THE EFFECTS OF COMBINATION OF BEHAVIOURAL INTERVENTION, NUTRITIONAL EDUCATION AND EXERCISE (COMBINE) PROGRAM ON HAEMOSTATIC MARKERS AMONG OBESE SUBJECTS

By

NORSUHANA BINTI OMAR

THESIS SUBMITTED IN FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

UNIVERSITI SAINS MALAYSIA

JUNE 2012

ACKNOWLEDGEMENTS

Praise to Allah (S.W.T), the most compassionate and most merciful, whose blessing has helped me through the entire completion of this research project and made all of this and everything possible to finish and complete this master study.

I would like to express my deepest appreciation and gratitude to my supervisor, Dr. Che Badariah Abd Aziz for her invaluable guidance, constant encouragement and contribution of her time throughout the study. It is a pleasure to thank to my co-supervisors PM Dr. Wan Zaidah Abdullah and Dr. Rohana Abdul Jalil for their guidance, advice, and contribution of their time for the research and completion of this thesis.

A special thanks to all subjects that had voluntarily participated in this study. I would also like to thank Prof Wan Manan, Pn Wan Suriati and Fiyyonna Lim for their full commitment during the intervention period. In addition to that, I would like to express my thanks to the staffs in Haematology Laboratory especially to Pn Wan Soriany binti Wan Md Zain, En. Norkhairul Putra and Pn Azilwati binti Azizan for their kind assistance, cooperation, and technical support during the period of this study.

This research cannot be completed without the help given by medical doctors and staff nurses in Obesity Clinic and Medical Clinic at Hospital Universiti Sains Malaysia (HUSM).

I would also like to thank all physiology staffs and for their endless prayers and support throughout my study. Last but not least, I wish to convey my warmest gratitude to my family members and my dearest husband, Dr Mohd Shahrulsalam Mohd Shah for their enormous amount of loves, understandings, sacrifices and support that have made the task of completing this master project possible. Thank you very much. May Allah bless each one of you abundantly.

LISTS OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION

˚C	:Degree celcius
µl	:Microliter
AT	:Antithrombin
AMM	:Academy of Medicine Malaysia
ARIC	:Atherosclerosis Risk in Communities
BIA	:Bioelectrical impedance analysis
BMI	:Body Mass Index
BMR	:Basal metabolic rate
CETP	:Cholesteryl ester transfer protein
CHD	:Coronory heart disease
CHO	:Carbohydrate
CLSI	:Clinical Laboratory Standard Institute
COMBINE	:Combination of behavioral intervention, nutritional education and exercise
DIC	:Disseminated intravascular coagulation
ELISA	:Enzyme-linked immunosorbent assay
Fib	:Fibrinogen
FAO	:Food and Agriculture Organization
FDP	:Fibrin degradation product
FFM	:Fat free mass
FVII	:Factor VII
FXIII	:Factor XIII
HDL	:High density lipoproteins
HC II	:Heparin Cofactor II

HUSM	:Hospital Universiti Sains Malaysia
kg	:Kilogram
LDL	:Low density lipoproteins
m	:Metre
MEMS	:Malaysian Endocrine and Metabolic Society
min	:Minute
ml	:Milliliter
mM	:Milimolar
NCEP	:National Cholesterol Education Program
NEFAs	:Non-esterified fatty acids
ng/ml	:Nanogram per microliter
OPD	:Ortho-phenylenediamine
PAI-1	:Plasminogen activator inhibitor type 1
PAP	:Plasminogen -antiplasmin
rpm	:Round per minute
SPSS	:Statistical Package for Social Sciences
TAFI	:Thrombin activatable fibrinolysis inhibitor
TEF	:Thermic effect of food
TF	: Tissue factor
TFPI	:Tissue factor pathway inhibitor
TG	:Triglyceride
t-PA	:Tissue-type plasminogen activator
u-PA	:Urokinase-type plasminogen activator
VLCD	:Very low calorie diet
vWF	:Von Willebrand factor

KESAN PROGRAM INTERVENSI KOMBINASI TINGKAH LAKU, PENDIDIKAN PEMAKANAN DAN AKTIVITI FIZIKAL (COMBINE) KE ATAS PENANDA HEMOSTATIK DALAM SUBJEK OBES

ABSTRAK

Obesiti merupakan keadaan pengumpulan lemak berlebihan yang menimbun di dalam badan seseorang, dan ia meningkatkan risiko penyakit jantung koronari. Kajian ini adalah bertujuan untuk mendapatkan data saintifik mengenai kesan program intervensi kombinasi tingkah laku, pendidikan pemakanan dan aktiviti fizikal (COMBINE) ke atas data antropometri, ujian darah biokimia dan penanda hemostatik. Kajian penurunan berat badan ini mengambil masa selama 12 minggu yang telah melibatkan 28 subjek obes. Semua subjek dikehendaki menandatangani borang keizinan menyertai program terlebih dahulu sebelum menyertai program. Data antropometri diukur ke atas semua subjek sebelum intervensi dan selepas intervensi. Sebanyak 15 ml darah diambil 2 kali iaitu sebelum intervensi dan selepas tamat program. Ujian-ujian penanda hemostatik adalah seperti tahap fibrinogen, factor VII, thrombin activatable fibrinolytic inhibitors (TAFI), tissue plasminogen activators (t-PA), plasminogen activator inhibitors-1(PAI-1), plasminogen dan Heparin Cofactor II. Manakala ujian biokimia termasuk ujian glukosa dan ujian lipid. Pakej penurunan berat badan ini termasuk berjalan kaki, senaman ‘dumb bell’, senamrobik dan diikuti pendidikan pemakanan yang dijalankan di Hospital Universiti Sains Malaysia pada setiap hari Khamis bermula jam 8 pagi hingga 1.00 tengahari. Semua data dianalisis menggunakan ujian ´paired t-test’, SPSS 18.0. Manakala ´Spearman`s rho’digunakan untuk mengetahui hubungkait diantara penanda

