Cardiovascular health effects of moderate weight loss.

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Abbreviations used in this thesis

ABPI	ankle brachial pressure index
AU	arbitrary units
BMI	body mass index
BMR	basal metabolic rate
BP	British Petroleum
CI	confidence interval
CV	coefficient of variation
DHA	docosahexaenoic acid
DPA	docosapentanoic acid
DHEAS	dehydroepiandosterone sulphate
e-mail	electronic mail
ECG	electrocardiogram
EDTA	ethylenediaminetetracetic acid
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
$HbA1_c$	haemoglobin $A1_c$
HDL	high density lipoprotein
HEBS	Health Education Board for Scotland
HRT	hormone replacement therapy
IDDM	insulin dependant diabetes mellitus
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
LDL	low density lipoprotein
MONICA	monitoring trends and determinants of cardiovascular disease
MRFIT	Multiple Risk Factor Intervention Trial
MI	myocardial infarction

n-3	omega three
n-6	omega six
NHANES	National Health and Nutrition Epidemiological Study
NIDDM	non insulin dependant diabetes mellitus
P/S	polyunsaturated / saturated
PAI	plasminogen activator inhibitor
PCOS	polycystic ovary syndrome
PVD	peripheral vascular disease
RCA	red cell aggregation
REE	resting energy expenditure
SHBG	sex hormone binding globulin
t-PA	tissue plasminogen activator
u-PA	urokinase plasminogen activator
UK PDS	United Kingdom Prospective Diabetes Study
VLCD	very low calorie diet
VLDL	very low density lipoprotein
WHR	waist to hip ratio
WI	weighed intake

Statement

I declare that I am the author of this thesis, and that no part of the work in this thesis has formed part of any other thesis.

Assays for PAI activity, t-PA antigen, factor activity, insulin, lipoproteins and measurements of plasma and whole blood viscosity, were conducted by the staff of the Coagulation Laboratory in the Department of Medicine and Department of Pathological biochemistry, as mentioned in the acknowledgements, for providing these facilities. I am familiar with all the procedures, and have conducted the majority of them under supervision. The electrocardiogram traces for the angina study were scored by Professor Peter McFarlane, Department of Medical Cardiology.

I alone completed all other work reported in this thesis.

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Summary

This thesis describes the results of dietetic led weight management for weight loss in three different groups of subjects: overweight; overweight with angina; and those whose body weight was close to the healthy upper BMI of 25 kg/m^2. It forms part of a growing literature examining moderate weight loss as a success outcome in weight management. The work in this thesis addresses an important general research question; whether the effect of modest weight loss *per se* on established risk factors for IHD was similar across a number of subject groups. The specific aims were to examine the effect of moderate weight loss on the established IHD risk factors, fibrinogen, factor VII activity, plasma and whole blood viscosity, PAI activity and t-PA antigen. The role of modest weight loss on the adrenal hormone DHEAS was also studied.

The established indices of IHD risk for rheology and haemostasis: plasma and whole blood viscosity, RCA, haematocrit, fibrinogen, factor VII activity and the fibrinolytic enzymes PAI activity and t-PA antigen are known from epidemiological studies to reflect IHD risk, and to be elevated in association with increased BMI. The serum DHEAS and plasma total cholesterol, in particular the LDL and HDL cholesterol fractions were of interest and also measured, although the available evidence suggested a more marginal influence on rheological and haemostatic factors.

Conventional dietary management of obesity and overweight usually results in body weight losses close to 5.0 kg over a three month period. However, the achievement of an "ideal body weight" has often been set as the target for success in those aiming to reduce their body weight. This approach has been considered to be flawed given the accumulating evidence that some benefits from moderate weight loss have been recognised with as little as 5% body weight loss.

The present studies examined the effects of moderate weight loss on risk factors for IHD using a non invasive dietetic led approach. The dietary approach used for all studies was an individualised energy deficit prescription within one to one consultations. The aim of the intervention was to provide a comparable effect from weight loss in all subjects and total compliance to the regimen a 6.0 to 7.0 kg weight loss should have resulted over 12 weeks. The use of a standard regimen permitted some group comparisons across the different studies.

Otherwise healthy, but overweight subjects, mean BMI 34.9 (SD 6.1) kg/m^2, succeeded in achieving a mean weight loss close to 5.0 kg (4.8%) over a twelve week period. Reported dietary intakes from 7 day WI diaries suggested that a diet composition in line with current targets was reached. The actual weight loss seen was around 80% of that planned. Some benefits in terms of IHD risk were shown with significant falls in the RCA, factor VII activity and plasma cholesterol concentrations. No other significant changes were found. Before and after intervention the biological measurements were within the established reference ranges.

The benefits of weight loss were also examined in a group of overweight subjects with established ischaemic disease, of mean BMI 30.8 (SD 4.5) kg/m^2. It was hypothesised that those who were overweight with angina pectoris would show more adverse haemostatic and rheological factors and show greater benefits in comparison with those who were also overweight but free from cardiovascular disease. The weight loss in this group, 3.5 (SD 2.6) kg, 4.2%, was similar to the healthy overweight subjects with comparable effects on established risk factors, a significant fall in RCA, factor VII activity and plasma total and LDL cholesterol. The remaining IHD risk factors were unchanged after weight loss.

The final study examined whether weight loss in a group of subjects, whose mean BMI was 25.3 (SD 1.3) kg/m^2, close to the healthy range of 18.5-25 kg/m^2, would influence

IHD risk factors. This study was completed in a work site setting to reflect the different population required. The weight loss achieved was close to 3.0 kg, representing a mean 4% weight loss. However, before and after intervention the biological measurements all remained within their reference ranges, no significant changes were found in haemostatic and rhelogical measures.

The conclusion of this thesis is that modest weight loss, (around 4%) which can be achieved through well planned dietetic management, does produce important reductions in IHD risk. The weight loss achieved was similar in groups with BMI > 28 kg/m^2, with or without IHD, but less in absolute terms in individuals with baseline weights near the top of the healthy (acceptable range). Reductions in factor VII activity and RCA were related to the amount of weight loss, but the reductions were not greater in those with higher baseline values and existing IHD. The falls in factor VII activity and RCA were accompanied by falls in other established IHD risk factors, plasma lipid concentrations and blood pressure.

Chapter 1: Overweight and obesity

1.1 The increased health risks attached to overweight and obesity

Both cross-sectional and longitudinal studies have demonstrated strong and consistent associations between indicators of overweight such as BMI, waist circumference or WHR, and a range of conditions affecting most systems of the body.

Debate about the health hazards of overweight and the benefits of weight loss have been dominated by the consideration of IHD, although many other obesity related conditions can contribute to the symptoms and distress suffered by the overweight. Early epidemiological studies failed to show the influence of overweight when analysed with multivariate statistical methods. These analyses removed many of the influences of overweight, as they "controlled" for the influences of the diseases known to be related to overweight such as hypertension, hyperlipidaemia and IGT. All these conditions are regarded as being reversible consequences of overweight which are intensified by the presence of a central fat distribution.

The most powerful analyses to demonstrate the effect of overweight on cardiovascular disease have resulted from large longitudinal studies such as Whitehall, Intersalt, the Boston Nurses, American Cancer Association and Framingham. The results of these principal studies are similar, but there are some aspects of the evidence that prevent simple conclusions being drawn. The crude relationships between BMI and mortality and morbidity are not linear but follow a J-shaped distribution (Lew and Garfinkel, 1979). The relative risks are greater when BMI is under 18.5 and are probably related to smoking. As overweight develops above a BMI of 25 kg/m^2 the risks increase gradually and then more markedly when BMI exceeds 30 kg/m^2. This relationship has been interpreted as indicating a lesser problem with coronary disease risk in the overweight. However, since underweight people are less common (BMI < 20 kg/m^2 in only 6% of the population) than

those overweight (BMI > 25 kg/m^2 in 53% of the population) (Bennett *et al,* 1995) the contribution of overweight to coronary disease in absolute terms (i.e. population attributable risk) is much greater.

The incidence of IHD becomes more common with increasing age, but there is a clear decrease in the IHD risk attached to overweight (Must *et al,* 1992), and the difference between the sexes becomes less, mostly due to effects of the menopause in women (Barrett-Conner *et al,* 1984). As the development of IHD takes place over a long period of time the duration of overweight is likely to be important in determining the reversibility. It is also possible that different mechanisms for IHD apply at different extremes of BMI. Thus, smoking and with it thrombotic risks, may dominate the cardiac risk profile of the underweight and in older people, while atheroma is likely to be more important as a primary factor in the overweight and in younger people.

It must be remembered that in terms of symptoms and total medical burden, the main health hazard of overweight is not IHD but a range of other related conditions which contribute importantly to ill health and health service costs. When IHD risk attributable to obesity is relatively small (e.g. in the elderly) these other problems dominate.

1.1.1 Hypertension

Studies have shown a higher risk of IHD with increases in arterial pressure (Kannel *et al,* 1987). A review of the Framingham study results revealed that overweight subjects had significantly raised blood pressure (Stamler *et al,* 1978). The influence of age on blood pressure was investigated by dividing the population into 20-39 year and 40-69 year age groups. The prevalence of hypertension in the younger age group was twice that in those of normal weight, and three times that in underweight, subjects. The prevalence of elevated blood pressure in the older overweight age group was double that of older normal

weight subjects. Those who were 125% or more above ideal body weight had double the prevalence of hypertension, defined as diastolic blood pressure above 95 mmHg or the use of anti-hypertensive therapy. These findings concur with another UK study (Elliott and Marmot, 1984). The Scottish Heart Health study results suggested that gender, age, alcohol consumption and BMI were all influential in a multiple regression model of factors influencing blood pressure (Smith *et al*, 1988). The second working party of the British Hypertension Association concluded that strong correlations existed between BMI and blood pressure and between raised BMI and the development of hypertension (Sever *et al*, 1993). The small influence of gender showed that men have higher blood pressure than women at all ages (Gregory *et al*, 1990). The mean values for systolic blood pressure were 125 vs. 118 mmHg and for diastolic pressure 77 vs. 73 mmHg for men and women respectively. The 52 communities of the Intersalt study found a direct positive correlation between BMI and blood pressure. Hypertension was defined as a systolic blood pressure greater than 140 and a diastolic greater than 90 mmHg or a controlled blood pressure with the use of regular anti hypertensive medication. Among all measured characteristics except age, body weight had the strongest relationship with blood pressure (Intersalt Research group, 1988). The Intersalt results showed significant correlations with both median systolic and diastolic blood pressure and BMI. Alcohol consumption and potassium but not sodium excretion elevated blood pressure.

In order to ensure accurate measurement of blood pressure in the overweight or those with a large upper arm circumference (30 cm or greater) a large sized measurement cuff should always be used (Wittenberg *et al*, 1994). The size of a small cuff is 23 x 12 cm and a large cuff is 34 x 15 cm. The use of a small cuff in those with an arm circumference of 30 cm or more has been shown to overestimate systolic blood pressure by between 6 - 9 mmHg. Similar findings concerning the overestimation of blood pressure when measured using an incorrectly sized cuff in the overweight was also found by Iyriboz *et al*, 1994. Both

studies concluded that the use of a large cuff in all subjects would result in more accurate blood pressure measurements and remove the majority of the overestimation. These findings suggest that the results of other studies which have examined the relationship between blood pressure and body composition may have been affected if incorrectly sized blood pressure measurement cuffs were used.

The influence of overweight in children on IHD risk factors including blood pressure was investigated in the Bogalusa Heart Study (Aristimuno *et al*, 1984). Paired comparisons showed that obese and very obese children had significantly higher systolic blood pressure than normal weight children. These respective differences were 110 vs. 98 mmHg systolic and 68 vs. 63 mmHg diastolic after a six year tracking period. Hypertension has also been described in approximately 1-2 % of adolescents, of which the obese account for 50% of all cases of adolescent hypertension (Rames *et al*, 1978).

It has been suggested that hypertension treatment should begin at diastolic blood pressure of above 90 - 100 mmHg in those aged up to 65 years and of above 105-115 mmHg in older subjects (Bannan *et al*, 1980, Sever *et al*, 1993). However, drug treatment of hypertension brings risks of serious side effects including heart failure (beta-blockers), postural hypotension and hypokalaemia (diuretics), as well as other lifestyle effects on feeling of well-being, which must be set against the benefits of blood pressure reduction of 5-10 mm Hg.

1.1.2 Impaired glucose tolerance

Strong positive relationships between insulin resistance, IGT, NIDDM and BMI have been found (Barret-Connor 1989, Zimmet *et al*, 1991). However, additional influences such as genetic traits which interact with BMI have also been suggested and are illustrated by cross sectional studies in Pima Indians (Knowler *et al*, 1981).

Weight gain was examined as a risk factor for the development of NIDDM in nurses aged 30-55 years as part of the Boston Nurses study of 114, 281 nurses (Colditz *et al,* 1995). After adjustment for age, BMI remained the dominant predictor for NIDDM and a higher BMI was related with an increased risk of developing NIDDM. Perhaps surprisingly, women of average BMI (24 kg/m^2) showed an elevated risk of NIDDM when compared with thinner women (BMI 22 kg/m^2). The study also compared women with stable body weight to those reporting weight loss or gain. A 1.9 and 2.7 increase in risk of developing NIDDM was associated with weight gains of between 5.0-7.9 kg and 8.0-10.9 kg respectively. In contrast, a weight loss of 5.0 kg reduced the risk of NIDDM by 50%. The Whitehall study of male civil servants examined the relationship between IHD, fasting blood glucose concentrations and mortality after five years of follow-up (Fuller *et al,* 1979). For those above the 98^{th} percentile for fasting blood sugar distribution (>110 mg/dL) a doubling of mortality was observed which was independent of raised blood pressure and overweight (Fuller *et al,* 1979). Another study of Pima Indians found that within this rather unusual population obesity itself was not an independent predictor of progression from IGT to NIDDM when insulin and glucose concentrations were controlled for (Saad *et al,* 1988). However, 83% of the Pima Indians with IGT were overweight (classified as a BMI > 27 kg/m^2) with an incidence of NIDDM 2.9 times that of their normal weight counterparts. In desert based Aborigines BMI was independently related to insulin response after glucose loading (O'Dea *et al,* 1988).

1.1.3 Raised serum lipids

Two studies found increased body weight associated with increased plasma lipids in both sexes (Kannel *et al,* 1971, Carlson and Bottiger, 1981). Some plasma lipids such as cholesterol were themselves found to be associated with increased IHD risk (Van Itallie, 1985, Denke *et al,* 1994). In young (20-44 years) and older women (45-59 years), raised total and LDL cholesterol and triglyceride concentrations and lowered HDL cholesterol

concentrations were associated with excess body weight. This pattern seen in post-menopausal women was also shown in men (Denke *et al*, 1993) and children where raised triglyceride concentrations have been noted in the obese and very obese (Arishmino *et al*, 1984).

As part of an eight year study of nearly 116, 000 women, mild to moderate overweight was found to increase the IHD risk in middle-aged women grouped according to BMI between 23-25, and 25-29 kg/m^2 (Manson *et al*, 1990). The increased BMIs were paralleled by elevations in IHD risk and these relationships were strengthened with increases in plasma cholesterol, NIDDM and hypertension.

The most characteristic lipid disturbance in obesity is elevated triglycerides and low HDL cholesterol (Bjorntorp, 1990). Total cholesterol is also frequently elevated in the overweight (Gregory *et al*, 1990). Decreased HDL concentrations have been observed with increased BMI and associated with increased risk of MI (Glueck *et al*, 1980, Lipid Research Clinics, 1980, Haffner *et al*, 1985). Elevated LDL lipoprotein cholesterol concentrations in obesity are common, but they probably reflect background lipid profiles in the general population, and not a specific effect of overweight (Barrett-Connor, 1985).

The Prospective Cardiovascular Munster study illustrated the relationships between BMI and plasma lipoproteins (Assman and Schulte, 1992). Elevated plasma triglycerides, coupled with an increased ratio of hepatic lipase to lipoprotein lipase in the overweight, lead to an excess of the small dense LDL (LDL_3) particles which are readily oxidised and highly atherogenic (Sattar *et al*, 1997).

Plasma triglycerides are an established independent risk factor for heart disease risk (Hokanson and Austin, 1995). The increased atherogenic combination of raised triglyceride and lowered HDL concentrations have been described in the overweight (BMI > 25 kg/m^2). The relationship between overweight and the increased risk of coronary

disease was considered in 1985 at the consensus development conference. Obesity was identified as a major influence in raising plasma lipids and in turn weight reduction was identified as being advantageous in normalising plasma lipids and lipoproteins for overweight individuals (Burton *et al*, 1985).

1.1.4 Haemostatic and rheological factors and overweight

Raised concentrations of the plasma protein fibrinogen have been shown to be associated with increased IHD risk and it becomes elevated in response to increased BMI, smoking, stress and family history of IHD (Ernst, 1991, Ernst and Resch, 1993). Strong relationships between obesity and raised fibrinogen concentrations, coagulation factor VII activity (Folsom *et al*, 1991) and PAI activity have been described (Meade *et al*, 1986, Meade *et al*, 1993). Measures of blood flow and RCA are higher in the overweight than normal weight subjects (Poggi *et al*, 1994). These findings suggest an elevated thrombosis risk in the overweight and possible mechanisms for this increased risk are an impairment in fibrinolytic response and an elevation in the coagulation indicators. The role of overweight and weight loss with respect to each rheological and fibrinolytic factor will be considered in greater depth in chapter 3.

1.1.5 Endocrine/metabolic diseases and dehydroepiandosterone sulphate

Obesity has been characterised by abnormal steroid secretion and a reduced SHBG (Kopelman *et al*, 1980). Reduced SHBG concentrations have been inversely correlated to hyperinsulinaemia and insulin resistance and to the presence of increased upper body fat (Weaver *et al*, 1990). The hyperinsulinaemia of obesity usually overcomes the insulin resistance. However, the increased plasma insulin concentrations may enhance renal retention of sodium and possibly cause hypertension as an undesirable secondary effect (Bray, 1989).

Rogers and Mitchell (1952) reported on the incidence of ovarian dysfunction in obesity. They reported that 43% of women with abnormal menses were obese, compared with only 13% of the control group of non obese women. The two most common menstrual disorders were secondary amenorrhea and dysfunction and uterine bleeding. A larger study, of 26,000 overweight women taking part in a slimming study, examined the incidence of abnormal menstruation. The incidence of menstrual disorders was directly associated with the degree of obesity (Hartz *et al*, 1979). The presence of hirsutism was related to the duration of the obesity which indicated hyperandrogenism as a correlate of obesity. The presence of PCOS has been strongly related to obesity and characterised by a chronic ovarian hypersecretion of androgens (Friedman and Kim, 1985, Yen, 1976).

Adrenal functions are altered in obesity, as the secretion rate of adrenal hormones is proportional to body weight (Jackson and Mowat, 1970). The increased cortisol production rate in obese humans has been reproduced in those made obese by overfeeding. A shortened plasma half life has been shown in association with the increased metabolism of cortisol in obesity (Migeon *et al*, 1963).

The C_{19} steroid DHEAS and the other principal steroids in this group are androstenedione and androst - 5- ene 3β, 17β-diol, are both produced by the adrenal glands. Lowered concentrations of these steroids are seen in obesity, particularly with an abdominal fat distribution (Tchernof *et al*, 1996). DHEAS holds a particular interest to those studying obesity, as it has been postulated as the possible link between hyperinsulinaemia and obesity. It is also thought to affect platelet activity and thus blood coagulation (Nestler *et al*, 1992). Recent evidence suggests that DHEAS exerts multiple anti-atherogenic effects and that hyperinsulinaemia may reduce serum DHEAS by decreasing production and enhancing clearance. Lower DHEAS is found in those with elevated coronary risk and the overweight (Barrett-Connor *et al*, 1986, Nestler *et al*, 1989, 1992). All of these

alterations in the production rate of adrenal hormones have been inter-linked with increased plasma insulin concentrations and insulin resistance.

Obesity has also been associated with a number of cancers. The American Cancer Society followed men and women aged over 30 for 13 years. The study included 336, 442 men and 419, 000 women free of cancer, heart disease, stroke and having only altered body weight by a maximum of 4.5 kg in the preceding 12 months. The results of the study clearly indicated that obesity was associated with infertility and cancers of the breast, endometrium and ovary in women, and an increase in the incidence of prostrate cancer in men (Garfinkel, 1985). The effect of overweight on increasing androgen and oestrogen metabolism may have underlying effects on the development of these conditions (Longscope *et al*, 1986). The influence of overweight on the risk of death from cancer was confirmed by the results of a large study by Manson *et al* (1985). The study found that mortality among women with BMIs of 32 kg/m^2 or greater was 2.1 times the relative risk of death of women with BMIs below 19.0 kg/m^2.

1.1.6 Smoking

Cigarette smoking has been strongly associated with all forms of coronary disease in both sexes (Doll and Peto, 1976, Mann *et al*, 1976). On average, middle aged smokers have about double the risk of a major coronary event, and this is compounded with increasing overweight (Pooling Project Research Group, 1978). The classic parallel curves of BMI against mortality, which have been represented for both smokers and non smokers, show that the progressive mortality increases with increasing overweight, an effect which is present at every level of BMI (Lew and Garfinkel, 1979). It has been shown in the UK population that smokers tend to be thinner than ex-smokers or those who have never smoked (Gregory *et al*, 1990). The role of smoking cessation in favouring weight gain was shown in the MRFIT trial (Shimokata *et al*, 1989). The MRFIT data suggested that

weight fluctuation may reflect unsuccessful attempts at smoking cessation. The health outcomes which included heart disease, MI and death, may be a consequence of past and current smoking habits. It has been suggested that nicotine may reduce food intake (Shimokata *et al*, 1989). The importance of remaining a non-smoker despite the regain of body weight must be emphasised in health promotion and all settings.

1.1.7 Weight change, weight cycling, mortality and life expectancy

Periods of "famine and plenty" are well recognised to have occurred throughout the evolution of man and something that humans have become adapted to. However, the modern conditions of overeating and inactivity with episodic dietary restriction mean weight cycling is a common occurrence with a baseline of obesity which tends to rise throughout life. Current statistics on the number of adults trying to follow a weight reducing diet are alarming. Figures for the UK suggest up to 35% of adults are engaged in some form of slimming behaviour at any one time (Kent and Bowyer, 1992) and in the United States up to 40% of adult women and 20% of adult men (Williamson *et al*, 1992). Given that a larger proportion of the UK population are becoming more overweight, this evidence warns that large numbers of adults are, repeatedly in adult life, within cycles of slimming and weight regain.

Retrospective studies suggest that potential hazards exist from "weight cycling" (i.e. reported fluctuations in adult weight) with increases in IHD risk and BMI. The evidence supporting these claims is fairly consistent, but probably confounded. In the Western Electric study of self-reported weight change 5% of the study group were considered as "weight changers" (a gain and a loss of 10% body weight) within a 10 year period (Hamm *et al*, 1989). The 98 men considered to be weight changers showed a significant 50% higher mortality from IHD and cancer than the remaining group. In contrast, Wing *et al*, (1992) found no effect of "weight cycling" on total body fat, fat distribution or REE, and

challenged the hypotheses that weight changes are related to metabolic changes or disease activity. Larger studies such as the NHANES study or the MRFIT trial also have data on body weights. Weights are again self reported and are without detail concerning the reasons underlying weight changes. Brownell and Rodin (1994), posed many as yet unanswered questions concerning weight cycling and suggested an equal prevalence of cycling in both males and females of both normal and increased body weight. They also proposed that weight cycling may be associated with negative psychological and behavioural influences on lifestyle, the principal factors being binge eating, depression and life dissatisfaction.

A number of epidemiological and cohort studies with longitudinal survival data have been used to assess the disease association of reported weight changes. The problem with this approach remains that weight loss may have been the result of intentional slimming, or it may have reflected the presence of a disease which would shorten survival. The reductions in all the major risk factors for cardiovascular disease would suggest that weight loss should ultimately lead to increased survival. Self reported weight loss, in a number of large observational studies appears to have adverse effects on survival, even amongst the overweight. Thus the NHANES study identified obesity as a risk factor for mortality but found no benefit from reported weight loss for the whole population. Pamuk *et al* (1992) followed the first NHANES study subjects (1971-1975) surviving to five years post-study for the cohort aged between 45-74 years at admission. For those with a BMI between 26-29 kg/m^2, weight loss increased their risk of death even after adjustment for age, race, smoking habit, parity and pre-existing illness. Those subjects who lost 15% or more of their maximum weight had twice the mortality of those who only reduced their body weight by 5%. At BMIs greater than 29 kg/m^2 mortality was increased in proportion with weight lost for women.

Lee and Paffenbarger (1992) investigated the effect of body weight change on longevity in a cohort of Harvard University alumni. Self reported weights and health and activity questionnaires were completed at two periods, 11 years apart. The lowest mortality was found in those maintaining a stable weight. Weight losses and gains were associated with significantly increased mortality from all causes. Blair (1993) examined the effect of weight changes on mortality in the MRFIT cohort of over 10,000 men of mean BMI 27.7 kg/m^2 over a 7 year period. Mortality once more was found to be lowest in those whose weight remained stable, irrespective of smoking habit. The strongest associations were seen with 1 SD of weight change and death in the two lowest tertiles of BMI. There was no association with weight change in the heaviest tertile of the group. Andres *et al* (1993) summarised the results of the principle studies examining the issue of weight loss and mortality, and concluded that the highest mortality rates do occur in the adult who has either gained or lost excessive amounts of body weight. The lowest mortality rates are usually associated with modest weight gains. The prevention of severe overweight may be more effective than weight loss in reducing mortality.

