertension	6.6	9.8	0.63
Cardiovascular disease	0	3.9	0.68

* = $p < 0.05$, ** = $p < 0.001$

2. Laboratory findings

It was found that there was no difference between groups in terms of serum cholesterol and LDL. However there was significantly increased of serum uric acid in obese group as compared to the non-obese group (4.1 ± 0.1 and 3.1 ± 0.1 mg/dL in obese group and non-obese group, respectively, $p < 0.001$). There was significantly increased of serum triglyceride in obese group as compared to the non-obese group (159.6 ± 7.7 and 126.3 ± 6.9 mg/dL in obese group and non-obese group, respectively, $p < 0.05$). There was significantly decreased of serum HDL in obese

group as compared to the non-obese group (41.4 ± 1.0 and 46.4 ± 1.9 mg/dL in obese group and non-obese group, respectively, $p < 0.05$). Moreover serum leptin level was significantly higher in obese group when compared with non-obese group (32.46 ± 2.4 and 11.9 ± 1.0 ng/dL in non-obese group and obese group, respectively. $p < 0.001$). There was no difference between groups in terms of WBC and RBC level. However Hb level was significantly increased in obese group when compared with non-obese group (13.1 ± 0.2 and 12.3 ± 0.2 $x10^6$ g/dL in obese group and non-obese group, respectively, $p < 0.05$). Hct level was significantly increased in obese group when compared with non-obese group (41.3 ± 0.6 and 38.9 ± 0.6% in obese group and non-obese group, respectively $p < 0.05$). There were no significant differences between groups with regard to MCV, MCH, MCHC and RDW, platelet, neutrophil, lymphocyte, eosinophil and basophil level. Only monocyte level was significantly decreased in obese group when compared with non-obese group (4.4 ± 0.2 vs. 5.9 ± 0.3% in obese group and non-obese group, respectively, $p < 0.001$). Table 7 summarizes the laboratory findings between obese and non-obese group.

Table 7 Laboratory parameters of the non-obese and obese group

Laboratory parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
Uric acid (mg/dL)	3.1 ± 0.1	4.1 ± 0.1**	<0.001
Triglyceride (mg/dL)	126.3 ± 6.9	159.6 ± 7.7*	0.004
Cholesterol (mg/dL)	167.0 ± 8.8	171.0 ± 4.4	0.65
HDL (mg/dL)	46.4 ± 1.9	41.4 ± 1.0*	0.01
LDL (mg/dL)	95.4 ± 7.5	97.5 ± 4.2	0.79
Leptin (ng/mL)	11.9 ± 1.0	32.46 ± 2.4**	<0.001
WBC (cell/cumm.)	5,530 ± 261.3	5,898 ± 217.2	0.29
RBC ($x10^6$ cell/μL)	5.0 ± 0.1	5.3 ± 0.0	0.05
Hb (g/dL)	12.3 ± 0.2	13.1 ± 0.2*	0.03
Hct (%)	38.9 ± 0.6	41.3 ± 0.6*	0.01

Table 7 (cont.)

Laboratory parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
MCV (fL)	78.6 ± 1.4	78.2 ± 0.9	0.81
MCH (pg)	24.7 ± 0.5	24.5 ± 0.4	0.76
MCHC (g/dL)	31.5 ± 0.2	31.3 ± 0.2	0.62
RDW (%)	14.9 ± 0.1	14.8 ± 0.1	0.79
Platelet count (cell/cumm.)	305,200 ± 10430	323,900 ± 10240	0.23
Neutrophil (%)	59.5 ± 1.4	60.4 ± 0.8	0.58
Lymphocyte (%)	31.3 ± 1.7	32.4 ± 0.8	0.51
Monocyte (%)	5.9 ± 0.3	4.4 ± 0.2**	<0.001
Eosinophil (%)	2.0 ± 0.2	2.7 ± 0.2	0.07
Basophil (%)	0.1 ± 0.0	0.1 ± 0.0	0.23

* = $p < 0.05$, ** = $p < 0.01$

3. Cardiac function assessed by 2D-conventional echocardiography

Left ventricular systolic function was determined in obese and non-obese participants. It was found that, mean LVEDV (biplane) was significantly increased in obese group when compared with non-obese group (120.10± 3.53 and 89.13 ± 3.32 ml in obese and non-obese group, respectively, $p < 0.001$) as well as mean LVESV (biplane) (42.29± 1.67 and 30.44 ± 1.35 ml in obese and non-obese group, respectively, $p < 0.001$). However, mean LVEF (Tei and biplane) and mean %FS did not differ significantly between two groups (67.32± 0.83 vs. 69.17 ± 0.93% for mean LVEF (Tei), 64.96 ± 0.82 vs. 65.79 ± 0.87% for mean LVEF (biplane) and 37.64± 0.66 vs. 38.82 ± 0.76% for %FS in obese and non-obese group, respectively). The assessment of diastolic function in obese and non- obese group demonstrated that there were no significant differences between groups regarding to mean MV E velocity, mean MV A velocity, mean MV E/A ratio, mean MV DecT, mean e' lateral, mean E/e' sepal and mean E/e' lateral. Only mean e' septal was significantly decreased in

obese group when compared with non-obese group (0.15 ± 0.00 vs. 0.16 ± 0.00 m/s, in obese and non-obese group, respectively, $p < 0.05$). The result was shown in Table 8.

Left ventricular dimensions and geometry were investigated and compared. Apparently, obesity affects many geometric parameters. The study demonstrated that, mean IVSDd was significantly increased in obese group when compared with non-obese group (0.90 ± 0.02 and 0.77 ± 0.02 cm in obese group and non-obese group, respectively, $p < 0.001$) as well as mean LVIDd (4.82 ± 0.05 and 4.43 ± 0.06 cm in obese group and non-obese group, respectively, $p < 0.001$). Furthermore, mean LVPWd, mean LV mass, mean LVMI and mean RWT were significantly higher in obese group compared with non-obese group. The result was demonstrated in Table 8. A representative image showing LV dimensions and geometry of obese adolescents was demonstrated in Figure 5. The LV geometry of participants was classified into normal geometry, concentric remodeling, eccentric hypertrophy and concentric hypertrophy. There were no significant differences between groups regarding to the proportion of concentric remodeling subjects, eccentric hypertrophy subjects, concentric hypertrophy subjects and normal geometry subjects. The proportion of concentric remodeling subjects was 7.8 and 10% whereas the proportion of eccentric hypertrophy subjects was 1.9 and 0% in obese group and non-obese group, respectively. The proportion of concentric hypertrophy subjects was 1.9 and 0% in obese group and non-obese group, respectively. The proportion of normal geometry subjects was 88.4 and 90% in obese group and non-obese group, respectively. The detail is in Table 8.

Additionally, mean LA/Ao ratio, mean AV Vmax and mean PV Vmax were not significantly different between groups. However, mean RVSP was significantly lower in obese group (as shown in Table 8).

Table 8 Conventional echocardiographic parameters in obese and non-obese adolescents

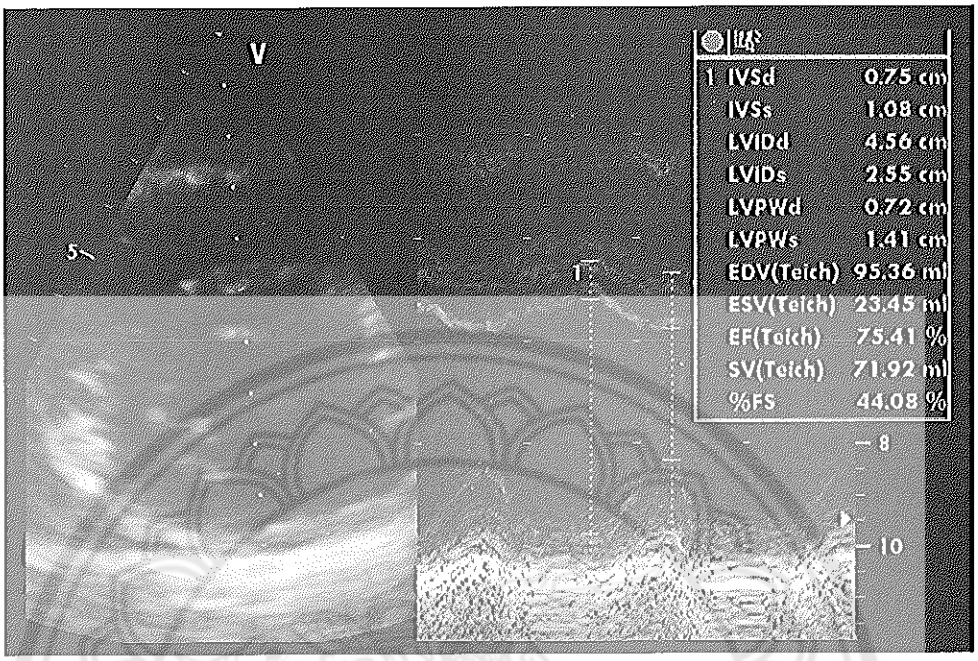
Parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
2D-mode			
LVEDV (biplane) (ml)	89.13 ± 3.32	120.10± 3.53 **	<0.001
LVESV (biplane) (ml)	30.44 ± 1.35	42.29± 1.67 **	<0.001
LVEF (biplane) (%)	65.79 ± 0.87	64.96 ± 0.82	0.51
M-mode			
FS (%)	38.82 ± 0.76	37.64± 0.66	0.26
LVEF (Teicholz) (%)	69.17 ± 0.93	67.32± 0.83	0.15
LA/Ao ratio	1.20 ± 0.02	1.28 ± 0.02	0.07
IVSDd (cm)	0.77 ± 0.02	0.90 ± 0.02 **	<0.001
LVIDd (cm)	4.43 ± 0.06	4.82 ± 0.05**	<0.001
LVPWd (cm)	0.72 ± 0.02	0.85 ± 0.01 **	<0.001
LV mass (g)	103.20 ± 4.60	140.80 ± 5.04**	<0.001
LVMI (g/m^2)	65.30 ± 2.27	74.35 ± 2.63*	0.02
RWT	0.32 ± 0.01	0.36 ± 0.00*	0.03
Doppler-mode			
MV E velocity (ms)	0.99 ± 0.02	0.96 ± 0.01	0.50
MV A velocity (m/s)	0.46 ± 0.01	0.47 ± 0.01	0.65
MV E/A Ratio	2.19 ± 0.09	2.11 ± 0.07	0.56
MV DecT (ms)	184.80 ± 6.63	187.80 ± 5.18	0.72
e' septal (m/s)	0.16 ± 0.00	0.15 ± 0.00*	0.03
e' lateral (m/s)	0.20 ± 00	0.20 ± 00	0.91
E/e' sep	5.93 ± 0.18	6.36 ± 0.18	0.12
E/e' lat	4.97 ± 0.16	4.88 ± 0.13	0.66
RVSP (mmHg)	19.74 ± 0.79	17.07 ± 0.62*	0.01
AV Vmax (m/s)	1.10 ± 0.02	1.10 ± 0.02	0.92
PV Vmax (m/s)	0.99 ± 0.03	1.00 ± 0.02	0.83

Table 8 (cont.)

Parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
LV geometry			
Concentric remodeling (%)	10	7.8	0.74
Eccentric hypertrophy (%)	0	1.9	0.80
Concentric hypertrophy (%)	0	1.9	0.80
Normal geometry (%)	90	88.4	0.89

* = $p < 0.05$, ** = $p < 0.001$

A.