hemostatik dengan lain-lain parameter. Paras $p<0.05$ dianggap signifikan bagi semua analisis. Bagi indek jisim tubuh (BMI), terdapat penurunan yang signifikan iaitu (33.22 ± 0.67 kg/m²) selepas intervensi berbanding (35.78 ± 0.82 kg/m²) sebelum intervensi. Begitu juga terdapat keputusan yang signifikan untuk lain-lain data antropometri seperti ukuran lilitan pinggang dan komposisi badan. Bagi ujian penanda hemostasis, terdapat perbezaan penurunan yang signifikan selepas tamat program ke atas lima penanda hemostasis iaitu ujian ke atas fibrinogen, factor VII, t-PA, PAI-1 dan TAFI, begitu juga dengan paras trigliserida, mengalami penurunan sebelum intervensi (1.73 ± 0.18 mmol/L) kepada 1.24 ± 0.07 mmol/L selepas intervensi, ($p<0.05$). Sementara itu, kenaikan signifikan didapati pada paras Heparin Cofactor II selepas tamat intervensi ($119.89 \pm 3.58\%$), berbanding sebelum intervensi ($105.22 \pm 4.42\%$) ($p<0.05$). Tetapi, tiada perubahan signifikan pada paras plasminogen, kolesterol, lemak tepu tinggi, lemak tepu rendah dan paras glukosa. Secara kesimpulannya, pendekatan intervensi tanpa melibatkan farmakologi ini boleh dianggap sabagai satu kejayaan berpandukan keputusan positif didapati dalam penurunan bahaya penanda hemostatik dan juga penurunan fizikal parameter serta ujian biokimia. Semua ujian ini perlu diuji dengan lebih mendalam lagi untuk dijadikan sebagai panduan keberkesanan dalam program penurunan berat badan pada masa akan datang.

THE EFFECTS OF COMBINATION OF BEHAVIOURAL INTERVENTION, NUTRITIONAL EDUCATION AND EXERCISE (COMBINE) PROGRAM ON HAEMOSTATIC MARKERS AMONG OBESE SUBJECTS

ABSTRACT

Obesity is a condition of excess body fat and it significantly increases the risk of coronary heart disease. Studies have shown that impaired haemostatic in obesity were found to predict future development of coronary heart disease and numbers of coronary events. The purpose of this study was to determine the effects of COMBINE program on the changes of anthropometry parameters, biochemical profile and haemostatic markers in obese subjects. The assessment was carried out on 28 obese subjects for 12-week duration. All subjects were required to give an informed consent before enrolling into the study. Anthropometric data were measured from the subjects selected before intervention (baseline) and after completing the program (post-intervention). About 15 ml of blood specimen was drawn from all subjects and tested for biochemical profile (e.g. fasting blood glucose and lipid profile) and haemostatic markers, such as fibrinogen, factor VII, thrombin activatable fibrinolytic inhibitors (TAFI), tissue plasminogen activators (t-PA), plasminogen activator inhibitors-1(PAI-1), plasminogen and Heparin Cofactor II at baseline and at post intervention. During this intervention period, the subjects were involved in a weekly program from 8.00 am until 1.00 pm on every Thursday at Hospital Universiti Sains Malaysia. The package of weight loss program consisted of brisk walking, dumb bell and easy-style of aerobic dance accompanied with nutrition education modules. Their food intake and physical activity were recorded in diary and they act as important tools in the behaviour modification method. Data analysis

for this study was carried out using paired t-test, SPSS 18.0. In order to correlate the haemostatic markers with other parameters, Spearman`s rho correlation analysis was used. The significant level, $p< 0.05$ was used. The results showed that there was a significant reduction in the BMI at post intervention (33.22 ± 0.67 kg/m²) when compared to pre intervention (35.78 ± 0.82 kg/m²). Similarly, there was a significant reduction ($p<0.05$) for other anthropometric data such as waist circumference and body composition analysis. Besides that there was also significant reduction in five haemostatic markers such as fibrinogen, factor VII, TAFI, t-PA and PAI-1 at post-intervention when compared to baseline. Similarly, there was a significant reduction in triglyceride level at the baseline (1.73 ± 0.18 mmol/L) compared to post-intervention, (1.24 ± 0.07mmol/L) ($p< 0.05$). Meanwhile there was a significant increased level of Heparin Cofactor II after the intervention (119.89 ± 3.58%) compared to baseline (105.22 ± 4.42%) ($p< 0.05$). But, there was no significant difference seen in plasminogen level as well as in cholesterol, high density lipoprotein, low density lipoprotein and fasting blood glucose levels. In conclusion, the non-pharmacologic approach as an intervention is considered a successful program based on the positive findings such as reduced haemostatic hazard markers and improved other parameters such as physical parameters and biochemical markers. These markers should be further explored to be utilised as predictors to determine the effectiveness of a weight loss program and to predict the risk to develop cardiovascular disease in obese subjects.

CHAPTER 1

INTRODUCTION

1.1 Background

Obesity is one of the most serious public health problems and is a leading preventable cause of death worldwide (Barnes et al., 2007). Obesity contributes to a shorter expectation of life, increased morbidity, or cost to the community in terms of both money and anxiety (Waterlow, 1976).

Obesity is a condition of excess body fat and in most cases results from energy intake in their diet over a period of time, has exceeded their energy expenditure for metabolism, physical activity and growth. Obesity continues to be a prevalent public health problem in the developed countries, while there is strong epidemiological evidence indicating that the prevalence of obesity in developing countries often increases in communities emerging from lifestyles of subsistence into affluence (Ismail et al., 2005).

Various studies have suggested that obesity per se is an independent cardiovascular risk factor, as well as predisposing to type 2 diabetes, hypertension, and dyslipidaemia (Gallist et al., 2000; Masahiko et al., 2004; Kristine et al., 2005). Obese subjects are at risk for the development of cardiovascular disease, which can in part be explained by disturbances in the haemostatics and fibrinolytic system. Indeed, obese subjects tend to

have higher values of fibrinogen, factor VII, factor VIII and plasminogen activator inhibitor -1 (PAI-1) compared to non-obese subjects (Mertens et al., 2002).

There are many health benefits and financial gains that can be achieved by reducing obesity. It is an essential need for all health care providers to work towards a common goal and to take an active role in addressing obesity. The National Heart, Lung, and Blood Institute of the National Institutes of Health (2007), recommends lifestyle modification as the primary intervention.

In relation to the obesity issues discussed above, a combination of behavioral intervention, nutritional education and exercise (COMBINE) program was introduced in USM in 1990. This is a safe method of weight reduction practice recommended to obese subjects attending the Obesity Clinic at Hospital Universiti Sains Malaysia.

1.2 Obesity overview

1.2.1 Definition of Obesity

Obesity is a condition of abnormal or excessive fat accumulation in adipose tissue, which is associated with a variety of health problems. However, obese individuals may differ with the degree of excess fat, and regional distribution of the fat within the body. Indeed, excess abdominal fat is a great risk factor for cardiovascular disease compared to excess body fat per se (WHO 1998).