These data highlight interpretational difficulties with self reported weight loss which may not have been intentional. Inadvertent weight loss usually indicates some sub-clinical disease process. The evidence on intentional weight loss appears more convincing, although it must be remembered that intentional weight loss may be a response to symptoms or advice for existing disease. Williamson *et al* (1995) studied reported weight loss in 28,000 healthy non smoking women and showed a reduction of >9.1 kg was associated with a 25% reduction in all-cause, cardiovascular and cancer mortality. However, in healthy women intentional weight loss less than 9.0 kg increased mortality. They also studied 15,000 women with existing disease and showed that weight loss of 9 kg or less reduced mortality from IHD (-10%), diabetes, (-30 to -40%) and obesity related cancers (-40 to -50%).

The health influences of reported fluctuations in body weight are probably dominated by the presence of disease - either as a cause of weight fluctuations or as a reason for intentional weight loss, but which did not completely reverse the associated risks. Weight loss in population studies is a product of many causes, many reflecting the presence of disease. However, the evidence on intentional weight loss or slimming under advice supports advocating moderate weight loss in both sexes of adult diabetic people. Conventional diets, when used in NIDDM subjects, lead to increased survival even in those with existing disease.

1.1.8 Fat distribution, overweight and health risks

An additional influence of obesity on the established risk factors for IHD remains the regional fat distribution or body shape. This has been shown to be influential on plasma lipid concentrations, blood pressure and glucose tolerance (Seidell *et al*, 1991). The presence of abdominal obesity, "an apple shape" carries greater overall risk than the presence of gluteal-femoral obesity (pear shaped). Although the causal mechanisms are not fully understood, those with abdominal rather than gluteal-femoral fat distribution are more likely to have hypercholesterolaemia, hypertension and IGT (Depres *et al*, 1990, Seidell *et al*, 1991). The relationship of waist circumference with these risk factors has been examined in some detail (Lean *et al*, 1995), since waist circumference predicts both total fat (Lean *et al*, 1996) and intra-abdominal fat (Han *et al*, 1997). The measurement of waist circumference was evaluated as a simple method to determine overweight and adverse fat distribution, to identify people with cardiovascular risk factors and a range of other conditions (Han *et al*, 1995, Han *et al*, 1996, Lean *et al*, 1998). People with waist circumference between 94-102 cm in men, 80-88 cm in women have increased cardiovascular risks. Those with waist circumferences greater than 102 cm in men and 88 cm in women have approximately triple the cardiovascular risk factors of those whose waist circumferences are below these limits.

1.2 The potential benefits and hazards from weight loss

1.2.1 Cardiovascular risk factors

There is evidence for health benefits from weight loss on most major cardiovascular disease risk factors, symptoms and possibly even on atheroma regression. Five principal risk factors for IHD are influenced by overweight and obesity: (1) hypertension, (2) IGT or NIDDM, (3) hyperlipidaemias (usually elevations in plasma triglyceride reduction in HDL cholesterol and elevations of small dense LDL (LDL_3), (4) altered haemostatic and (5) rheological factors. Recent information also links overweight with increased LDL oxidisability (van Gaal *et al,* 1997) probably reflecting the increased proportion of LDL_3 in the overweight (Sattar *et al,* 1997). During weight loss all these factors are improved, and there may be additional improvements if the individual is then able to take more physical activity. A consensus in the literature has reached the conclusion that weight loss significantly reduces the global risks of IHD.

1.2.2 Blood pressure

Weight loss reduces blood pressure in thin and obese, normotensive and hypertensive individuals. A retrospective analysis of blood pressure in military recruits to the Danish Armed Forces demonstrated that those who lost weight for whatever reason showed reductions in blood pressure (Sonneholm *et al,* 1989).

Virtually all of the many studies reporting the effects of weight loss on blood pressure show a marked and predictable reduction, even with the relatively low weight losses (2-5 kg) achieved by conventional dietary means. The effect of weight loss in upper arm circumference can be predicted from published equations (James *et al,* 1994). Each 1 cm of arm circumference is equivalent to about 1.2 kg/m^2 in both men and women, or about 4 kg loss for a patient of BMI 30 kg/m^2. The loss of arm circumference would reflect body

weight loss and reduce blood pressure. However, the ability of investigators to measure this difference would depend on the use of the correctly sized blood pressure cuff.

The degree of blood pressure reduction from conventional weight loss is similar to that from widely used antihypertensive drugs. Possible interactions between anti-hypertensive drug therapy and weight loss for blood pressure reduction were studied by Oberman *et al* (1990) in hypertensive individuals with body weight of more than 110% ideal. Weight loss of 4.5 kg led to blood pressure changes of 20 mmHg systolic and of 15 mmHg diastolic in patients treated with either atenolol or chlorthalidone. The possible influences of antihypertensive drugs on weight loss have also been studied (Davis *et al,* 1993). In an atenolol treated group mean weight loss was 2.7 kg, chlorthalidone 6.9 kg and placebo 4.9 kg. Decreases in diastolic blood pressure were 2, 4 and 2 mmHg respectively. Long term weight loss after 5 years monitoring reduced the rate of failure in blood pressure control for those receiving placebo, low dose diuretic or β blockers by 23%. The results were similar in each drug group. This study is a reminder that β blockers tend to impede weight loss, and can cause weight gain. Weight reduction, sodium restriction and physical activity training can supplement the effects of pharmacological treatment to lower blood pressure, with the advantage that all have no side effects. For individuals who remain significantly hypertensive despite weight loss, the addition of antihypertensive drug therapy will continue to be effective.

Practically all dietary approaches to weight loss involve a lowering of sodium intake, which will also contribute to blood pressure lowering. The meta-analyses of Law *et al* (1991a and b) draw the very clear conclusion that a decrease in sodium intake of 50 mmol/day will reduce systolic blood pressure significantly, by about 5 mmHg in normotensive or 7 mmHg in hypertensive individuals aged 50-59. Reductions in diastolic blood pressure are approximately half these values. The separate effects of weight loss

and sodium restriction on blood pressure have also been studied. A weight loss of 11 kg produced a 20% decrease in both systolic and diastolic blood pressure in hypertensive subjects (Reisin *et al*, 1978). This was a fall from a mean initial blood pressure of 160/90 mmHg. These differences were maintained even when sodium intakes were kept constant by encouraging the maintenance of usual salt intakes by the consumption of salty foods, demonstrating the independent effect of weight loss. The long term effects on blood pressure of weight and salt reduction programmes were compared using 12 month programmes of weekly / monthly group sessions focused on weight reduction, salt restriction or both (Rissanen *et al*, 1985). Mean weight reductions of 7.0 kg and 5.0 kg were achieved in the weight reduction, and the weight reduction and salt restriction groups respectively after three months. These weight losses were maintained at 12 months. Negligible weight changes were observed in the salt restriction group. Systolic and diastolic blood pressure significantly fell in the weight loss groups. Systolic blood pressure fell from 159 to 147 and 150 to 143 mmHg for the weight loss and the weight loss and sodium restriction groups, respectively. Diastolic blood pressure fell from 101 to 94, and 98 to 93 mmHg respectively. The salt restriction only group showed no significant fall in blood pressure, though 24 hour sodium excretion fell from 50 to 35 mmol.

The practicality of applying weight reduction or salt reduction programmes for hypertension management was studied in the Trial of Antihypertensive Medication study (Wassertheil-Smoller *et al* 1992). Nine diet / drug combinations in 878 mildly hypertensive moderately obese participants were evaluated. The greatest changes in blood pressure were observed in the weight reduction / chlorthiadone treated group. A weight loss of 6.8 kg reduced blood pressure by 15 mmHg. In the weight reduction / atenolol group 3.0 kg loss was accompanied by 14.8 mmHg fall in blood pressure. The diet / placebo groups all exhibited a smaller decrease in blood pressure of 8.9 mmHg with a weight loss of 4.4 kg. The weight reduction intervention lowered blood pressure more

than the low sodium / high potassium diets. Additional comparisons were made to determine whether diet therapy enhanced the impact of pharmacological therapy. Weight reduction with chlorthalidone yielded a significantly lower diastolic blood pressure (an additional fall of 4.3 mmHg). Weight reduction in combination with atenolol enhanced the impact of diet therapy by 2.4 mmHg.

In summary, it is clear that weight loss of an order achievable in clinical practice of 5-10 kg will reduce normal blood pressure by a valuable amount. The expected reduction per kg weight loss in normotensive overweight subjects is around 1.0 - 1.7 mmHg systolic and 0.8 - 1.0 mmHg diastolic, or 1.8 - 2.0 mmHg per 1% weight loss. Blood pressure reduction to this degree will confer benefits in reduction of cardiovascular disease, in particular stroke.

1.2.3. Glucose tolerance, insulin sensitivity and diabetes

In adults, an effect of age is to produce a decline in insulin sensitivity. The detailed reasons are unknown, but overweight has a compounding action reducing insulin sensitivity at any age. Those who are pre-disposed, probably for genetic reasons or through acquired pancreatic disease, demonstrate an accelerated progression to reach the diagnostic criteria for IGT and NIDDM, so this occurs at a younger age in the overweight.

The great majority of NIDDM patients are overweight, around 75% in most studies (Hadden *et al*, 1975, Holbrook, 1989, Lean *et al*, 1990). Physical inactivity seems to be a key factor influencing the development of diabetes (Colditz *et al*, 1995). Physical activity training improves insulin sensitivity separately from any effect of body weight (Koivisto and Defronzo, 1984).

An underlying pre-disposition to IGT and NIDDM is identifiable from a number of relatively simple markers including family history of NIDDM in a first degree relative, IGT

during pregnancy, high waist circumference, and southern Asian origins. The UK PDS suggested that a minimum weight loss of around 18 kg is needed for newly presenting patients to achieve normoglycaemia, defined as a fasting blood glucose below 6 mmol/L (UK PDS, 1990). However, lesser degrees of weight loss improve glucose tolerance significantly in people with diabetes, and also other risk factors such as elevated lipids and blood pressure (Vessby *et al,* 1984). Another study in a primary care setting showed a reduction in plasma glucose from 8.2 to 6.5 mmol/l with a 3.2 kg (2%) reduction in body weight (Bitzen *et al,* 1988).

Limited information from a retrospective survival analysis in NIDDM patients with BMI above 25 kg/m^2 suggests that 15-20% weight loss in the first year after diagnosis could reverse the elevated mortality risk of elderly NIDDM. An increase in life expectancy of about 3-4 months was found in a group of NIDDM patients with a mean age of 64 at diagnosis (Lean *et al,* 1990). Williamson *et al,* (1995) found that an intentional weight loss of 9 kg or more led to 30-40% reduction in diabetes-related mortality.

A special problem when studying NIDDM patients is that the altered metabolic state improves rapidly with energy restriction, so the effects of treating newly diagnosed compared with established NIDDM patients will be very different. This phenomenon makes interpretation of results from studies using a VLCD in NIDDM difficult to interpret. A VLCD was used to achieve 10.5 kg weight loss in 30 subjects over 40 days (Henry *et al,* 1985). A sub-group of 12 subjects were further evaluated during 40 days re-feeding. Blood glucose fell from 297 mg to 158 mg after 20 days and further to 138 mg/L at day 40, a total decrease of 40%. Re-feeding to maintain weight losses resulted in a secondary 80% increase in fasting blood glucose, though this remained markedly lower than before weight loss (254 vs. 167 mg/dL glucose).

The improvement in diabetic control with weight loss can be very marked in newly diagnosed patients who have symptomatic diabetes. For the majority of NIDDM patients weight loss is sufficient to remove symptoms. A 10% weight loss (8.2 kg) has been shown to reduce fasting blood glucose from 12 to below 8 mmol/L which represents the diagnostic threshold for diabetes (Hadden *et al,* 1975). In a primary care setting, a reduction in plasma glucose from 8.2 to 6.5 mmol/L was shown with a 3.2 kg (2%) reduction in body weight (Bitzen *et al,* 1988).

Once body weight is stabilised in the newly diagnosed diabetic patient, insulin sensitivity continues to decline with age (UK PDS, 1995). Attempts to check this progress with further dietary interventions have usually met with limited success in terms of weight loss when conventional dietary interventions are used. The benefits have been shown to be greatest in subjects categorised as having poorly controlled diabetes (Wing *et al,* 1990). For patients whose body weight was greater than 120% ideal body weight, as little as 4.4% weight loss led to a significant 4.3% decrease in $HbA1_c$ and a 15% decrease in fasting blood glucose.

In contrast, plasma glucose concentrations do not always improve after weight loss. A study of newly diagnosed NIDDM patients followed each subject until they achieved a weight loss of 9.1 kg (Watts *et al,* 1990). A total of 55 subjects (41%) showed improvements in metabolic control (responders), but the remainder failed to demonstrate benefits (non responders). Responders showed a 50% fall in blood glucose from 14.0 to 7.0 mmol/L. The non responders had an increase in fasting blood glucose from 14.0 to 18.0 mmol/L (22%). No predictive differences between these groups were seen in age, body weight or initial fasting blood glucose. Although the plasma glucose response to weight loss cannot be forecast by initial clinical parameters the success or failure of diet therapy can be predicted from the plasma glucose level after a weight loss of 2.3 - 4.5 kg.

The authors concluded that moderately obese patients with NIDDM who remain hyperglycaemic after a weight loss of 2.3 - 4.5 kg are unlikely to improve with further weight loss and should be considered swiftly for treatment with either oral hypoglycaemic agents or insulin.

The impacts of diet restriction and of weight loss are separate on glycaemic control and lipid changes. Active energy restriction appears to have a marked but transient effect on blood glucose and insulin sensitivity. When active weight loss ceases, there is a tendency for insulin sensitivity to return gradually towards its starting point, reaching a new point defined by the new body weight. Diet composition also influences insulin sensitivity and may be changed during weight management.

In practical terms, weight loss of the order 5-7 kg is usually fairly easily achieved in most newly diagnosed patients, with conventional dietary advice in a routine clinical setting, following advice administered by a dietitian. People with NIDDM are not more resistant to weight loss for any metabolic reason, and initial weight loss may sometimes be better maintained in NIDDM patients rather than those with simple obesity. Body weight changes in NIDDM patients with BMI greater than 25 kg/m^2 showed that patients whose BMI was under 25 kg/m^2 at diagnosis had on average gained 1.3 kg one year after diagnosis, those with BMI 25-30 kg/m^2 at diagnosis had lost 2.6 kg, and those with BMI greater than 30 kg/m^2 at diagnosis had lost 6.8 kg one year after diagnosis. These results indicate that dietary intervention, at least in the first year after diagnosis of diabetes, is relatively rewarding (UK PDS, 1995).

It has been established that avoiding weight gain in adults would have a major effect on delaying or avoiding completely the development of IGT and NIDDM. The opportunity exists for targeting advice concerning both diet and exercise, at those identified with a predisposition for IGT or NIDDM through family history of NIDDM, IGT in pregnancy,

high waist circumference or WHR, or origins in South Asia. Weight loss of 5% body weight leads to clinically important improvements in diabetic control. Loss of 10-20% body weight in overweight NIDDM patients can normalise metabolic control and possibly also increase life expectancy. Long term follow up results do not support the routine use of VLCD because interventions to ensure weight maintenance are not well developed.

Obesity and NIDDM are both associated with insulin resistance (Olefsky and Kolterman, 1981) a less than expected biological response to the hormone usually characterised by hypercholesterolaemia (Bonadonna and De Fronzo, 1991). Hyperinsulinaemia has been shown to be a vascular risk factor in healthy normoglycaemic normotensive individuals, related to the presence of obesity (Winocour *et al*, 1991). Obesity is frequently accompanied by hyperinsulinaemia both in the fasting situation and after a glucose challenge (Olesfsky and Kolterman 1981). Enhanced VLDL synthesis, an atherogenic plasma lipid profile and hypertriglyceridaemia are all associated with elevated plasma insulin concentrations (De Fronzo and Ferraninni, 1991). In obese subjects and in those with NIDDM, weight loss has been shown to improve insulin sensitivity (Henry *et al*, 1986). The decreased insulin sensitivity in those with NIDDM and its exacerbation in the obese NIDDM indicate why weight reduction is one the most effective treatments for these NIDDM patients (Campbell and Carlson, 1993).

1.2.4 Blood lipids

The effects of weight reduction on blood lipids and lipoproteins were examined in a meta-analysis of both hyperlipidaemic and normolipidaemic subjects (Dattilo and Kris-Etherton, 1992). Results from 70 studies within the meta-analysis indicated that weight reduction was associated with a decrease in plasma cholesterol of -0.79 mmol/L per kg weight loss. The change in LDL cholesterol concentrations was -0.39 mmol/L/kg, HDL cholesterol concentrations was +0.14 mmol/l/kg and triglyceride concentrations -0.66 mmol/L/kg.

These results, although derived from studies of different duration, are convincing that weight reduction through dieting is a viable approach to correct plasma lipids in overweight individuals (Weinsier, 1992).

The benefits of weight loss on plasma lipids of obese women (BMI 36.2 kg/m^2) were examined by Anderson *et al* (1995). An eight week slimming period resulted in an 11% decrease in body weight and a fall in plasma cholesterol concentrations and triglyceride concentrations of 23% and 16% respectively. Weeks 8 to 24 produced another 4.7 kg weight loss representing an overall reduction in body weight reduction of 16.5%. During the later phase of the study, cholesterol concentrations increased by 5.2%, LDL cholesterol concentrations by 4.5% and triglyceride concentrations by 5.1%. These findings suggest that improvements in plasma lipids after weight loss can be divided into two periods, the effects of energy restriction and weight loss. In most studies with duration under 3 months, subjects are still actively losing weight, and in a state of negative energy balance at the end of the study. When study duration is longer, most subjects will have stopped losing weight and become weight stable and some may be regaining weight.

During acute energy restriction, all blood lipids fall. When weight destabilises they tend to rise again towards the pre-weight loss value. Plasma HDL cholesterol tends to rise to a higher value than pre-weight loss over several weeks after weight destabilisation.

The effect of longer term weight fluctuations on plasma cholesterol concentrations can be examined using data from the Framingham study. This has suggested that there are small benefits for plasma cholesterol as well as blood glucose concentrations in subjects whose body weight fell over a 10 year period (Higgins *et al*, 1988). Over a 30 year period the Framingham study (Garrison *et al*, 1985) suggested some long term improvement in plasma cholesterol concentrations with weight loss. In addition, some of the spontaneous

weight loss may possibly be linked with a cholesterol lowering effect from underlying disease within the population (Higgins *et al*, 1993).

1.2.5 Weight loss at different levels of body mass index and waist to hip ratio

A few studies of weight loss have categorised the fat distribution of subjects by WHR (Lean *et al*, 1995). Cut off values of WHR, above which health risks increase appreciably, (a WHR > 0.95 in men and > 0.80 in women), have emerged from prospective studies. Metabolic risk factors particularly serum concentrations of triglycerides and HDL cholesterol improve most with weight loss in subjects with WHR above these values (den Besten *et al*, 1988, Sonnichsen *et al*, 1992, Wing *et al*, 1992, Kanaley *et al*, 1993, Houmard *et al*, 1994). The beneficial effect of reduction in waist circumference on cardiovascular risk factors has been shown (Han *et al*, 1997). A waist reduction of 5 cm led to a close to 10% improvement in at least one of the risk factors for cardiovascular disease, these included total and LDL cholesterol concentrations, triglyceride concentrations or systolic blood pressure. Waist circumference can thus act as an indicator of obesity and the need for weight management, and also as a monitor of change in weight and risk.

1.2.6 Cardiovascular symptoms: angina

Angina is the most reliably diagnosed symptom of IHD, provided standard criteria are used. Epidemiological evidence from the Rochester Coronary Heart Disease Project has shown women at the 75th percentile for BMI had a 50% increased risk of suffering from angina when compared with women at the 25th percentile (Beard *et al*, 1992). The work of Ornish *et al* (1990) in patients with known and stable angina suggested a low fat diet of close to 10% energy from fat, together with a number of other interventions can reduce angina frequency by 91% after a one year dietary education programme. The intervention group lost 10.0 kg weight (from 91.0 to 81.0 kg) over a 12 month period and had a 17%

reduction in total serum cholesterol together with evidence of atheroma regression. Over this period of weight reduction another improvement was an increase in frequency of physical activity (33%). Interpretation of these findings was complicated since the control group, who had no improvements in angina, were very much lighter than the intervention group. It has recently been reported that close to 40% of obese angina patients do not have significant coronary artery disease, so benefit from weight loss might be expected without invoking atheroma regression (Bahadori *et al*, 1996). It seems likely that moderate intentional weight loss will improve the frequency of anginal pain, though further studies are required.

1.2.7 The endocrine system particularly dehydroepiandosterone sulphate

A very wide range of endocrine changes are associated with overweight, and many have been shown to undergo some degree of reversal following weight loss. In particular the reduced SHBG in PCOS shows a tendency towards normalisation which was associated with improved insulin sensitivity (Friedman and Kim, 1985, Kopelman *et al*, 1981). Improvements in endocrine and ovarian function in obese women with PCOS have been demonstrated: 13 of 24 women with mean weight 91.5 kg reduced their body weight by 5% or more, and 11 of these achieved normal menstruation patterns (Kiddy *et al* 1992). Those reducing body weight by less than 5% showed no improvements.

The impact of intentional weight loss on a wide range of diseases have been studied (Williamson *et al*, 1995). The findings showed a striking reduction in incidence of the hormone related cancers: prostate in men, uterus and breast in women. A weight loss of 1-19 lb. decreased fully adjusted mortality by close to 55%, and a loss of greater than 20 lb. reduced fully adjusted mortality by 40%. Decreased aromatase activity after weight loss, reducing oestrogen in women and testosterone in men is the likely mechanism (Wade *et al*, 1981).

Caloric restriction has been established as being beneficial in extending life-span and retarding ageing in laboratory animals (Pahlavani *et al*, 1994). More recently, DHEAS has been proposed as a bio-marker of ageing and of caloric restriction (Lane *et al*, 1997). In this primate study, reduced caloric restriction slowed down the decline in DHEAS with age. These findings are the first evidence that nutritional intervention has the ability to alter aspects of ageing in a long lived species.

Male survivors of MI have been shown to have reduced serum DHEAS in comparison with healthy matched controls (Slowinska-Srzednicka *et al*, 1989, Mitchell *et al*, 1994). In women however, a prospective study failed to find any relationship between DHEAS concentrations and IHD (Orentreich *et al*, 1984). In males high serum DHEAS concentrations have been related to central fat distribution and inversely with BMI and plasma insulin levels (Herranz *et al*, 1995). A significant inverse correlation was found between BMI and DHEAS in a study of pre-menopausal women of high mean BMI (41.8 SD 10.3) (De Pergola *et al*, 1995), although age has been established as the principal influence on plasma DHEAS (Gray *et al*, 1991).

Experimentally induced hyperinsulinaemia lowers serum DHEAS concentrations (Nestler *et al*, 1989) and weight loss decreases plasma insulin concentrations (UK PDS, 1995). A reduction in weight may therefore be expected to result in a decrease in insulin and thereby an increase in DHEAS, and so contribute to the ultimate reduction in IHD risk. Substantial weight loss of an order seldom achieved in routine clinical practice (13.0 SD 4.0 kg) in healthy pre-menopausal women and men significantly increased DHEAS concentrations (Leenan *et al*, 1994). This study, published while the present thesis was in progress, used a 4.2 MJ (1000 kcal) per day dietary plan in overweight pre-menopausal women and men aged below 51 years (Leenen *et al*, 1994). These subjects BMI range was 28-38 kg/m^2 and a 14% weight loss (12-13 kg) resulted in acute increases in DHEAS

concentrations (32% and 36% for men and women respectively). Corresponding 35% and 45% reductions in plasma insulin concentrations in men and women were also reported. Another study of weight loss in hyper-insulinaemic pre-menopausal hirsute women achieved 5.8% weight loss in using a conventional dietary approach of 1500 kcal daily (Crave *et al,* 1995). The effect of weight loss increased serum DHEAS concentrations by 15-20% and decreased insulin by 15-20%. However, this potentially beneficial effect has not been investigated in older subjects who have lower circulating DHEAS concentrations (Gray *et al,* 1991, Haffner *et al,* 1994).