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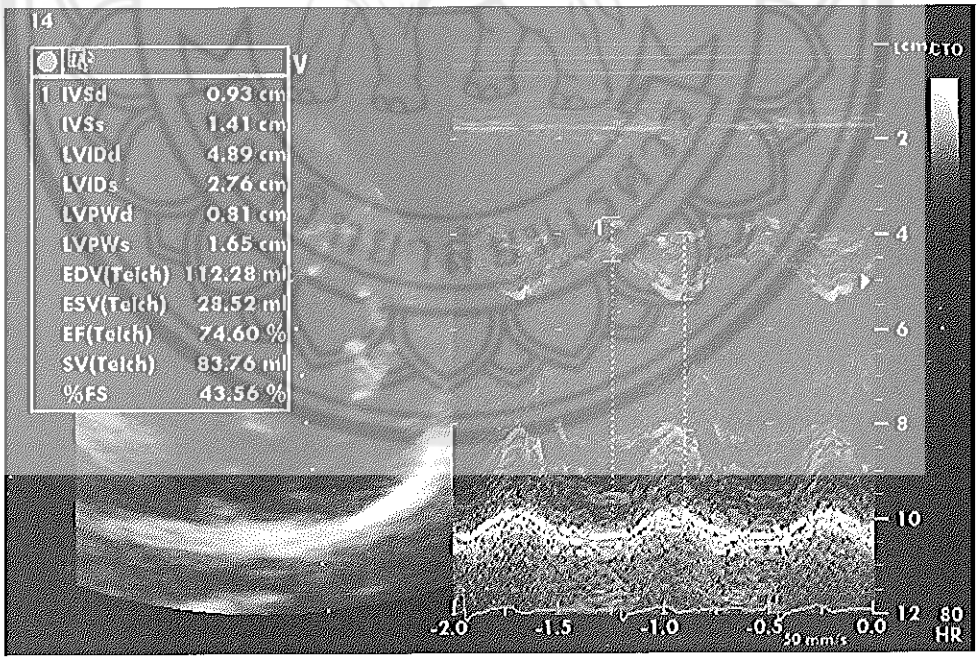

Figure 6 A representative figure of LV dimension of non-obese group (A) and obese group (B)

4. Cardiac function assessed by 2D-Speckle-tracking echocardiography

In this study, longitudinal deformation properties of the left ventricle were further investigated using 2D-STE. The study showed that, absolute average. longitudinal strain in A2C was significantly lower in obese group when compared with non-obese group (19.5 ± 0.3 and 21.2 ± 0.4%, respectively, p = 0.006) as same as absolute average longitudinal strain in A4C (19.1 ± 0.3 and 20.6 ± 0.5% in obese group and non-obese group, respectively, p = 0.01). Absolute average longitudinal strain in APLAX was also reduced in obese group (18.8 ± 0.4 and 20.7 ± 0.7% in obese group and non-obese group, respectively, p = 0.01). Absolute GLS was significantly lower in obese group when compared with non-obese group (19.1± 0.3 and 21.1 ± 0.3% in obese group and non-obese group, respectively, p < 0.001). The 2D-STE parameters of the non-obese and obese group are presented in Table 9. A representative of longitudinal deformation property of an obese participant was shown in Figure 6-7.

Table 9 2D-Speckle-tracking echocardiographic longitudinal strain in non-obese and obese group

Parameters	Non-obese (n= 30) Mean ± SD	Obese (n=51) Mean ± SD	p-value
Absolute avg. long. strain in A2C (%)	21.2 ± 0.4	19.5 ± 0.3*	0.006
Absolute avg. long. strain in A4C (%)	20.6 ± 0.5	19.1 ± 0.3*	0.01
Absolute avg. long. strain in APLAX (%)	20.7 ± 0.7	18.8 ± 0.4*	0.01
Absolute GLS (%)	21.1 ± 0.3	19.1± 0.3**	<0.001

* = p < 0.05, ** = p < 0.001

Avg. = average, A2C = apical 2 chamber view, A4C = apical 4 chamber view, APLAX= apical long axis view, GLS = global longitudinal strain.

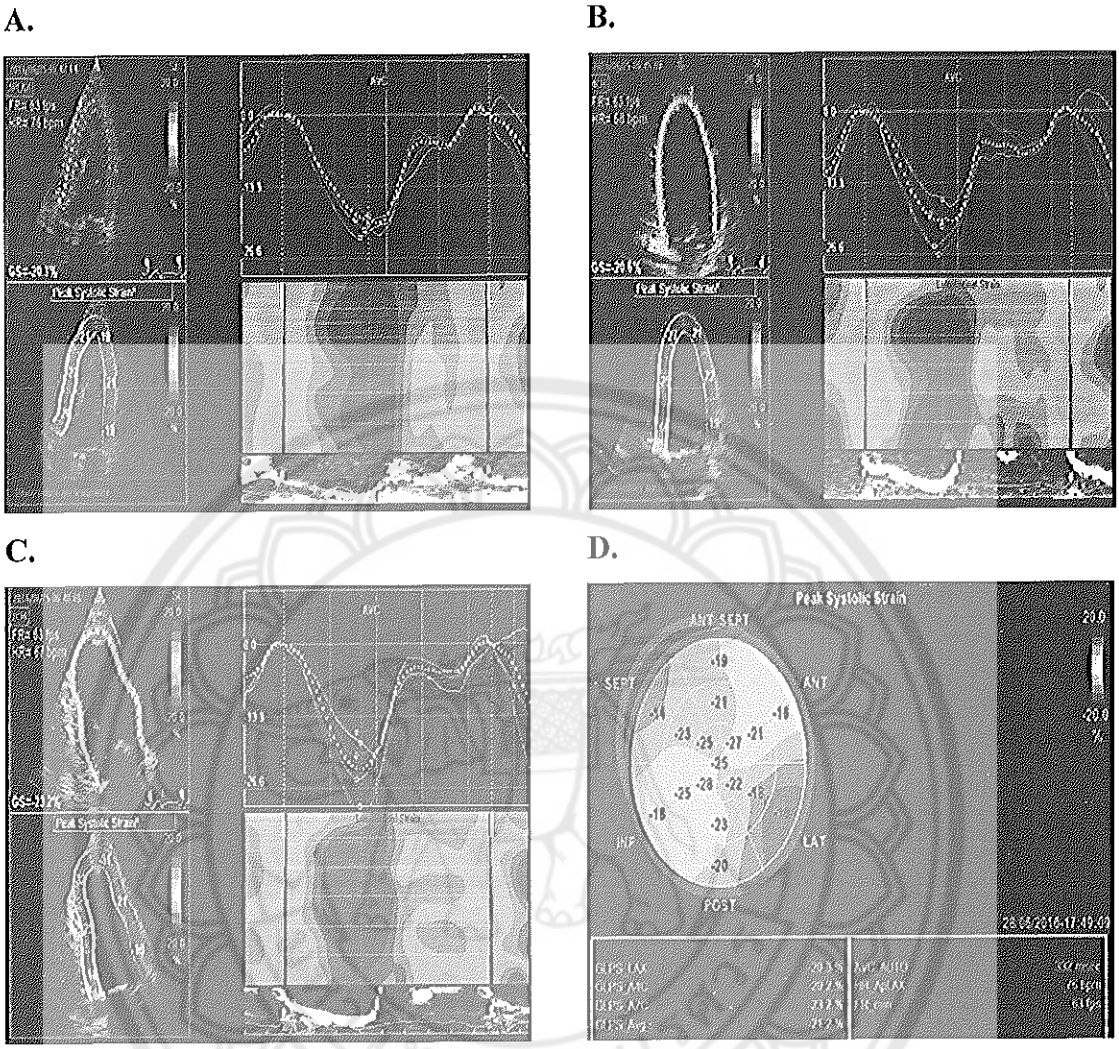

Figure 7 A representative figure of LV longitudinal strain of non-obese group from (A) apical long axis view, (B) apical 4 chamber view, (C) apical 2 chamber view and (D) Diagram- showing the calculation of LV global longitudinal strain

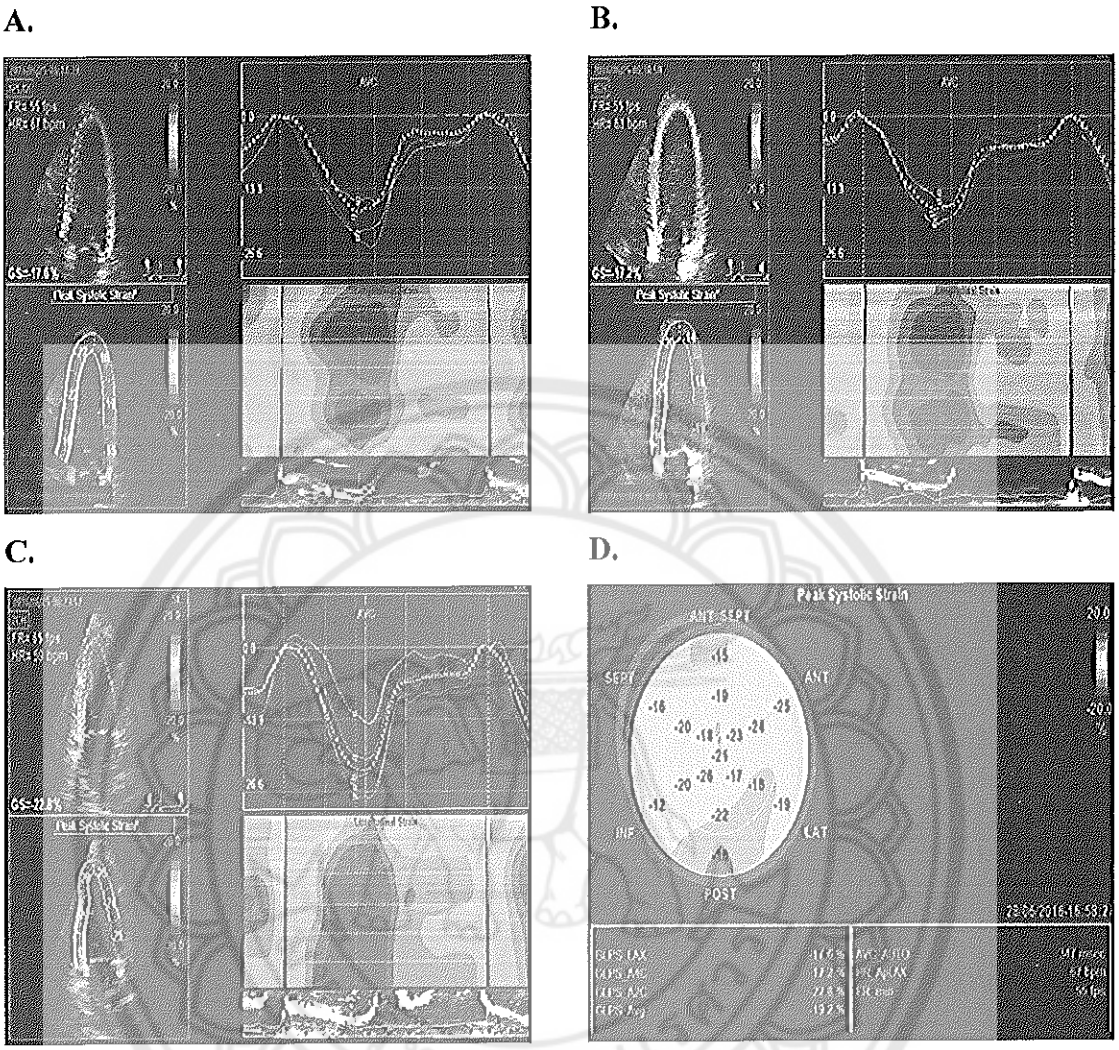

Figure 8 A representative figure of LV longitudinal strain of obese group from (A) apical long axis view, (B) apical 4 chamber view, (C) apical 2 chamber view and (D) Diagram-showing the calculation of LV global longitudinal strain

Cardiac function of the obese adolesents with hyperleptinemia and with normo-leptinemia

The previous study indicated that cardiac function assessed by 2D-STE of obese adolescents was altered. We further evaluated the impact of hyperleptinemia on that alteration. Fifty-one obese participants were divided into 2 groups according to their blood leptin level, i.e.; obese with normo-leptinemia (Obese NL) and obese with hyperleptinemia (Obese HL) group. Totally, there were 25 participants assigned for

obese with normo-leptinemia group and 26 participants for obese with hyperleptinemia group.

1. Basic characteristics

The mean age of the studied population was 18.0 ± 0.2 and 17.6 ± 0.2 years in the obese with hyperleptinemia group and obese with normo-leptinemia group, respectively. There were no differences between groups regarding to mean age and sex. Body fat-related parameters, i.e., body weight, BMI, waist circumference and body fat percentage varied significantly between obese with hyperleptinemia group and obese with normo-leptinemia group. There were no significant differences between groups with regard to mean SBP, mean DBP, mean PP and HR.

Family history for hyperlipidemia, DM, hypertension, and cardiovascular diseases did not differ significantly between obese with hyperleptinemia group and obese with normo-leptinemia group. The detail was shown in Table 10.

Table 10 Baseline characteristics of the obese with hyperleptinemia group and obese with normo-leptinemia group

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
Age (years)	17.6 ± 0.2	18.0 ± 0.2	0.30
Sex (%)	48	73	0.06
Weight (kg)	77.1 ± 2.3	81.9 ± 2.7	0.18
Height (cm)	166.4 ± 1.8	164.3 ± 1.2	0.35
BMI (km/m^2)	27.7 ± 0.5	30.2 ± 0.7*	0.01
Waist circumference (cm)	91.0 ± 1.4	94.2 ± 1.9	0.19
Body fat (%)	26.4 ± 1.1	31.3 ± 1.0*	0.002
SBP (mmHg)	125.2 ± 2.1	121.8 ± 1.8	0.24
DBP (mmHg)	75.8 ± 1.5	73.6 ± 1.7	0.35
PP (mmHg)	49.3 ± 1.8	48.1 ± 2.1	0.68
HR (beats/min)	82.6 ± 2.6	79.2 ± 2.0	0.30

Table 10 (cont.)