1.2.2 Body Mass Index (BMI)

Body Mass Index is defined as body weight in kg divided in meter squared as shown below.

BMI = Weight (kg)/Height (m^2)

This indicator is commonly utilized in epidemiological studies, to predict obesity-related morbidity and mortality in adults. A BMI of 30 kg/m^2 is considered the threshold of obesity. BMI however, does not distinguish between weight associated with muscle and weight associated with fat. BMI is considered to provide the most useful, albeit crude, population-level measure of obesity. The classification of weight status in adults as proposed by WHO (1998) is shown in Table 1.1

The current classification has been questioned because of possible population difference in the risk of co-morbidities and attempts have been made to redefine the classification of obesity using BMI for Asian (World Health Organization, International Association for the Study of Obesity, International Obesity Task Force, 2000 (WHO/IASO/IOTF)) population based on studies by Ko et al., (1999) and Deurenberg-Yap et al., (2000).

However the WHO Expert Consultation Committee 2004 has recommended to retain the current WHO classification of BMI (WHO 1998) and acknowledged the need to have public health action as recommended (Table 1.2).

Table 1.1: Classification of weight status in adults according to Body Mass Index (BMI)

Classification	BMI (kg/m^2)	Risk of co-morbidities
Underweight	< 18.5	Low (but risk of other clinical problems increased)
Normal range	18.5 – 24.9	Average
Overweight:	≥ 25	-
Pre-obese	25 – 29.9	Increased
Obese class I	30.0 – 34.9	Moderate
Obese class II	35.0 – 39.9	Severe
Obese class III	≥40.0	Very severe

Source: World Health Organization (WHO), 1998.

Table 1.2: Classification of weight status according to BMI in Asian Adults

Classification	BMI (kg/m2)	Risk of co-morbidities
Underweight	< 18.5	Low (but risk of other clinical problems increased)
Normal range	18.5 – 22.9	Average
Overweight:	> 23.0	-
At Risk	23.0 – 24.9	Increased
Obese class I	25.0 – 29.9	Moderate
Obese class II	> 30.0	Severe

Source: World Health Organization, International Association for the Study of Obesity, International Obesity Task Force (WHO/IASO/IOTF) 2000.

1.2.3 Waist Circumference

Regarding the health risks related to obesity, we have to take into account not only the magnitude of obesity but also, and perhaps more relevant, the body fat distribution. There are two main types of obesity regarding the fat distribution pattern: android or central type obesity with the majority of fat depots located in the abdominal area, both subcutaneous and visceral, and gynoid or peripheral obesity in which the fat depots are mainly located subcutaneously in the lower body (hips and lower extremities). The difference between both types is fundamental, because the metabolic and cardiovascular complications of obesity are almost exclusively related to visceral fat depots.

The BMI does not provide any indication of the distribution of fat in the body. Waist circumference is positively correlated with abdominal fat. Hence, waist circumference is a valuable additional alternative method in identifying individuals at increased risk. Waist circumference (WC) is a convenient and simple measurement which is unrelated to height and correlates closely with BMI and Waist-Hip Ratio. It is an approximate index of intra-abdominal fat mass. Thus, based on current evidence, the following waist circumference is associated with an increased risk of co morbidities:

- Men $\geq$ 90cm
- Women $\geq$ 80cm

(WHO 1998).

1.2.4 Waist-Hip Ratio

Waist Hip Ratio (WHR) is another simple measurement that has been used in epidemiological studies in the past but does not provide additional information compared to WC. The values that are associated with an increase abdominal fat and increased risk of hypertension, diabetes and ischaemic heart disease are:

- WHR > 0.90 for men
- WHR > 0.85 for women

However, waist circumference is the preferred measure of abdominal obesity compared to the WHR (WHO 1998).

1.2.5 Body Composition

Body composition is used to describe the percentages of fat, bone and muscle in human bodies. Because muscular tissue takes up less space in body than fat tissue, the body composition, as well as weight, determines leanness. The National Institute of Health (2011) recommends that a healthy adult male's body should have between 6 to 24 percent fat and a female should have between 14 to 31 percent fat. Levels higher than the recommended value may indicate excess body fat.

Body composition can be measured in several ways. The common method is by using a set of measurement calipers to measure the thickness of subcutaneous fat in multiple places on the body. Another common method is bioelectrical impedance analysis (BIA), which uses the resistance of electrical flow through the body to estimate body fat.

Besides that, others reference methods are available to estimate body composition accurately at the individual level. Multicompartment models, underwater weighing, air displacement plethysmography, labeled water techniques and dual-energy X-ray absorptiometry (DXA) are the most reliable methods to obtain accurate measures of total body fat (Parker et al., 2003). Computed tomography and magnetic resonance imaging have been shown to provide information about body fat distribution (Brambilla et al., 1994). However, these reference methods are still not suitable for use because they are costly. Therefore, anthropometry and bioelectrical impedance are more widely used especially when the size of the population is large and they are also cheaper.

The bioelectrical impedance analysis (BIA) is a method for estimating body composition, meaning it measures how much of body weight is fat and how much is nonfat. The general principle behind BIA: two conductors are attached to a person's body and a small electric current is sent through the body. The resistance between the conductors will provide a measure of body fat, since the resistance to electricity varies between adipose, muscular and skeletal tissue. Fat-free mass (FFM) is a good conductor as it contains a large amount of water (approximately 73%) and electrolytes, while fat is anhydrous and a poor conductor of electric current. FFM is composed of all non-fat tissues and represents the main active component from the metabolic point of view. A main function of fat mass is as an energy reserve, because it consists of triglycerides, which has a high caloric power (Claude et al., 2000). Factors that affect the accuracy and precision of this method include instrumentation, subject factors, technician skill, and the prediction equation formulated to estimate the FFM. Another variable that can affect the amount of body fat measured by this method is the amount of liquid an individual

has consumed before the test. As electricity travels more easily through water, if a person has consumed a large amount of water before the test, the results will show a lower percentage of body fat consumption. Less water will increase the apparent percentage of body fat (Parker et al., 2003).

1.3 Prevalence of obesity

The global burden of overweight (BMI 25.0 – 29.9) and obesity (BMI ≥ 30.0) is estimated at more than 1.1 billion (Ismail et al 2005). The prevalence of obesity is rapidly increasing in the United States (U.S.) from 14% in 1971 (Flegal et al., 2002) to 30.6 % in 2002 (Hedley et al., 2004).