1.2.8 Potential health hazards of weight loss

The sequence of adverse developments during starvation were described classically by Keys *et al* (1950), and included depression, apathy, inactivity and cognitive impairment. A number of physical changes also occur during enforced weight reduction. Changes in the skin and hair loss are common, particularly with extreme dietary regimens such as VLCD. Starvation regimens have been largely dismissed as potentially dangerous, and have received little attention in the recent literature. Starvation entailing a daily energy deficit of 2500-2700 kcal alone will achieve a weight loss of about 3-4 kg per week. Tissue composition lost in starvation represents about 50% lean muscle and 50% adipose tissue, and thus presents difficulties through alteration in body composition (Garrow, 1988). Starvation runs increased risks of unexpected death even before body weight reaches a minimal healthy weight (Garnett *et al,* 1969). The extreme psychological effects of starvation were recorded by Keys *et al* (1950). Underlying heart disease exacerbated by the loss of heart muscle was the most likely causes of sudden death with the use of starvation diets (Garnett *et al,* 1969) or the development of ventricular arrhythmia secondary to the elevation of free fatty acids characteristic in starvation situations (Garrow *et al,* 1989, Drott and Lundholm, 1992).

Everhart (1993) recently produced a comprehensive review of the evidence regarding the development of gallstones. After weight loss through dietary intervention the development of gallstones increased up to 22%. Above 24% weight loss following diet there was a three fold increase in gallstone development. With weight loss greater than 45%, 60% patients developed either gallstones or gallbladder sludge (Shiffman *et al*, 1991).

Kaufman *et al* (1990) discussed in the light of three clinical cases, each of whom were described as being at risk of seizure by electroencephalography, the possible link between weight reduction and seizures, principally with respect to the use of VLCD regimes. The weight loss preceding the incidence of seizure was 5%. Caution must be advised in the use of VLCD in individuals with histories of seizures.

Obesity and overweight increase bone mass as it is responsive to changes in body mass. It has been demonstrated that bone mass falls with weight loss; as overweight subjects returned to their pre-diet weight, the bone mass followed these patterns (Compston *et al*, 1992). Another study suggested that weight regain was not accompanied by a complete restoration of bone mass (Avenell *et al*, 1994). This may put those who "weight cycle" at an increased risk of osteoporosis.

A number of potential hazards have been associated with weight loss, but they are relatively uncommon, and likely to be out-weighed by benefits, or to account for a general impairment of quality of life.

1.2.9 Psychological and "quality of life" effects

Weight gain is the result of relative overeating in response to a highly complex system of psychological and social pressures, and reversing this process without resolving the psycho-social issues introduces stress. Brownell and Rodin (1994) proposed that the process of weight cycling, may be associated with negative psychological and behavioural

influences on lifestyle. The principal factors which have been identified include binge eating, depression and life dissatisfaction. No consistent findings relating weight cycling behaviour, which indicated a group of subjects who regularly challenged their overweight, and psychological characteristics have emerged. A greater number of psychopathologies are apparent in the overweight in comparison with those who were weight stable (Foreyt *et al,* 1995). Two recent reviews, the National Task Force on the Prevention and Treatment of Obesity (1994) and Rebuffe-Scrive *et al* (1994) supported these findings.

A recent review of this area (Wadden and Stunkard, 1992) has suggested the existence of complex emotional disturbances in those who are overweight and obese. The effect of parental psychological characteristics on eating behaviour and dietary compliance in overweight children has been considered (Favaro and Santonasto, 1995). Their study of 49 couples with children suffering from obesity found that the characteristics of the mother were more important than those of the father in cases of obesity and weight loss. The mothers who had a more serious psychiatric symptomology and a more disturbed personality were associated with more severe obesity in their children. Weight loss was lower in children with mothers who showed neurotic tendencies.

Recent assessments of indicators of quality of life in those who are either obese or morbidly obese have suggested that these people have (or portray themselves as having) as poor a quality of life as others diagnosed with terminal cancer (Sullivan *et al*, 1993). In addition, it has been recognised that emotional and psychological problems specific to obese persons do exist but they are now seen as consequences of the prejudice and discrimination which are directed at the obese and the overweight. The benefits of weight loss with regard to psychological measures are now required to be considered in greater depth.

1.2.10 Benefits of weight maintenance at body mass index below 25 kg/m^2

By recent convention a BMI range 18.5-25 kg/m^2 has become considered acceptable for good health based on mortality data (WHO, 1996). It has been reported that those individuals whose BMI are within the upper part of the "healthy range" showed a greater incidence of diabetes and IHD than those with BMI 21-22 and a 5 times increased risk has been reported (Colditz *et al*, 1995, Willett *et al*, 1995, Williamson *et al*, 1995). The lowest risk of NIDDM is in people with BMI <21 kg/m^2 (Chan *et al*, 1994).

The ideal BMI which has indicated minimum risks to health was shown to be 20-21 kg/m^2 (Lew and Garfinkel, 1979, Willett *et al*, 1995). This has been principally derived from the J-shaped curve which described the relationship of body weight and mortality (Folsom *et al*, 1993). A recent quantitative analysis has succeeded in combining all principal studies that have investigated body weight and mortality, suggesting that the BMI associated with increased mortality risk is below 23 kg/m^2 and above 28 kg/m^2 (Troiano *et al*, 1996). A limitation of this analysis is the failure to consider the risk of specific diseases, such as IHD and diabetes. Data from the Boston Nurses study (Manson *et al*, 1990) examined the risk of coronary heart disease with obesity. From BMI <21 kg/m^2, with a relative risk of 1.0 this rises to 1.3 at BMI 21-23, 1.3 BMI 23-25 and 1.8, BMI 25-29 kg/m^2. These data, where cigarette smoking was controlled for, reflect the importance of obesity as a determinant for coronary heart disease risk.

With increasing age and using data for entire populations it has been suggested that older people who gain small amounts of body weight have showed benefits as measured by improved mortality risk whilst the opposite situation been associated with an increased mortality risk (Lew and Garfinkel, 1979). However, these data reflect the influences of sectors within entire populations who are unwell. It has been reported that in healthy people a decreased mortality was associated with a lower body weight, and an increased mortality was associated with a slightly higher body weight or BMI (Lissner *et al*, 1991).

A study of weight loss in males whose mean BMI was 24.6 (SD 1.8) kg/m^2 achieved losses of 7.4 kg improved fibrinolytic factors (Velthuis *et al,* 1995). These data have suggested benefits from the maintenance and achievement of a BMI of between 22-23 kg/m^2.

1.3 Conclusions

The health consequences from being overweight affect almost every system of the body. Weight loss as a result of intentional slimming results in predictable reduction in risks for ischaemic and coronary diseases. Benefits and health improvements are seen from a wide range of other specific weight related conditions as well as more "minor" symptoms including excess sweating and dyspepsia/indigestion.

A wide range less frequently studied physical disorders have been associated with being overweight and obese. The relative scarcity of reports on these conditions in relation to body weight change may reflect both the difficulty in measurements and possibly the considerable time period required to trace physical changes in these systems. Some common physical symptoms associated with obesity such as breathlessness, dyspepsia and profuse sweating are rarely described other than anecdotally, but often represent the earliest and most predictable benefit to patients.

Overall, good clinical evidence supports anecdotal reports in justifying weight loss to improve health and reduce health costs. The evidence with particular reference to thrombosis risk and indices of clotting are based on the results of slimming studies, the majority of which have used either surgery or very low calorie diets. One of the main aims of the work presented in this thesis was to examine further the effect of weight loss, achieved using a moderate daily energy deficit (600 kcal / 2510 kJ) on these measures. It is hoped to extend our understanding of the value, or limitations, of moderate weight loss achievable from routine dietetic intervention.

Chapter 2: The results of different treatments in the management of overweight and obesity and the assessment of dietary intake.

2.1 Dietetic approaches to weight loss

The management of obesity by the health care professions has developed along separate lines for the main health professional groups potentially involved, who are dietitians, doctors, nurses, physiotherapists and psychologists. Dietitians are professionals who address nutrition related diseases primarily on a clinical basis. Within hospital services they offer both inpatient and outpatient services in most general hospitals and in the community and see the majority of internal referrals usually with little or no input from specialist health professionals. Physician-led obesity clinics exist in only five UK National Health Service centres, with only a few surgeons performing surgery in cases of morbid obesity, usually gastroplications (COMA, 1994, SIGN, 1996). However, these latter approaches represent the fringes of clinical circumstance, and the most usual scenario in the first instance is a dietary approach. Practice nurses offer weight management advice to many overweight clients, although this appears to be on an *ad hoc* basis (Hoppe and Ogden, 1996). Other relevant health professionals, e.g. physiotherapists, clinical psychologists rarely have a major input into current obesity and overweight management.

The target for success, or "good compliance" with slimming regimens in the past has usually been the achievement of a BMI within the healthy range i.e. weight normalisation (below 25 kg/m^2) (Bjorntorp, 1992). For this reason the medical interpretation of more limited weight loss has tended to be negative. In the context of a general population whose weight increases steadily between the ages of 18 and 60, simply arresting or slowing weight gain should be considered a therapeutic success, and the health benefits will be large if this is done at an early stage particularly in younger patients. When weight loss is sought for medical reasons, the criterion for success should be of the order 5-10%

body weight and ideally maintained for at least 12 months (SIGN, 1996). The achievement of moderate weight loss (5-10%) body weight is known to have beneficial effects in a number of chronic diseases (Goldstein, 1993). The results of weight management approaches are increasingly being evaluated in line with these reasonable targets.

2.1.1 Dietary methods: conventional weight reducing diets

The use of a reduced energy regimen for self directed slimming, without guidance from either lay or professional people has been estimated to be effective in achieving a 10% decrease in overweight in only 10% of the population attempting to lose weight (Nezu and Perri, 1992). It is against data of this nature, with considerable interpretational limitations, that the success of weight loss programs must be judged.

Most conventional dietetic approaches providing published evidence use one-to-one interviews, but groups are used by commercial organisations. Conventional dietary restriction is usually considered to be based on foods, not on the use of nutritional supplements (Garrow and Webster, 1992) and provide a minimum of 800 kcal / 3347 kJ per day. For many years the 1000 kcal (4.2 MJ) diet was used as standard dietary advice, but compliance was recognised to be poor. Pearson *et al* (1991) report that this type of regimen has a less than 40% success rate. The success criteria in this case were defined as the achievement of a 50% reduction of the overweight, as defined by the subjects personal target weight (Pearson *et al*, 1991).

Weight loss on a 1200 kcal regimen was found to be a third of that predicted for subjects if they complied completely for 3 months (Frost *et al*, 1991). The value of moving away from more severe energy restriction was endorsed by Frost *et al*, (1991) who confirmed that a greater energy restriction does not lead to a greater weight loss in free living

subjects. The Lean and James (1986) formula together with a daily energy deficit close to 500 kcal below estimated energy expenditure was used to create individual diet prescriptions. A mean prescription of 1700 kcal/day over three months produced a 5.0 kg weight loss, compared to a 3.0 kg loss achieved with a standard 1100 kcal/day approach. These results contrast with predicted weight losses of 6.0 kg and 13.2 kg respectively which were calculated assuming a 100% compliance with prescription by all subjects.

In 1992, Dwyer surveyed the current scientific literature for the regimens for weight reduction in most common use, and found those providing limits of 1200 kcal daily for women and 1800 kcal per day for men were most frequently used. These regimens demand a deficit ranging between 500-1000 kcal/d below estimated energy requirements, and given adequate compliance, can reduce body weight by 0.5-1.0 kg per week. Dyer (1994) described the use of conventional weight reducing regimes as being potentially destructive, given their failure to incorporate long term support for adjustments in food choice, eating behaviour and lifestyles. The majority of weight loss treatments in medical clinics consist of referral to a local dietetic clinic, rather than use of more innovative team based approaches (Young, 1992). As the sample populations, intervention, attrition, duration and outcome measures vary widely in dietary interventions, a formal meta-analysis was not attempted.

Few clinical audits of the assessment and management of body weight are available in the scientific literature. A review of the outpatient management of obesity by junior medical practitioners indicated that the most obese subjects were categorised easily. However, children who were obese and those patients with additional cardiovascular risk factors were less likely to be reported as being obese and be given dietary advice (Denen, 1993). Other audits of weight management are even more general in nature and report the difficulty in measuring the effects of intervention in these settings. An exception was a

cross sectional survey of a specialist obesity clinic (Pacy *et al*, 1987). The most common reason for seeking weight loss among all subjects was to improve their appearance, although other physical symptoms including shortness of breath, and pain in weight bearing joints were ranked second in importance. The results in terms of weight loss were described by treatment duration, 1-3 months, 4-6 months, 7-12 months and greater than this period. The treatment methods were dietary advice alone, with 17 subjects (11%) being given jaw wiring. The losses were 5.0 (SD 6.2), 12.4 (SD 11.0), 12.4 (SD 10.2) and 12.0 (SD 5.2) kg respectively. These very encouraging results are likely to have been improved by the inclusion of the jaw wired subjects.

The mean weight loss that was most often achieved in the majority of studies was close to 5.0 kg, with a typical study duration of 12 weeks. It is likely that a 12 week study duration is moat usually chosen because maximum weight loss is achieved in this period (Nezu and Perri, 1992). However, a common failing in almost all conventional approaches for weight loss has been to leave weight maintenance to chance, so that regain is usual and most often almost complete.

2.1.2 Attrition Rates

Attrition or dropout rates vary in the various dietary interventions, given their different source populations, though most are around 20% (Tauberlassen *et al*, 1990). A reluctance of many researchers to report attrition rates, by excluding those who did not complete the study from their final analyses has the effect of reporting exaggerated weight losses (assuming that unaccounted drop outs fail to lose weight). Pratt (1989) evaluated the various reasons for attrition from dietary studies, and found the individual's perception of the personal value of the programme, together with self reinforcement and commitment were the most influential factors.

2.1.3 Effect of macronutrient diet composition on weight reduction

There are consistent findings from metabolic studies and epidemiological observations, for favouring high carbohydrate / low fat diets to assist weight loss and prevent regain (James *et al*, 1990). However, experimental evidence is not so persuasive for weight loss.

A 12 week randomised trial compared two sets of dietary advice designed for weight reduction. Each provided 1200 kcal/day, but was either low in carbohydrate (approximately 32% energy) or high in carbohydrate (approximately 58% energy) (Baron *et al*, 1989). Weight loss was 5.0 kg in the low carbohydrate and 3.7 kg in the high carbohydrate diet groups with no significant differences in the lipoprotein patterns between groups. Another study comparing two diets of different composition in free living subjects was conducted using 1200 kcal/day diets for 110 women with mean body weight of 84.0 kg (Lean *et al*, 1997). Weight loss was 5.2 kg over 3 months, with no significant difference on diets providing either 35% or 58% energy from carbohydrate. Similar weight losses were achieved using low or high carbohydrate diets in obese persons in a metabolic ward setting randomly assigned to diets with either 15% energy from carbohydrate and 53% energy from fat or 45% carbohydrate and 26% energy from fat (Golay *et al*, 1996). The weight loss in response to these diets was 8.9 or 7.5 kg respectively. The results of this study showed that energy intake not nutrient composition determined weight loss in response to low energy diets over a short time period, in this case 6 weeks of hospitalisation.

Several dietary intervention studies, exchanging low fat and high fat diet compositions have been investigated without specific weight loss advice. On average, weight loss of 1-3 kg was seen. This may be of significance for preventing long term gain.

2.1.4 Behavioural modifications: the principles in weight reduction

Behaviour modification has been described as a process which first identifies unproductive habits, which are then replaced with adaptive behaviour patterns (Beales and Kopelman, 1994). The behaviour therapist seeks to support a process of change in behaviours associated with weight gain, with reappraisal after a defined period (Blundell, 1984). Rewards or penalties can be used (Jeffery *et al*, 1983).

A strengthening of behavioural modification strategies can be achieved with the use of contingency contracting which employs legally styled, enforceable, agreements negotiated between therapist and client, usually over a defined period (Wing and Greeno, 1994). An element of this approach can be seen in the use of commercially organised slimming programmes, when a commitment to continue in a particular programme is given.

Behaviour therapy in body weight management addresses two main aims: 1) a reduction in the food energy consumed, 2) an increase in physical activity and thus total energy expenditure. In practice, a mixed approach of dietary advice and eating behaviour therapy are most often used (Wing and Greeno, 1994). Thus food intake and eating patterns may be monitored and assessed to identify eating behaviour cues before introducing control methods. Self-monitoring has been considered vital to the success of these approaches as the subjects then contribute to and define their own programme of care with which they feel most comfortable. Wing (1993) proposed that a change in peoples own individual environmental prompts for eating activity was required. Wing suggests that it helps people to develop a better insight into their own actions, and even perhaps clarify their particular reasons for being and remaining overweight. Cognitive approaches are often used in parallel with behavioural methods, to combat barriers to weight loss which exist within the patients' framework of beliefs. The administration of behaviour therapy can be either through group teaching or individual counselling. The success of each method being very

dependent on the individual preferences of patients (Perri and Nezu, 1992).

Wadden (1993) reviewed the results of treatments which included behavioural therapy for weight reduction in the years 1974-1978 and 1984-1990 (table 2.1). Sixty five randomised weight reduction studies were published in four behavioural journals over these periods were reviewed. Weight loss for the 1988-1990 period was 8.5 kg in 21.3 weeks, whilst in 1974-1978 a 3.8 kg weight loss was achieved over a mean period of 8.4 weeks, and attrition rate was about 20% in all cases. In 1990 compared to 1974, subjects were more overweight and were studied over longer periods. The rates of weight loss with treatments designated as principally behavioural are generally a little greater than with diet therapy alone.

The need to treat obesity as a chronic disease and to offer a continuous ongoing programme of care with the need to consider the differing individual needs of patients and changes over time has been stressed (Perri *et al,* 1993). A range of professional therapists have been proposed to be present in care teams to incorporate many components such as exercise, social support and coping strategies and post-treatment patient therapist contact. Perri *et al* (1993) showed that in overweight subjects a maximum initial weight loss of 9.0 kg was achieved after 9 months, and 5.0 kg loss remained 27 months post-treatment. A more standard behavioural approach without a care team or flexibility in the components achieved 7.0 kg weight loss after 9 months, and only 1.0 kg weight loss was maintained 27 months later. Perri *et al* (1988) found that after 18 months follow-up behaviour therapy alone had decreased body weight by 3.0 kg and 33% of this was maintained. Other more comprehensive approaches which included long term behaviour follow up, a social influence programme, and aerobic exercise resulted in 12.0 kg loss and 82% of the weight lost of this was maintained over 24 months. A number of studies have achieved weight losses 10-15 kg over 12 months, with about 30% regain at 2 years, 60% regain at 5 years,

(Jeffrey *et al*, 1983, Wolfe 1983, Kramer *et al*, 1989, Graham *et al*, 1992).

2.1.5 Physical activity strategies in the treatment of obesity

The established health benefits of physical activity include reduced blood pressure (Kostis *et al*, 1992) improved lipids (especially HDL cholesterol and triglyceride concentrations) and increased insulin sensitivity (Siscovick *et al*, 1985) and have been discussed in brief in the behaviour modification section. The potential mechanisms linking exercise with weight include enhanced resting metabolic rate, preservation of lean tissue during weight loss, increased total daily energy expenditure and post-exercise oxygen consumption (Hill *et al*, 1994). The influences of physical activity on hunger and satiety are conflicting, with both increases and decreases being reported (Bray *et al*, 1990). A J-shaped relationship with appetite, involving a paradoxical increase in appetite with complete inactivity has been suggested.

When physical activity or exercise alone is used in the treatment of obesity, weight losses are modest and average 2-3 kg (King and Tribble, 1991). However, evidence does exist that exercise alone can produce much larger reductions in weight when exercise is of sufficient frequency, intensity and duration (Gwinup, 1975). There appears to be a dose response relationship between level of exercise and degree of weight lost (Gwinup, 1975). One study which compared the effects of dieting versus exercise on weight loss and lipoproteins, in a one year randomised controlled trial, found that both intervention groups lost significant weight compared to controls. Dieters lost an average of 7.2 kg after 12 months compared to exercisers who lost an average of 4.0 kg (Wood *et al*, 1988).

A combination of diet and exercise generally produces greater weight losses than diet alone (Pavlou *et al*, 1989a, b, Kempen *et al*, 1995, Skender *et al*, 1996) although this is not always observed (Grilo, 1994). One study which randomised dieters to an exercise or

no exercise group found that the exercise group lost an average of 10.8 kg during the 12 week intervention period compared to the diet only group who lost an average of 8.1 kg. Perhaps more importantly, subjects in the exercise group adhered to the prescribed diet better than the no exercise group (Racette *et al*, 1995).

One encouraging finding in the study of exercise in the treatment of obesity is the effect on weight maintenance. During one study, subjects who had participated in a 12 month randomised controlled trial of the effects of weight loss through exercise or diet were randomly assigned to either a weight maintenance treatment or an assessment only control group (King *et al*, 1989). The maintenance strategy involved regular mailed information packs and telephone contacts. The initial amount of weight lost that was regained in intervention was different for exercisers compared to dieters when compared with control groups. Although dieters had lost more weight than exercisers after 12 months, dieters regained 42% of weight lost compared to exercisers who only regained 17.4% of weight lost. Similar findings occurred in the study by Skender *et al* (1996) who also followed subjects for 2 years. After 12 months, dieters had lost an average of 6.8 kg, exercisers 2.9 kg and the diet plus exercise group 8.9 kg. At 2 years, dieters had reached a weight 0.9 kg above baseline, the diet plus exercise group 2.2 kg below baseline and the exercise only group 2.7 kg below baseline, only 0.2 kg greater than that at one year. However, this meant that the total weight change at 2 years was not significantly different between either group.

An 18 month follow up data of a study which randomly assigned subjects to different dietary interventions and to exercise or no exercise, showed no difference in weight regained between the different dietary regimens. However, comparisons between exercisers and non-exercisers showed a significant difference in the maintenance of weight loss. All non exercise groups regained nearly all of the weight lost during the 8 week

treatment period compared to the exercisers who maintained most of the weight lost (Pavlou *et al*, 1989b).

Two of the few studies to follow subjects who had taken part in commercial weight loss programmes both found that maintenance of weight lost was associated with frequency of exercise (Holden *et al*, 1992, Grodstein *et al*, 1996).

The combination of physical activity and diet limits the amount of lean tissue lost (Skender *et al*, 1996), maintains reductions in plasma cholesterol (Svedsen *et al*, 1994) and limits weight regain (Garrow and Summerbell, 1995). Analysis of physical activity schemes within these publications have consistently shown the benefits of physical activity on both psychological and physical well being. Advice to increase regular appropriate physical activity should be incorporated in all weight management programmes. Reviews of the literature indicate that physical activity increases additional short term weight losses by around 1-2 kg when used in combination with a conventional reduced energy regimen (Brownell and Jeffery, 1987, Wood *et al*, 1991).

2.1.6 Financial incentives

Within the area of behavioural therapy for overweight, financial incentives have frequently been used to achieve agreed targets. Commercial organisations often relate their perceived success to the personal financial commitment made by clients.

Phillips and Philbin (1992) used a 12 week competitive behavioural programme (Brownell *et al*, 1984) which comprised a personal pledge of $10 and allocation to one of two slimming teams. After weight reducing advice the team to achieve the greatest weight loss kept the money. Over 12 weeks this achieved a weight loss of 3.5 vs. 3.0 kg per person in the winning and losing teams respectively. Other advice concerning nutritional principles was given in a learn and lunch format, offering food and nutritional advice.

A range of financial commitments ($30, $150, and $300) were investigated by Jeffery *et al* (1983) in a study of obese middle-aged men, allocated to be treated on a group or individual basis and with a 12 month follow up period. After 15 weeks, weight loss was 14.6 kg for individuals with highest financial commitment, 11.3 kg for the middle group and 10.6 kg for those with the lowest financial commitment (table 2.2).

Thus greater financial commitment and group therapy both favoured greater weight loss at 15 weeks, but at one year all the three groups differed by only 2.0 kg body weight.

In conclusion, it is evident that behavioural approaches to slimming can only operate via changes in energy expenditure or energy intake. No single package of behavioural interventions exist, but the application of the basic general principles of behavioural techniques seem to offer hope for greater weight reduction and better maintenance than simple dietary advice. The cost implications may also be favourable as a mixture of lay and professional leaders may feature in the use of behavioural treatments. The wide range of successes from behavioural treatments points to differences between individual skills, and also depend heavily on the response of relatively small subjects groups recruited in different parts of the developed world, all components which are hard to evaluate. Since studies of behavioural therapy often do not specify the exact methods used in detail it is seldom clear how much dietary advice is incorporated in behavioural strategies. Conversely, many studies of dietary advice have failed to describe simple behavioural tips which are usually included in good dietetic practice.

Many health professionals are unfamiliar with the terminology of behavioural therapy, and lack confidence in its use. The 5 core components or stages are self monitoring, stimulus control, modifying the topography of eating, adjunctive behaviour strategies and cognitive restructuring (Perri *et al*, 1992). It is appropriate for all weight loss programmes to include some behavioural measures and for all health professionals to be trained in simple

behavioural modification techniques (chapter 5, table 5.1).

2.1.7 Starvation regimens

These have been largely dismissed as potentially dangerous, and have received little attention in the recent literature. Total starvation entailing a daily energy deficit of 2500-2700 kcal will potentially achieve a weight loss of about 3-4 kg per week. The composition of tissue lost in starvation represents about 50% lean muscle and 50% adipose tissue initially (Garrow, 1988). Starvation leads to increased risks of death, even before body weight reaches a minimal healthy weight (Garnett *et al*, 1969). This can be related to underlying heart disease exacerbated by the loss of heart muscle by starvation (Garnett *et al*, 1969) or to ventricular arrhythmia, secondary to elevated free fatty acids (Garrow, 1988, Drott and Lundholm, 1992). The other psychological and physical effects of starvation were documented in detail (Keys, 1950). Healthy subjects who starve voluntarily die after 6-7 weeks, when fat stores have been expended (Keys *et al*, 1950).