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
Family history (%)			
Hyperlipidemia	0	3.8	0.70
DM	4	15.4	0.17
Hypertension	12	7.7	0.61
Cardiovascular disease	0	7.7	0.86

* = $p < 0.05$, ** = $p < 0.001$

2. Laboratory findings

Blood biochemistry laboratory, lipid profile and uric acid, of the two groups did not differ significantly. Only leptin level was significantly increased in obese HL group when compared with obese NL group (47.1 ± 2.2 vs. 17.1 ± 0.8 ng/ml in obese HL group and obese NL, respectively, $p < 0.001$). Hematologic parameters, Hb and Hct was significantly lower in obese HL group (12.5 ± 0.3 vs. 13.7 ± 0.3 $x10^6$ g/dL, in obese HL and obese NL, respectively, $p = 0.02$ for Hb and 40.0 ± 0.8 vs. 42.7 ± 0.9% in obese HL and obese NL, respectively, $p = 0.03$). Whereas others hematologic parameters were similar. Details are presented in Table 11.

Table 11 Laboratory parameters of the obese with hyperleptinemia group and obese with normo-leptinemia group

Laboratory parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
Uric acid (mg/dL)	4.4 ± 0.2	3.8 ± 0.2	0.10
Triglyceride (mg/dL)	159.4 ± 11.2	159.8 ± 10.7	0.98
Cholesterol (mg/dL)	172.0 ± 6.2	170.2 ± 6.3	0.84
HDL (mg/dL)	41.7 ± 1.3	41.1 ± 1.6	0.79
LDL (mg/dL)	98.1 ± 6.2	96.9 ± 6.0	0.88
Leptin (ng/ml)	17.1 ± 0.8	47.1 ± 2.2 **	<0.001
WBC (cell/cumm.)	5,700 ± 369.1	6,088 ± 237.6	0.37
RBC ($x10^6$ cell/μL)	5.4 ± 0.1	5.1 ± 0.0	0.07
Hb (g/dL)	13.7 ± 0.3	12.5 ± 0.3 *	0.02
Hct (%)	42.7 ± 0.9	40.0 ± 0.8 *	0.03
MCV (fL)	78.6 ± 1.4	77.8 ± 1.2	0.67
MCH (pg)	24.7 ± 0.5	24.3 ± 0.5	0.69
MCHC (g/dL)	31.4 ± 0.3	31.2 ± 0.3	0.74
RDW (%)	14.9 ± 0.1	14.7 ± 0.1	0.43
Platelet count (cell/cumm.)	323,100 ± 15400	324,700 ± 13870	0.93
Neutrophil (%)	61.4 ± 1.3	59.4 ± 1.0	0.25
Lymphocyte (%)	31.6 ± 1.2	33.3 ± 1.2	0.31
Monocyte (%)	4.1 ± 0.3	4.6 ± 0.3	0.26
Eosinophil (%)	2.7 ± 0.3	2.7 ± 0.3	0.98
Basophil (%)	0.1 ± 0.0	0.1 ± 0.0	0.95

* = $p < 0.05$, ** = $p < 0.001$

3. Cardiac function assessed by 2D-conventional echocardiography

To investigate the impact of leptin on left ventricular function, 2D-conventional echocardiography was analyzed. It was shown that left ventricular systolic parameters mean LVEDV (biplane), mean LVESV (biplane), mean LVEF (both from biplane and Tei) and mean %FS were not different between groups. Similarly, left ventricular diastolic parameters, mean MV E velocity, mean MV A velocity, mean MV E/A ratio, mean e' septal, mean e' lateral, mean E/e' sepal, mean E/e' lateral and mean IVRT were not different between groups. The result was shown in Table 12.

Left ventricular dimensions and geometry were investigated and compared. The study demonstrated that, left ventricular dimensions and geometry parameters, mean IVSDd, mean LVIDd, mean LVPWd, mean LV mass, mean LVMI, mean RWT were not different between groups. Similarly, the proportion of concentric remodeling subjects, eccentric hypertrophy subjects, concentric hypertrophy subjects and normal geometry subjects were not different between groups (as shown in Table 12).

Additionally, mean LA/Ao ratio, mean AV Vmax, mean PV Vmax and mean RVSP were not significantly different between groups. The detail was shown in Table 12.

Table 12 2D-conventional echocardiography of the obese with hyperleptinemia group and obese with normo-leptinemia group

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
2D-mode			
LVEDV (biplane) (ml)	125.90 ± 5.57	114.50 ± 4.23	0.10
LVESV (biplane) (ml)	42.78 ± 2.56	41.82 ± 2.23	0.77
LVEF (biplane) (%)	66.26 ± 1.21	63.71 ± 1.07	0.12

Table 12 (cont.)

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
M-mode			
FS (%)	38.39 ± 0.94	36.93 ± 0.92	0.27
LVEF (Teicholz) (%)	68.21 ± 1.17	66.46 ± 1.17	0.29
LA/Ao ratio	1.30 ± 0.03	1.25 ± 0.04	0.36
IVSDd (cm)	0.88 ± 0.03	0.92 ± 0.03	0.35
LVIDd (cm)	4.89 ± 0.07	4.76 ± 0.07	0.21
LVPWd (cm)	0.83 ± 0.02	0.87 ± 0.02	0.25
LV mass (g)	141.40 ± 6.86	140.30 ± 7.48	0.92
LVMI (g/m^2)	75.00 ± 3.29	73.73 ± 4.13	0.81
RWT	0.35 ± 0.01	0.36 ± 0.01	0.57
Doppler-mode			
MV E velocity (ms)	0.93 ± 0.02	0.99 ± 0.02	0.12
MV A velocity (m/s)	0.48 ± 0.02	0.47 ± 0.02	0.97
MV E/A Ratio	2.05 ± 0.11	2.18 ± 0.10	0.39
MV DecT (ms)	189.50 ± 8.42	186.20 ± 6.29	0.75
e' septal (m/s)	0.15 ± 0.00	0.16 ± 0.00	0.36
e' lateral (m/s)	0.20 ± 0.00	0.19 ± 0.00	0.11
E/e' sep	6.32 ± 0.26	6.41 ± 0.27	0.81
E/e' lat	4.63 ± 0.18	5.12 ± 0.16	0.05
RVSP (mmHg)	17.11 ± 0.86	17.03 ± 0.92	0.95
AV Vmax (m/s)	1.10 ± 0.02	0.09 ± 0.03	0.78
PV Vmax (m/s)	1.01 ± 0.03	0.99 ± 0.02	0.70

Table 12 (cont.)

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
LV geometry			
Concentric remodeling (%)	12	7.7	0.96
Eccentric hypertrophy (%)	4	0	0.77
Concentric hypertrophy (%)	0	3.8	0.68
Normal geometry (%)	84	88.5	0.93

* = $p < 0.05$, ** = $p < 0.001$

4. Cardiac function assessed by 2D-Speckle-tracking echocardiography

In this study, we evaluated longitudinal deformation properties of the left ventricle between obese NL group and obese HL group by using 2D-STE and the following indices of longitudinal strain, absolute average longtudinal strain in A2C, absolute average longtudinal strain in A4C, absolute average longtudinal strain in APLAX and absolute GLS were used. The study showed that, absolute average longtudinal strain in A2C was 18.6 ± 0.4 and 20.4 ± 0.5% in obese HL group and obese NL group, respectively. Absolute average longitudianl strain in A4C was 18.8 ± 0.4 and 19.5 ± 0.4% in obese HL group and obese NL group, respectively. Absolute average longitudianl strain in APLAX was 18.2 ± 0.5 and 19.5 ± 0.6% in obese HL group and obese NL group, respectively. Absolute GLS was 18.5 ± 0.3 and 19.8 ± 0.4% in obese HL group and obese NL group, respectively. Statistical analysis revealed that absolute average longitudianl strain in A2C and absolute GLS were significantly lower in obese HL group when compared with obese NL ($p = 0.02$ and 0.03, respectively). There were no significant differences between groups regarding to absolute average longitudinal strain in A4C and absolute average longitudinal strain in APLAX. The 2D-STE parameters of the obese HL group and obese NL group are presented in Table 13. A representative of longitudinal deformation property of an obese HL participant was shown in Figure 8-9.

Table 13 2D-Speckle-tracking echocardiographic (longitudinal strain) results of the obese with hyperleptinemia group and obese with normo-leptinemia group

Parameters	Obese NL (n= 25) Mean ± SD	Obese HL (n=26) Mean ± SD	p-value
Absolute avg. long. strain in A2C (%)	20.4 ± 0.5	18.6 ± 0.4 *	0.02
Absolute avg. long. strain in A4C (%)	19.5 ± 0.4	18.8 ± 0.4	0.28
Absolute avg. long. strain in APLX (%)	19.5 ± 0.6	18.2 ± 0.5	0.10
Absolute GLS (%)	19.8 ± 0.4	18.5 ± 0.3*	0.03

* = $p < 0.05$, ** = $p < 0.001$

Avg. = average, A2C = apical 2 chamber view, A4C = apical 4 chamber view, APLAX= apical long axis view, GLS = global longitudinal strain.

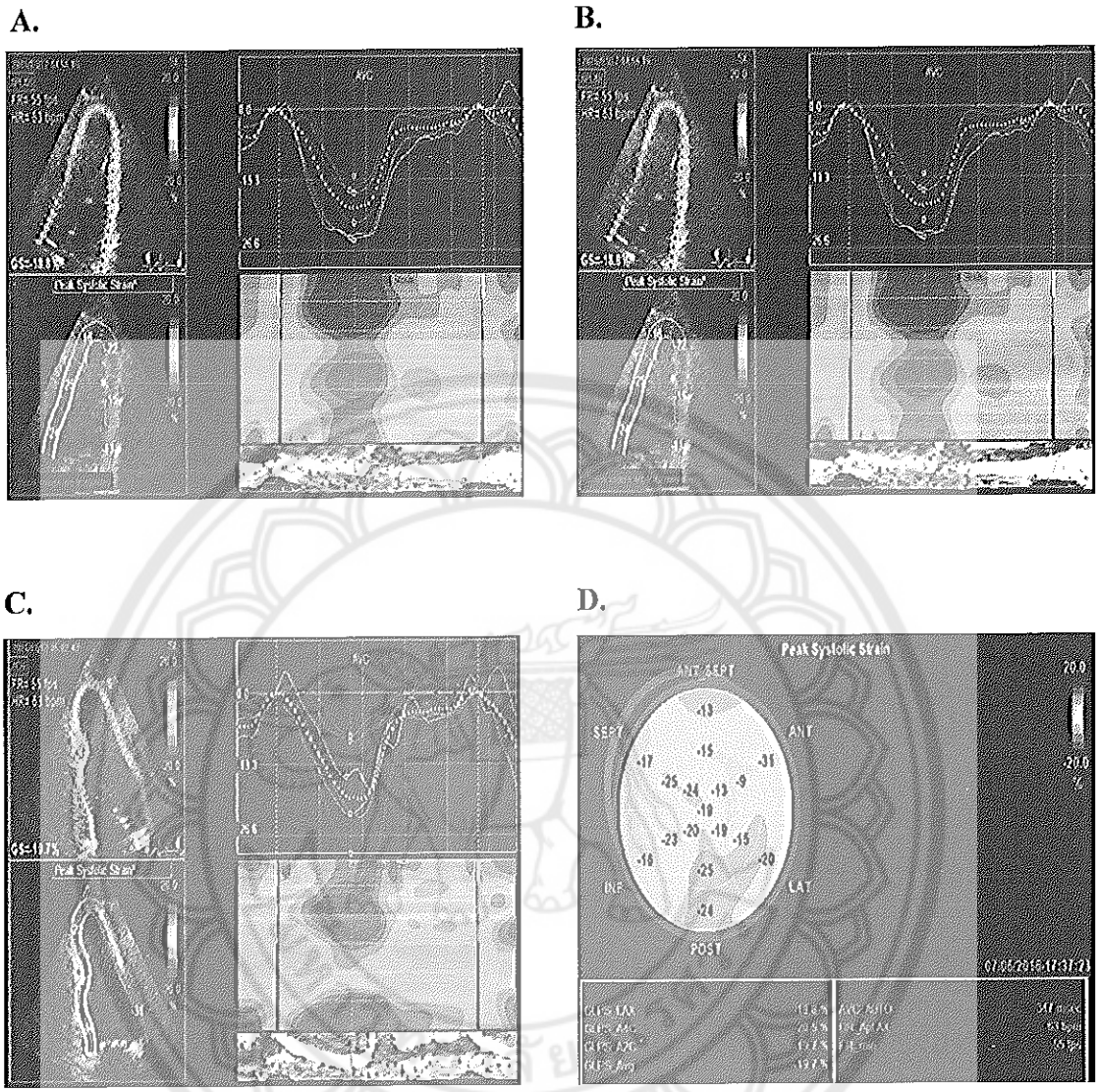

Figure 9 A representative figure of LV longitudinal strain of obese with normo-leptinemia (obese NL) group from (A) apical long axis view, (B) apical 4 chamber view, (C) apical 2 chamber view and (D) Diagram- showing the calculation of LV global longitudinal strain