Obesity has been reported to be more common in minority populations, such as African-Americans and Hispanics, than Caucasians (Cossrow and Falkner 2004). For example, a study found that 39% of African-Americans, 33% of Hispanics, and 29% of Caucasians meet the criteria of obesity (Hedley et al., 2004).

In the Asia Pacific region, the prevalence of obesity in men is between less than 1% in China to about 58% in urban Samoa. In women, obesity prevalence is between less than 2% in China to about 77% in urban Samoa (WHO/IASO/IOTF, 2000). Local data on prevalence of obesity reveals that the problem faced in Malaysia is more serious than those reported in other Asian countries (Figure 1.1).

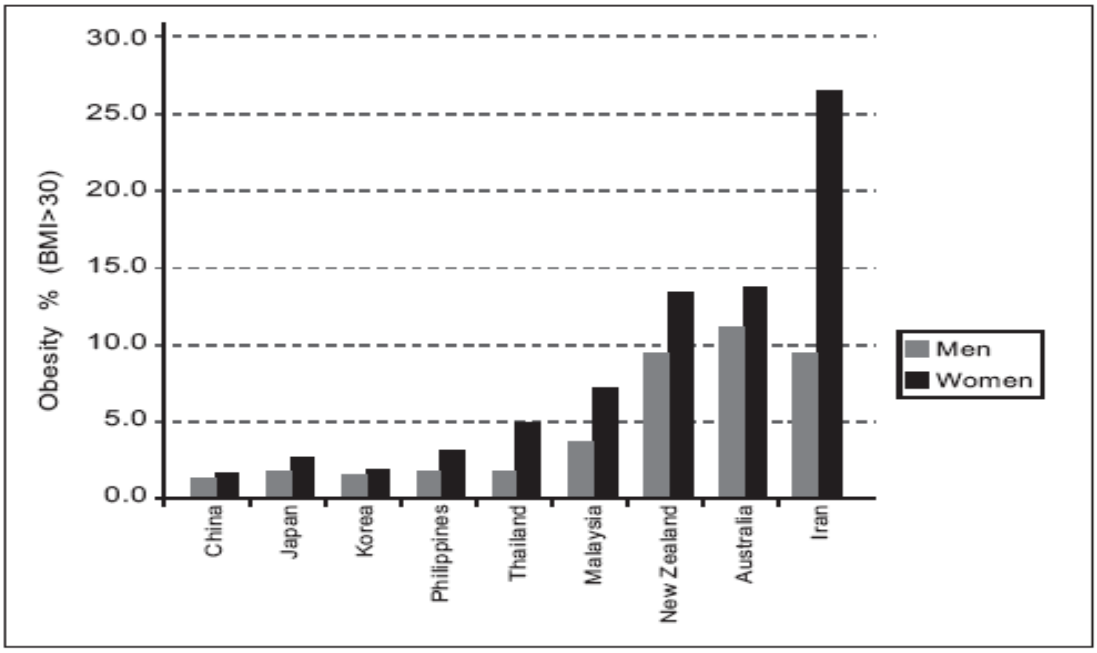

Figure 1.1: Prevalence of obesity in Asia Pacific region. (Source: WHO/IASO/IOTF, 2000).

In Malaysia, the National Health Morbidity Survey (1996) reported that in adult males, 15.1% were overweight and 2.9% obese; while in adult females, 17.9% were overweight and 5.7% obese. It was also reported that the prevalence of obesity is almost similar between rural and urban populations. In a nationwide study of 4,600 rural villagers throughout Peninsular Malaysia, (Khor et al.,1999) reported a prevalence of 19.8% overweight among men and 28.0% among women. The prevalence of obesity was 4.2% amongst men and 11.1% among women in the rural community studied. Obesity was more common in Malays and Indian compared to Chinese (Lim et al., 2000).

Overweight and obesity are also a concern among the older populations in this country. In a study among 945 elderly people, mostly Malays, from major functional groups in Peninsular Malaysia (Zaitun et al., 1999), the prevalence of overweight was 18.2% and obesity was 4.3%. In another study by Suzana et al., (2003) among 820 elderly Malays from four rural areas of Peninsular Malaysia, the prevalence of overweight and obesity were 24.7% and 11.4%, respectively. But the prevalence of obesity amongst those aged ≥ 18 years old has markedly increased by 280% since the last National Health and Morbidity Survey in 1996 (Lekhraj et al., 2007).

1.4 Cardiovascular impact in obesity

The effects of increased body fatness on cardiovascular function are predictable. The event affecting the cardiovascular system is initiated by an increase in body weight. A higher lean tissue mass as well as increase in oxidative demands of metabolically active adipose tissue leads to increase in body oxygen consumption and this is accompanied by an absolute increase in cardiac output. However the values are within the normal range when they are normalized to body surface area (Peter G Kopelmen, 2002).

Obesity produces an increment in total blood volume and cardiac output which is partly due to increased metabolic demand induced by excess body weight (Alpert MA, 2001). Thus, at any given level of activity, the cardiac workload is greater for obese subjects compared to lean individuals (Poirier et al., 2001).

The increased demand for cardiac output is achieved by an increase in stroke volume while the heart rate remains comparatively unchanged. The obesity-related increase in

stroke volume results from an increase in diastolic filling of the left ventricle. The volume expansion and increase in cardiac output lead to structural changes of the heart. The process results in re-modeling of the left ventricle with eccentric hypertrophy (Messerli et al., 1986). If there is a concomitant increase in systemic blood pressure, the process is followed by concentric hypertrophy, a typical form of hypertensive heart disease (Figure1.2).

Left atrial enlargement may also occur in normotensive obese individuals but typically in the setting of increased left ventricular mass. Left atrial enlargement may not be mediated solely through left ventricular diastolic dysfunction impairment but may simply reflect a physiological adaptation to the expanded blood volume (Sasson et al., 1996). As a consequence, left atrial dilation may mediate the excess risk of atrial fibrillation associated with obesity (Backman et al., 1979). However, left ventricular hypertrophy in long-standing obesity and/or the effects of concomitant hypertension may also be contributing factors to left atrial enlargement.