2.1.8 Very low calorie diet regimens

The VLCD regimens are usually in the form of liquid drinks but which often allow a daily meal based on food. The term is used when energy provision from the entire regimen is less than 800 kcal per day (Royal College of Physicians, 1983, Committee on Medical Aspects of Food Policy, 1987, National Task Force on Obesity Prevention and Treatment, 1993).

The use of VLCD was extensively reviewed by the United States Task Force on Obesity treatment (National Task Force on the Prevention and Treatment of obesity, 1993). The committee concluded that supervision by a physician and the selection of patients with BMI of >30 kg/m^2 should provide appropriate and adequate safeguards to VLCD use.

The recommended VLCD diet composition includes 1g protein per kg ideal body weight per day to minimise muscle breakdown. Cholelithiasis has been shown as the most frequent clinical complication associated with the use of VLCD. Yang *et al* (1992) reported a 10% incidence of clinically recognisable symptoms of gallstones in 16 weeks of VLCD therapy. Potential dangers with VLCD are increased if there is underlying heart disease present, and through prolonged use. A 12-20 week period is acceptable (National Task Force on Obesity Prevention and Treatment, 1993).

Mean weight losses with VLCD range from 1.5-2.5 kg per week, so that their use over 12-16 weeks should produce close to a 20.0 kg weight loss. This compares with an average weight loss of 0.5-0.6 kg per week using a conventional 1200 kcal diet, leading to a weight loss of 6-8 kg over the same time. Basal metabolic rate is suppressed by a mean of 15% on VLCD (Bray, 1969, Apfelbaum, 1971, Garrow and Webster, 1989) tending to slow the speed of weight loss.

In practice, VLCD compliance is rarely complete with patients tending to revert to their usual diet and life style using VLCDs intermittently. Acceptability of VLCD regimens has been investigated in comparison with a similar energy provision to that achieved using food from either fish and fowl to supply dietary protein (Wadden *et al*, 1985). No differences in actual weight loss were seen between the two groups although the "food" based diet was rated as being more acceptable by the subjects.

The use of VLCD regimens in many short term weight reducing studies, but fewer consider their long term use (Hymans *et al*, 1993). Either an intermittent 500/1200 kcal regimen, or a constant 1200 kcal daily regime for 20 weeks in two overweight patient groups achieved weight losses of 9.5 or 8.0 kg respectively (Foster *et al*, 1992). Using estimates of energy requirements the expected weight losses would have been 27.5 and 22.0 kg, respectively, so both values suggested shortfalls in dietary compliance. Actual

weight losses were 34% and 36% of those estimated for each group if patients had followed advice completely. The authors concluded that there were no benefits in weight loss associated with VLCD use.

2.1.9 Maintenance of weight loss after very low calorie diet

Maintenance of weight loss continues to be of crucial concern for all types of weight control programmes including VLCD. Flynn and Walsh (1993) enrolled overweight but otherwise healthy patients on a VLCD regimen for a period of 26 weeks. After this period the weight loss achieved was 10% body weight for 90% of the study population, and this was maintained by 33% of subjects in a 30 month follow up. The initial mean weight loss was 21.4 kg at 26 weeks, and the maintained mean weight loss was 6.5 kg at 30 months. The entire programme was completed by 44% of subjects completed, the others having all withdrawn before the completion of the follow up period.

Weight maintenance following VLCD in NIDDM patients was examined by Wing *et al* (1994). There was greater weight loss with VLCD when compared with conventional hypocaloric diet (14.5 kg vs. 10.5 kg) at 50 weeks. These differences were due to differences in weight loss after the first 12 weeks of dietary treatment. Interestingly, the authors did not consider these weight losses important enough to justify the use of a VLCD regimen over a conventional reduced energy dietary regimen. Actual weight losses are comparable with the use of VLCD, whatever the duration of the study. Caprio-Shovic *et al* (1993) examined the effectiveness of a 16 week VLCD programme and rigorously followed up the recruits. Weight loss was 15.0 kg (15% body weight) for men and 15.0 kg (9% body weight) for women at 16 weeks, but at 1.5 years baseline weights were regained by two thirds of the remaining of subjects.

A structured programme was tested which combined a VLCD (12 weeks / 2500 kJ) with a

period of relaxing the dietary regime to 5000 kJ for 6 weeks and finally a period of weight maintenance on a step 1 national cholesterol education programme diet for 7 further weeks (Osterman *et al*, 1992). At 25 weeks, weight loss was 25.0 kg, providing evidence for improved compliance on this structured programme, though the long term weight maintenance post-programme was not evaluated. Reviews of the long term maintenance of weight loss after VLCD concluded that after one year post-slimming 75% of dieters regain most of weight lost (Andersen *et al*, 1988, Safer *et al*, 1991). After 2 years this figure rose to 85-90% (Sikand *et al*, 1988, Wadden *et al*, 1989). A study of VLCD followed by Dexfenfluramine showed the value of medication, in the maintenance of weight loss. After eight weeks VLCD, the patients then continued on a diet providing 75% of estimated energy requirements. They were randomised to receive either the medication or placebo. Those patients who received placebo showed a mean weight regain of 2.9 kg, whereas those receiving the medication reduced weight by 5.8 kg (Finer, 1992).

2.1.10 Milk diet

A milk based regimen was pioneered by Garrow as an alternative to the more expensive commercial VLCD. A typical milk diet is based on 3 pints of whole milk providing 59g protein and close to 50% energy from fat, with appropriate multivitamin therapy, ferrous sulphate supplements and an inert bulk laxative if necessary (Garrow, 1981, Garrow *et al*, 1988). Weight losses on the milk regimen containing 1170 kcal per day are comparable with those achieved with proprietary VLCD. A study designed to compare a commercially available VLCD (Cambridge diet), and the milk diet resulted in similar weight losses of 1.5-2.0 kg per week over 8 weeks (Garrow and Webster, 1989). The same limitations probably exist with the milk as with the commercial VLCD in terms of long term weight maintenance, but its low cost and universal availability make this approach preferable.

2.11 Conclusions

There are a range of different clinical approaches towards the achievement of weight loss. The outcome of the majority of these interventions are moderate weight losses, which have been acknowledged to lead to health benefits. The use of the VLCD regimes are proven to achieve good weight losses over the short term, but their value in long term weight maintenance depends on effective weight maintenance strategies. The moderate energy deficit approach has been shown to lead to a weight loss of close to 5.0 kg over 12 weeks, and this approach could offer promise for weight maintenance over the longer term. The value of this approach may lie with an emphasis on eating conventional foods, and introducing regular meal patterns. The effect of diet composition on weight loss is minimal, although the consumption of a high carbohydrate diet in the longer term should be advocated to reduce risk of long term chronic disease. Physical activity in addition to dietary intervention can offer opportunities for improved maintenance of weight loss, though physical activity programmes alone result in minimal weight losses.

2.2 Physical interventions

2.2.1 External mandibulo-maxillary fixation

External mandibulo-maxillary fixation, jaw or tooth wiring, for obesity is not currently offered by any major UK centres, probably due to the lack of evidence to prove that weight loss is maintained. Jaw-wired subjects are only able to consume liquids (Garrow, 1988). Jaw wiring together with a milk diet has resulted in remarkable weight loss of 36.0 kg over 9 months and 42.0 kg over 11 months in obese patients of BMI above 40 kg/m^2 (Harding, 1980). The published results of jaw-wiring indicate profound weight loss. Where this therapy is practical and acceptable, renewed efforts to maintain weight loss might make jaw-wiring worthy of re-appraisal, and to reduce surgical risks prior to

gastroplication surgery, it would seem justified. Limitations to the use of jaw-wiring are a lack of teeth, or the loosening of teeth. A period of unwiring can resolve the loosening of teeth.

2.2.2 Waist cords

In an attempt to prevent weight regain after wire removal, use of a waist cord has been studied (Garrow, 1981). The inextensible waist cord was developed for use after considerable weight reduction (e.g. with jaw-wiring) to prevent weight regain. In one study, (Garrow *et al*, 1986), 14 subjects from 36 whose jaws were wired were given a waist cord. In the 14 subjects mean weight loss immediately after jaw-wiring was 43.0 kg over 11 months and the weight regain was only 10.0 kg at one year (Garrow and Webster, 1986). The waist cord has also been studied to maintain weight loss between periods of intermittent protein sparing fast treatment (Simpson *et al*, 1986). Subjects with a waist cord lost an additional 5.0 kg weight over 11 months follow up compared with those without a waist cord who regained 7.0 kg weight over the same period.

The acceptability of the waist cord differs widely between patients, perhaps limiting long term use in some patients (Jung and Chong, 1991). For practical reasons the waist cord can only be used when there is an identifiable waist, excluding most patients with BMI >30 kg/m^2. The BMI of Garrow's subjects was 46.0 kg/m^2 before wiring, and 29.8 after wiring when the waist cord was first used. A waist cord has been shown to be a cheap and effective aid to restrict weight regain.

2.2.3 Gastrointestinal surgery

There is a long and varied history of surgical attempts to cure obesity. In practice a multidisciplinary team is required including a surgeon/physician, nutritionist/dietitian and

clinical psychologist. Pre-operation psychological assessment is usually advised, and post-operative respiratory problems may make availability of intensive care ventilatory facilities desirable (Beales and Kopelman, 1994).

The most widely used stomach surgery is the vertical banded gastroplications (Pories *et al*, 1992a). Weight loss after gastric restriction surgery has been reviewed by Halverston (1985). The average loss is 4.5 kg per month for the first 6 months, which has been associated with improvements in the major accompanying diseases of overweight. Final weight loss following surgery is usually around 50% of the excess above ideal body weight, but is determined by the diameter of pouch outlet (Kral, 1992). The regain following weight loss is much less than with other interventions with a failure rate at around 10% (Pories *et al*, 1992b). There are potential nutritional, emotional and psychiatric complications, though the greatest benefits are for patients with greatest health risks, and peri-operative mortality may be as low as 0.1% (Deitel *et al*, 1992, National Institutes for Health, 1992).

For those grossly overweight people who suffer some of the many health complications of their obesity, surgery may be life saving, and this applies particularly to sleep apnoea syndrome (Dyer 1994, Loube *et al*, 1994). NIDDM has been described as a surgical disease (Pories *et al*, 1992a) as post-surgical weight loss succeeded in resolving NIDDM. A total of 80% of the 163 subjects with IGT achieved normoglycaemia. The impact on health and quality of life of gastroplication is currently under review from the Swedish Obesity Study. Early evidence suggests improved outcomes compared to conventional dieting approaches (Sjostrom, 1994) although it might be argued that the control group does not represent best conservative practice.

2.2.4 Intra-gastric balloon

Initial impressive weight losses over the initial three month period of up to 21.0 kg have been shown using the intra-gastric balloon (McFarland *et al,* 1987, Ramhandany and Baird, 1988). All studies showed regain of the weight lost, due to the ability of the stomach to expand. Potential dangers in the use of balloons include rupture and intestinal blockage (Garrow, 1988). The evidence does not support the use of an intra-gastric balloon in obesity treatment.

2.2.5 Liposuction

The use of cosmetic surgery to remove adipose tissue deposits known as liposuction or liposculpture has not been used widely except in the field of cosmetic surgery (Fischer, 1991). The practical use of this therapy is difficult to assess and it has side effects, including the relocation of adipose tissue deposits to their original sites and to some new ones leading to further deformations of body shape (Coleman, 1991). Current evidence suggests that this surgery is of no value in long term clinical management of obesity and overweight unless the psychological motivations for overeating are resolved (Coleman, 1991). A role for cosmetic surgery may be to remove the folds of skin resulting from excessive weight loss e.g. after surgery.

2.2.6 Conclusion

A conventional weight reducing diet remains the most valuable intervention in the treatment of overweight given the considerations of both safety and nutritional adequacy and efficacy, and usually results in around 4 -5 kg weight loss. Do-it-yourself dieting is only successful for 10% of slimmers. The results for the use of VLCD (or the cheaper milk diet) suggest they can be valuable in inducing short term weight loss of 10-15 kg. Medical supervision is required, together with a minimum BMI of 30 kg/m^2, and long term

management in these cases. The use of jaw-wiring, waist cords and surgery provide a variety of alternative treatments available to the clinician. With careful selection procedures and an established multidisciplinary team for peri-operative care and long term supervision, gastroplication may be the most useful therapy in those patients with high health risks associated with their obesity, and for whom less drastic interventions have proved unsuccessful.

2.3 Anti-obesity medication

There are a number of anti-obesity medications, either under development, or available for prescription by physicians. The main classes of medication available, or under development for weight management operate through appetite suppression (or satiety promotion), inducing malabsorption (anti-nutrients) or by thermogenesis to increase energy expenditure. The use of anorectic medications have received particular criticism for their poor long term effectiveness (Garrow, 1991). The older amphetamine-like drugs can lead to dependence, and have been abused in the past. Case reports have linked some centrally active appetite suppressants including dexfenfluramine with the very rare condition primary pulmonary hypertension and dexfenfluramine has recently been withdrawn from use in the UK.

2.3.1 Malabsorption

Lipase inhibition induces a partial malabsorption of dietary fat by the inhibition of triglyceride hydrolysis. The chemically synthesised hydrogenated derivative of lipostatin (tetrahydrolipostatin) is a potent inhibitor of gastric, pancreatic and carboxylester lipase (Hadvary *et al* 1988). Drent *et al* (1993) found that the 12 week use of tetrahydrolipostatin (Orlistat) together with a hypocaloric diet (500 kcal daily energy deficit) achieved a weight loss of 4.3 kg compared to 2.1 kg in people receiving diet alone. Beales and Kopelman (1994) have suggested that the principles by which the medication

operates are sound, although there are possible risks, possibly correctable, of fat soluble vitamins A, D, E and K depletion. Predictable side effects from malabsorption, altered bowel habit, and also bacterial overgrowth of the small bowel have led to around a 10% patient withdrawal from therapy. Alpha glucosidase inhibitors such as acarbose, delay digestion of starch and sucrose. These drugs reduce blood glucose, but do not produce weight loss.

2.3.2 Appetite Adjusters

These medications have been divided into groups by Silverstone (1992). Those acting via catecholamine pathways, those acting via serotoninergic pathways (5-hydroxtryptophan, 5HT), serotonin precursors and other agents such as opiate receptor blockers.

2.3.3 Dexfenfluramine

The only serotoninergic drug licensed for use in obesity treatment in the UK was dexfenfluramine, which has very recently been withdrawn from use. The medication reduced the subjective levels of hunger and food intake (Silverstone and Goodhall, 1992), and large clinical trials showed an average weight loss of 3.0 kg more than placebo after 12 months of treatment (Guy-Grand, 1992). Stunkard *et al* (1980) compared the use of dexfenfluramine with a behaviour programme, or a behavioural programme alone for one year. The behaviour modification alone achieved a weight loss of 10.0 kg, the use of behaviour therapy and Dexfenfluramine achieved a weight loss of 15.0 kg respectively. In a one year follow-up a third of all weight lost was regained despite the continued use of behavioural therapy.

A recent Scottish study (Manning *et al,* 1995) showed the best outcome in the treatment of overweight NIDDM was with dexfenfluramine for three months followed by conventional diet therapy. However, after a year of treatment this difference, compared

with other available therapies, was lost. Another study compared dexfenfluramine with dietary advice to encourage compliance with healthy eating principles (Mathus-Vliegen *et al*, 1993). Over a 6 and 12 month intervention weight loss was 10.0 and 11.0 kg for placebo and dexfenfluramine treated groups respectively over the one year study period suggesting no benefit of medication above the influence of dietary counselling.

2.3.4 Sibutramine

Sibutramine is a serotonin and noradrenaline uptake inhibitor which was launched in USA and Brazil in 1998. A study of depressed patients who were not actively seeking to reduce their body weight, found that in 8 weeks a weight loss of 2.1 kg was achieved (Kelly *et al*, 1995). The result of a double blind placebo controlled trial found that when given with dietary advice, the same dose (30 mg /day) achieved a weight loss of 6.1 kg over 12 weeks, while the placebo group lost 0.9 kg (Bray *et al*, 1995). Side effects of sibutramine were recognised including a dry mouth, dizziness and headaches. Pulse and blood pressure are elevated by sibutramine, although this is usually counteracted by weight loss. A recent study in France found that 24 week treatment with sibutramine reduced weight by 6.5 kg in the treatment group and 3.1 kg in the placebo group. Fifty seven percent of treatment compared with 25% of placebo subjects lost at least 5% of body weight. The study also found beneficial effects on plasma glucose and insulin. Further long term studies of the continued effectiveness of sibutramine are in progress.

2.3.5 Fluoxetine

This antidepressant operates in a very similar manner as the dexfenfluramine and fenfluramine as a seratonin reuptake inhibitor but it does not have a licence for weight loss. Effectiveness has been demonstrated in use with diabetic patients (O'Kane *et al*, 1993) where a 4.3 versus 0.8 kg weight loss was achieved for treatment versus placebo group and hence an overall drug effect of 3.5 kg weight loss. In addition, Potter Van Loon *et al*

(1992) found an increase in insulin action in NIDDM subjects taking Fluoxetine, aside from any effect on reducing body weight. A long term trial of Fluoxetine (Darga *et al,* 1991) reported the results after one year, with a 41% and 27% dropout rate for the drug and placebo group respectively. Weight loss was 8.5 kg and 4.5 kg respectively for the treatment and control groups. Connelly *et al* (1995) used dietary management of obesity in a double blind study of the effectiveness of fluoxetine. Weight loss in the drug treated group loss was 3.9 kg and 1.3 kg in the placebo, over 12 weeks in obese elderly NIDDM patients. The drug was also evaluated in terms of achieving weight loss against placebo (Sayler *et al,* 1994). The drug showed improvements of 10% against placebo for systolic blood pressure reduction, an 8% improvement in BMI and a 10% improvement in total cholesterol and fasting blood glucose. An uncontrolled weight maintenance study was designed for the evaluation of fluoxetine (Goldstein *et al,* 1993). Over an eight week period a weight loss of 3.6 kg was achieved.

2.3.6 Phentermine

This anorectic appetite suppressing drug has been widely used with overweight patients. Its effectiveness was found to be unrelated to dietary habit, body weight and age in a randomised controlled study (Munro *et al,* 1968). Over 16 weeks weight loss was 4.0 kg with placebo and 7.0 kg with drug, a drug effect of 3.0 kg. The use of phentermine was extensively evaluated together with fenfluramine (Weintraub *et al,* 1992a, 1992b). Although the results were difficult to interpret due to the complex experimental design, the studies spanning a 4 year period do suggest a value in the long term use of anorectic agents with few side effects. Over stage one, a period of 3 months, weight loss was 10.0 kg, and overall weight loss for the whole study was 50.0 kg. However, the study used a range of dosages and combinations of the two medications, and this may account for the programme's success (Weintraub *et al,* 1992a, 1992b).

2.3.7 Conclusion

The range of drugs for weight management reflect both the lack of an ideal medication and the strength of mechanisms maintaining and governing appetite and body weight. Most available drugs, used alongside conventional diet advice, produce an average weight loss of 2.0-5.0 kg (5% body weight), better than placebo over a 3 month period, and this is maintained for as long as the diet is followed and the drug is taken. In routine practice drugs are introduced as therapeutic trials so the "non-responders" can be identified and the medication withdrawn. In "responders" weight loss of 8-12 kg should be expected. There is a wide range of efficacy between individuals and the reasons for this variation are unknown, however some individuals can achieve remarkable responses. Side effects reported in well documented controlled trials may be less problematic than might be imagined from anecdotal reports. Evidence on drugs which can be taken effectively for long periods are urgently required, since the main role of a drug in weight management is probably to prevent long term weight gain, rather than to induce massive weight loss in patients who already have become obese.

2.4 Weight reducing diet therapies in specific diseases

2.4.1 Non insulin dependent diabetes mellitus

Most NIDDM patients are overweight and the disease can be completely controlled by weight loss in many cases (Nutrition Sub-Committee of the British Diabetic Association, 1992). The recommendations for diet composition for other aspects of diabetes management are entirely compatible with weight loss (Nutrition Sub-Committee of the British Diabetic Association, 1992). The aims and methods of dietary interventions in overweight NIDDM patients are similar to that offered to other overweight people as a population, but given the much greater health risks in those with NIDDM, a greater motivation to achieve acceptable weights might be anticipated.

2.4.2 Weight loss in newly diagnosed non insulin dependent diabetes mellitus

The UK PDS examined the effects of dietary management on newly diagnosed NIDDM diabetics (UK PDS, 1990). A weight loss of 18.0 kg was required to normalise blood glucose to 6 mmol/l or below, and this was achieved by 16% of patients. In a primary care setting a weight loss of 3.2 kg, achieved using nurses competent with the principles of dietetics, resulted in significant falls in blood glucose (Bitzen *et al,* 1988). In 1990, newly diagnosed NIDDM patients in a routine clinic setting (Lean *et al,* 1990), those with BMI 25-30 kg/m^2 lost 2.6 kg, those with BMI >30 kg/m^2 lost 6.8 kg at one year. Weight loss in the first year of treatment was found to improve the prognosis of NIDDM. Expected survival time for their group of diabetics was 8 years at a median age of presentation of 64 years. The value of group treatment for weight reduction in newly diagnosed NIDDM patients has been examined (Heller *et al,* 1988). Group treatment achieved greater weight loss at 6.0 kg compared to 3.5 kg from individual dietetic advice at three months, 7.2 kg versus 2 kg at 6 months and 5.5 versus 3.0 kg at one year. A cost benefit was reported from group therapy as a result of the more efficient and effective use of the resources of the health professionals, nurse, doctor and dietitian.

2.4.3 Weight loss in established non insulin dependent diabetes mellitus

A recent study investigated 4 widely used weight reduction strategies in NIDDM patients attending routine diabetic clinics (Manning *et al,* 1995). The study closely reflected the clinical situation, recruiting subjects who had failed in earlier dietetic treatments to reduce body weight adequately. A control group was followed with no intervention offered beyond routine care. The three active interventions were standard dietetic intervention, behavioural group therapy and dexfenfluramine for 3 months followed by a mixture of home and clinic visits over one year. For all treatments at 3 months a 1-2 kg weight loss was achieved and this was maintained for one year. Home visits showed no additional

benefits. This study demonstrated the difficulty in achieving weight reduction in established NIDDM patients. The weight losses achieved were rather less than those achieved in overweight non diabetic subject groups. Similar results were demonstrated in a comparison of conventional dietary intervention with a more intensive intervention which included group behaviour therapy (Blonk *et al*, 1994). Weight loss was 2.2 kg at 3 months and 1.3 kg at 24 months. No significant differences were seen between conventional and intensive interventions.

The effect of a VLCD on metabolic control and cardiovascular risk factors in the treatment of obese NIDDM diabetics was evaluated (Uusitupa *et al*, 1990). A 3 month VLCD resulted in a 14.0 kg weight loss, and fasting blood glucose fell from 12.3 to 10.5 mmol/l. No effects were seen on serum cholesterol or triglyceride concentrations, however systolic and diastolic BP were significantly reduced. A recent review of the benefits of using VLCD in overweight NIDDM patients concluded that as weight loss improved the medical problems associated with NIDDM the use of VLCD regimens may have a place in long term treatment. Even short term VLCD for weight control could offer value (Henry and Gumbiner, 1991).

2.4.4 Heart disease and hyperlipidaemia

The results for the majority of treatments for hyperlipidaemia are shown in table 2.3 which is devoted to hyperlipidaemia and weight loss. The results in terms of weight loss are comparable with the results of weight management in other overweight subjects. The range of different interventions used in this subject group are comparable with those described for diabetes. It is usually assumed that a medical diagnosis, and feedback of medical information will increase the motivation for weight loss. A study of nurse-delivered dietary advice in a primary care setting in addition to information concerning the

plasma cholesterol concentration was not found to have any motivational effect (Robertson *et al,* 1992). The attitudes of patients towards adherence to a multiple restriction cardiac diet was examined by Barnes and Terry (1991). The researchers found that simple messages and concrete examples of diet advice were regarded as being of value.

The effects of weight reduction on blood lipids and lipoproteins were examined in a meta-analysis (Dattilo and Kris-Etherton, 1992) which included studies in both hyperlipidaemic and normolipidaemic subjects. Results from 70 analysed studies indicated that weight reduction was associated with a decrease in plasma cholesterol concentrations of 0.79 mmol/L per kg weight loss. The fall in LDL cholesterol concentrations was 0.39 mmol/L/kg, HDL cholesterol concentrations was 0.14 mmol/L/kg and triglyceride concentrations 0.66 mmol/L/kg. These results, although derived from studies which differed in duration, are convincing that weight reduction through dieting is a valuable approach to reduce plasma lipids in overweight individuals (Weinsier, 1992).