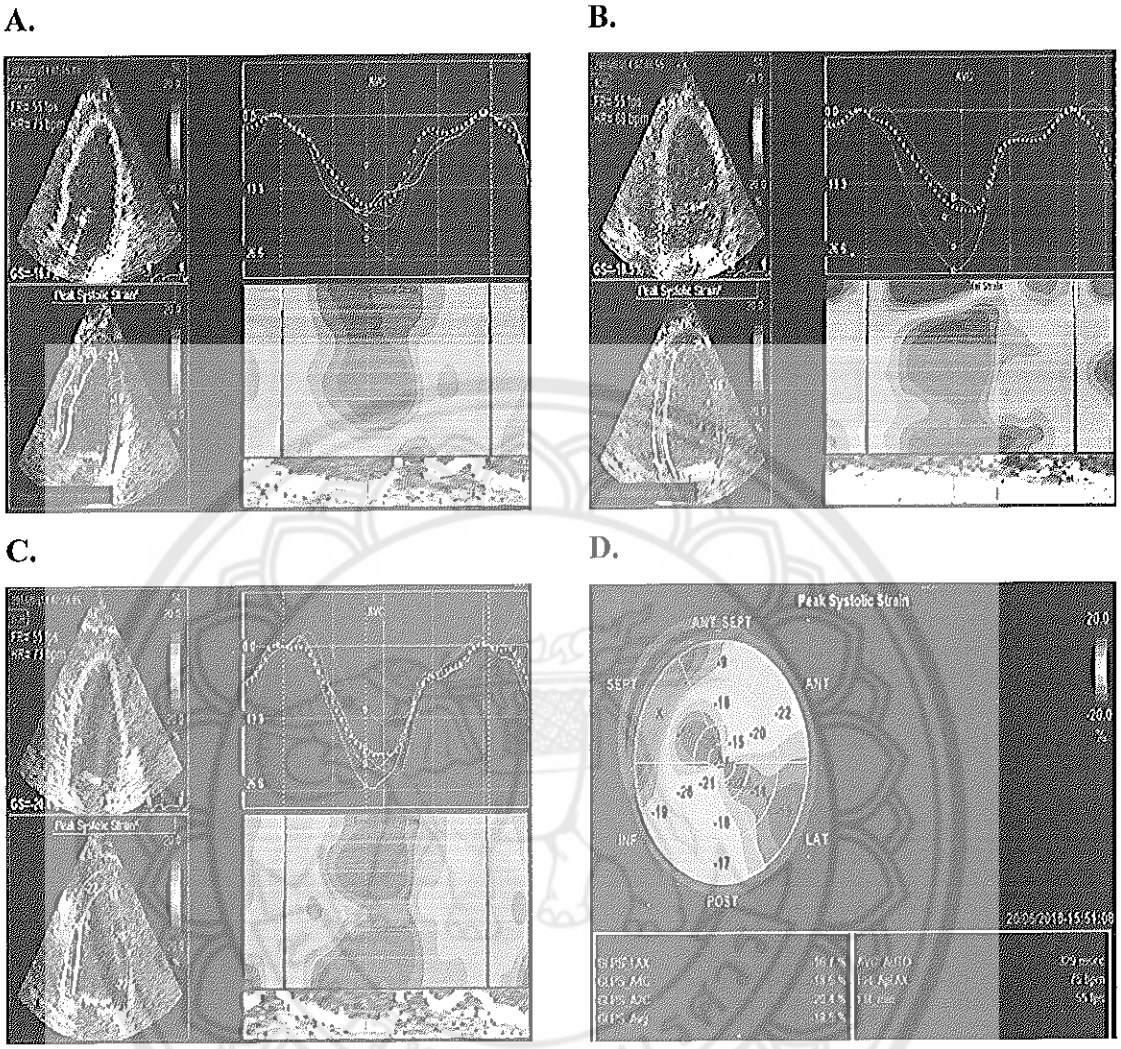

Figure 10 A representative figure of LV longitudinal strain of obese with hyperleptinemia (obese HL) group from (A) apical long axis view, (B) apical 4 chamber view, (C) apical 2 chamber view and (D) Diagram showing the calculation of LV global longitudinal strain

Association of leptin levels and other clinical parameters and absolute GLS

1. Univariate correlation analysis

To determine the association leptin levels and other clinical parameters on absolute GLS, the correlation of leptin levels and other clinical parameters and absolute GLS was evaluated by means of univariate regression analysis (The Pearson's Correlation Coefficient test), The result presented that, absolute GLS correlated negatively with BMI ($r = -0.39$, $p < 0.01$), Hb ($r = -0.24$, $p < 0.1$) and leptin level ($r = -0.23$, $p < 0.1$). Leptin level correlated positively with age ($r = 0.28$, $p < 0.05$), sex ($r = 0.37$, $p < 0.01$) and BMI ($r = 0.31$, $p < 0.05$) and correlated negatively with Hb ($r = -0.48$, $p < 0.01$). Univariate correlation analysis results of leptin levels and other clinical parameters and absolute GLS in obese participants are shown in Table 14. Figure 10-Figure 12 showed the Pearson's correlation scatter plot between absolute GLS and BMI, Hb level and leptin level ($r = -0.39$, $p < 0.01$, $r = -0.24$, $p = 0.08$, $r = -0.23$, $p = 0.09$, respectively).

2. Multivariate correlation analysis

Multivariable analysis was further performed. The study shown that leptin, Hb, BMI, age and sex can predict the absolute GLS with 32% ($R^2 = 0.32$) at p-value < 0.001 and $SE_{est} = \pm 1.86$. The strongest predictors of GLS were Hb ($\beta = 0.55$, $p < 0.05$), BMI ($\beta = 0.32$, $p < 0.05$) and leptin ($\beta = 0.35$, $p < 0.05$), respectively. The results are shown in Table15.

Table 14 Univariate correlation analysis in obese participants (n=51)

	Factors	1	2	3	4	5	6
1. Age:	Pearson correlation	1					
	p-value	-					
2. Sex:	Pearson correlation	0.15	1				
	p-value	0.29	-				
3. BMI:	Pearson correlation	0.01	-0.02	1			
	p-value	0.93	0.84	-			
4. Hb:	Pearson correlation	-0.16	-0.76***	-0.05	1		
	p-value	0.23	<0.01	0.71	-		
5. Leptin:	Pearson correlation	0.28**	0.37***	0.31**	-0.48***	1	
	p-value	0.04	<0.01	0.02	<0.01	-	
6. Absolute GLS:	Pearson correlation	0.05	0.13	-0.39***	-0.24*	-0.23*	1
	p-value	0.69	0.34	<0.01	0.08	0.09	-

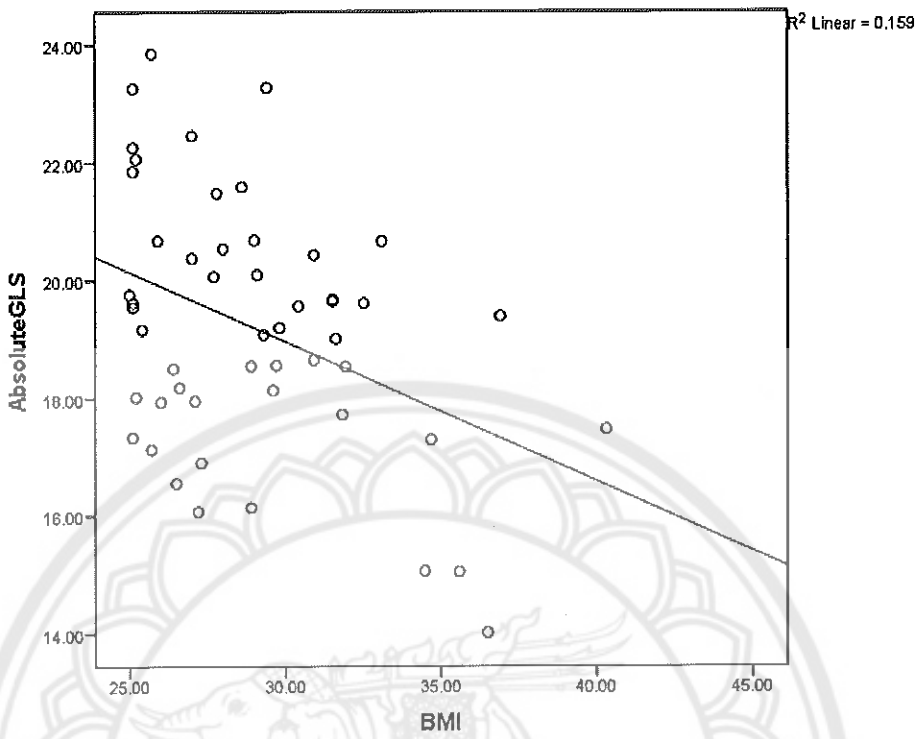

Figure 11 Correlation between absolute GLS and BMI in obese participants

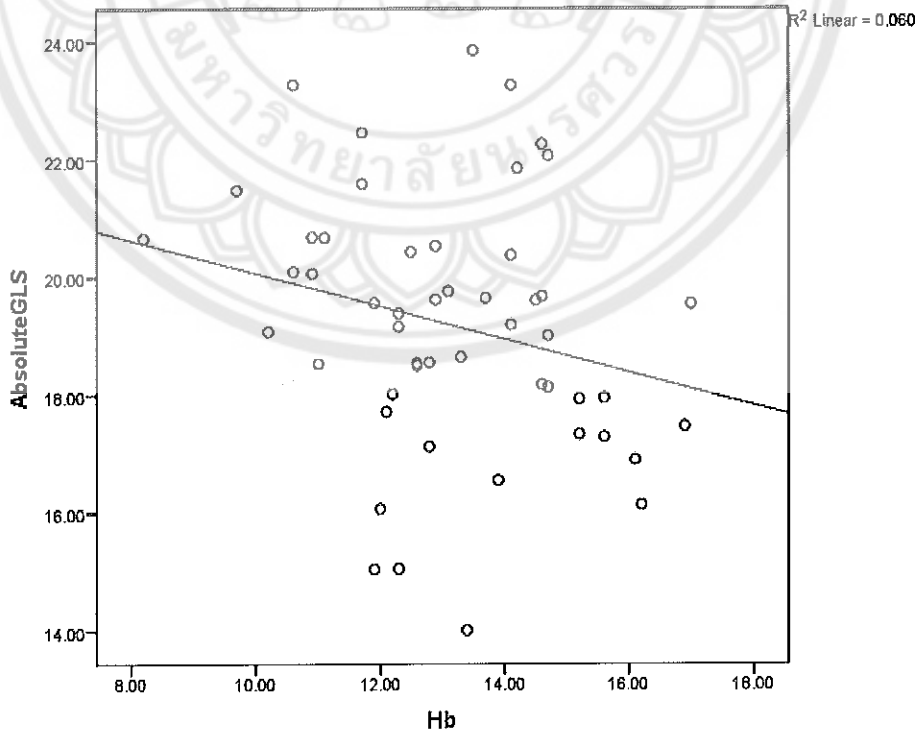

Figure 12 Correlation between absolute GLS and Hb levels in obese participants

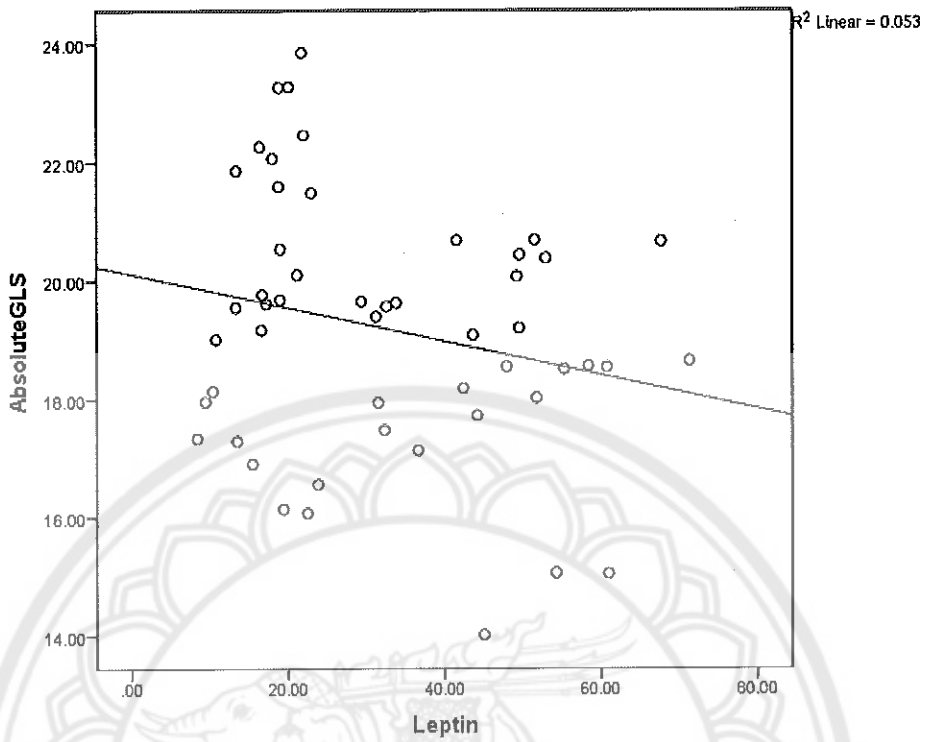

Figure 13 Correlation between absolute GLS and leptin level in obese participants

Table 15 Association of absolute GLS and leptin level and other clinical variables in obese participants

Factors	b	S.E.	β	t	p-value
1. Leptin	**-0.04**	**0.01**	**-0.35**	**-2.35**	**0.02**
2. Hb	**-0.63**	**0.22**	**-0.55**	**-2.75**	**0.008**
3. BMI	**-0.19**	**0.07**	**-0.32**	**-2.46**	**0.01**
4. Age	0.15	0.20	0.09	0.75	0.45
5. Sex	-0.76	0.83	-0.17	-0.91	0.36

$R = 0.57$, $R^2 = 0.32$, R^2 adj = 0.25, F (degree of freedom) = 4.39, Sig. of $F < 0.05$

$SE_{est} = \pm 1.86$, b = unstandardized beta coefficient, β = standardized beta coefficient.