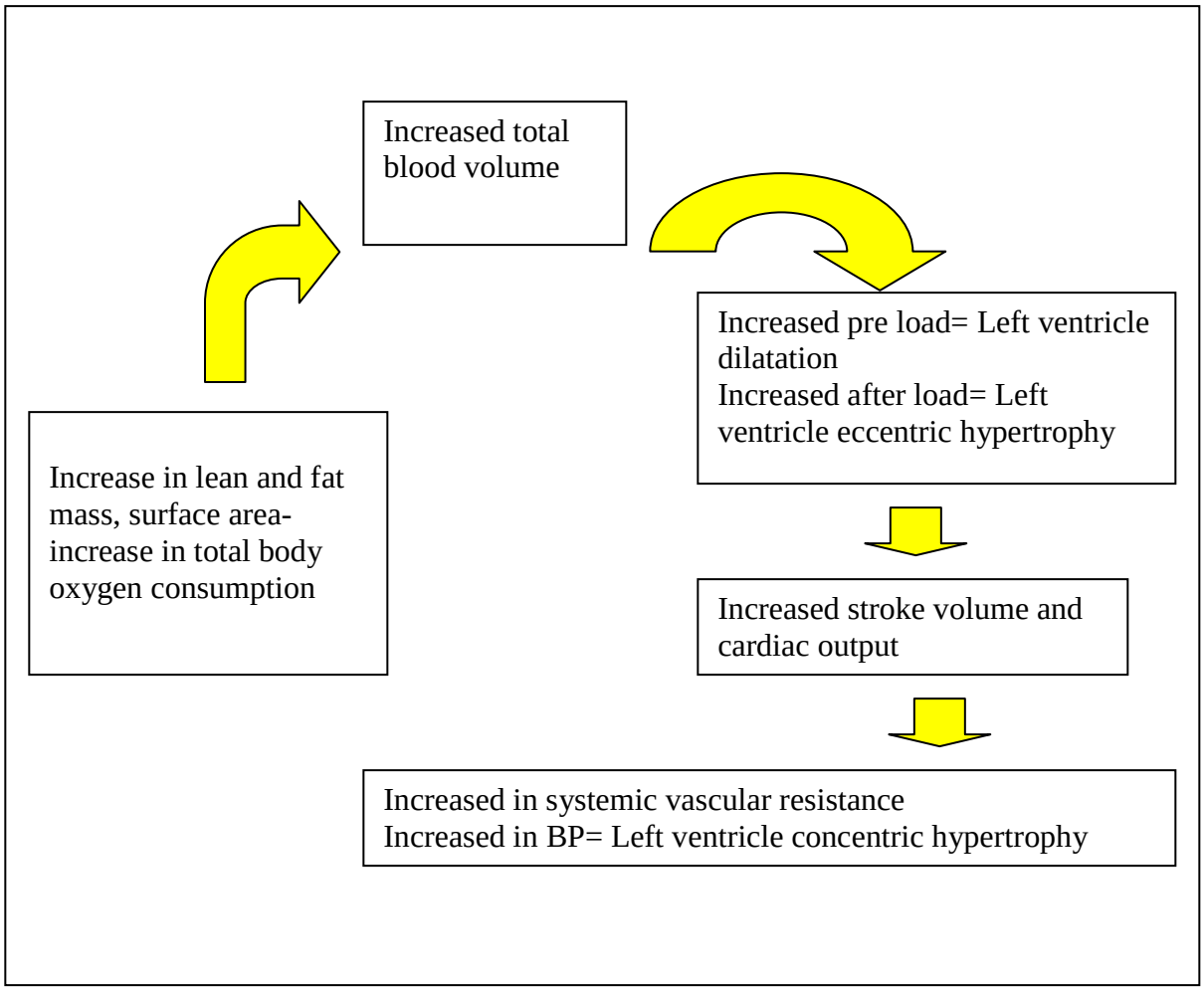

Figure 1.2: Summary of events affecting the cardiovascular system.

Studies such as Framingham Heart Study, the Manitoba Study, and the Harvard School of Public Health Nurses Study, have documented that obesity is an independent predictor of clinical coronary heart disease (CHD) (Rabkin et al., 1977, Hubert et al., 1983 and Manson et al., 1990). The conclusion made by these studies result from following up each obese subject for more than two decades.

CHD is narrowing of the blood vessels that supply blood and oxygen to the heart; it is usually due to atherosclerosis (Mosca et al., 2007). Atherosclerosis is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a hardening of the arteries. It is caused by the formation of multiple atheroma (Maton et al., 1993). In the context of heart or artery matters, atheroma is commonly referred to as atheromatous plaques. Atherogenesis is the developmental process of atheromatous plaques. It is characterized by a remodeling of arteries leading to subendothelial accumulation of fatty substances called plaques. The build up of an atheromatous plaque is a slow process, developed over a period of several years through a complex series of cellular events occurring within the arterial wall, and in response to a variety of local vascular circulating factors (Maton et al., 1993).

The blood coagulation system has been alleged to determine the onset and outcome of atherosclerosis (Cooper et al., 2000). There are two principal reasons for this

presumption. The first reason is that cleavage products from the coagulation system, e.g. thrombin, FXa and FVIIa may exert pro and anti-inflammatory effects on endothelial cells and leucocytes (Esmon, 2000). Endothelial injury starts the process of atherogenesis by the effects of platelets deposition. The ultimate result is narrowing of the blood vessels. The second reason is that formation of small blood clots that occlude major arteries (in the pre-existing narrowed vessels), has been documented in myocardial infarction or ischaemic stroke. This represents the leading complication of atherosclerosis (Fuster et al., 1996).

Platelets are involved in the initiation of early atherosclerotic lesions, together with other factors they contribute to the progression of plaque growth and may lead to deleterious clinical events if a pro-thrombotic state is present at the time of plaque fissuring or rupture (Fuster et al., 1992).

There are two main types of measurements related to haemostatic disturbances in the investigation of the pro-thrombotic state in cardiovascular disease: measurement of haemostatic factors levels and measurement of activation products of haemostasis. In the former type of measurements, the most promising factors identified have been fibrinogen, factor VII (FVII) and the fibrinolytic variables i.e tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) (Fareed et al., 1998).

1.5 Overview of Haemostasis

Haemostasis is derived from the Greek meaning “The stoppage of blood flow”. The haemostatic system is a vital protective mechanism that refers to the process for preventing blood loss by sealing sites of injury in the vascular system. The components of normal haemostasis are: the blood vessels, platelets, blood coagulation factors, inhibitors of coagulation, and the fibrinolytic system. However, this system must also be controlled so that the blood loss does not coagulate within the vasculature and restrict the normal blood flow. In other words, the integrity and patency of the haemostatic systems depends upon balance between coagulation activation, inhibition and fibrinolytic activity. Haemostasis is determined by the interaction of endothelial and subendothelial cells, platelets, leucocytes, coagulation factors and coagulation inhibitors (Curry & Pierce, 2007).