The benefits of weight loss on plasma lipids of obese women (BMI 36.2 kg/m^2) were examined in a 24 week study (Anderson *et al,* 1995). The first 8 week slimming period resulted in an 11% decrease in body weight and a fall in plasma cholesterol and triglyceride of 23% and 16% respectively. Weeks 8 to 24 produced another 4.7 kg weight loss representing an overall reduction in body weight reduction of 16.5%. During the later phase of the study, cholesterol and triglyceride increased both by 5.2% and LDL by 4.5%. These findings suggest that improvements in plasma lipids after weight loss can be divided into two periods, the effects of energy restriction and weight loss, or weight stability.

2.4.5 Conclusions

The dietary principles which are recommended for NIDDM and hyperlipidaemia are

compatible with those advocated for the general population (COMA, 1991). An additional reduction in energy intake will achieve weight loss in both. The case for using VLCD or surgery is stronger in the conditions where risks to health are greater and where long term medical follow-up is already required. Efficacy of dietary approaches in newly diagnosed diabetic patients is improved in comparison with simple obesity. In NIDDM patients weight losses of between 6.0-10.0 kg are more likely to be maintained for a year (table 2.4). Overweight hyperlipidaemic patients appear to lose weight at the same rate as patients with simple obesity. Benefits on blood pressure are also seen in these patients (table 2.5)

2.5 Settings and sources of advice

2.5.1 Group versus individual therapy

The value of a group approach has been described as offering a cost effective environment for effective weight management (Bitzen *et al*, 1988). Whilst the group environment is undoubtedly unsuitable for some shy or reluctant socialisers, for those agreeable to this approach, it allows additional harnessing of structure and support not accessible to the subject undergoing individual one-to-one dietary counselling.

The Harrow slimming club uses an approach similar to that used by commercial organisations, with frequent meetings as a group, usually in the evenings. However, it is run under the National Health Service, by State Registered Dietitians and although clients were fee paying, it is non profit making (Bush *et al*, 1988). The programme of 10 session runs over 10 weeks, with individual weighing, dietary advice and education. An identical dietary approach based on a 5000 kJ/1200 kcal dietary prescription was used for all-comers. An audit of the club showed a weight loss of 5.4 kg for those who attended 8 of

the 10 sessions. This was reduced to 3.8 kg in those attending around seven sessions. Another group approach to weight management is the Finnish Primary Health Care programme (Hakala *et al*, 1993). This Finnish study used a 1200 kcal diet group, and a control group who were followed up, but offered no advice. After the six week weight reduction course, which included behavioural advice, follow-up meetings were arranged at monthly and twice monthly intervals. Men reduced their weight by 10.9 kg at one year, and women 5.4 kg over the same time period. After a lengthy 7 year follow-up, men has maintained weight losses of 8.7 kg and women 3.5 kg.

Advantages for group therapy in comparison with conventional one-to-one therapy in newly diagnosed diabetic patients have been described (Heller *et al*, 1988). No improvement in diabetic control in established overweight patients was found by Blonk *et al* (1994) following group treatment. The impressive results of Jeffery *et al.* (1983) favoured group therapy (table 2.2).

Group therapy offers obvious and important benefits for decreasing cost and improving professional resource management (Chenoweth, 1990). Good weight losses of 11.3% of starting weight over 6 months were seen in this setting and described (Ashwell and Garrow 1975). Several studies have made direct comparisons between group therapy and conventional one to one counselling for weight loss, with contrasting results.

In the treatment of overweight patients who had recently survived MI weight reducing advice was compared in the individual and group situation. Patients were allocated to receive weight reducing nutritional advice on a group or individual basis. The use of either individual dietetic intervention, group advice, or a diet sheet directly handed to patients was compared (Wright *et al*, 1981). No significant differences were seen with weight losses of 8.1, 7.5 and 8.9 kg respectively after 6 months.

The "Pawtucket Weigh In" was a randomised controlled heart health programme designed to reduce obesity by offering either individual advice or group treatment. In the pilot study the 10 week program achieved a weight loss of 3.5 kg in the group education and 3.9 kg in the individual advice group (Lasater *et al,* 1991). Group or individual treatments in severely overweight subjects were compared by subjects being randomly allocated to group counselling, 2 weeks weight reduction in a rehabilitation centre and then group sessions for 2 years, or individual follow up by a physician, also for 2 years (Hakala *et al,* 1993). Mean starting BMI was 43.5 kg/m^2 in women and 42.2 kg/m^2 in men. With individual counselling weight losses were 8.0 and 17.0 kg after 3 months, 3.0 and 13.0 kg at 5 years in women and men respectively. The group counselling team achieved 15.5 and 15.0 kg losses at 3 months and then 2.1 and 3.0 kg losses at 5 years for women and men, respectively. The Australian "Gutbuster waist loss" programme was a group programme designed for men who were recruited to a 6 week course, and then followed for a year. The programme involved advice on exercise, dietary fat, and alcohol intake. Average waist losses of 4% were achieved and lifestyle advice led to lowered fat and alcohol intakes. A more advanced programme which included 6 additional fortnightly sessions falls in waist circumference were 10% (Egger *et al*, 1996). These programmes highlight the value of weight management group programmes in men.

2.5.2 Commercial slimming groups

Commercial slimming organisations have, to date, failed to publish the findings of their work, though the findings from non commercially run groups are reported in the literature. The commercial clubs themselves suggest that their treatments, together with a measure of financial commitment, are effective, but attrition rates are a major problem (Hymens *et al*, 1993). The compliance with commercial slimming organisations varies from 10 to 50 weeks (Garb and Stunkard, 1974, Volkamar *et al*, 1981). The findings that a 9.3 kg

weight loss (11.3% of starting weight) over 6 months in a slimming organisation are impressive, though these figures are based on self reported body weights provided by willing, successful subjects (Ashwell and Garrow, 1975). The American TOPS (take off pounds sensibly) programme reported a similar degree of success (Kahn and Williamson, 1991). Weight loss was found to be related to the length of attendance, and to the initial degree of overweight (Garb and Stunkard (1974). Between 25-30% of TOPS members lost more than 20 lb. in their survey.

2.5.3 Family/partner

A family based slimming programme was compared to a diet sheet, or dietary advice given on an individual basis in overweight subjects, or a non commercial slimming group approach (Cousins *et al,* 1992). The entire family slimming group achieved losses of 4-5 kg, in comparison to those allocated to individual treatment, which was either individual advice or a diet sheet. Those who received the group treatment in both the diet sheet and individual advice groups lost 1-2 kg over the one year study period.

The value of a partnership of "buddy system" in weight loss interventions where a commitment or partnership between people is made has been examined. In practice, the "buddy" or supporter may be a friend or relation of a subject who themselves do not require weight management. A weight loss programme in a work-site found no effect of partnership compared with slimming alone on final weight loss (Zandee and Oberman, 1996). The meta-analysis of Black *et al* (1990) examined weight loss programmes of couples, versus subject alone analyses. They supported the inclusion of couples in programmes up to the first 12 weeks of slimming, but not beyond that period.

2.5.4 Self-help groups

Self help management of obesity was the approach examined by Garb and Stunkard (1974). A nation-wide self help group was examined to measure weight loss achievements and also attrition rates. The greater the degree of overweight, the more likely the subjects were to remain in the study, and the more likely to achieve weight loss. Attrition rates were 47% after one and 70% after two years. Mean losses for groups were 14 (SD 16) lb. and 30% of subjects lost more than 20 lb. whilst 6% lost more than 40 lb.

The use of church-based behavioural therapy programs in religious communities to whom the church provides a central meeting point has also shown benefits. The Baltimore church high blood pressure programme offered 8 diet counselling and exercise sessions in an 8 week programme. Team incentives, nutrition education and counselling sessions for black women were evaluated (Kumanyika *et al,* 1992). Weight loss was 3.0 kg after the programme and a 6 month follow up suggested that this weight loss was maintained or exceeded by 65% of subjects.

2.5.5 Workplace

Health awareness amongst employers has led to successful work place programmes for weight loss (Brownell, 1986). However, a need to provide single sex programmes because of the different physical requirements of men and women and the fact that women in general tended to have less manual employment was highlighted by Teufal (1992). The needs of the individual work-site were also examined with group therapy being combined with financial intervention (Philips and Philbin 1992). Twenty eight Texan police employees of mean body weight 96 kg, 146% of ideal body weight were recruited to join a 12 month study. This comprised nutrition counselling, seminar attendance on eating behaviour and monitoring of body weight and blood lipid levels (Briley *et al,* 1992).

Plasma cholesterol but not triglycerides showed significant decreases with a weight loss of 2.5 kg over 52 weeks (a decrease in 2.4% in body weight). Re-entry was offered to programme to consolidate achieved benefits. The effectiveness of work-site wellness programmes was examined by Erfurt *et al* (1991). Their review defined effective programmes as those that had achieved and maintained a 3 lb. weight loss at 3 years. Another criteria of effectiveness they proposed was the recruitment of at least 72% of the population of overweight employees to any weight management programme (Erfurt *et al*, 1991). The effectiveness of contingency contracting towards weight loss in a work-site setting was considered by Zandee and Oermann (1996). They compared weight loss using contingency contracting alone, with a partner or no contracting. Weight loss was close to 7.0 kg for all groups over a 10 week period however improvement was shown with contracting.

A recent article review examined work-site based weight management studies (Hennrikus and Jeffery, 1996). They found that the majority of weight management studies were mostly of an uncontrolled design. However, a unique feature of these studies was that they reached a median of 39% of the overweight populations in their respective work-sites, and achieved good weight losses over the short term of 1-2 lb. per week. However, the literature failed to demonstrate long term reductions in site wide obesity and overweight or other improvements in health and productivity benefits.

2.5.6 Conclusion

Group therapy for obesity provides an appealing setting to some subjects but is an uncomfortable situation for others. Most studies have shown greater benefits using groups rather than individual treatments. Group therapy is more widely used in otherwise healthy overweight subjects rather than in those with a particular diagnosis such as established NIDDM, heart disease or hyperlipidaemia. Group therapy represents a much more

effective use of professional time and resources, although greater commitment from patients is usually required.

The setting that weight management treatment is given is crucial in determining the outcome. The target population, the setting and in turn the flexibility and frequency of the follow-up are all important. A wide range of settings and a variety of programmes described are all valuable in providing a range of weight management programmes. The work-site setting has not been fully exploited and may offer longer term opportunities for weight management and dietary intervention studies.

2.6 Overall conclusion

A wide range of treatments for overweight have been tested, and the majority can achieve a modest (5-10%) weight loss, sufficient to improve clinical outcomes for NIDDM, plasma lipids, hypertension and other risk factors for IHD. This weight loss is usually achieved over 12 weeks, after which it is difficult to achieve further loss. Greater weight loss may be possible for patients with major diseases, e.g. diabetes on diagnosis which can be improved or controlled by weight loss or dietary modification.

Greater weight loss in the region of 10.0-15.0 kg, can be achieved using VLCD or milk diets over 20 weeks, offering advantages when preparing for surgery and can be used to form the start of a structured programme. Weight regain is usual without long-term professional or counsellor contact, so weight management programmes should include provision for a long term maintenance programme. Simple behavioural and cognitive approaches offer advantages for long term weight maintenance which are currently under-utilised, largely through ignorance. Exercise has a value, though this appears to be important in the maintenance of weight loss, and has only small effects on weight loss.

Group treatment leads to greater weight loss than individual treatment in most settings, and has major resource advantages. Group approaches should be adopted as the principal method for weight management. Family based weight management for overweight and adolescent children, focusing on behavioural methods and physical activity, principally to check weight gain, should be given priority.

2.6.1 Important unanswered research question

A dietary intervention incorporating an energy deficit approach to weight loss would achieve losses close to the theoretical 0.5 kg per week in obese volunteers, those with a diagnosis of coronary disease, and those with a close to healthy BMI, but a desire to lose weight.

Table 2-1 Wadden's summary analysis of sixty five studies of weight loss which included behavioural modification

Variable	1974	1978	1984	1985-87	1988-90
studies (n)	15	17	15	13	5
Sample size (n)	53.1	54.0	71.3	71.6	21.2
Initial wt (kg)	73.4	87.3	88.7	87.2	91.9
Initial overweight /%	49.4	48.6	48.1	56.2	59.8
Weeks of study	8.4	10.5	13.2	15.6	21.3
Wt loss (kg/study)	3.8	4.2	6.9	8.4	8.5
Loss per week (kg/wk)	0.5	0.4	0.5	0.5	0.4
Attrition %	11.4	12.9	10.6	13.8	21.8
Follow up (weeks)	15.5	30.3	58.4	48.3	53
Weight loss on follow up	4.0	4.1	4.4	5.3	5.6

From Wadden (1993)

Table 1 provides a summary of randomised trials in which moderate weight reduction was combined with behaviour modification. All values are means, hence the majority of values are given to one decimal place.

Table 2-2 Weight losses following financial commitment

Financial commitment	Weight loss (kg) (individual approach)		Weight loss (kg) (group approach)
	15 weeks	52 weeks	52 weeks
High	14.6	6.3	14.4
Middle	11.3	7.4	15.4
Low	10.6	5.4	12.6

From Jeffery *et al* (1983)

Table 2-1 Effects of weight loss in dyslipidaemia

Study	Design / duration	Population	Treatment groups	No. patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Effect on lipids mg/dl or % Chol	TG	HDL	LDL	Comments
Wing *et al*, 1985	16 weeks randomised unblinded 6 month follow-up	20m and 33 f patients with NIDDM ≥ 20% IBW	16 week completers	53	96.4 kg	-2.8 kg (-2.9%)	+9.3	+9.0	+2.5	-	Weight loss of 4.6 kg to 13.6 kg produced significant improvements in lipid profile & glycaemic control. Systolic bp fell by 4.8 mm Hg
McMahon *et al*, 1985	21 weeks randomised placebo controlled	42m 14f patients with diastolic blood pressure 90-109 mmHg on 4 occasions at baseline BMI >26.0 kg/m2	Weight reduction	26	95.5 kg	7.4 kg (-7.7%)	-5.6%	-7.7%	+6.1%	0%	Antihypertensive medication discontinued at least 4 weeks prior to baseline.Fall in bp associated with weight loss
			metoprolol	18	99.7 kg	+2.0 kg (+2.2%)	+4.0%	+56.4%	-10.4%	+5 %	
			placebo	18	96.4 kg	+ 0.5 kg (+0.5%)	+2.6%	+7.8%	+1.7%	+5.3%	
Bremer et al, 1994	8 week run in , 12 week randomised double blind trial with dex.	obese dyslipidaemic subjects	dex. treatment & diet 12	30.7 SD 0.8	-4.2 kg (-2.6%)		-13%	-22%	+11%	-15%	Weight loss with medication has benefits on plasma lipids
			control 14	32.0 SD 0.9	-2.5 kg (-3.2%)		+1%	+17%	+2 %	-6%	

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss on dyslipidaemia (part 2)

Study	Design / duration	Population	Treatment groups	No. patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Effect on lipids mg/dl				Comments
							Total chol.	TG	HDL	LDL	
Litchtenstein *et al*, 1994	15 week diet study, 5 week run in diet, 5 week 29% energy from fat, 5 week period 17% energy from fat weight reducing, same composition fed *ad libitum*	11 subjects, 6 women, 5 men (metabolic unit study)	baseline diet	11	27.0 SD 4.5	No change	226 SD 33	110 SD 32	48 SD 11	158 SD 28	First actual values, percentage values relative to these
			lower fat weight stable diet	11		-0.3 kg SD 2.7 (0.4%)	-14%	+4%	12.5%	--19%	
			lowest fat weight reducing diet	11		-3.0 kg SD 0.47 (-4%)	-15.9%	-12.5%	-20%	-24%	
			lowest fat adlibitum diet	11		-0.14 SD 0.2(-0.9%)	-8%	-19%	-27%	-15%	
Neil *et al*, 1995	6 month randomised study of dietary advice to improve raised lipids and reduce body weight	163 men and 146 women cholesterol > 6.0 >8.5 mmol/l	Dietitian diet advice (DRV)	103	26.64 SD 4.1	26.40 SD 4.0	-0.98%	+18%	-0.8%	-2.2%	Dietary advice to improve weight loss & plasma lipids has most effect on LDL cholesterol
			FFQ, nurse administered	104	26.31 SD 3.93	26.24 SD 4.2	-2.5%	-20%	+4.1%	-3.48%	
			Diet leaflet by post	102	26.32 SD 4.32	26.08 SD 4.29	-1.8%	-24%	+1.6%	+3.6%	
Kaplan *et al*, 1985[6]	3 months randomised	28m 37 f patients with FBG>1490 mg/dl or confirmed NIDDM	Diet,	16	-	-3.0 kg	-15.0	-35.0	-13.0	+5.5	
			Exercise	18	-	-0.6 kg	+4.0	-8.0	+5.0	+0.8	
			Diet and exerc	16	-	-0.3 kg	+3.0	+5.0	+3.0	+1.6	
			Education control	15	-	+0.05 kg	+1.0	+0.8	+10.0	-3.7	

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss on dyslipidaemia (part 3)

Study	Design / duration	Population	Treatment groups	No. patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Effect on lipids mg/dl				Comments
							Total	TG	HDL chol.	LDL	
Sopko *et al*, 1985	Two 18 week periods, randomised	21m sedentary obese (110% IBW) volunteers age range 19-44 years	Control	8	82.5 kg	< 0.5 kg (-0.6%)	0.0%	-2.6%	-0.5%	+0.5%	
			Exercise weight stable	10	100.3 kg	< 0.5 kg (-0.5%)	+2.6%	+0.6%	+ 2.0%	+0.2%	
			Inactive weight reduction	9	99.3 kg	-6.1 kg (-6.1%)	+2.0%	+2.6%	+2.4%	+0.4%	
			Exercise weight reduction	4	81.2 kg	-6.2 kg (-7.6%)	1.2%	+-9.9%	+5.5%	-3.8%	
Leighton *et al*, 1990	26 weeks	66 patients with cholesterol screens >40 mg/dl, screened from 1024 by LDL>75th percentile, tg<4.5 mmol/l poor aerobic capacity	Diet counselling	33	70 kg	0 kg (-0%) (n=26)	-7%	-11%	-12%	-4%	
			Diet counselling with exercise	33	70 kg	-2 kg (-2.9%) (n=24)	-11%	-6%	-13%	-11%	
Wing *et al*, 1987	12 month behavioural control programme	33m and 81 f patients with NIDDM≥20% above IBW, age 31-71 years	Behavioural control	114	-	-5.6 kg					inverse relationship to falls in plasma cholesterol according to weight loss

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss on dyslipidaemia (part 4)

Study	Design/ Duration	Population	Treatment Groups	No. patients	Mean weight or Baseline BMI	Change in BMI or weight (mean±SD)	Effect on lipids mg/dl				Comments
							Total chol	TG	HDL	LDL	
Wood *et al*, 1991	52 weeks	132 m 132 f	1. Control	79	85 SD 9.0 for all subjects	1.7 SD 4.8	-0.14 SD 0.6 (-2.5%)	0.2 SD 0.5 (+12.5%)	-0.05 SD 0.05 (-4.5%)	0.15 SD 0.15 (-5.5%)	No benefits were seen in subjects overall. But males exercise and diet improved triglyceride and HDL chol. In women this improved trig & total chol
			2. Hypocaloric diet	71		4.5 SD 5.7	-0.2 SD 0.6 (-7.0%)	-0.41 SD 0.60 (-7.8%)	0.1 SD 0.5 (+8.0%)	-0.01 SD 0.22 (-7.0%)	
			3. + exercise	81		7.2 SD 5.4	-0.33 SD 0.7 (-33%)	-0.3 SD 0.50 (-33%)	0.1 SD 0.18 (+12%)	-0.3 SD (-7.3%)	
Wolf & Grundy, 1982	Formula diet within a metabolic unit, 13-15 weeks	Obese non diabetics	All given liquid formula diet 4-5 weeks	15	152.6% IBW	09.7% IBW (-27%)	-33mg/dl (-15%)	-20 (-12%)	+8 (+21%)	+9 (+6%)	
		Obese IDDM	1000 kcal 4-5 weeks	4	139.5% IBW	113.3% IBW (-20%)	-18 mg/dl (-8%)	-123 (-45%)	+11 (+31%)	+16 (+12%)	
Rosenth *et al*, 1985	26 days uncontrolled study of low fat & chol diet & daily exercise	Obese subjects with heart disease	Diet and exercise group	21	99.3 kg	5.2 kg (5%)	48 (-21%)	-150 (-49%)	-28 (-16%)		Weight together with exercise has improved lipid profile and body weight

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure, exerc = exercise

Benefits of weight loss on dyslipidaemia (part 5)

Study	Design/ Duration	Population	Treatment groups	No. patients	Mean weight or baseline BMI	Change in BMI or weight (mean±SD)	Total chol.	Effects on lipids mg/dl			Comments
								TG	HDL	LDL	
Phinney *et al*, 1991	2 months – until wt. loss of 25-35 kg, VLCD then weight maintenance	6 obese women	1000 kcal/day, then VLCD 400-600 kcal, food or 420 kcal commercial VLCD	6	97.7 kg	30.3 SD 3.7 kg (-31% BW)	-1.87 mmol/l (-34%) rose to 4.92 mmol/l (+36%) + 2 ths				Major weight loss was associated with late rise in serum cholesterol, perhaps due to mobilisation of adipose tissue
Wing & Jeffrey, 1995	18 month study of behaviour therapy for weight loss	159 overweight subjects	Calorie goal 1000-1500 kcal, mild exercise, slimming advice by post	131 28	88.9 SD 9.0 90.7 SD 9.8	0.6 kg (-0.6%) -6.0 kg (6%)	No change chol.	+0.23 (+18%)	+0.16 (+14%)		10-15% weight loss sustained over 18 months result in CHD risk factor improvement
Shinkai *et al*, 1994	12 week diet & exercise programme	Mildly obese women	Control group Diet & exercise group	15 17	26.6 SD 27.2 SD 2.0	25.2 SD 26.3 SD 1.9					Improvement in exercise ability with moderate weight losses
Stewart *et al*, 1990		Overweight hyperlipid-Aemic Normal weight hyperlipid-aemic	Hyperlipidaemic Normolipiaemic	20 6	26.5 SD 0.52 23.3 SD 1.0	24.4 SD 0.47 (↓8%) 23.3 SD 1.1	↓ to 6.3 mmol/l SD 0.3 (↓3.2%)	↓2.1 mmol/l ↓trig 36%	+18% (1.1 mmol/l)	↓4.4 (↓8.8%)	Dietary changes and weight loss improves serum cholesterol & triglyceride levels

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure, exerc = exercise

Table 2-2 Benefits of weight loss in non insulin dependant diabetes

Study	Design/ duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Changes in diabetic control and other measures	Comments
Anderson *et al*, 1994	Randomised weight reducing diet. Two hypocaloric treatments, 1. diet with food one 2. Diet with liquid supplement: 12 weeks	Obese subjects with NIDDM duration of diabetes greater than 1 year	Two experimental diets, both hypocaloric and providing 800 kcal	40	30-40 kg/m2 104.7 kg SD 3.3	-15.7 kg±2.8 -14.7 %	Decrease of : 28% in FBG, 20% in HbA_{1c}, 18% chol., 30% in trig.	Similar effects of both weight loss
Doar *et al*, 1975	2-6 months	Patients with newly diagnosed DM, mean age 56 years	Diet	118	-	- 5.1 ± 4.0 kg		Change in oral glucose tolerance and plasma insulin predicted by change in weight: 95% patients improved
Hadden *et al*, 1975	6 months	Patients with maturity onset diabetes, 40-70 years, 98 enrolled, 78 completed six month follow up.	intensive diet therapy, reduced energy and fat dietary advice	57	83.5 kg	- 8.2 kg (-9.8 %)	36% decreasec in FBG from 11mmol/l No change in chol and trig	Mean fasting plasma glucose was less than 8.0 mmol/l for 42 patients.
Heitzman *et al*, 1987	18 months unblinded	22m and 24f NIDDM. FBG>140 mg/dl or IGT duration of diabetes was not given	Relaxation control Behaviour modification Cognitive modification behaviour modification	14 13 13 15	77.1 kg 83.5 kg 78.0 kg 82.6 kg	+0.5 kg -2.7 kg -3.8 kg -2.0 kg	Patients who lost greatest amounts of body weight showed ↑ improved	Men achieved a greater weight loss than women (-3.6 vs. +0.04 kg) Sig corr wt loss at 18 months and glycated Hb.