CHAPTER V

DISCUSSION AND CONCLUSION

The present study demonstrated that there were significant differences in mean age, mean weight, mean BMI, mean waist circumference, mean body fat percentage, mean SBP and mean DBP between non-obese and obese adolescents. These changes may relate to obesity. The higher mean SBP and DBP in obese group found in this study are consistent with other studies Koebnick, et al. (169) showed that the number of hypertension in overweight adolescents was multiple when compared with lean adolescents. Furthermore, stepwise progression result was increased according to the higher weight for all subgroups. There were a number of studies indicating chances of hypertension in overweight youths are essentially higher compared to lean individuals (170-174). Hypertension and others cardiovascular involvement (i.e., higher LVM, cardiac dysfunction) recognized in beginning stages of obesity. The mechanisms may be from volume overload or humoral activation (46). It is thought that obesity may affect the renin-angiotensin-aldosterone system resulting in alterations of blood pressure. Cooper, et al. (175) discovered that the angiotensin-converting enzyme and angiotensinogen were higher in obese participants. It is also found that renin-angiotensin-aldosterone system has been found in adipocytes (176-178), resulting in an increased angiotensin II (Ang II). Ang II induces vasoconstriction and inotropic effects in the cardiovascular system (179). Therefore, increased Ang II level may be a possible pathway of obesity-related hypertension. The other parameter which may involve in increased blood pressure in obese is lipid profile. It was apparently shown that serum HDL was significantly lower whereas serum triglyceride was significantly higher in obese adolescents as compared to the non-obese group. However, there was no difference between groups in terms of serum cholesterol and serum LDL. This finding was in accordance with Freedman, et al. (180) and Reinhr, et al. (181) discovered that overweight is highly related to the triglyceride and HDL level and weakly related to the LDL level. Moreover, Chinali, et al. (15) found a significantly higher total triglyceride and lower HDL in obese adolescents. In addition,

there was showed that an increase in BMI was associated with increase in prevalence of diabetes mellitus, hypertension and dyslipidemia (182). Furthermore, hyperlipidemia itself can cause hypertension.

Hyperlipidemia causes endothelial dysfunction and loss of vasomotor activity (183-184). This damage may present as higher systemic blood pressure. Several studies have showed a correlation between hyperlipidemia and hypertension (185-186). Few prospective studies have demonstrated the relationship between plasma lipids and the future development of hypertension (187). The pathophysiology of endothelial dysfunction in hyperlipidemia is complex and involves multiple mechanisms. One of the most important mechanisms is the alterations of releasing vasodilator substances, that is, nitric oxide (NO) (188) and maybe involved in ROS production (189). It is possible that the higher mean SBP and mean DBP in this study may be a result of overweight, obesity and hyperlipidemia.

Interestingly, the present study showed that some of hematologic parameters varied between obese and non-obese group. Hemoglobin and hematocrit level was significant higher in obese as compared to non-obese adolescents. Previous studies have also reported higher hemoglobin levels among overweight and obese participants compared with normal weight participants. Elmugabil A., et al. (190) found that obese pregnancy women had higher WBC count and higher hemoglobin levels when compared with normal weight pregnancy woman. Whereas, Kordas, et al. (191) reported that obesity were related with a lower probability of anemia among non-pregnant Colombian women in the reproductive age, and that overweight and obese women had higher estimates of hemoglobin. Moreover, the study of Hanafi M. I., et al. (192) revealed that anemia was more prevalent among normal and underweight students with no significant correlation between hemoglobin level (anemia) and BMI among studied population. Bagni U. V., et al. (193) also revealed the lower Hb levels in obese when compared with overweight in adolescent girls. This discrepancy needs to be confirmed. An important factor which affects hemoglobin level, iron deficiency, should be investigated.

Our present study found that monocyte level was significantly lower in obese group when compared with non-obese group whereas other parameters did not vary. This finding is conflict with several studies, which demonstrated a chronic inflammation

state in obesity (194-195). The higher leukocyte and subpopulation counts (neutrophils and monocytes) were observed in obese adolescents (196-197), adults (198) and children (199).This controversy may be due to differences between groups in the number of participants and need more investigated.

Another finding of our study was that the obese group had statistically higher serum uric acid level (UA) when compared to the control group (4.1 ± 0.1 vs. 3.1 ± 0.1 mg/dL, $p < 0.001$). This was in accordance with Oyama, C., et al. (200) who found the positive correlation between serum UA levels and obesity-related parameters (BMI and percentage of overweight (POW)). Serum uric acid levels of the subjects with high POW were higher than low POW group, suggesting that serum UA levels were higher in obese subjects and could be used as one of obesity-related marker. This characteristic can be attributed to the following factors: 1) obese individuals have reduced renal clearance of uric acid, which can result in higher serum levels (201); 2) adipose tissue, similar to the liver and intestine, has abundant activity of xanthine oxidase (enzyme responsible for catalyzing purines and uric acid); and 3) obesity is associated with elevated activity of xanthine oxidase and increased production of uric acid by adipose tissue (202) .Moreover increased serum UA is correlated with metabolic syndrome, endothelial dysfunction, hypertension, diabetes, cardiovascular disease and all-cause mortality (203-210). The correlation of uric acid and cardiac dysfunction in obese adolescents need to be further investigated.

Leptin plays a critical role in controlling appetites, reproduction, and immune system (211). Leptin has been shown to be an important metabolic hormone which actions throughout the body (212). Similarly to insulin, plasma leptin levels are positively related with adiposity. However, obese individuals often have high plasma leptin levels, which result in a failure to respond to exogenous leptin and from reduced leptin receptor signaling. This leptin resistance limits the therapeutic use of leptin (213).

Clearly, serum leptin level was significantly higher in obese group when compared with non-obese group in this study. This finding was in accordance with Willers S. M., et al. who found correlation between BMI and leptin level (214).

One of the main purposes of this study was to investigate cardiac function of obese adolescents by 2D-conventional and Speckle-tracking echocardiography. Our study showed that, there were significant differences in some LV dimension and function parameters assessed by 2D-conventioanl echocardiography including LV mass, LVMI, RWT, IVSDd, LVIDd, LVPWd, LVEDV, LVESV and e' septal between obese and non-obese adolescents.

Apparently, cardiac morphological change (LV mass, LVMI, RWT, IVSDd, LVIDd and LVPWd) was found in obese participants. This finding supports previous studies (13,20,77,215) demonstrating that obesity was related with a higher prevalence of LV hypertrophy and increased LVM in adolescents. Moreover, Fribery, et al. (216) showed a higher LVM in obese adolescents by using cardiac MRI. It was confirmed that the increase in LVM was positively correlated with BMI. The structural changes may be due to higher vascular resistance or may be involved in hormonal changes like leptin or adiponectin (45-47). In the current study, global LV systolic function was assessed. It was found that there were increased LV end diastolic volume and end systolic volume (LVEDV and LVESV) in obese subjects, although almost all within the normal range. The evaluation of global LV systolic function assessed by LVEF and %FS was not different from that of non-obese teenagers. This finding is relevant to many studies showing normal systolic function in obese subjects (217-219). However, some studies indicated impaired systolic function in obese adolescents (15, 220). This discrepancy may be explained by Chinali, et al. (15) and Pascual, et al. (220) who revealed that a LV systolic dysfunction was only found in a severely obese subjects proposing that LV function is effected in late obesity. It is possible that the alteration of cardiac function might be subclinical heart disorder.

In the present study, there was no significant difference in main diastolic function indices, E/A ratio, Dec T, mitral early (E) or late diastolic (A) flow between the obese and non-obese adolescents. However, e' septal was initially reduced in obese group when compare to that of the non-obese adolescents. The E/e'sep ratio also showed a trend to be higher in obese adolescents ($p = 0.12$). This finding is relevant to a previous study that showed impaired early diastolic function (e') in obese children (87). However, the certain mechanism is not well investigated.

It was noted that the decreased of mitral annular velocities (e') indicated a pathological process rather than physiological adaptation of hypertrophic heart in obesity (221). In spite of many studies, there is still controversy regarding the impact of obesity on left ventricular diastolic parameters. The study of Kadappu, et al. suggested that one of tissue Doppler parameters (peak early diastolic annular velocity) measured on the level of mitral annulus may be clinically useful as early markers of global heart dysfunction in obese subjects (221). Moreover, Wang, et al. reveals that a reduction in e' velocity had prognostic significance in 518 subjects with a variety of cardiac vascular etiologies. Reduction in e' velocity and increased left atrial dimensions were the strongest predictors for cardiac mortality (222). Tissue Doppler imaging is capable to measure regional and global myocardial systolic and diastolic velocities. The velocities derived from the annulus or LV base primarily reflect longitudinal motion, due to the longitudinally directed fibers, which are found in the sub-endocardium (223-224). This may explain why these measurements are so useful for the assessment of the consequences of ischemia, to which the sub-endocardium is particularly sensitive (224-226). Therefore, e' appear to be good independent indices of diastolic function (227-228), and although e' is marginally superior as a prognosticator they are intrinsically linked as systolic function determines to some extent LV relaxation in early diastole (229).

Two-dimensional Speckle tracking echocardiography (STE) is a new method for investigating cardiac function. It can evaluate longitudinal, radial and circumferential strains, and quantitatively evaluate twist, rotation and torsion movements.The data obtained from STE shows better sensitivity for detection of systolic dysfunction even in the presence of normal LVEF (230-231).Therefore, it was considered to use STE to investigate subclinical cardiac dysfunction of obese adolescents in our study. It was found that all LV longitudinal strain indices including absolute average longitudinal strain in apical 2 chamber view, absolute average longitudinal strain in apical 4 chamber view, absolute average longitudinal strain in apical parasternal long axis view and absolute global longitudinal strain (GLS) were significantly lower in the obese adolescents. This result demonstrated subclinical LV systolic dysfunction in obese adolescents. Our finding is support the study of Share B. L., et al. that found worsen longitudinal strain and strain rate (SR) in obese women

adolescents. These results might be explained by altered relaxation and contraction properties of myocardial fibers in obesity which resulting from a higher ventricular and atrial filling pressure (232).

The second aim of this study was to assess cardiac function in obese adolescents with and without hyperleptinemia. Several studies have indicated that hyperleptinemia is a powerful risk factor for cardiovascular disease related with obesity. Leptin receptors have been discovered in atherosclerosis subjects and several studies demonstrated the correlation between plasma leptin level and atherosclerosis (38-39). Moreover, several studies revealed a more leptin-sensitive platelets and increased platelet aggregation in obese subjects when comparing with their lean subjects (44). Leptin also affects both cardiac structure and function. Some studies showed the positive correlation between plasma leptin levels and cardiac hypertrophy. In vitro study, leptin can induce myocytes hypertrophy in neonatal rat (45-47) and also in human cardiomyocytes (48). Cardiac function assessed by echocardiography in leptin- deficiency mice was altered. Samuelsson et al. assessed the effect of exogenous leptin administration on parameters of cardiovascular function in neonatal rats. Echocardiography indicated impaired left ventricular morphology and systolic function including increased LVIDd, LVIDs, LVPWd, increased LV volume at systole, increased LV mass, decreased IVSd with decreased LVEF and %FS in leptin-treated rats when compare with saline treated rats (49). These results provide information supporting the alteration of cardiac function in hyperleptinemia. It was questioned that whether subclinical dysfunction of obese adolescents is related to plasma leptin level. The obese adolescents categorized to normo-leptinemia group (obese NL) and hyperleptinemia group (obese HL) were investigated. Interestingly, our present study showed that over fifty percent of the obese adolescents had hyperleptinemia. This prevalence is consistent with the study of Popruk S., et al. (233) and Tungtrongchitr R., et al. (234), which found that hyperleptinemia were found in over sixty percent in overweight and obese Thai males and over eighty percent in Thai females age between 18-60 years old. However, Foschini D., et al. (37) reported the presence of hyperleptinemia in only 25.92% of obese Portuguese adolescents. This controversy may be due to ethnic differences of leptin level among adolescents (235).