1.5.1 Coagulation system

The three major steps of coagulation process are the initiation, amplification and propagation phases. Vascular injury leads to intravascular exposure of tissue factor (TF). Tissue factor binds circulating factor VII to form TF-VII complexes. This initial step results in activation of factor X and IX on TF-bearing cells (initiation phase). Platelets are localized to the site of injury by adhesion to the subendothelial matrix mediated by interaction between collagen, von Willebrand factor (vWF) and GP 1b receptors on the surface of platelets. During initiation phase, activated factor X (FXa) generates small amount of thrombin, which is not high enough to produce a haemostatic sufficient fibrin

clot, but leads to an activation of platelets and further enzymatic coagulation factors (factor XI, VIII and V) (amplification phase).

Activated platelets release thromboxane and their granular contents (serotonin, vwF, calcium and coagulation factors) which stimulate aggregation of platelets. Platelets also alter their surface by expressing negative charged phospholipids to facilitate calcium-mediated coagulation factor binding. Activation of coagulation factors and subsequent thrombin generation takes place on the surface of activated platelets (propagation phase). Thrombin itself potentiates its generation by activation of factor XI, VIII, and V, which results in a thrombin burst, sufficient to cleave fibrinogen and activate factor XIII as well as thrombin activatable fibrinolysis inhibitor (TAFI). Soluble fibrin monomers polymerize and are cross-linked by FXIII. A mixture of fibrin and platelets form a stable clot which is anchored at the extracellular matrix due to the cross-linking of fibrin with adhesive protein (Gerlach et al., 2009). The steps in the coagulation cascade are simplified in Figure 1.3.

Fibrin is then cross linked by FXIII to be stabilized further by incorporation of the fibrinolysis inhibitors alpha-2-antiplasmin and TAFI (thrombin activatable fibrinolysis inhibitor) and binding to several adhesive proteins of various cells (Muzbek et al., 2008).

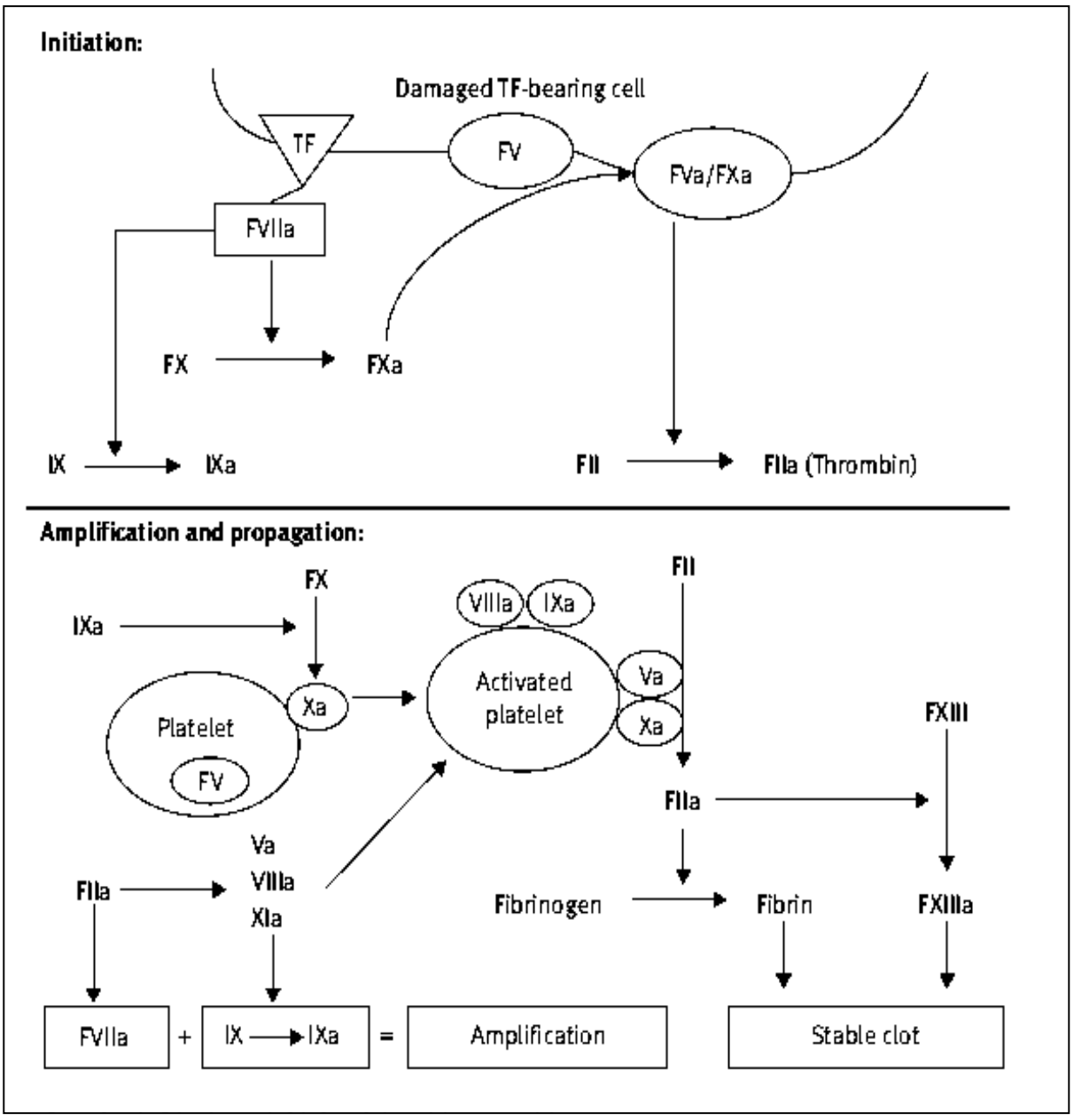

Figure 1.3: Summary of steps in coagulation cascade (Adapted from Contin Educational Anesthesiology Critical Care Pain article, 2007, Curry A & Pierce JM, 2007).

1.5.2 Fibrinolytic System

The fibrinolytic system is responsible for the degradation of the solid-phase fibrin network that constitutes the major protein component of thrombus to fibrin degradation product (FDP). As a consequence, the fibrinolytic system facilitates the dissolution of blood clots after repairing of a locally injured vessel wall and thus ensures an unobstructed circulation. The component of the system includes plasminogen and plasmin, several endogenous and exogenous plasminogen activators and their inhibitors.