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss on non insulin dependant diabetes (part 2)

Study	Design/ duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Changes in diabetic control and other measures	Comments
Henry *et al*, 1985	40 days	3M 27F NIDDM, patients taken off medication duration 9.5 SD 6 years	330 kcal daily	30	96.8 kg	-10.5 ± 0.4 kg (-10.8 SD 0.4%)	FBG - 159.0 g/dl	40 days of refeeding 12 patients had lower FBG than baseline & remained medication free
Hughes *et al*, 1984	6 months, diet or surgical intervention	12 obese diabetics	surgery protein supplement	6 6	85.8 SD 10.9 (↓ 27%) 99.3 SD 11.1 kg (↓ 19%)	85.8 SD 10.9 79.9 SD 9.6	FBG 321-166 mg/dl (↓ 48%) 253-171 mg/dl (↓ 32%)	Wt loss results in improved NIDDM control with caloric restriction alone HDL chol ↑ 19.5%
Kelley *et al*, 1993	Intervention study, baseline diet & low calorie diet, 6 weeks	Obese NIDDM diabetes duration < 5 years	VLCD 800 / 400 kcal, 7 day crossover period	7	BMI 32.8 SEM 1.9 kg/m2	BMI decrease 5.3 kg/m2 (16%↓)	HbA_{1c} 8.8 SD 0.5 % to 6.3 SD 0.5 (↓28%), trig. decreases 186 to 178 mg/dl.(↓4.3%)	Weight loss, & kcal restriction improves NIDDM control & plasma lipids
Lean *et al*, 1990	Audit of weight change year 1 of Δ mean 8 years	263 patients with NIDDM, 189 obese, 233 lived > 1 year. Mean age 65	Conventional 1:1 dietary advice	66 115 71	< 25 25>30 > 30	+ 1.3 kg - 2.6 kg -6.8 kg		Weight loss of 1 kg ass. with 3-4 months greater survival when BMI > 25
Liu *et al*, 1985	1 month	20 established obese NIDDM on except OHA only	Hypocaloric diet hypocaloric diet & glipizide	10 10	90.8 kg 93.6 kg	-6.5 ± 0.6 kg (-7.2 SD 0.6 %) - 6.4 ± 0.5 kg (-6.8% SD 0.5%)	FBG -61.0 mg/dl -129.0 mg/dl	-23mg.dl (chol.) - 75 mg (trig) - 30 mg/dl (chol) - 37 mg/dl trig)

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure,

Benefits of weight loss on non insulin dependant diabetes (part 3)

Study	Design/duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Changes in diabetic control and other measures	Comments
Long *et al*, 1994	Non randomised controlled trial bariatric surgery for weight loss, 10 years follow-up	Obese subjects with impaired glucose tolerance duration of diabetes 4.8 SD 2.5 years	Experimental group control group	106 27	48 SD 8 kg/m2 51 SD 9 kg/m2	< in overweight by 60%	FBG decreased from 6.0 to 4.8 mmol/l	In the control group DM developed in 6 subjects, and one subject in the experimental group
O'Kane *et al*, 1993	Randomised double blind placebo controlled trial 1 year duration	Obese NIDDM duration of diabetes 4.3, range 1-12 years for drug group & 3.3 range 1-8 years in the control subjects	Total either Fluoxetine Placebo	19 9 10	BMI 38 range 30-57 BMI 35.8 Range 30 - 43	-4.3 kg (-11.1 - -3.6) (-4.5%) +1.5 (-2.3 - +4.4) (+1.5%)	Improvement in blood glucose (-25%) and HbA_{1c} (-9%) at 6 months for treatment group, not maintained at 1 year	
Pories *et al*, 1992	Longitudinal study, follow-up after 132 months	morbidity obese patients	Greenville gastric bypass	515 patients, 55.9% IGT (12.2% NIDDM)	Mean 216% ideal body weight range: 140-327% IBW	mean body weight 162% (139-212%) 42% excess body weight reduced after 132 months	30 from 288 patients remained diabetic	Drastic surgery to achieve weight loss can result in resolution of NIDDM
Reaven, 1985	Weight loss weight maintenance programme	15 overweight patients with NIDDM >65 duration unknown	Study completers	12	–	-9 kg		Elderly patients with poorly controlled NIDDM who loose weight successfully improve DM control

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss on non insulin dependant diabetes (part 4)

Study	Design/duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Changes in diabetic control and other measures	Comments
Singh *et al*, 1992	Audit of delayed NIDDM diagnosis	newly diagnosed NIDDM	Men Women	36 54	Na	Na	Na	Weight loss noticed 24 months before Δ NIDDM, & 3 months before in 20% patients
Stanik & Marcus 1990	4 -12 weeks	6M 1F obese patients glucose >300 mg/dl. >70% IBW, age 48-67 years, NIDDM duration 1-8 years	Severe caloric restriction	7	107.3 kg	-11 kg (10.2%)	Hyper glycaemic obese patients response to oral glucose	Weight loss improved insulin secretory response & plasma glucose
Tauber-Lassen, *et al* 1990	1 year randomised double blind placebo controlled trial	40 obese patients with NIDDM	Dexfenfluramine Placebo	20 20	98.6 kg 94.9 kg	- 6.3 ± 1.4 % - 2.9 ± 0.8 %	-2.2 -0.7	Abstract
Willey *et al*, 1991	Randomised double blind placebo controlled study, 12 weeks	Obese NIDDM duration not given	Dexfenfluramine or placebo	34 patients treatment group placebo group	BMI 34.3 SE 1.1 35.0 SE 1.2 33.8 SE 1.4	BMI 33.6 SE 1.5 (2%↓) 33.6 SE 1.9 (4%↓) 33.6 SE 1.3 (-0.6%↓)	Significant decrease in fructosamine (12.6%) & in HbA_{1c} (16%) & systolic bp (6.5% & in diastolylic by 14% for both groups	No correlation with of weight loss with improvements in DM control
Wilson *et al*, 1980	6 month treatment & 36 month follow-up in obese NIDDM	58 newly diagnosed NIDDM	low sugar low fat & low calorie diet	58	81.0 SD 1.8	71.8 SD 1.4 (11.3%)	FBG 10.4 SD 0.5 to 7.0 SD 0.3 (↓32%)	Improved body weight, FBG & tg

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure, tg = triglyceride

Benefits of weight loss on non insulin dependant diabetes (part 5)

Study	Design/duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ± SD)	Changes in diabetic control and other measures	Comments
Watts *et al*, 1990	Retrospective review, 4 year interviews	Obese patients with NIDDM> 130% above IBW. Mean 165% above IBW	Behavioural weight loss program with one year maintenance	178	99.5 kg	≥ 9.1 kg loss (-9.1%)		Patients not showing benefits in glycaemic control with between 2.3-9.1 kg weight loss were unlikely to show clinical improvement
Wing *et al*, 1994	Randomised single blind study, 30 weeks	Obese NIDDM duration not given	400 kcal 1000 kcal	36 17	BMI 36.9 SD 0.9 35.7 SD 1.2 kg/m2	-11 kg body weight -(-11%) (-13%)	Both groups had improved insulin sensitivity, fasting bg	Weight loss and degree of calorie restriction improve diabetic control
Wing *et al*, 1994	Randomised single blind study, 30 weeks	Obese NIDDM duration not given	400 kcal 1000 kcal	36 17	BMI 36.9 SD 0.9 35.7 SD 1.2 kg/m2	-11 kg body weight -(-11%) (-13%)	Both groups had improvements in insulin sensitivity, fasting bg	Weight loss & degree of calorie restriction improve diabetic control
Wing *et al*, 1990	12-20 weeks uncontrolled	5M and 123 F > 20% IBW, mean age 52.6 years: DM duration 6.2y	Behavioural weight loss prog with one year maintenance	178	99.5 kg	-4.8 ± 7.2 kg (-4.8 SD 7.2%)	FBG +10.3 HbA_{1c} -0.4 %	Patients poorly controlled at start best 1 year outcome

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure, bg = blood glucose, prog = programme

Benefits of weight loss on non insulin dependant diabetes (part 6)

Study	Design/ duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI	Change in BMI or weight (mean ±SD)	Changes in diabetic control and other measures	Comments
Wing *et al*, 1987	12 month behavioural control programme	33M 81F with NIDDM, >20% IBW, age 31-71, mean 53.5, duration DM 6.5 years range 6 months to 25 years	Non responders >bw < 0-2.3 kg Responders 1. < 2.4 - 6.4 kg 2. <6.9-13.6 kg 3. > 13.6 kg	23 22 42 27	97.5 kg 97.2 kg 97.1 kg 97.3 kg	0 - 2.4% 2.5 - 6.5% 7.0 - 14% >14% Mean wt change 5.6 kg	Fasting bg = 0.14 -0.29 -0.28 -0.35	Patients with > than 5% body wt reduction had significant reductions in glycosylated Hb (-0.1%/kg)
Wing *et al*, 1985	16 weeks randomised unblinded 16 months follow up	20m & 33w with NIDDM ≥ 20% IBW mean duration NIDDM 5.9 years	16 week completers	53	96.4	-2.8 ± 0.8 kg (-2.9%)	FBG HbA_{1c} -7.2 +0.3 mg/dl	Decrease in insulin by 6.6 ± 2.2. Trig. < 9.0 mg/dl. Weight loss 4.6 > 13.6 kg produced benefit
Wolffenbuttel *et al*, 1989	Diet review at 3 months (slim) & 6 months (obese)	30 m & 31 f NIDDM (20 new NIDDM) duration 5 years	obese BMI >27 kg/m2 non obese BMI<27 kg/m2	33 28	31.9 kg/m2 24 kg/m2	-1.6 kg/m² (-5.0%) +0.1 kg/m² (+0.4%)	FBG HbA_{1c} -0.7 -1.5 -0.8 -0.1	8 of 33 obese on OHA. Weight reduction improved metabolic control in obese but not non obese patients.

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Table 2-3 Weight loss and hypertension

Study	Design/ duration	Sample	Treatment groups	No patients	Mean weight or BMI	Change in BMI or weight	Mean changes in blood pressure	Comments
Dustan *et al*, 1990[1]	5 months calorie restriction	24f obese hypertensive normo glycaemic	diet therapy	24				Abstract. Weight loss reduced blood pressure independent of dietary restriction & composition
Eliahou *et al*, 1981	Low calorie reducing diet	123 patients with essential hypertension & > 10% above IBW, BP > 150/90 mmHg on at least two occasions	No antihypertensive therapy 167/110 Antihypertensive therapy held constant 180/112 Antihypertensive therapy altered 188/121	46 56 21	135.5% IBW 132% IBW 136.3% IBW	For each 1% reduction in IBW the following BP reductions were:	-1.9/-1.3 -2.5/-1.4 -2.3/-1.4	Results are for the 123 compliant patients who lost at least 5% of their weight at any time & who had a least 5 visits. Over $^2/_3$ of these patients achieved normal blood pressure with loss of $^1/_2$ of their excess weight

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss and hypertension (part 2)

Study	Design/ duration	Sample	Treatment groups	No patients	Mean weight or BMI	Change in BMI or weight	Mean change blood pressure	Comments
Haynes *et al*, 1984	6 months randomised	> 10% IBW, 21-60 years old, mean 46.5 , diastolic bp 85-104 mmHg	Control 134/89 Behavioural programme 135/91	27 24	88.8 94.9	-4.1 kg -0.8 kg	+4.8/1.4 -0.2/-0.1	Large difference between baseline weights between study groups. No difference in bp between treatment groups
MacMahon *et al*, 1985	21 weeks randomised placebo controlled trial of metoprolol to weight loss	42m 14f patients 20-55 years diastolic bp of 90 – 109 mmHg 4 times during baseline period. BMI>26, no anti hypertensive medication for 4 weeks	Weight reduction 149/101 Metoprolol 151/100 Placebo 150/110	26 18 18	95.5 kg 99.7 kg 96.4 kg	-7.4 kg +2.0 kg +0.5 kg	-13/-10 -10/-6 -7/-3	Improved plasma lipids in weight loss group
Oberman *et al*, 1990	6 months randomised	878 patients randomised: mean age 48.6 years (21-64) years: mean bp 143.4/93.9 mm Hg: bp> 100 mm Hg for medicated compared to 90-104 mmHg for those non medicated	9 groups including placebo chlorthalidone, atenolol, usual diet, low na^+/high k^+, weight loss & combinations bp 143/94	692	88kg	-2.0 kg	-16/-12 (mean arterial)	For weight loss alone weight change -4.35 kg, bp change was 111.89/-9.48 mm Hg & total cholesterol change was + 0.11 mmol/l. Weight loss reduced overall cardiovascular risk

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss and hypertension (part 3)

Study	Design/duration	Sample	Treatment groups	No patients	Mean weight or BMI	Weight change (kg)	Mean change blood pressure	Comments
Ramsey *et al*, 1978	1 year randomised: unblinded	67 patients, 49 completed; obese patients not requiring a special diet. Mean baseline bp in completers 166.8/102.3 mm Hg	Dietitian Diet sheet Physican advice to lose weight	15 14 20	mean body weight 115% ideal	-5.1 -2.6 -2.2	-11.9/-6.9 - 5.7/-3.0 -11.2/-5.3	Mean body weight > 15% IBW: patients who increased treatment were 3 (dietitian) , 1 (diet sheet), 10 advice
Rissanen *et al*, 1985	12 months sequentially allocated to treatment groups	Patients with > 2 year history essential hypertension < 60 years > 20% above IBW	Weight reduction salt restriction Combination	24 17 23	97.6 kg 90.2 kg 87 kg	- 6.9 (0.7) 0 -5.0 (0.6)	-11.5/7.0 -6.5/5.0 -4.0/2.5	Weight reduction of greater benefit than salt restriction for bp reduction
Rocchini *et al*, 1987	20 weeks randomised	50 adolescents weight for height > 75% for age & sex; mean age 12.4 years, 10-16.	Control 126/73 Diet group 125/79 Diet & exercise 128/78	17 15 18	72.6 kg 72.0kg 71.2 kg	+3.8 -1.9 -2.8	+5/4 -10/10 -14/10	Patients averaged 12.4 ± 3.2 years & were in >75 th % in weight for sex, age & height, weight 72.5 (13) kg
Rocchini *et al*, 1988	20 weeks, calorific restriction	34 m 38 f, mean age 12.6 (10-17) years weight for height >75%	Control 126/13 No exercise 127/14 Exercise 129/79	22 26 25	73 kg 73 kg 72 kg	+4 -2.5 -2.4	4/-1 -10/-12 -16/-13	Best effect from exercise & weight loss

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Benefits of weight loss and hypertension (part 4)

Study	Design/duration	Sample	Treatment groups	No patients	Mean weight or Baseline BMI kg/m²	Change in BMI or weight	Mean changes in systolic diastolyic pressure	Comments
Sonneholm *et al*, 1989	6 months randomised	Retrospective analysis of medical records; Danish men registered as in the military 3207 males including 1651 obese males and 1504 controls; 964 obese & 1134 controls	Severely obese 144/90 Random controls 126/75	964 1134	31 kg/m²	0.069 ± 0.004 kg/m² 0.141± 0.050 kg/m²		For any current BMI subjects who had previously lost weight had a < prevalence of hypertension than in weight stable subjects who remained weight stable. Subjects who had previously gained weight had > risk of hypertension than those who remained weight stable
Schotte & Stunkard, 1990	Analysis of three weight reducing studies in which patients received min 16 weeks active therapy. Treated +/- behaviour therapy and or Fenfluramine	32m & 269 female mean age 44.7 years (19-69 years) > 20% over IBW BP 149/95 Males were 152 ± 34% overweight; females were 164 ± 25% overweight.	Non responders & responders (those who remained weight stable)	36 265	men 164% IBW women 152% IBW	<-2.5 kg -10.4 ± 4.7 kg	-10.3/0.80	Independent effect on bp of weight loss. Greatest effect on bp occured early. Bp remained below baseline levels despite weight regain

Abbreviations: IBW = ideal body weight, m = male, f = female, bp = blood pressure

Chapter 3: Review of haemostasis, rheology, fibrinolysis and haemorheology. The relationship of ischaemic heart disease risk, overweight, weight loss and changes in dietary composition

3.1 Factor VII

Factor VII a single chain glycoprotein is synthesised in the liver (Radcliffe and Nemerson, 1975). It exists in plasma at low concentrations (0.13-1 mg/L) (Bajaj and Mann, 1973). Almost all of factor VII is present as the inactive zymogen (99%) and activation is achieved through the actions of serine proteases. These hydrolyse factor VII into a two chain form, increasing the activity 85 fold. The conversion of factor VII to factor VIIa involves the cleavage of an arginine bond resulting in a light chain and a heavy chain containing the active site linked by two disulphide bonds. In contrast with other zymogens of other serine proteases, factor VII possesses significant catalytic activity and coagulation can be initiated by the association of factor VII with membrane bound tissue factor (Rao *et al*, 1986). The activation of factor VII, factor XIIa, IXa and thrombin all activate factor VII and factor IX.

3.1.1 The role of factor VII in the intrinsic and extrinsic coagulation cascade

Factor VII is a key component in the promotion of the coagulation cascade. There are two systems operative in humans both of which activate the coagulation cascade, the intrinsic or the extrinsic systems (Bloom *et al,* 1994) (figure 3.1).

The extrinsic pathway of blood coagulation is thought to be the main pathway for the initiation of thrombin and fibrin formation. After injury to the endothelial lining of the blood vessel wall, sub-endothelial fibres become exposed initiating the platelet component of haemostasis. On adherence to the collagen fibrils, platelets are changed

and allow platelet products such as thromboxane to be released. This promotes of vasoconstriction resulting in an impaired blood flow. A complex number of interactions then take place which consolidate the platelet plug, allowing the process of repair to proceed.

Tissue factor, a membrane-bound protein also becomes exposed to blood after endothelial damage. Coagulation factor VII becomes bound to tissue factor in the presence of free calcium ions and the complex becomes highly active. Activated factor VII, or factor VII_a has a high affinity for factor X which itself becomes activated (Xa). The peptide is attached to the endothelial and platelet surfaces. Activated factor X is an endo-peptidase enzyme which has the effect of activating prothrombin to thrombin. Factor V is another essential co-factor and is present in both platelets and plasma. This becomes bound to the factor VII_a, X_a, Ca^{2+} complex, and is then activated by thrombin or VX_a. This can then convert more prothrombin to thrombin.

The intrinsic, or contact system which initiates blood coagulation involves the action of factor XII. Specific steps in the cascade are not fully understood nor is the precise mechanism for the initiation of contact activation. There is some overlap with the extrinsic pathway. The start of the intrinsic system is with the Hageman factor (XII), prekallikrein (also known as Fletcher factor) and high molecular weight kininogen (HMWK) which react together. This results in the generation of XIIa which is then able to activate factor XI, to factor XI_a. Factor XI_a then activates factor IX, which can also be activated by the enzyme complex of IXa, thrombin modified factor VIII, negatively charged phospholipid and Ca^{2+}. Factor IX can also be activated by the factor VII_a, tissue factor complex. As with the extrinsic system, factor VII complex activates prothrombin to thrombin, before fibrinogen is converted to fibrin.

3.1.2 Factor VII and factor VII activity as risk factors for ischaemic heart disease.

Factor VII activity has been measured within large epidemiological studies that have sought, among their other aims, to prospectively examine any relationships between risk factors for IHD and coronary events. The factor VII activity (also known as factor VIIc) has been the form of factor VII most widely measured and described and as such the remainder of this chapter will focus on it. In 1980, the Northwick Park Heart study showed that coagulation factor VII was a predictor of fatal IHD but no association was observed with non fatal events (Meade *et al,* 1980). The Northwick Park Heart study also showed that the strongest association of factor VII activity with fatal IHD events occurred soon after measurement (Meade *et al,* 1980). However, factor VII activity was still associated with fatal IHD events over the study's 16 year follow up period (Ruddock and Meade, 1994).

Results from the six year follow up of the Prospective Cardiovascular Munster study of 19,698 subjects, demonstrated a similar though less marked distinction between the factor VII activity in those who suffered either fatal or non fatal events (Heinrich *et al,* 1994). Factor VII activity in the 23 subjects who suffered fatal coronary events tended to be higher than the concentrations in those who suffered non fatal events ($p=0.06$), though no differences were seen between men who suffered IHD events and those who remained well.

The Northwick Park Heart study and the Prospective Cardiovascular Munster study are very similar in their findings concerning the relationship between factor VII activity and fatal events. It has been proposed that the factor VII activity at the time of thrombogenesis initiation is important for the determination of the size and stability of the thrombus, through the fibrin deposition and the platelet aggregating property of thrombin (Meade *et al,* 1993).

Considerable debate surrounds the different assays for factor VII activity (Miller *et al,* 1994, Meade *et al,* 1991, ECAT, 1993). An international comparison of the different factor VII activity assay methods was carried out by Miller *et al* (1994). The assay used for the Northwick Park Heart study was reported to have a greater sensitivity than either the Prospective Cardiovascular Munster study assay or that used in the Atherosclerosis in Communities study (Wu *et al,* 1990). These findings have important implications for the comparison of results between studies, particularly the Northwick Park Heart study and Prospective Cardiovascular Munster study results.

3.1.3 Major influences on factor VII activity

Positive relationships have been shown between factor VII activity and a number of other risk factors such as fibrinogen, smoking habit, total cholesterol and blood pressure. These positive associations were reported by the Gothenburg Heart Study (Rosengren *et al,* 1990), Framingham study (Kannel *et al,* 1987), the Caerphilly and Speedwell studies (Yarnell *et al,* 1991) and the Prospective Cardiovascular Munster study (Balleisen *et al,* 1987).

Another prospective multicentre study, the Progetto Lombardo Atero-Trombosi study (Cortellaro *et al,* 1992) examined the association of haemostatic variables, including factor VII activity, with conventional risk factors and atherothrombotic events in four groups of patients with pre-existing vascular disease. From the entire cohort, sub-groups were identified including survivors of MI, angina pectoris patients, those suffering transient ischaemic attacks, and those with PVD. In the MI and PVD groups raised plasma factor VII activity was positively associated with risk of coronary events.

The importance of the factor VII genotype has recently been discussed in relation to risks of coronary events (Humphries *et al,* 1994). An estimated 20% of the UK population

have the polymorphic factor VII genotype which favours a lower plasma factor VII activity, and thus a reduced likelihood of experiencing thrombotic ischaemic events.

The Atherosclerosis in Communities study of 12 000 men and women aged 45-64 years (Folsom *et al,* 1991) reported that factor VII activity was elevated in women compared with men. An increase in factor VII activity of 4% per decade was seen only in women. Increases in body size, triglycerides, LDL and HDL cholesterol concentrations were all accompanied by higher factor VII activity in all subjects.

The relationship between factor VII concentrations and coronary artery disease severity was examined in patients with similar degree of atheroma, some of whom had survived a prior MI (Broadhurst *et al,* 1990). Those who had suffered prior infarction had significantly higher concentrations of factor VII activity.

Seasonal influences affect factor VII activity (Woodhouse *et al,* 1994), and the elevation of factor VII activity in older people throughout the winter months has been related to their increased risk of cardiovascular death in this period. The influence of habitual alcohol intake is unclear. Alcohol intake has been shown to have no significant effect on factor VII activity in the Scottish Heart Health study (Lee *et al,* 1995), but was negatively related with factor VII activity in the Atherosclerosis in Communities study (Folsom *et al,* 1991).

3.1.4 Relationship between factor VII activity and plasma lipids

The Northwick Park Heart study was important as it identified the strong associations between factor VII activity and the incidence of coronary artery disease (Meade *et al,* 1980, 1986). Raised plasma cholesterol concentrations were also related to the development of IHD, but the associations were stronger for factor VII activity and IHD. A positive correlation was observed between factor VII activity and both plasma

triglyceride and cholesterol concentrations. Epidemiological studies have also found similar relationships (Prospective Cardiovascular Munster study, Atherosclerosis in Communities study, MONICA, Caerphilly). The strongest associations in Northwick Park Heart study were seen with triglyceride rich chylomicron and VLDL (Mitropoulos *et al,* 1989). Plasma factor VII activity is determined largely by the circulating concentrations of factor VII antigen (a measure of total factor VII protein) which is also associated with plasma lipids. A cause and effect relationship has been seen between factor VII activity when lipid lowering is achieved, by using either diet or medication (Simpson *et al,* 1983, Wilkes *et al,* 1992).

A study of 3,000 patients with angiographically determined angina pectoris reported that factor VII activity and plasminogen increased in parallel with concentrations of cholesterol and triglycerides (ECAT, 1993). The role of dietary manipulation on factor VII activity and plasma lipids will be considered later.

3.1.5 Factor VII activity, body mass index and physical activity

The results from the Northwick Park Heart study have been very important in identifying the positive link between factor VII activity and body weight (Meade *et al,* 1986). Changes in factor VII activity were correlated with weight changes after the study population had undergone repeated measures (Meade, 1987). The Atherosclerosis in Communities study also related increased factor VII activity with increased BMI (Folsom *et al,* 1991).

A positive association was found between BMI with factor VII activity. The difference in factor VII activity between subject groups of mean BMI of 23 kg/m^2 and those of mean BMI > 30 kg/m^2 was close to 10% (Balliesen *et al,* 1987). Clinical studies have confirmed these epidemiological findings (Yang *et al,* 1993, Avellone *et al,* 1994, Poggi

et al, 1994). However, the relationship of increasing factor VII activity with BMI was not seen in subjects with angina (ECAT, 1993). The reasons for this are unclear, although the positive relationships between plasma cholesterol and triglyceride observed in the other studies were still present.