The basic characteristic results showed that there were differences between obese NL and obese HL participants in mean BMI, mean %body fat, uric acid, and hemoglobin level. It was not surprised that the obese with hyperleptinemia had higher BMI than the normoleptinemic obese. Blum W. F., et al. found a strong exponential relationship between leptin level and body mass index (BMI) in healthy children and adolescent subjects (236). This finding may be explained by 'leptin-resistant' model. To describe this phenomenon, results from several studies showed that elevation of blood leptin level may be form excess amount of adipocyte in obese subject and chronically increased plasma leptin level resulting a decreases leptin receptor expression and reduces leptin signaling (237). Moreover, leptin resistance can increase s vulnerability to obesity (238). In conclusion, the increased leptin level in obese individuals leads to leptin resistance which contributes to more obesity, prompting to a vicious cycle of more metabolic disorders (239-240).

Another finding of our study was that the obese HL group had statistically lower Hb level and Hct level when compared to the obese NL group ($p < 0.05$). This finding is relevant to a study of Togo M., et al. which found a negative correlation between the levels of leptin and hemoglobin in Japanese men (241) and in Thai overweight and obese subjects (234). Erythropoiesis is regulated by erythropoietin and in response to hypoxic state (242). Leptin production occurs mainly in adipocytes which not found a sensor for hypoxia. Although the effect of leptin on hematopoiesis may be reserve, the results of our study and pior study (234,241), demonstrate that leptin may play a part in hematopoiesis. The relationship between leptin and hematopoiesis and its mechanism needed to be further investigated.

Although, there was no significant difference in 2D-conventional echocardiographic parameters of left ventricular systolic function, left ventricular diastolic function and left ventricular dimensions and geometry between the obese with hyperleptinemia and obese with normo-leptinemia adolescents, LV longitudinal strain indices were found significantly lower in the obese with hyperleptinemia group. These results support the role of STE in investigation of subclinical cardiac dysfunction in obese adolescents.

The positive correlation between obesity and increased risk of cardiovascular disease is well recognized. Obesity causes alterations in myocardial structure and function. Even though a mechanism is unclear, leptin has been thought as an important

factor or a contributing factor for obesity-induced cardiovascular disease. Several studies have demonstrated that leptin is able to regulate a variety of cardiac and vascular effects that include hypertensive (243), atherosclerotic effects (244), endothelial dysfunction (245) and thrombosis induction (246). At cardiac level, although some data are contradictory, leptin seems to contribute to the modulation of metabolism, hypertrophic effects, and turnover of ECM, so hyperleptinemia is independently related to poorer cardiovascular outcome. In vitro study, leptin can induce hypertrophy in rat myocytes (45-47) and human cardiomyocytes (48). Cardiac function assessed by echocardiography in leptin- deficiency mice was altered. Samuelsson AM., et al. assessed the effect of exogenous leptin administration on cardiovascular function in rats. 2D-conventional echocardiographic parameters indicated impaired left ventricular morphology and systolic function including increased LV internal diameter at diastole (LVIDd), LV internal diameter at systole (LVIDs), LV posterior wall at diastole (LVPWd), increased LV volume at systole, increased LV mass, decreased intraventricular septal thickness at diastole (IVSd) with decreased ejection fraction (LVEF) and fractional shortening in leptin-treated rats when compare with saline treated rats (49). These results confirm the alteration of cardiac structure and function by hyperleptinemia. However, these parameters did not change in obese participants in our study. This difference may be form the different species and leptin level in rats model may be higher than that in our study.

To the best of our knowledge, our study is the first to demonstrate the influence of leptin on the 2D-speckle-tracking echocardiographic parameters in disease-free obese adolescents. However, it was noted that other parameters, Hb and BMI, maybe involved in this alteration. To test that, we used univariate regression and multivariable regression analysis. Multivariable analysis showed that Leptin, Hb, BMI, age and sex can predict the absolute GLS with 32% (R^2= 0.32) at p-value < 0.001 and $SE_{est} = \pm 1.86$. The serum leptin level is independently correlated absolute GLS.

The association between mean BMI and GLS has been addressed recently (5). Our result is relevant to that observation. It was shown that the increase in BMI related to a worsen GLS (lower absolute GLS). Therefore, it is suggested that losing weights in young obese is necessary and routine checking up may be needed.

Becoming obese is an anabolic event. An increased body size leading to a larger circulation system resulting in increased blood volume, myocardial fibers hypertrophy and chamber enlargement. Several studies from autopsy showed a significantly higher in heart weight, ventricular enlargement and eccentric hypertrophy leading to congestive heart failure. Moreover, the strongly correlation between left ventricular mass, chamber size and wall thickness with severity and duration of obesity was discovered (72). In the study in obese children and adolescents the results showed that obese children and adolescents had significantly higher LV mass (indexed with height or surface area) than normal-weight subjects (75). Many studies, revealed a positive correlation between BMI and cardiac output and stroke volume in obese female adolescents (78) and in obese adolescents subjects (80-81). Although these results demonstrated an increased cardiac size and output but obese subjects often presented evidence of myocardial dysfunction, which is positively associated with the severity and duration of obesity (86).

Several factors have been proven to account for the cardiac dysfunction in obese subjects. Myocardial dysfunction in obesity may be the result of long-term volume overload. This is called 'high output' state which leading to congestive heart failure. Another possible mechanism may promote myocardial dysfunction is 'insulin resistance' which can cause an alteration in myocardial substrate utilization leading to an increased myocardial fatty acid oxidation and oxygen consumption consequence to an altered in cardiac function (88). Interestingly, leptin which secreted by adipocytes has been indicated to cause myocardial dysfunction in rats (89).

One of the parameters that correlated with absolute GLS is hemoglobin. Higher hemoglobin showed significant relation to worsen cardiac strains (lower absolute GLS).These results provides information supporting the effects of increased hemoglobin on cardiac dysfunction of obese adolescents. This is consistent with Guglin M., et al. that found that lower hemoglobin and hematocrit level had significant effects to improve LV and RV ventricular systolic function in heart failure patients. A possible mechanism is that higher hemoglobin can leads to increased blood viscosity and increased the cardiac workload, which leads to worsen contractility. Likewise, several animal studies demonstrated that a decreased in blood viscosity significantly increased in coronary blood flow, increased in left ventricular systolic function,

increased in left ventricular stroke volume and cardiac output (247-248). All these findings support our finding that an increased in Hb correlates with worsen LV systolic function.

The association of serum leptin level and compromised cardiac function has been found in our study. Hyperleptinemia is independently related to poorer cardiac strains. In the past decades, there have been limited data supporting the impact of hyperleptinemia on cardiac function in human. The mechanism by which leptin alters cardiac function is poor understood. It is thought that leptin may induce cardiac hypertrophy via autonomic nervous system (249) or may act directly to cardiomyocytes (48,250). Moreover leptin provides some effects on the vascular system. It leads to vascular remodeling and angiogenesis. Leptin also determined an significant factor in hypertension and leading to cardiac hypertrophy (251).

Leptin can induce cardiomyocyte hypertrophy by its signaling via the PI3K-AKT and MAP kinases (48,250,252-253). Moreover hyperleptinemia can cause an increase protein synthesis and increased expression of actin and myosin which up regulated in cardiac hypertrophy (48). In addition chronic leptin administration induces higher blood pressure and heart rate by stimulation of sympathetic nervous system (249,254) leading to left ventricle hypertrophy (255).

Leptin can induce vascular remodeling and leading to hypertension (240). The possible mechanisms may be involved in the RhoA/ROCK pathway, PI3K/AKT pathway, and the MAP Kinases (105-106). In addition, leptin increases the production of proinflammatory cytokines, such as TNF-α, IL-6 and reactive oxygen species (ROS) in endothelial cells (256-257). Leptin has also increases the secretion of the vasoconstrictor (endothelin-1 ((ET-1)) (258). Leptin has also stimulating platelet aggregation (246), monocytes (259) and effecting the immune response (260-261). This leads to the vascular dysfunction, hypertension and resulting in cardiac hypertrophy. Moreover, it is found that the effect of leptin may be involved the myocardial extracellular matrix (ECM). From studied in ob/ob mice (109) and diet-induced obesity (DIO) mice (110), the results showed an increased fibrosis in ob/ob mice and DIO mice, suggesting that leptin signaling might contribute to the alteration of the cardiac ECM in obesity (109-110). Leptin enhanced MMP-2 expression and activity in neonatal rat fibroblasts (112) and primary human pediatric ventricular

myocytes (111). To date, studies of the effect of leptin on ECM remodeling are few. Leptin's effects on cardiac remodeling in obesity needed to be confirmed. Another possible mechanism is that, leptin may contribute to an alteration in metabolism substrate utilization leads to cardiac dysfunction. Study in isolated rat hearts, a showed that leptin administration increases FA oxidation and triacylglycerol lipolysis via stimulated STAT3-NO-p38 MAPK pathway (64,114) In an in vitro study, leptin infusion in murine cardiomyocytes can cause increases in FA uptake (115). These studies support that leptin may alter the cardiac metabolism resulting in cardiac dysfunction. It is possible that a worse longitudinal systolic strain in obese with hyperleptinemia participants in our study may be result from these mechanism.

Conclusion

In conclusion, subclinical LV systolic dysfunction found in obese adolescents and found in obese adolescents with hyperleptinemia indicates that leptin could be a link between obesity and the subclinical LV systolic dysfunction. It was also found that hyperleptinemia was associated to worsen longitudinal myocardial deformation even in the absence of comorbidities in early stages in obese adolescents. These results should provide the obese individual and physicians to better management of obesity in adolescents.

Limitations and strengths

The strengths of this study are our study is the first to demonstrate the influence of leptin on the 2D-speckle-tracking echocardiography in disease-free obese Thai adolescents and our study use the proper cut off value for obesity for Asian population which can truly classify subjects as non-obese or obese. However, there are some limitations in this study. Our study group is a relatively small and also the single-center study. The number of women participants which is larger than men should be considered. Further studies in a larger population and multi-center research may be giving more benefits. Additionally, many evidence has demonstrated a strongly correlation between hyperleptinemia and hyperinsulinemia which have not investigated in our study. Moreover, the renin-angiotensin-aldosterone system may also play an important role which also not investigated. Therefore, insulin level and

the role of leptin in mediating the vascular, cytokines, sympathetic nervous system activity and renin–angiotensin–aldosterone system activity needed to be investigated for certain mechanisms.

There is also a limitation of strain analysis in this study. Although the reproducibility was excellent in longitudinal strain, it was fair in circumferential and radial strain. Therefore, we did not use these two strains in our study. It is suggested that these strains, including twist, rotation and torsion should be performed in further investigation.

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APPENDIX

APPENDIX A ETHICS APPROAVAL FORM

COA No. 030/2016
IRB No. 796/58

คณะกรรมการจริยธรรมการวิจัยในมนุษย์
มหาวิทยาลัยนเรศวร
99 หมู่ 9 ตำบลท่าโพธิ์ อำเภอเมือง จังหวัดพิษณุโลก 65000 เบอร์โทรศัพท์ 05596 8642

เอกสารรับรองโครงการวิจัย

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยนเรศวร ดำเนินการให้การรับรองโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นมาตรฐานสากล ได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guideline และ International Conference on Harmonization in Good Clinical Practice หรือ ICH-GCP

ชื่อโครงการ	:	การประเมินการทำงานของหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเทคนิคสเปคเคิ้ลแทรคกิ้งในวัยรุ่นอ้วนที่มีเลปตินในเลือดสูง
Study Title	:	Cardiac function assessed by speckle tracking echocardiography in obese adolescents with hyperleptinemia.
ผู้วิจัยหลัก	:	ด.ร.ดวงเดือน สิริวิทยาวรรณ
Principal investigator	:	Dr. Duangduan Siriwittayawan
สังกัดหน่วยงาน	:	คณะสหเวชศาสตร์
ผู้ร่วมวิจัย	:	นางสาวชมเลวรรณ อิ่มเอิบธรรม
วิธีทบทวน	:	แบบเร่งรัด (Expedited Review)
รายงานความก้าวหน้า	:	ส่งรายงานความก้าวหน้าอย่างน้อย 1 ครั้ง/ปี หรือ ส่งรายงานฉบับสมบูรณ์หากดำเนินโครงการเสร็จสิ้นก่อน 1 ปี