Fibrinolysis is initiated by either one of the serine protease urokinase-type plasminogen activator (u-PA) or tissue-type plasminogen activator (t-PA). The activity of the t-PA is dependent on the presence of the obligatory cofactor fibrin. Binding of t-PA to fibrin increases the affinity of t-PA for the zymogen plasminogen by two or three orders of magnitude, resulting in a significant acceleration of the conversion of the substrate into the active enzyme plasmin. Plasmin is rather a specific serine protease that recognizes and digests lysyl and arginyl-peptide bonds and converts insoluble, intact fibrin polymers into a distinct series of soluble degradation products. This fibrinolytic process is mainly controlled by two homologous proteins that exert a similar mechanism of action:

(1) Plasminogen activator inhibitor type 1 (PAI-1), a 50 Kd glycoprotein that rapidly forms inactive, 1:1 equimolar complexes with t-PA (and with the non – fibrin specific u-PA). (2) The activity of plasmin is controlled by α2-antiplasmin, a glycoprotein that is present at high concentration in plasma (Geoffrey et al., 2005). The overall fibrinolytic activity is summarized in Figure 1.4.

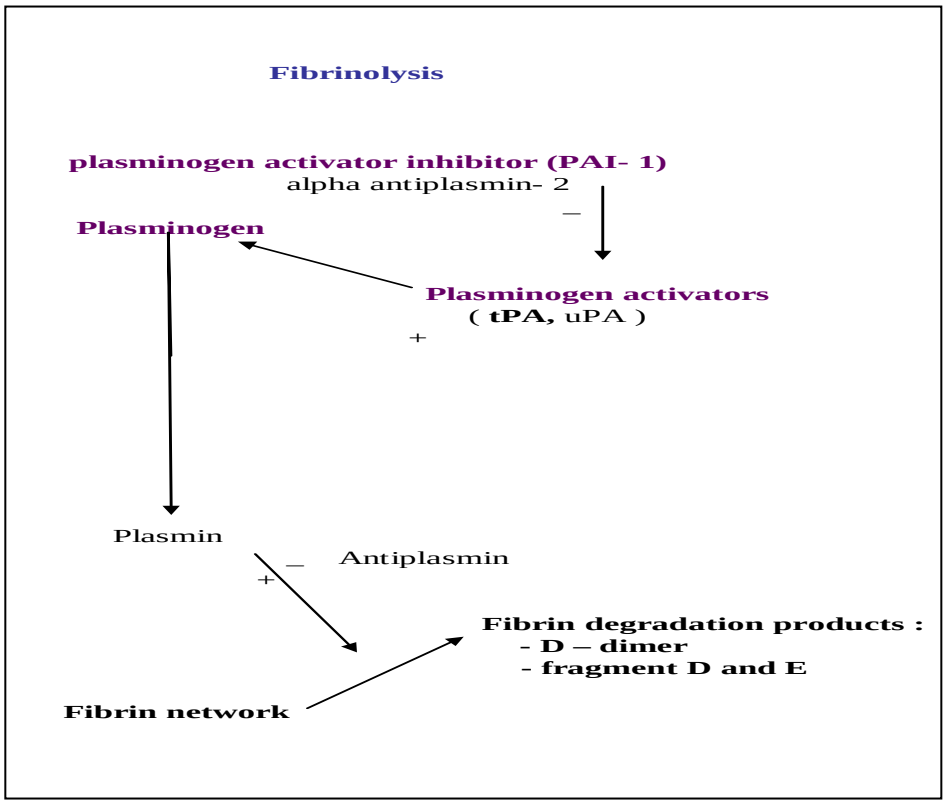

Figure 1.4: Schematic illustration of physiologic fibrinolysis

1.5.3 Natural inhibitors/ natural anticoagulant of coagulation system.

The coagulation pathway is also regulated via inhibitory systems. The inhibitory systems consists of tissue factor pathway inhibitor (TFPI), antithrombin (AT), heparin cofactor II (HC II) activity and protein C activity. TFPI targets the initiation of coagulation, AT and HC II activity blocks thrombin generation and thrombin activity, and protein C inhibits the propagation of coagulation, as shown in Figure 1.5.

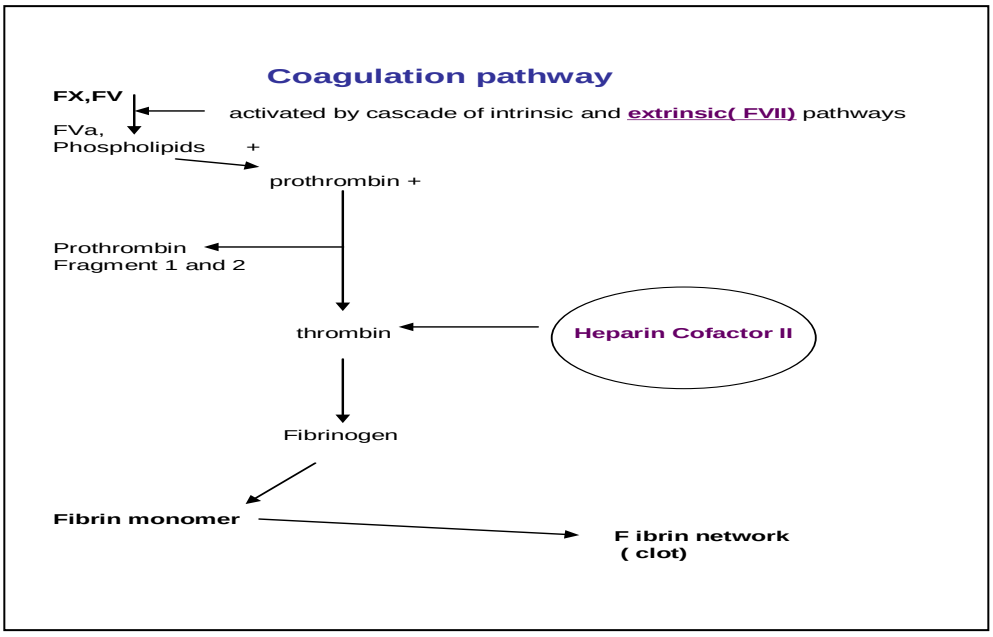

Figure 1.5: Schematic diagram of regulation of coagulation. Formation of a clot is highly regulated by natural anticoagulant mechanisms that confine the haemostatic process to the site of injury in the vessel.

The overall relationships between coagulation, fibrinolytic, platelet and endothelial cell components of the haemostatic system are described in Figure 1.6.