The role of central obesity on factor VII activity, as measured by an elevated WHR has been investigated (Licata *et al,* 1995). Nineteen overweight subjects, with a raised WHR > 0.81 for women, > 0.92 for men and mean BMI of 36.3 kg/m^2 and 20 lean subjects (mean WHR 0.75) mean BMI 23.3 kg/m^2 were examined. Factor VII activity was elevated in the obese group with the elevated WHR compared with the lean group. The differences between the two values for factor VII activity were significant 79 SD (11%) and 105 SD (18%) (Licata *et al,* 1995).

Physical activity as a regular part of lifestyle has shown important effects on factor VII activity. The higher the frequency of reported physical activity the lower the factor VII activity (Connelly *et al,* 1992). The beneficial effect of regular moderate physical activity on factor VII activity was also noted in the Caerphilly prospective study (Elwood *et al,* 1993). In both cases the factor VII activity measurements were not made immediately after physical activity and thus did not reflect acute changes. This was important as immediately post physical activity an acute increase in fibrinolysis and coagulation has been observed (Schobersberger *et al,* 1996).

3.1.6 Weight loss and factor VII activity

Some dietary intervention studies have examined the impact of weight loss on factor VII activity using a number of different approaches. They have all shown an elevation in factor VII activity with increased BMI. Folsom *et al,* (1993) used an energy restricted diet to achieve weight loss in overweight men and women of mean BMI 31 kg/m^2. After

a 6 month period a weight loss of 9.5 kg was accompanied by an 11% fall in factor VII activity.

Weight loss achieved using a short term VLCD approach has shown more acute effects on factor VII activity. A 4.3% weight loss over a 7 day intervention period lowered factor VII activity by 26% in subjects whose starting BMI was 32.7 kg/m^2 (Slabber *et al,* 1992). Longer term use (3 month) of a VLCD in 52 subjects whose mean BMI was 35 kg/m^2 in women and 40 kg/m^2 in men, improved mean body weight by 17% and factor VII activity by 23% (Palareti *et al,* 1994). However, not all studies of weight loss have significantly altered factor VII activity. The use of VLCD in 20 women with polycystic ovary disease whose mean BMI was 34 kg/m^2 achieved an 8.0 kg weight loss and factor VII activity was unchanged (Andersen *et al,* 1995). A 3.5 kg weight loss at 1 month and 4. 2 kg was achieved loss over a 3 month period, using a dietary intervention of 1000-1200 kcal daily in 135 subjects (Baron *et al,* 1989). Factor VII activity decreased at 1 month, but rose back to baseline levels at 3 months. These results suggest weight loss alone does not explain the relationship between obesity and factor VII activity (table 3.1).

3.1.7 Factor VII activity and physical activity intervention for weight loss

A combined physical activity and smoking cessation intervention was used in young men and achieved a weight loss of close to 4.0 kg, with a significant fall in factor VII activity (Gris *et al,* 1990). The results were not adjusted for the effect of smoking. In contrast, no improvement in factor VII activity was seen after an intervention to improve the frequency of physical activity and lower dietary fat intake in a study of healthy middle aged men (Rankinen *et al,* 1994a). The authors concluded that any benefit from physical activity is likely, at best, to reflect a temporary change in coagulation measures. However, the subjects within the latter study remained weight stable, and the benefit of

physical activity may have been effective through weight loss (Gris *et al,* 1990, Rankinen *et al,* 1994a).

3.1.8 Factor VII activity and surgical intervention for weight loss

Surgical means to achieve weight loss result in much greater weight loss than dietary interventions (Pories *et al,* 1992). A 50.0 kg weight loss was achieved by subjects of mean weight 154 (SD 27) kg after a 6 month period, a further 14.0 kg weight loss was achieved after 12 months. Factor VII activity was decreased by 20% at 6 months, from 113 to 90% but increased to 99% at 12 months despite the continued weight loss (Primrose *et al,* 1992). These studies point to an effect of acute energy restriction to reduce factor VII activity, independent of sustained effects from weight loss.

3.1.9 Factor VII activity and dietary fat composition

Factor VII activity is increased by hyperlipidaemia and by consumption of a high fat diet. It has been well established that diets that are high in saturated fatty acids raise total plasma cholesterol (Hegsted *et al,* 1965, Keys *et al,* 1965) and are associated with increased IHD risk (Schonfeld *et al,* 1982). The increased intake of saturated fatty acids has also been shown to elevate factor VII activity. A randomised cross over study compared 2 controlled iso-energetic diets which provided either 20% or 50% of energy from fat on the effect on factor VII activity (Bladberg *et al,* 1994). The two diets were served on consecutive days to 17 young volunteers of mean BMI 21.8 kg/m^2 and factor VII activity was measured at 1.5 hour intervals postprandially throughout the day. The high fat diet elevated factor VII activity by 6 to 15% when compared to the low fat diet.

The effect of a low fat high fibre diet on factor VII activity was examined in a longer study (Marckmann *et al,* 1992). Young healthy men were randomised to receive either a

low fat, high fibre diet for 8 months or a "control" diet which was the usual Danish diet and provided close to 40% energy from fat. However, while remaining weight stable the factor VII activity in the group following a low fat diet (26% energy from fat) decreased by 5-10% during the first two and final months of the study. In the intervening months factor VII activity displayed considerable variation across the study.

Miller *et al,* (1986) examined the effect of a diet providing 13% or 62% energy from fat on factor VII activity. Sixty two middle aged men were allocated to receive each weight maintenance diet in turn using a crossover design and both factor VII activity and total factor VII antigen were measured. Each diet was followed for 3 weeks after which the plasma factor VII activity was 16% higher on the high fat diet, though total factor VII antigen concentrations were unchanged. Plasma cholesterol was significantly decreased and triglyceride increased after the high carbohydrate low fat intervention. To examine a more acute effect of dietary fat on factor VII activity Miller and colleagues (1986) examined individual day to day fluctuations in both dietary fat intake and factor VII activity. The values for factor VII activity were examined as standard deviations about individual mean values measured across the duration of the study. This representation of the findings demonstrated a strong positive association between factor VII activity and total dietary fat consumption.

A larger study also examined the influence of dietary fat intake on plasma lipids and factor VII activity (Miller *et al,* 1989). The results indicated that dietary fat and plasma triglyceride concentrations were both independently and positively related with factor VII activity. For the highest and lowest dietary fat intakes factor VII activity differed by 13%. This difference was comparable to the that seen between factor VII activity in those who suffered MI in the Northwick Park Heart study and those who remained well (Meade, 1987).

The influence of fat type on total factor VII antigen has also been investigated. Five healthy adults were asked to consume either a high fat diet, high in unsaturated fat (63% total energy from fat, and of that 68% from unsaturated fatty acids) or a high saturated fat diet (62% total energy from fat, and of that 85% from saturated fatty acids) or a low fat diet which provided 15% energy from fat (Mitropoulos *et al,* 1994). The diets were each fed for 4 weeks using a crossover design and included a 12 week washout period between diets. Factor VII activity was raised by 13.1% following the high saturated fat diet and by 6.5% after the unsaturated high fat diet and the results from both interventions were compared to the factor VII activity after following the low fat dietary regimen. Plasma stearic acid was particularly strongly associated with factor VII activity, suggesting an *in vivo* role for saturated fatty acids in increasing the coagulability of blood. Interestingly, factor VII antigen was significantly increased only after the consumption of the high saturated fat diet, suggesting a role for unsaturated fatty acids in reducing total factor VII protein.

A long term cholesterol-lowering diet was implemented in premenopausal women (Brace *et al,* 1994). The subjects were advised to consume sufficient energy in order for them to maintain their body weight. Dietary energy from fat was decreased to 30% from 36% and the change was maintained for 20 weeks. An 11% improvement in factor VII activity was observed over the study period though no change was observed in total factor VII antigen. These findings suggest a benefit from a diet low in both saturated and total fat on IHD risk.

The habitual consumption of a high fat diet has been established as leading to elevated total factor VII activity and antigen (Marckmann *et al,* 1990). An increase in factor VII antigen was shown in habitual consumers of a high compared with low fat diets (Miller

et al, 1994). A diet which provided less than 28g day $^{-1}$ /m^{-2} per day fat was defined as low fat while a diet providing >39.9 g day $^{-1}$ /m^{-2} high fat.

3.1.10 The influence of polyunsaturated to saturated fat ratio and specific fatty acids on factor VII activity

The effect of different P/S ratios on factor VII activity was examined using a low (0.3) and a high P/S ratio diet (3.0). The usual UK diet has a P/S ratio close to 0.7 (Gregory *et al,* 1990). The study subjects were each given experimental dietary advice in line with their usual dietary energy intake. Each diet was followed for 7 days including a 7 day washout period between the diets. No changes were seen in factor VII activity, or in total factor VII antigen (Miller *et al,* 1989). Two diets with widely differing P/S ratios 0.28 or 0.89 have been compared (Marckmann, *et al* 1990). Each diet was consumed by healthy non obese volunteers for a period of 2 weeks, both provided 32% from fat. Both dietary regimens significantly lowered factor VII activity by 11% and 14% respectively compared with the usual dietary intakes comprising 40% energy from fat. No effect from fat type on factor VII activity and antigen was observed. Lipid profiles were improved with a significant fall in triglyceride concentrations on both regimens, however plasma cholesterol concentrations fell only in the group consuming the high P/S ratio diet.

Early work by Hegsted (1965) examined the cholesterolaemic effects of different fatty acids. Stearic acid, a significant contributor to saturated fatty acid intake, was found not to affect total plasma cholesterol. However, other fatty acids rich in foods such as lauric, palmitic and mystic acids were hypercholesterolaemic (Hegsted *et al,* 1965). The role of specific fatty acids on factor VII activity has also been considered. Early studies have indicated effects of various fatty acids on thrombogenesis, as well as elevating plasma lipoproteins. Saturated fatty acids have been reported to shorten thrombus time though

actual thrombogenicity was lowered with decreasing carbon chain length (Hegsted *et al*, 1965).

The effects of high intakes of specific saturated fatty acids on factor VII activity have been considered (Tholstrup *et al,* 1994a) using carefully prepared diets rich in stearic, palmitic, mystic and lauric acids. Slim, healthy, young male subjects were randomised to one of 3 experimental diets within a controlled metabolic feeding study. One diet provided 42% stearic acid, one 43% palmitic acid, and a third 10% mystic acid and 30% lauric acid. For all diets the total percentage energy from fat was 40%, with 90% of the total fat energy provided from the test fats. The diet high in stearic acid lowered factor VII activity by 13% over a 3 week intervention. Factor VII activity was unaffected by the two other diets. Another study by the same authors compared the effect of mystic and palmitic saturated fatty acids on factor VII activity (Tholstrup *et al,* 1994b). The study design required that each fat was given in a synthetic form in order to provide 40% energy from total fat, and 41% of the total fat from either palmitic or mystic acids. Both of the saturated fatty acids significantly raised factor VII activity by 2% and 4% respectively, with no differences in their cholesterolaemic effects. A more recent study (Tholstrup *et al*, 1996) examined the short term effects (over 24 hours) of a high fat meal containing either stearic or mystric acid. Stearic but not mystric acid tended to cause some increase in factor VII coagulant activity. These results are at odds with another study which suggested that mystic acid was mainly responsible for the hypercholesterolaemic effects of saturated fatty acids (Hayes *et al,* 1991).

3.1.11 The role of the consumption of fish and omega three fatty acids on factor VII activity

Cross-cultural (Armstrong *et al,* 1975) and prospective studies (Kromhout *et al,* 1985, Shekelle *et al,* 1985, Norell *et al,* 1986, Burr *et al,* 1989) have suggested an inverse

relationship between fish intake and IHD. One of the possible theories to explain this relationship has been "hypercoagulability" as measured by increased plasma factor VII activity and fibrinogen concentrations. Others theories have included the effects of fish intake on the fibrinolytic system, improvements in the lipid profile, or decreased platelet aggregation.

The influence of fish intake and dietary n-3 fatty acid intake on factor VII activity were examined in the Atherosclerosis in Communities study (Shahar *et al,* 1993). The three principle n-3 fatty acids within the diet are EPA, DHA and DPA. Usual intakes were estimated using a FFQ method. No significant associations were seen between either of the fatty acids and factor VII activity, or between the dietary polyunsaturated fatty acids derived from the intake of fish and other seafood and factor VII activity. Muller *et al* (1989) examined the effect of supplementing the diet of 40 healthy volunteers with mackerel (intervention) and meat paste (control) for a 6 week period. Factor VII antigen or VII activity were unaffected by dietary supplementation.

3.1.12 Conclusion

Total fat intakes influence strongly both factor VII activity and protein. The long term consumption of a diet high in saturated fatty acids has been associated with increases in both factor VII activity and factor VII protein, though in the short term factor VII activity is principally affected. The role of polyunsaturated fatty acids on factor VII is conflicting and some studies have reported a beneficial effect on factor VII activity. In addition, specific saturated fatty acids, such as stearic, have been shown to have varying effects on both factor VII activity and factor VII antigen. However, the reduction of total dietary fat intake results in a significant improvement in factor VII activity and total plasma cholesterol and triglyceride concentrations. The effect of manipulating dietary

fat composition appears much less influential. Weight reduction has at least as great an effect as lowering dietary fat intakes. However, in the majority of intervention studies weight loss has been achieved by changes in dietary composition and energy intake and moderate physical activity. Though it is the intervention as a whole which must be seen to have influenced these coagulation measures as it is impossible to separate the influences of both change in diet composition and decrease in energy intake.

3.2 The fibrinolytic system in haemostasis

The coagulation of blood is a carefully controlled and reversible process which prevents the loss of blood from injured vessels. The fibrinolytic system is the back up or fail safe system which becomes initiated when fibrin is being formed (Kay, 1988). The fibrinolytic system (figure 3.2) comprises three major protease enzymes, plasmin, t-PA and u-PA and the rapid inactivator PAI. The three fibrinolytic enzymes are homologous and the inactive zymogens exist as single chains whilst their active forms have two carbon chains held together by disulphide bonds.

Plasminogen is a single chain glycoprotein with a plasma concentration of 1.5-2.0 μmol/l and a half life of approximately two days. Plasminogen becomes adsorbed onto either fibrinogen or fibrin molecules at their lysine binding sites. Only fibrin molecules possess a specific binding site for t-PA. The plasminogen bound to fibrinogen then becomes unavailable for t-PA activation and very little plasminogen activation occurs (Hoylaerts *et al,* 1982).

The plasma concentrations of t-PA are produced almost entirely by the vascular endothelial cells, whilst the less abundant endothelially produced activator u-PA is present in urine, tears, saliva and plasma (Bachman, 1994). Unlike t-PA, u-PA does not

have a specific fibrin binding site and can activate plasminogen in its absence. It is likely that u-PA is valuable in maintaining clear ducts and tubules, such as in the kidney, and limiting the amount of insoluble fibrin which remains in the circulation and tissues (Kay, 1988). In normal plasma, the concentration of t-PA is 70 pmol/l with a half life of 5 minutes (Booth *et al,* 1990). About 15% of total t-PA is in the active form and the remainder exists complexed together with PAI. An acute rise in t-PA concentrations results from a number of stimuli such as physical activity, venous occlusion and catecholamines (Prowse and MacGregor, 1988).

PAI is a rapid inactivator of both t-PA and u-PA and is present in the plasma with a half life of 7 minutes (Booth *et al,* 1990). The presence of the inhibitor PAI was first confirmed using immunohistochemical techniques and is now known as PAI-1, as another inhibitor PAI-2 has been detected (Chmielewska and Wiman, 1986). Almost 90% of the PAI-1 in the blood is present within platelets whilst the remainder is present within endothelial and liver tissues (Sprengers and Kluft, 1987). In the tissues almost all the PAI-1 is active compared with only 3% - 5% in platelets (Booth *et al,* 1990). PAI-1 is released from platelets by thrombin, which increases platelet aggregation, limits plasmin formation and favours fibrin deposition. The normal plasma concentrations of PAI-1 are 20 - 100 pmol/l.

The fibrinolytic activity of plasma varies throughout the body, depending on the site and the response from different stimuli but in general, fibrinolytic activity is greatest in the arterial system. Altered fibrinolysis has been observed during pregnancy, where high levels of PAI -1 and PAI-2 are measured in the neonate. Increased PAI-1 concentrations are also observed following trauma and surgery. Reduced fibrinolytic activity has been noted in obese subjects, those on prolonged bed rest and in women using the oral contraceptive agent. Post surgically the reduction in fibrinolysis probably represents the

exhaustion of fibrinolytic components following their activation in response to thrombus formation.

Tests to measure fibrinolytic potential after stimuli such as physical activity or venous occlusion have been developed. Only a small increase in t-PA is required to completely overwhelm the low levels of PAI-1 normally present (Prowse and MacGregor, 1988). Using the venous occlusion test it has been shown that some people who are termed "non-responders" have elevated PAI-1 activity (Nilsson *et al,* 1985). The inhibition of released t-PA in this situation results in increased PAI-1. PAI-1 behaves as an acute phase reactant (Juhan-Vague *et al,* 1985, Kluft *et al,* 1985) and is raised in a number of pathological conditions including diabetes and IHD (Juhan-Vague *et al,* 1984, 1988). However, impaired fibrinolysis is usually a result of either a decrease in t-PA synthesis or an inactivation of t-PA and to a lesser extent u-PA (Kay, 1988). The analysis of different components of the fibrinolytic system such as PAI-1 and t-PA are relatively recent. They supersede more crude earlier measures of overall fibrinolytic activity such as clot lysis time or euglobulin lysis measured by a fibrin plate method (Meade *et al,* 1980, Wilhelmsen *et al,* 1984). Epidemiological studies such as Northwick Park Heart study and the Gothenburg study have continued using these methods to ensure long term follow-up data from their populations remains comparable. Some studies report the antigen or activity of both t-PA and PAI-1, and others report overall clot lysis time. Table 3.3 includes the results of studies of weight loss and the fibrinolytic system.

3.2.1 Fibrinolytic system and ischaemic heart disease risk

Several cross-sectional studies have shown an association between increased fibrinolytic activity and IHD but they cannot establish which comes first (Chakrabarti *et al,* 1968, Hamsten *et al,* 1986, Francis *et al,* 1988, Jannson *et al,* 1993). Early reports from the

Northwick Park Heart study suggest an association between impaired fibrinolytic activity and increased IHD incidence, albeit not as strong as that for fibrinogen and factor VII activity. This is perhaps due to the few incidents recorded in younger men (Meade *et al,* 1980, 1986). Longer term follow-up of the Northwick Park Heart study reports a strong and independent relationship between low fibrinolytic activity and IHD incidence, whilst still supporting the other positive relationships between fibrinogen and factor VII activity (Meade *et al,* 1993). In the Gothenburg study, euglobulin lysis time was used to establish global fibrinolytic capacity and this was found not to be related to IHD incidence at baseline (Wilhelmsen *et al,* 1984). In contrast, another study which examined euglobulin lysis time in survivors of acute MI found a reduced lysis in those who suffered reinfarction (Pedersen *et al,* 1993).

Raised PAI activity has been found in those with diabetes (Juhan-Vague *et al,* 1989a) hypertension (Landin *et al,* 1990) and angina pectoris (Yudkin *et al,* 1987, Juhan-Vague *et al,* 1989b) which are sub groups of the population all known to be at increased risk of IHD. The European Concerted Action on Thrombosis study reported an association between decreased fibrinolytic activity due to high levels of PAI-1 and increased coronary stenoses (ECAT, 1993). In atherosclerotic patients, fibrinolytic activity is probably decreased due to an increase in PAI-1 and therefore a decrease in t-PA (Collen and Juhan-Vague, 1988). PAI-1 has been reported to be a risk factor for recurrent MI (Hamsten *et al,* 1987). A recent review concluded that raised PAI-1 concentrations and hypertriglyceridaemia may predispose diabetics to coronary thrombosis and an increased incidence of atherosclerosis and IHD (Rocha and Paramo, 1994).

Circulating t-PA in the fibrinolytic system has generally been thought to offer protection against IHD. However, the findings of a recent study in those with angina have indicated that a greater t-PA mass was associated with a greater risk of mortality. These

contrasting observations probably reflect the increased t-PA concentrations due to the raised concentrations of t-PA / PAI-1 complexes which was seen in subjects with increased total concentrations of inhibitors (Jansson *et al,* 1991, 1993). In a random healthy population, raised insulin concentrations have been associated with lowered t-PA and raised PAI-1 concentrations (Eliasson *et al,* 1994a). This relationship has previously been proposed as the missing link between insulin resistance and atherothrombosis (Juhan-Vague *et al,* 1991). A positive relationship between IHD risk factors has been reported with factor VII activity, fibrinogen and PAI-1 activity being positively related to each other and to the sex hormones testosterone and oestrodiol (Yang *et al,* 1992).

3.2.2 Other influences on the fibrinolytic system

Dyslipidaemias have been associated with alterations in factor VII activity (see section on lipid and coagulation and haemostasis) and PAI-1 activity (Zitoun *et al,* 1996). Smoking has been associated with increased PAI activity, BMI and plasma triglyceride concentrations (Oudenhoven *et al,* 1994). Regular physical activity improves fibrinolysis, though this has also been associated with reductions in body weight (Gris *et al,* 1990).

3.2.3 The fibrinolytic system and body weight

Concentrations of PAI-1 correlate closely with BMI (Vague *et al,* 1986, Sundell *et al,* 1989) and are greatest in those with a central fat distribution measured by WHR (Vague *et al,* 1989). The role of fat distribution was also examined in obese and lean middle aged women with either a high or low WHR. Raised WHR was associated with raised PAI-1, and negatively related to insulin sensitivity (Landin *et al,* 1990). Both euglobulin lysis time and PAI-1 were positively correlated with BMI (Urano *et al,* 1993). The European Concerted Action on Thrombosis study demonstrated an increase in PAI-1 activity with increased BMI in angina subjects (ECAT, 1993).

The long term effect of major weight loss on the concentrations of fibrinolytic factors t-PA and PAI-1 was examined in 45 subjects 14-20 years post jejuno-ileal bypass surgery. The PAI-1 and t-PA concentrations in post surgical patients (mean BMI 30.2) were compared with those in other 10 obese subjects (mean BMI 44.0). The PAI-1 and t-PA antigen concentrations were raised in the obese subjects compared with the post surgical groups by 74% and 40% respectively (Sylvan *et al*, 1992).

3.2.4 The fibrinolytic system and weight loss

Weight loss in overweight subjects improves fibrinolytic capacity by decreasing PAI-1 activity (Andersen *et al,* 1988). A physical activity regimen for men aged 20-30 years with mean BMI of 26.5 achieved a 5% weight loss and a 42% reduction in PAI-1 activity. These changes were the result of exercise as dietary advice was not included in the study (Gris *et al,* 1990). A moderately restricted energy regimen in males (mean BMI 24.6 kg/m^2) showed benefits on both PAI-1 and t-PA antigen, and PAI activity (Velthuis-te-Wierik *et al,* 1995). Other studies of the effects of body weight reduction on t-PA antigen and PAI-1 activity have been inconclusive (Palareti *et al,* 1994).

3.2.5 The fibrinolytic system and diet composition

The fibrinolytic system could theoretically be influenced by nutritional factors in a number of ways, either by the dietary components which affect circulating constituents of the system, or by becoming adsorbed onto the vascular endothelium (Ogston, 1985). The reported studies have concentrated on changes of diet composition and examined the effects on the fibrinolytic factors. The effect of different fat loads (saturated fat, n-6 polyunsaturated fats) on fibrinolytic activity has also been examined (Salomaa *et al,* 1993). Increased PAI-1 activity and antigen were associated with total and VLDL triglycerides during fasting and during post prandial lipaemia, though no effect of fat

type was observed. These results concur with findings from other similar studies (Heinrich *et al,* 1990, Tholstrup *et al,* 1994a, b, Rankinen *et al,* 1994a, b). A moderate reduction in dietary fat intake (from 35 to 30% energy) was not associated with any significant changes in the components of the fibrinolytic system (Marckmann *et al,* 1992). However, an 8 month study of a low fat diet (26% energy from fat) was associated with a 50% improvement in fibrinolytic capacity measured by increased t-PA. The beneficial effects of a high carbohydrate dietary intake were shown in a study of the relationship between habitual diet and cardio-respiratory fitness (Rankinen *et al,* 1994). Positive relationships between PAI-1 and t-PA antigens and dietary fat intake, in contrast with negative relationships with increased carbohydrate intake were found.

The effect of fruit and vegetable consumption were examined using a FFQ in a cross section of healthy subjects (Nilsson *et al,* 1990). The highest tertile of fruit consumption was associated with lowered PAI-1 activity in comparison with the 2 lower tertiles. The concentrations of t-PA antigen were unchanged across groups showing improvements in the fibrinolytic system. Although dietary fat composition does not significantly improve the fibrinolytic system, moderate alcohol intake was beneficial and increased t-PA antigen concentrations (Lee *et al,* 1995).

A 6 month supplementation study (a daily supplement of 4 g ω-3 fatty acids) in hypertriglyceridaemic post coronary bypass patients examined their effect on fibrinolysis (Eritsland *et al,* 1994). An increase in PAI-1 activity was the only significant change which concurs with the findings of a previous study (Hellsten *et al,* 1993). A crossover study compared the effects of a fish supplemented diet (210 g fatty fish daily) with a diet in which the fish was replaced by an equivalent amount of lean meat. Fish increased PAI-1, PAI-1 antigen and reduced t-PA activity and increased t-PA antigen, whereas with the meat diet they remained unchanged (Marckmann *et al,* 1991).