เอกสารรับรอง

1. AF 01-10 เวอร์ชั่น 1.0 วันที่ 27 พฤศจิกายน 2558
2. AF 02-10 เวอร์ชั่น 1.0 วันที่ 27 พฤศจิกายน 2558
3. AF 03-10 เวอร์ชั่น 2.0 วันที่ 13 มกราคม 2559
4. AF 04-10 เวอร์ชั่น 2.0 วันที่ 06 มกราคม 2559
5. AF 05-10 เวอร์ชั่น 2.0 วันที่ 06 มกราคม 2559
6. สรุปโครงการเพื่อการพิจารณาทางจริยธรรมการวิจัยในมนุษย์ เวอร์ชั่น 1.0 วันที่ 27 พฤศจิกายน 2558
7. โครงการวิจัย เวอร์ชั่น 2.0 วันที่ 06 มกราคม 2559
8. ประวัติผู้วิจัย เวอร์ชั่น 1.0 วันที่ 11 พฤศจิกายน 2558
9. งบประมาณที่ได้รับ เวอร์ชั่น 1.0 วันที่ 11 พฤศจิกายน 2558
10. แบบเก็บข้อมูล เวอร์ชั่น 1.0 วันที่ 11 พฤศจิกายน 2558

ลงนาม
(นายแพทย์สมบูรณ์ ศันสุภหวัสดิกุล)
ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์
มหาวิทยาลัยนเรศวร

วันที่รับรอง	:	20 มกราคม 2559
Date of Approval	:	January 20, 2016
วันหมดอายุ	:	20 มกราคม 2560
Approval Expire Date	:	January 20, 2017

ทั้งนี้ การรับรองนี้มีเงื่อนไขดังที่ระบุไว้ด้านหลังทุกข้อ (ดูด้านหลังของเอกสารรับรองโครงการวิจัย)

APPENDIX B DATA COLLECTION FORM

Version 1.0
11 พ.ย. 58

แบบเก็บข้อมูล : การประเมินการทำงานของหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเทคนิคสเปคเคิ้ลแทรคกิ้งในวัยรุ่นอ้วนที่มีเลปตินในเลือดสูง

ส่วนที่ 1 ข้อมูลทั่วไป

เพศ ☐ ชาย ☐ หญิง อายุ.....ปี

ประวัติการสูบบุหรี่ ☐ ไม่สูบ ☐ สูบ จำนวน.....มวน/วัน

☐ เคยสูบ เลิกสูบมาแล้ว .....เดือน/ปี

ประวัติการดื่มแอลกอฮอล์ ☐ ไม่ดื่ม ☐ ดื่ม ระยะเวลา.....เดือน/ปี

☐ เคยดื่ม เลิกดื่มมาแล้ว.....เดือน/ปี

โรคประจำตัว ☐ ไม่มี ☐ มี ระบุ.....

ท่านมีภาวะหยุดหายใจขณะหลับหรือไม่ ☐ ไม่มี ☐ มี

ยาที่ใช้เป็นประจำ ☐ ไม่มี ☐ มี ระบุ.....

ประวัติความเจ็บป่วยในครอบครัว ☐ ไม่มี ☐ มี ระบุ.....

พฤติกรรมการออกกำลังกาย ☐ ออกกำลังกายเป็นประจำ (≥ 3 ครั้ง/สัปดาห์)

☐ ออกกำลังกายบ้าง แต่ไม่ค่อย (< 3 ครั้ง/สัปดาห์) ☐ ไม่ออกกำลังกาย

ประเภทการออกกำลังกาย (ออกกำลังกาย ด้วยวิธีใด) .....

ส่วนที่ 2 การตรวจร่างกาย

น้ำหนัก .....กิโลกรัม ส่วนสูง .....เซนติเมตร

BMI .....กก./ม.^{2} เส้นรอบเอว .....เซนติเมตร

Body fat .....% Body fat .....กิโลกรัม

ความดันโลหิต

วัดครั้งที่ 1..... mmHg วัดครั้งที่ 2..... mmHg วัดครั้งที่ 3..... mmHg

เฉลี่ย..... mmHg

อัตราการเต้นของหัวใจ ครั้งที่ 1..... ครั้งที่ 2..... ครั้งที่ 3.....

ผลการตรวจเลือด

Hemoglobin..... g/dl Hematocrit..... %

RBC..... $x10^6$/ul MCV..... fl

MCH..... pg MCHC..... g/dl

WBC..... $x10^3$/ul Platelet..... $x10^3$/ul

Neutrophils.....% Lymphocytes.....%

Monocytes..... % Eosinophils.....%

Basophils.....%

Total cholesterol ..... mg/dL Triglycerides ..... mg/dL

HDL ..... mg/dL LDL..... mg/dL

VLDL ..... mg/dL Fasting blood glucose ..... mg/dL

Leptin ..... ng/ml

ผลการตรวจคลื่นเสียงสะท้อนความถี่สูง

M-mode		2D-echocardiography	
IVSDd	..... mm	LVEDV	..... ml
LVIDd	..... mm	LVESV	..... ml
LVPWd	..... mm	LVEF	..... %
LVMI	..... g/m^2		
Ao/LA ratio	.....		
Doppler		**2D-strain**	
MV E velocity	..... m/s	GLS	..... %
MV A velocity	..... m/s	GCS	..... %
MV E/A Ratio	.....	GRS	..... %
MV DecT	..... ms		
IVRT	..... Ms		
E' septal	..... m/s		
E' lateral	..... m/s		
E/E'	.....		

แบบคัดกรอง

ภาวะนอนกรนและปัจจัยเสี่ยงต่อภาวะอุดกั้นทางเดินหายใจขณะหลับ (OSA)

ส่วนที่ 1

คุณเคยนอนกรนหรือไม่	☐ไม่เคย	☐เคย
คุณรู้สึกเหนื่อย ไม่สดชื่นหลังตื่นนอนไหม	☐ไม่เคย	☐เคย
คุณรู้สึกว่าตัวเองอ้วนหรือไม่ (BMI ≥ 25 kg/m^2)	☐ไม่รู้สึก	☐รู้สึกว่าอ้วน
คุณเป็นโรคความดันโลหิตสูงหรือไม่ (BP≥140/90 mmHg)	☐ไม่เป็น	☐เป็น

ส่วนที่ 2	ช่องนี้สำหรับเจ้าหน้าที่
1. คุณเคยนอนกรนไหม	
☐เคย ☐ไม่เคย ☐ไม่ทราบ	(1) (0) (0)
2. ถ้าคุณนอนกรน เสียงกรนของคุณเป็นอย่างไร	
☐เสียงดังกว่าเสียงหายใจเล็กน้อย	(0)
☐เสียงดังเท่าๆกับเสียงพูดคุย	(0)
☐เสียงดังกว่าเสียงพูดคุย	(0)
☐เสียงดังมาก คนในห้องที่อยู่ติดกันสามารถได้ยิน	(1)
☐ไม่ทราบ	(0)
3. คุณนอนกรนบ่อยแค่ไหน	
☐แทบทุกคืน	(1)
☐ 3-4 คืน / สัปดาห์	(1)
☐ 1-2 คืน / สัปดาห์	(0)
☐ 1-2 คืน / เดือน	(0)
☐ไม่เคย / แทบไม่เคย / ไม่ทราบ	(0)
4. ภาวะนอนกรนของคุณรบกวนบุคคลอื่นไหม	
☐ใช่	(1)
☐ไม่รบกวน / ไม่ทราบ	(0)
5. เคยมีใครบอกคุณไหมว่าคุณมีอาการหยุดหายใจขณะหลับ เช่น กระสับกระส่ายอยู่บนเตียง เสียงกรนหยุดไปชั่วขณะแล้วกลับมากรนเสียงดังอีกเป็นระยะๆ	
☐แทบทุกวัน	(2)
☐ 3-4 ครั้ง / สัปดาห์	(2)
☐ 1-2 ครั้ง / สัปดาห์	(0)
☐ 1-2 ครั้ง / เดือน	(0)
☐ไม่มี / แทบไม่มี / ไม่ทราบ	(0)

		ช่องนี้สำหรับเจ้าหน้าที่	
ส่วนที่ 3			
6.	หลังจากตื่นนอนคุณรู้สึกเหนื่อยและอ่อนเพลียแค่ไหน		
	☐แทบทุกวัน		(1)
	☐ 3-4 ครั้ง / สัปดาห์		(1)
	☐ 1-2 ครั้ง / สัปดาห์		(0)
	☐ 1-2 ครั้ง / เดือน		(0)
	☐ไม่ทราบ		(0)
7.	ในระหว่างทำงานคุณรู้สึกเหนื่อยและอ่อนเพลียบ่อยแค่ไหน		
	☐แทบทุกวัน		(1)
	☐ 3-4 ครั้ง / สัปดาห์		(1)
	☐ 1-2 ครั้ง / สัปดาห์		(0)
	☐ 1-2 ครั้ง / เดือน		(0)
	☐ไม่เคย / แทบไม่เคย		(0)
8.	ในระหว่างทำงานหรือขับรถ คุณเคยสัปหงกหรือหลับไหม		
	☐เคย	(1)	
	☐ไม่เคย		(0)
9.	ถ้าในข้อ 8 คุณตอบ เคย คุณเป็นบ่อยแค่ไหน		
	☐แทบทุกวัน		(1)
	☐ 3-4 ครั้ง / สัปดาห์		(1)
	☐ 1-2 ครั้ง / สัปดาห์		(0)
	☐ 1-2 ครั้ง / เดือน		(0)
	☐ไม่เคย / แทบไม่เคย		(0)
ส่วนที่ 4			
10.	คุณเป็นโรคความดันโลหิตสูงหรือเป็นโรคอ้วนหรือไม่		
	☐เป็น ☐ไม่เป็น	(1)	(0)

การแปลผลแบบคัดกรอง

ส่วนที่ 1 ถ้าตอบว่าไม่เคยทั้ง 4 ข้อ แสดงว่า ไม่มีภาวะเสี่ยง

แต่ถ้ามีอย่างน้อย 1 ข้อ อาจมีภาวะเสี่ยง ให้มารวมคะแนนในส่วนที่ 2-4

ส่วนที่ 2 จากข้อที่ 1-5 ถ้ารวมคะแนนได้ ≥2 คะแนน ถือว่า Positive

ส่วนที่ 3จากข้อที่ 6-9 ถ้ารวมคะแนนได้ ≥2 คะแนน ถือว่า Positive

ส่วนที่ 4จากข้อที่ 10 ถ้ารวมคะแนนได้ 1คะแนน ถือว่า Positive

ดังนั้น จากส่วนที่ 2-4 ถ้า Positive ≥2 ส่วน ถือว่า มีความเสี่ยงต่อการเกิดภาวะอุดกั้นทางเดินหายใจขณะหลับ

APPENDIX C INFORMED CONSENT FORM

Version 1.0
19 พ.ย. 58

AF 05-10/3.0

Naresuan University Institutional Review Board	หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย (Informed Consent Form)

การวิจัยเรื่อง การประเมินการทำงานของหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเทคนิคสเปคเคิ้ลแทรคกิ้งในระยะอิ่วน
ที่มีเลปติดในเลือดสูง

วันให้คำยินยอม วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า นาย/นาง/นางสาว.....
ที่อยู่.....ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยที่วิจัยที่ลงนามฉบับวันที่..... และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนาม และ วันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย ความเสี่ยง หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย และแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจให้ปิดไว้ข้อมูลจนข้าพเจ้าพอใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผล และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคหรือสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่นในนามของคณะกรรมการพิจารณาจริยธรรมการวิจัยในคน อาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของข้าพเจ้า ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการลงนามที่จะเข้าร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของข้าพเจ้าได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการวิจัยและต้องการให้ทำลายเอกสารและ/หรือ ตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถเชื่อมโยงถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อ จะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตเพื่อการวิจัยทางด้านเภสัชภัณฑ์เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

.....ลงนามผู้ให้ความยินยอม
(.....) ชื่อผู้ยินยอมตัวบรรจง
วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด ให้ผู้เข้าร่วมในโครงการวิจัยอ่านข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย

(.....) ชื่อผู้ทำวิจัย ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน

(.....) ชื่อพยาน ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

Naresuan University Institutional Review Board	หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย สำหรับผู้แทนโดยชอบธรรม/ผู้ปกครอง (Informed Consent Form)

โครงการวิจัยเรื่อง การประเมินการทำงานของหัวใจด้วยคลื่นเสียงละห้อนความถี่สูงเทคนิคสเปคเคิ้ลแทรคกิ้งในวัยรุ่นอ้วนที่มีเลปตินในเลือดสูง

วันให้ความยินยอม วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า นาย/นาง/นางสาว..... (ชื่อ-นามสกุล ผู้แทนโดยชอบธรรม/ผู้ปกครอง) ที่อยู่..... ซึ่งมีความสัมพันธ์เป็น .....ของ ด.ช./ด.ญ./นาย/นาง/นางสาว..... (ชื่อ-นามสกุล ของผู้เข้าร่วมการวิจัย) ได้อ่านรายละเอียดจากเอกสารข้อมูลคำชี้แจงสำหรับผู้เข้าร่วมการวิจัยที่เขียนเข้าใจฉบับวันที่..... แล้ว ข้าพเจ้ายินยอมให้ ด.ช./ด.ญ./นาย/นาง/นางสาว.....(ชื่อ-นามสกุล ของผู้เข้าร่วมวิจัย) เข้าร่วมในโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนาม และ วันที่ พร้อมด้วยเอกสารข้อมูลคำชี้แจงสำหรับผู้เข้าร่วมในโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมเข้าร่วมในการวิจัยนี้ ข้าพเจ้าและผู้เข้าร่วมการวิจัยได้รับการอธิบายจากผู้วิจัยเกี่ยวกับวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย ผลประโยชน์ หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยและแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้าและผู้เข้าร่วมการวิจัยมีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยทั้งหมดจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ที่ข้าพเจ้าและผู้เข้าร่วมการวิจัย สงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าและผู้เข้าร่วมการวิจัย พอใจ