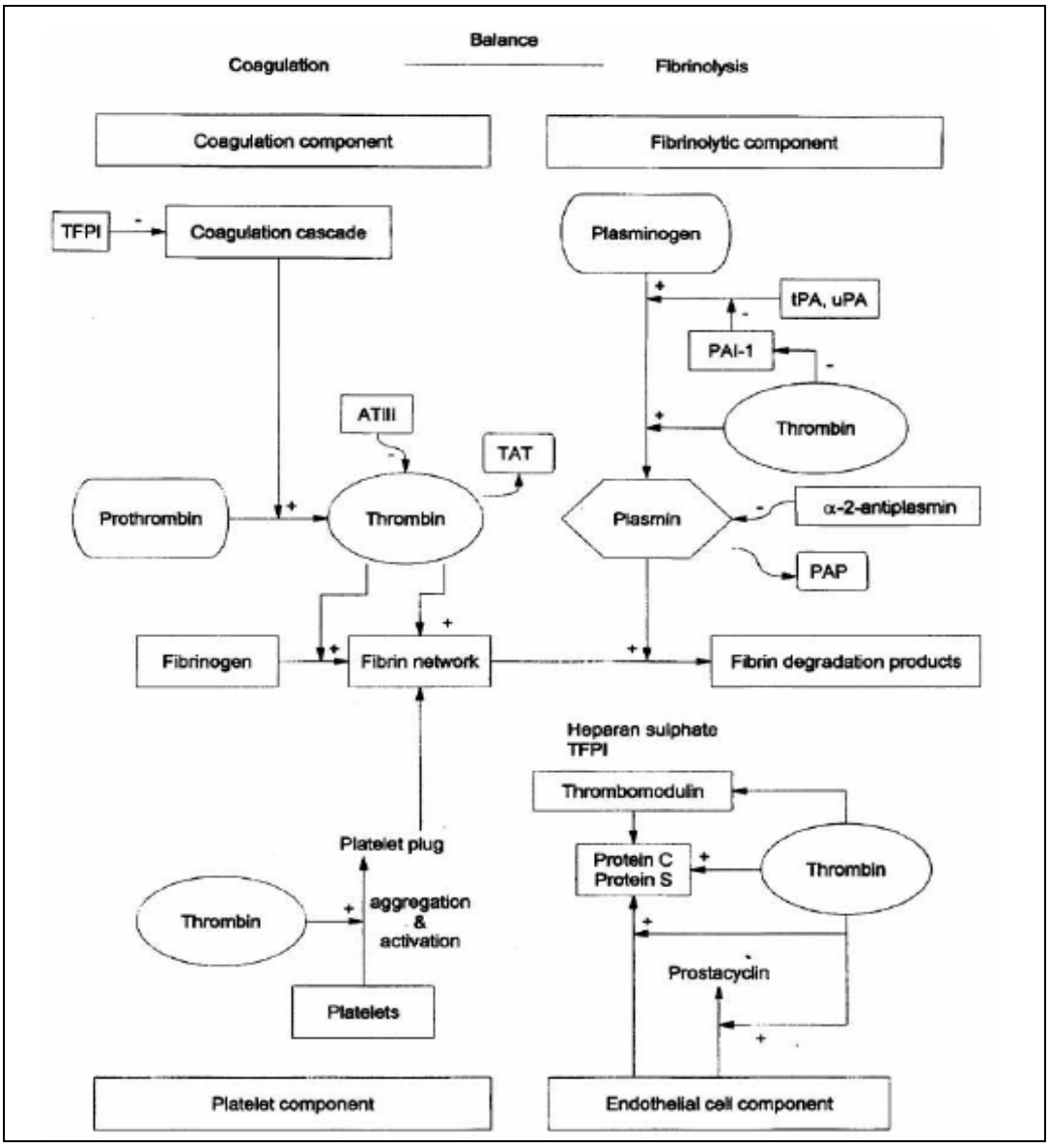

Figure 1.6: Interrelationships between the coagulation, fibrinolytic and endothelial cell components of the haemostatic system. Thrombin generated in the coagulation component influences events in other component: Antithrombin, PAI-1, PAP, plasmin-antiplasmin complex (marker of plasmin generation); TAT; thrombin-antithrombin complex (marker of thrombin generation); TFPI; t-PA; uPA, urokinase type plasminogen activator. (Adapted from Hester et al., 1997)

1.6 Justification of the study

Haemostatic markers contribute to the pathogenesis of obesity-related co morbidities such as cardiovascular disease and even modest weight loss reverses endothelial dysfunction and restores arterial homeostasis stress markers which potentially decrease the cardiovascular risk (Poobalan et al., 2007). However the levels of haemostatic markers need to be serially assessed to prove its reversibility to normal levels following the weight loss intervention.

Even though there were many studies on weight loss program, majority of them were done in the western settings. Relatively very few studies were published in the Asian region, especially in Malaysia. To date, very few studies looked at the effects of weight loss program on both the fibrinolytic and pro-thrombotic markers of hemostatic system and none has assessed on extensive haemostatic markers for evaluation of the weight loss program. In this COMBINE program, participants were encouraged to set modest (reasonable) weight loss goals instead of striving for the ideal body weight for their height (Rohana et al., 1997, 1999 & 2007). This practice was based on the result from other studies (Foster et al., 1995) that have suggested losing 5% to 15% of body weight within 3 to 6 months is associated with substantial improvements in obesity-related conditions such as metabolic syndrome, cardiovascular disease and hypertension.

This study was done to fill the research gap related to obesity and to explore the effect of weight loss on the hazard biomarkers, with special interest on haemostatic parameters. The effectiveness of a specific weight loss program therefore can be evaluated in this

intervention study prescribed in Obesity clinic, Universiti Sains Malaysia. This study investigates both physical and biochemical parameters of the subjects and their correlations with the levels of haemostatic markers. Thus the findings will be useful in future to be used as predictive markers for evaluation of the weight loss program. It is hoped that this specific program will help obese subjects to reduce their body weight and decrease the cardiovascular risks and eventually lead to a healthier lifestyle.

1.7 General objectives

To study physical, biochemical parameters and levels of haemostatic prothrombotic markers predisposing to cardiovascular risks in obese subjects participating in the COMBINE weight loss program.

1.8 Specific objectives

1. To compare physical parameters; BMI, waist circumference and body fat composition at pre and post COMBINE weight loss program.

2. To compare levels of haemostatic markers pre and post COMBINE weight loss program.

3. To determine correlation between haemostatic parameters and physical- biochemical parameters among the subjects participating in COMBINE program.