ข้าพเจ้าและผู้เข้าร่วมในโครงการวิจัยได้รับทราบจากผู้วิจัยว่าหากเกิดอันตรายใด ๆ จากการวิจัยครั้งนี้ ผู้เข้าร่วมวิจัยจะได้รับการรักษาพยาบาล โดยไม่เสียค่าใช้จ่าย (และระบุว่า จะได้รับการชดเชยจากผู้สนับสนุนการวิจัยหรือไม่)

ข้าพเจ้าเข้าใจดีถึงสิทธิที่จะบอกเลิกการเข้าร่วมการวิจัย เมื่อใดก็ได้โดยไม่จำเป็นต้องแจ้งเหตุผลและการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาใดๆหรือสิทธิอื่นๆ ที่ผู้เข้าร่วมการวิจัยจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวของผู้เข้าร่วมการวิจัยเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่น ในนามของสถิติหรือผู้สนับสนุนการวิจัย คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน และสำนักงานคณะกรรมการอาหารและยา อาจจะได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลส่วนตัวของผู้เข้าร่วมการวิจัย ทั้งนี้ระดับการทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการตกลงที่จะเข้าร่วมการศึกษาวิจัยนี้ข้าพเจ้าได้ให้ความยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของผู้เข้าร่วมการวิจัยได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ ของผู้เข้าร่วมการวิจัย เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการวิจัยและต้องการให้ทำลายเอกสารและ/หรือตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวผู้เข้าร่วมการวิจัย

ข้าพเจ้าเข้าใจว่า ข้าพเจ้าและ ผู้เข้าร่วมการวิจัยมีสิทธิที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของผู้เข้าร่วมการวิจัย และสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของผู้เข้าร่วมการวิจัยได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ที่ไม่มีการเปิดเผยชื่อของผู้เข้าร่วมการวิจัย จะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางด้านเภสัชภัณฑ์ เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้น และมีความเข้าใจดีทุกประการแล้ว ยินดีให้ ด.ช./ด.ญ./นาย/นาง/นางสาว.....(ชื่อ-นามสกุล ของผู้เข้าร่วมวิจัย) เข้าร่วมในโครงการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารใบยินยอมนี้

.....ลงนามผู้แทนโดยชอบธรรม/ผู้ปกครอง
(.....) ชื่อผู้แทนโดยชอบธรรม/ผู้ปกครอง(ตัวบรรจง)
.....ความสัมพันธ์ของผู้แทนโดยชอบธรรม/ผู้ปกครองกับผู้เข้าร่วมการวิจัย

วันที่ .....เดือน.....พ.ศ .....

ส่วนเพิ่มเติมโครงการวิจัย

ข้าพเจ้า ☐ ยินยอม
☐ ไม่ยินยอม

ให้เก็บตัวอย่างชีวภาพที่เหลือของ ด.ช./ด.ญ/นาย/นาง/นางสาว.....(ชื่อ-นามสกุล ของผู้เข้าร่วมวิจัย)ไว้เพื่อการวิจัยในขนาด

.....ลงนามผู้แทนโดยชอบธรรม/ผู้ปกครอง
(.....) ชื่อผู้แทนโดยชอบธรรม/ผู้ปกครองตัวบรรจง
.....ความสัมพันธ์ของผู้แทนโดยชอบธรรม/ผู้ปกครองกับผู้เข้าร่วมการวิจัย

วันที่ .....เดือน.....พ.ศ.....

หมายเหตุ กรณีไม่ยินยอม หรือไม่ยินยอมเข้าร่วมวิจัยในส่วนเพิ่มเติมนี้ของโครงการ

- ผู้วิจัยได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย อาการไม่พึงประสงค์ หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด ให้ผู้แทนโดยชอบธรรม/ผู้ปกครองของผู้เข้าร่วมการวิจัยตามหนังสือแสดงเจตนาเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย
(.....) ชื่อผู้ทำวิจัย ตัวบรรจง
วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน
(.....) ชื่อพยาน ตัวบรรจง
วันที่ .....เดือน.....พ.ศ.....

APPENDIX D NORMAL VALUES FOR 2D-ECHOCARDIOGRAPHIC PARAMETERS OF LV DIMENSION AND FUNCTION

Table 16 Normal values for 2D-echocardiographic parameters of LV size and systolic function

Parameters	Interpretation
IVSd, LVPWd > 1.3 cm	Hypertrophy
LVIDd > 55 cm	Dilate
LVEF ≥ 55%	Normal contraction
LVEF 45 – 50%	Mild impairment
LVEF 30 – 44%	Moderate impairment
LVEF < 30%	Severe impairment

Source: Modified from Lang, et al. (262)

Table 17 Normal values for 2D-echocardiographic parameters of left ventricular diastolic function by Mitral flow velocity

Measurement	Normal	Abnormal diastolic function (Grade I)	Pseudonormal diastolic function (Grade II)	Restrictive diastolic function (Grade III)
E/A ratio	≥ 0.8	< 0.8	0.8 – 1.5	> 2
DT (ms)	160 - 200	> 200	160 – 200	< 160
IVRT	50 – 100	≥ 100	60 – 100	< 60

Source: Modified from Nagueh, et al. (263)

Table 18 Normal values for 2D-echocardiographic parameters of left ventricular diastolic function by Mitral flow velocity

Parameters	Normal	Abnormal
Septal e'	≥ 0.08	< 8
Lateral e'	≥ 0.1	< 10
Septal E/e'	< 8	≥ 15
Lateral E/e'	< 8	> 13
Average (septal – lateral)	< 8	> 14

Source: Modified from Nagueh, et al. (263)

Table 19 Normal values for 2D-echocardiographic parameters of left ventricular diastolic function by Mitral flow velocity in subjects aged 16 to 20 years

Measurement	Age group (years)	
	16-20	**21-40**
E/A ratio	0.98 - 2.78	0.73 - 2.33
DT (ms)	104 -1	138 - 194
IVRT	32 - 68	51 - 83
Septal e'	0.10 - 0.19	0.10 - 0.20
Lateral e'	0.13 - 0.28	0.14 - 0.25

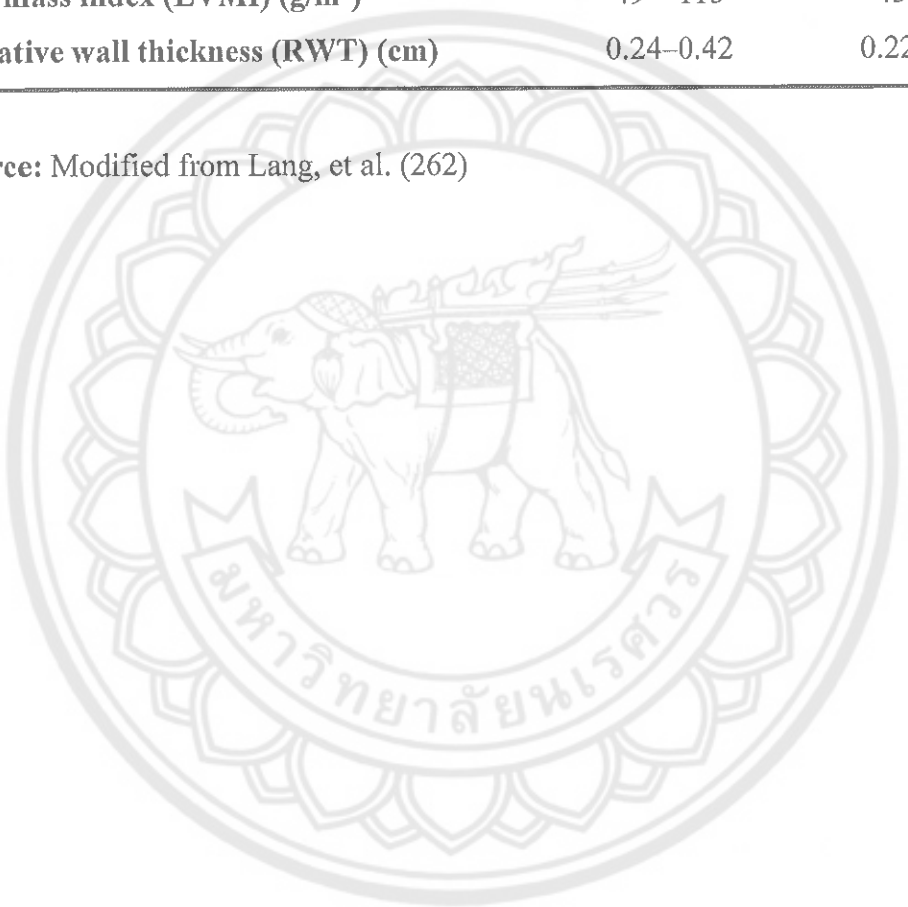
Note: For e' velocity in subjects aged 16 to 20 years, values overlap with those for subjects aged 21 to 40 years. This is because e' increases progressively with age in children and adolescents. Therefore, the e' velocity is higher in a normal 20-year-old than in a normal 16-year-old, which results in a somewhat lower average e' value when subjects aged 16 to 20 years are considered.

Source: Modified from Nagueh, et al. (264)

Table 20 Normal values for 2D-echocardiographic parameters for LV mass and LV mass index

Measurement	Male	Female
Linear method: M – mode tracing		
LV mass (g)	88 – 224	67 – 162
LV mass index (LVMI) (g/m^2)	49 – 115	43 – 95
Relative wall thickness (RWT) (cm)	0.24–0.42	0.22–0.42

Source: Modified from Lang, et al. (262)



APPENDIX E NORMAL VALUES FOR SPECKLE-TRACKING ECHOCARDIOGRAPHY (GLS)

Normal values for GLS depend on the definition of the measurement position in the myocardium, the vendor, and the version of the analysis software. Differences among vendors and software packages are still too large to recommend universal normal values and lower limits of normal. However, several meta-analysis studies revealed that, a peak GLS in the range of -20% can be expected in a healthy person (265-267). Moreover, there is evidence that women have slightly higher absolute values of GLS than men and that strain values decrease with age (265-266).

GLOSSARY

GLOSSARY

White blood cell count (WBC count) : is a count of the number of white blood cells per volume of blood

Red blood cell count (RBC count) : is a count of the number of red blood cells per volume of blood

Hemoglobin (Hb) : measures the amount of oxygen – carrying protein in the blood

Hematocrit (Hct) : measures of the percentage of red blood cells in a given volume of whole blood

Platelet count : is the number of platelets in a given volume of blood

Mean corpuscular volume (MCV) : is a measurement of the average size of your red blood cell

$$\text{MCV (fl)} = \frac{\text{Hct \% x 10}}{\text{RBC (x } 10^6 \text{ /µl)}}$$

Mean corpuscular hemoglobin (MCH) : is a calculation of the average amount of oxygen - carrying hemoglobin inside a red blood cell

$$\text{MCH (pg)} = \frac{\text{Hb \% x 10}}{\text{RBC (x } 10^6 \text{ /µl)}}$$

Mean corpuscular hemoglobin concentration (MCHC) : is a calculation of the average concentration of hemoglobin inside a red cell

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL) x 100}}{\text{Hct \%}}$$

Red cell distribution width (RDW) : is a calculation of the variation in the size of your red blood cell

Femtoliter (fl) : is a prefix in the metric system denoting a factor of 10^{-15} or 0.000 000 000 000 001

Microliter (µl) : is a prefix in the metric system denoting a factor of 10^{-6} or 0.000 001

Milliter (ml) : is a prefix in the metric system denoting a factor of 10^{-3} or 0.001

GLOSSARY (CONT.)

Picogram (pg)	:	is a prefix in the metric system denoting a factor of 10^{-12} or 0.000 000 000 001
Leptin	:	is a hormone genesis from adipose cells that regulate energy balance.
Speckle-tracking echocardiography	:	is a new ultrasound method that provides a quantitative assessment of global and regional myocardial function.
Strain	:	is the degree of myocardium deformation of interesting segment in association to its initial dimensions.
Strain rate (SR or ε′)	:	is the myocardial deformation rate.
Longitudinal strain	:	is myocardial deformation in the base to the apex direction.
Radial strain	:	is myocardial deformation in direction of getting on to the center of the LV cavity on a short-axis view. It is refers to the LV thickening and thinning through the cardiac cycle.
Circumferential strain	:	is the shortening of LV myocardium in the circular direction on a short-axis view.