3.2.6 Conclusion

The fibrinolytic system and components have been established as risk factors for IHD, and are also affected by a number of chronic diseases. For overweight subjects, a reduction in body weight is associated with increased fibrinolytic activity. The effect of dietary changes, particularly the reduction in fat intake, and also increases in fruit, vegetables and fish consumption have been associated with increased fibrinolytic activity. There is sufficient evidence that large weight losses improve fibrinolytic activity, although the effect of moderate weight loss remains unclear. The effect of moderate weight loss on t-PA antigen and PAI activity in overweight subjects requires further study.

3.3 The haemostatic and fibrinolytic systems and plasma lipids

3.3.1 Relationship between plasma lipids and coagulation factors

The association between raised plasma lipids, most frequently triglyceride, and risk factors for IHD have been well established (Meade *et al*, 1980). However, the exact nature of the relationships between the haemostatic factors and IHD risk are less clear. The presence of a positive relationship between plasma factor VII activity and triglyceride in middle aged subjects has been reported (Miller *et al*, 1986, Mitropoulos *et al*, 1989, Mitropoulos, 1994). A recent large survey of cardiovascular risk factors confirmed these relationships in an elderly population (Cushman *et al*, 1996). Raised triglyceride concentrations have been shown to be associated with increased body size and LDL cholesterol concentrations (Folsom *et al*, 1993). Other researchers have identified a positive relationship between triglyceride concentrations and factor VII activity (Mitropoulos *et al*, 1989). The liver, the source of the majority of the haemostatic factors and lipids, may mediate any links, although scant evidence exists of a causal relationship between the coagulation and lipid systems. This relationship is

thought to have become influential on the incidence of MI when the presence of atheromatous plaques or sites of vascular injury were present in addition to elevations in factor VII activity (Krauss *et al*, 1991). The hepatic synthesis of both the coagulation factors including factor VII activity, and the lipid fractions could be related. The relationship between factors noted here has been known to be open to confounding by factors including age and cigarette smoking.

3.3.2 Relationship between plasma lipids and plasma fibrinogen concentrations

Plasma fibrinogen concentrations and LDL cholesterol fractions have been positively associated (Wilhelmsen *et al*, 1984, Balleisen *et al*, 1985, Lee *et al*, 1990). Although, once more there are no findings to support this relationship as being causal. Weaker associations than for fibrinogen concentrations have been seen between LDL cholesterol and factor VII activity (Balleisen *et al*, 1985, Miller *et al*, 1986, Baron *et al* 1989). Falls in factor VII activity, but not fibrinogen concentrations, have been associated with weight loss (Folsom *et al*, 1993) and therefore any direct link between LDL cholesterol and fibrinogen appears unlikely.

3.3.3 Relationship between plasma lipids and fibrinolytic factors

The significant correlations between the changes in LDL cholesterol and the fibrinolytic factors are interesting although they must be interpreted with care, as the causality of these relationships cannot be identified. The LDL cholesterol fraction has been well established as the most atherogenic major cholesterol fraction (Pyorala *et al*, 1994). Raised LDL and lowered HDL concentrations have been associated with increased risk of MI (Castelli, 1996). The concentration of LDL cholesterol has been shown to increase with advancing age particularly in post-menopausal women (Hallberg *et al*, 1967). Obesity and overweight are also well established factors in raising LDL and other lipid fractions (Dattilo and Kris-Etherton, 1992). Plasma triglyceride concentrations

have also been related with increased plasma PAI activity (Tikkanen, 1996). The development of atherosclerosis has been described as the route by which the LDL promotes IHD (Castelli, 1996). The LDL cholesterol fraction has been proposed as the lipid fraction related most closely with haemostatic factors (Lowe, 1996 personnel communication).

The relationship between PAI activity, risk factors for IHD and the metabolic syndrome have been supported by research findings (Eliasson *et al*, 1994a). PAI activity has been correlated with low HDL concentrations, hyperglycaemia and hyperinsulinaemia. Concentrations of PAI have been shown to decrease with falls in insulin concentrations (Vague *et al*, 1986) and in WHR (Landin *et al*, 1990). Falls in PAI activity have also been associated with weight loss (Folsom *et al*, 1993). No relationship was found between the changes in HDL or total cholesterol or triglycerides with PAI, in contrast to the findings of a larger study (Eliasson *et al*, 1994b). The small range in the triglyceride concentrations already discussed may partly explain the non significant relationship between triglycerides and PAI. Thus, the mechanism by which weight loss results in the lowering of both LDL cholesterol and PAI activity are unlikely to be shared, and the relationships not causal. The relationship between t-PA and PAI activity has been described as very close, possibly as they are usually found complexed together *in vivo* (Pearson *et al*, 1997). The measurement of t-PA antigen has been associated with reduced fibrinolysis (Thompson *et al*, 1985). The majority of points which have been considered for PAI activity remain relevant to t-PA antigen.

3.3.4 The use of hormone replacement therapy and lipid lowering therapy to examine inter relationships between lipids, fibrinolytic and haemostatic systems

The use of exogenous hormones as part of HRT treatments in post-menopausal women has provided some insight into the relationship between the haemostatic and lipid

systems. The use of synthetic oestrogen and progesterone therapy has been associated, in some cases, with increased liver synthesis of the haemostatic factors, factor VII and plasma fibrinogen concentrations and plasma triglyceride (Alkjaersig *et al*, 1980). The area has recently been reviewed (Tikkanen, 1996) and it was concluded that exogenous HRT therapy has separate effects on the haemostatic and lipid systems. However, no effects on LDL cholesterol or total cholesterol concentrations were seen. The use of lipid lowering therapy was another setting where the relationship between the lipid and haemostatic system has been considered. Simvastatin, (20 or 40 mg / daily), was given to hypercholesterolaemic subjects using a randomised controlled double blinded study design (Mitropoulos *et al*, 1997). Haemostatic measurements were made following 2 years of lipid lowering therapy. There were significant falls in LDL and total cholesterol and triglyceride in those receiving medication compared with control subjects. However, PAI-1 increased significantly on lipid lowering therapy, whilst factor VII antigen fell significantly. The general failure of simvastatin therapy, which lowered plasma lipids, to affect haemostatic and rheological factors suggests the relationship between the haemostatic and lipid pathways to IHD may not be closely linked. In contrast, fibric acid derivatives, such as bezafibrate, lower fibrinogen by close to 20% and also lower plasma triglycerides and cholesterol concentrations (Almer *et al*, 1988). In this latter case, it was suggested once more that it was impossible to separate the effects on fibrinogen from those on plasma lipids. However, early results from a still ongoing bezafibrate study suggest that the changes in fibrinogen are only weakly correlated with those changes in plasma cholesterol concentrations (Meade, 1995).

3.3.5 Conclusion

Alterations in the fibrinolytic and coagulation systems often coexist alongside an altered lipid system. Raised triglycerides are seen alongside altered concentrations of

fibrinolytic and coagulation factors. Both components have been established as risk factors for IHD, and also are raised in a number of chronic diseases. Plasma LDL cholesterol concentrations are particularly associated with altered concentrations of fibrinolytic and coagulation factors, changes which are shown in both overweight and high risk coronary risk subjects. The reduction of body weight results in improvements in both these areas, although no evidence concerning whether these relationships may, or may not, be causal exists. The relationship between moderate weight loss on lipid and haemostatic pathways may be used to clarify the overlaps between these important physiological systems.

3.4 Haemorheology

Rheology is the branch of physics concerned with the flow and change of shape of matter, especially the viscosity of liquids. The branch of rheology study known as haemorheology concerns the influence of blood cellular and plasmatic components on the circulation (Dintenfass, 1971). The flow of blood is remarkable. At its minimum it has a viscosity of only 1.8 times that of water even though close to half of the volume is composed of cells (Dintenfass, 1971). The processes of cellular concentration, aggregation, deformation and hence viscosity are all components of haemorheology.

Blood rheology with emphasis on the relationship between viscosity and flow in arterial disease was reviewed using laminar flow principles to describe the flow in a tubular blood vessel (Lowe, 1986). Laminar flow exists when parallel "layers" of blood have different velocities relative to each other (figure 3.1). Each layer undergoes shearing when passing over another. The outer layers have a low velocity and high shear rate compared with the high velocity and low shear rate of the inner layers. The frictional resistance to blood flow (its viscosity) due to molecular interactions falls with increases in temperature. Viscosity in laminar flow is the ratio of shear stress (the applied force per unit area) to shear rate (the velocity gradient between adjacent layers) (Lowe, 1986).

Thus, the greater the viscosity of the liquid, the greater the shear stress required to maintain the shear and flow rates. Viscosity and flow rate have been represented as:

$$\text{Viscosity (mPa.s)} = \frac{\text{Shear stress (mPa)}}{\text{Shear rate } (s^{-1})}$$

$$\text{Flow rate } \alpha = \frac{\text{pressure gradient x tube radius}}{\text{tube length x fluid viscosity}} \qquad \text{(Lowe, 1986)}$$

Flow in a tube depends on both driving pressure and a resistance factor which is affected by the tube length, radius and the intrinsic blood viscosity. Erythrocyte defomability and plasma viscosity are the main determinants of blood viscosity when measured at high shear rates (Dintenfass, 1971). *In vitro* viscosity measurements can be made at a variety of shear pressures which can mimic the *in vivo* situation when shear stresses are lowered in the larger compared with the smaller vessels (Lowe, 1987, Schmid-Schonbein, 1981).

A variety of different shear rates can be applied. The measurement of whole blood viscosity at high shear rates (over 300 s^{-1}) at 37° C becomes an asymptotic value, where viscosity measurements reach a plateau and further rises in shear rate were no longer influential (Lowe *et al,* 1996) (personal communication). The measurement of viscosity in a viscometer has limited *in vivo* application given the many different sizes and types of vessels through which blood flows (Schmid-Schonbein, 1981). The additional and complementary measurements of haematocrit, plasma viscosity and RCA allow a fuller interpretation of viscosity measurements.

3.4.1 Blood viscosity

The haematocrit or packed cell volume has been identified as the principal determinant of viscosity although the presence of plasma proteins and erythrocyte defomability are important. Within perfused capillaries erythrocytes deform and adopt a cigar shape, and travel more rapidly than plasma to lower the apparent haematocrit. The RCA due to

rouleaux and associated networks provide blood's structural framework and strength of the RCA may increase the viscosity of the blood.

The shear stresses throughout the human circulation vary widely. They are low in arteries under conditions of normal arterial pressure (2.0 Pa), higher in the arterioles and highest in the smallest nutritive capillaries (10 Pa) (Lowe, 1988). Blood that has flowed from the arterioles to the post capillary venules (0.2-0.5 Pa) is subjected to increasing shear stresses in the larger veins and back to the heart. However, in all normal blood vessels the shear stresses are such that the blood becomes a low viscosity fluid (1.2-4.0 mPas) (Schmid-Schonbein, 1982).

The hyperviscosity syndromes of paraproteinaemias and polycythaemias have allowed some insight into the clinical significance of blood viscosity. Retinal circulation times show close relationships with increased viscosity, circulation times are improved when viscosity is lowered (Luxenberg and Mausolf, 1970). Other diseases which influence viscosity are trauma, inflammation, malignancy, obesity, diabetes, vascular disease, hyperlipidaemia, and arthritis (Chien *et al,* 1986, Lowe, 1987, 1988, Ehrly, 1991). The development of peripheral arterial disease and venous and arterial thrombosis are favoured by raised viscosity, increased RCA and haematocrit (Lowe, 1988). The stagnation of blood flow in a diseased vessel allows the activated coagulation factors to remain *in situ* and favours thrombin generation (Lowe, 1988). Poorly perfused tissues with low shear rate conditions favour locally increased viscosity and increased risk of rheological obstruction. Viscosity increases with age by a maximum of 10%.

3.4.2 Plasma viscosity

Plasma is the liquid part of the human blood in which erythrocytes, leucocytes and platelets are suspended. Blood flow in the smallest vessels is more influenced by plasma viscosity, in contrast with whole blood viscosity where haematocrit is the principal determinant of the flow of blood in the larger vessels (Harkness, 1981, Schmid-Schonbein 1981). The sizes and shapes of plasma proteins determine their influence on plasma viscosity. The small cigar shaped protein albumin contributes only 30% of the difference in viscosity between plasma and water. In contrast, the larger proteins such as fibrinogen, LDL cholesterol and immunoglobulins have shown greater influences on plasma viscosity (Lowe, 1987). Fibrinogen accounts for 22% of the difference in viscosity between plasma and water, despite only making up 4% of the total protein weight (Lowe, 1988).

3.4.3 Influence of whole blood and plasma viscosity on ischaemic heart disease risk

The conventional risk factors for IHD namely the plasma concentrations of cholesterol and triglyceride, overweight, male gender, high sodium intake and increasing age all increase with both whole blood and plasma viscosity (Chien, 1986, Lowe, 1986 , Lowe *et al,* 1986, 1988, de Simone *et al,* 1990,). Women have lower blood viscosity than men though this gender difference becomes reduced after the menopause (Lowe *et al,* 1988, Small *et al*, 1989). Premenopausal women using oral contraceptive agents have elevated blood, but not plasma, viscosity compared with those who do not use oral contraceptive agents. The effect is sufficient to remove the sex differences in blood viscosity. Smokers have reversible increases in blood viscosity due to increases in haematocrit and plasma viscosity (Lowe *et al,* 1980, 1988, 1992). The reversible increase in plasma viscosity has been partly explained by increased plasma fibrinogen concentrations in smokers (Lowe *et al,* 1991). Increased blood viscosity has been observed in those with

peripheral arterial disease when compared with healthy control subjects (Lowe *et al,* 1986, Ciuffetti *et al,* 1989). Plasma viscosity was used as a comparison between areas of low (Augsberg, Germany) and high (Glasgow, Scotland) incidence of IHD (Koenig *et al,* 1994). The higher incidence of IHD events was associated with higher blood viscosities.

3.4.4 Effect of body weight on whole blood and plasma viscosity

The relationship of overweight and obesity with raised whole blood viscosity is seen at every shear rate and is attributed to the positive relationship between viscosity and haematocrit and plasma viscosity (de Simone *et al,* 1990). A similar pattern was seen by Koenig *et al* (1987) where increased plasma viscosity was observed with increasing age and overweight. Overweight accompanied by a central fat distribution has been shown to raise whole blood viscosity in comparison with overweight without a central fat distribution (Wysocki *et al,* 1991). The majority of studies that have identified elevations in plasma fibrinogen concentrations have also reported an increase in blood and plasma viscosity.

3.4.5 Effect of weight loss on whole blood and plasma viscosity

A number of clinical studies have examined whether raised plasma and whole blood viscosities are raised with increased BMI and are reversible after intentional weight loss. These studies are summarised (table 3.3 and 3.4). They range in duration from 15 days to 12 months and use a variety of dietary methods from a VLCD to a 1300 kcal regimen. The consensus finding was that weight loss did not significantly lower fibrinogen (Ernst *et al,* 1993). However, RCA, an influential factor governing whole blood viscosity has been shown to be significantly lowered after weight loss (Ernst and Matrai, 1987, Poggi *et al,* 1994). Both moderate amounts of weight loss (approximately 5% body weight) and increased weight loss (15% body weight) has been shown to either lower (Parenti *et al,* 1988, Fanari *et al,* 1993, Poggi *et al,* 1994) or have no effect on plasma viscosity

(Craveri *et al,* 1990, Tozzi *et al,* 1994). This discrepancy may be the result of studying subjects with different BMI.

3.4.6 Conclusions

The effect of intentional weight loss on whole blood viscosity has been examined in the same studies as plasma viscosity (table 3.4). Whole blood viscosity was shown to be either unchanged (Parenti *et al,* 1988, Ernst, 1989, Craveri *et al,* 1990, Fanari *et al,* 1993) or lowered with weight loss (Ernst and Matrai, 1987, Tozzi *et al,* 1994). There are no published studies which have examined the effect of changes in dietary composition alone on blood or plasma viscosities.

3.5 Red Cell Aggregation

3.5.1 The physiological role of red cell aggregation

The aggregation of red blood cells, RCA, is a reversible process caused by macromolecules present in the plasma, such as fibrinogen and globulins, bridging between one cell and another (Lowe, 1987). The physiological relevance of this process is complex and at present remains incompletely understood.

Conditions of low blood flow favour RCA, enabling the erythrocytes to form linear aggregates or rouleaux. The rouleaux then form elastic networks (Fahraeus, 1929). Fibrinogen becomes adsorbed onto the red cells and favours bridging between cells. In static blood, a stress known as the yield stress is required to disrupt the elastic networks and to re-initiate blood flow.

The RCA is overcome and the cells become entirely dispersed at a minimum shear rate of $50s^{-1}$. In the large vessels RCA favours erthyrocyte migration to the vessel walls with the effects of increasing blood fluidity and improving oxygen transport. In low flow

conditions RCA favours the formation of local obstacles to vessel perfusion. Increases in fibrinogen concentrations greatly increase both RCA and whole blood viscosity especially when measured at low shear rates.

3.5.2 Red cell aggregation in overweight, diabetes, angina pectoris and hypertension

The RCA measured in overweight subjects, free of any associated pathological conditions, was significantly increased in comparison with that found in slim control subjects (Le Devehat *et al,* 1992). Increased plasma viscosity and fibrinogen concentrations have both been associated with an elevated RCA. Raised fibrinogen concentrations were proposed as a partial explanation for this increase seen in the overweight (Le Devehat *et al,* 1992). The presence of IHD and angina pectoris result in elevated RCA when compared with measurements made in healthy subjects (Rainer *et al,* 1987).

Diabetes has been associated with increased in RCA and plasma and whole blood viscosities. These rheological alterations favour reduced blood flow, and promote thrombosis. The elevated blood viscosity seen at low shear rates is probably due to elevated plasma proteins, in particular plasma fibrinogen concentrations which are frequently raised in diabetes (Barnes *et al,* 1988). Increased RCA has been reported in both IDDM and NIDDM diabetes (MacRury, 1990, MacRury *et al,* 1992). The raised RCA was positively correlated with the plasma concentrations of triglycerides and VLDL, and inversely correlated with HDL cholesterol. Smoking habit and BMI were both positively related to RCA. These findings led to the conclusion that RCA was a possible mechanism by which some cardiovascular risk factors promote disease (MacRury *et al,* 1992). A higher RCA has been shown in hypertensive patients and has been positively correlated with diastolic pressure (Rampling *et al,* 1989).

3.5.3 Relationship between red cell aggregation and plasma lipids

The effect of lipoprotein sub-fractions on RCA has been investigated (Simon *et al,* 1995). These researchers examined the aggregability of erythrocytes in the presence of fibrinogen, with either the HDL or the LDL cholesterol sub-fractions. The HDL sub-fraction known to be protective against IHD, was negatively correlated with RCA, though the presence of LDL, known to increase IHD risk, interacted with erythrocytes and enhanced fibrinogen induced RCA. These findings suggest raised plasma LDL cholesterol, and lower plasma HDL cholesterol and may favour increased IHD risk by enhancing RCA.

3.5.4 Red cell aggregation, body mass index and weight loss

The relationship between RCA and overweight has been established. Increased values of RCA have been reported in the overweight when compared with healthy weight control subjects (Le Devehat *et al* 1992). The effect of simple overweight, IGT, and NIDDM on RCA was also examined (Caimi *et al,* 1991). The overweight subjects (BMI 35.5 kg/m^2) had a lower RCA when compared to the IGT group (BMI 34.4 kg/m^2) and the highest RCA was measured in the NIDDM group (BMI 38.8 kg/m^2). The improvement in RCA with intentional weight loss was shown in two studies (table 3.5) (Ernst and Matrai, 1987, Poggi *et al,* 1992) which used low energy regimens. Both showed the beneficial effects of large (10-15% body weight) weight losses on RCA after 3 months intervention. The effect of changes in dietary composition on RCA, or the effect of dietary supplementation are unknown.

3.5.5 Conclusions

RCA has been shown to be influenced both by the presence of obesity and overweight and by the presence of metabolic disease. Preliminary data have suggested that weight

loss may be of benefit to lower RCA. A wide variety of measurements of RCA can be made, which makes the available data difficult to compare. Further studies are required to clarify the role of dietary modification and weight loss on RCA.

3.6 Haematocrit

The haematocrit of blood is also known as the packed cell volume. This is the ratio of the volume occupied by cells, especially the red cells, to the total volume of blood, expressed as a percentage. A linear increase in haematocrit over the range 35-55%, which includes most human values, is accompanied by a linear increase in blood viscosity (Chien, 1975, Lowe, 1987). Increased haematocrit has been related to the increased shear dependence of blood viscosity. The greatest influence of haematocrit was seen at the lower shear rates (Lowe, 1987, Stuart and Nash, 1990). However, erythrocyte deformability, in response to flow forces, facilitates cells of 7-8 μm diameter to flow through vessels with diameters of 3-5 μm (Schmid-Schonbein, 1981). The erythrocyte deformability is increased with increased shear stress, haematocrit and plasma viscosity. This partly compensates for the increased blood viscosity arising from increases in haematocrit or plasma viscosity under high shear conditions. In two random Scottish population samples (Lowe *et al,* 1988, 1992) blood viscosity was lower in women than in men. This was especially evident in pre-menopausal women and was largely due to their lower haematocrit. These gender differences were almost entirely removed in post-menopausal women. These studies (Lowe *et al,* 1988, 1992) also reported a correlation coefficient of 0.7 between blood viscosity and haematocrit. They concluded that around 50% of the community variation in blood viscosity was accounted for by variations in haematocrit, and 40% of the variation was due to changes in

erythrocyte deformabilty. The presence of peripheral arterial disease has also been significantly associated with increased haematocrit (Lowe *et al,* 1992).

3.6.1 Haematocrit and ischaemic heart disease risk

Patients with polycythaemic disease also have elevated haematocrit and elevated IHD risk compared to healthy subjects (Chievitz and Thiede, 1962). It is likely that this reflects the influence of haematocrit on blood. Smoking has also been associated with raised haematocrit (Lowe *et al,* 1992, Wannamethee *et al,* 1994). The influence of physical activity habit and physical fitness as measured by forced vital capacity on haematocrit are small (Sorlei *et al,* 1981, Carter *et al,* 1983). A number of epidemiological studies have examined the relationship between haematocrit and IHD events. The majority have reported inconclusive results, though they did report a positive association between increased haematocrit and an increased risk of MI (Sorlei *et al,* 1981, Carter *et al,* 1983, Campbell *et al,* 1985). In the majority of studies, a linear relationship between haematocrit and outcome of MI has been assumed, although this has been challenged recently (Knottnerus *et al,* 1988, Wannamethee *et al,* 1994). Their results have indicated that a haematocrit of 46% or greater is associated with increased risk of MI (Knottnerus *et al,* 1988, Wannamethee *et al,* 1994). These independent effects of haematocrit on coronary risk remained, even after adjustment for the principal IHD risk factors.

3.6.2 Effect of increased body mass index and weight loss on haematocrit

A positive relationship between raised haematocrit and BMI was shown in the Glasgow MONICA study (Lowe *et al,* 1992), by a study in healthy employed adults (de Simone *et al,* 1990) and in a population of Hawaii-resident Japanese migrants (Carter *et al,* 1983). No difference in haematocrit was seen between healthy and obese subjects (Poggi *et al,*

1994) but when males and females were grouped separately, males showed a significantly raised haematocrit. In contrast, no significant differences in haematocrit were reported by other studies which compared overweight with slim control subjects (Rillearts *et al,* 1989, Craveri *et al,* 1990, Le Devehat *et al,* 1992). Haematocrit was increased by 3% in obese subjects with a central body fat distribution and a similar BMI in comparison to obese without a central fat distribution, (Wysocki *et al,* 1991). Only 2 slimming studies that examined haematocrit (Ernst and Matrai, 1987, Poggi *et al,* 1994) achieved a significant fall in haematocrit after weight loss 15% weight loss in obese subjects after a three month intervention. The remaining studies reporting no change in haematocrit (Parenti *et al,* 1988, Craveri *et al,* 1990, Fanari *et al,* 1993, Tozzi *et al,* 1994) (table 3.6). No studies within the literature have reported that alterations in dietary composition have influenced haematocrit.

3.6.3 Conclusion

Risks of IHD are increased with increased rheological indices. The flow and viscosity of blood and plasma are significantly influenced by the presence of disease including IHD, diabetes, hypertension, angina pectoris and overweight. Available evidence suggests that weight loss in the overweight can be beneficial in lowering the measurements of blood flow and related indices. The majority of studies have been concerned with VLCD and surgical approaches. The influence of changes in dietary composition on haemorheology have yet to be established.

3.7 Fibrinogen and coagulation

3.7.1 Physiological role of fibrinogen in coagulation

Fibrinogen is a large plasma protein (340 KD) and is found in the form of a dimer, each half of which comprises three non identical peptide chains, (α, β and γ). It is synthesised

in the parenchymal cells of the liver, and its concentration in normal plasma is between 2-4 g/L with a half life of approximately 3 days (Poller *et al,* 1990). It is held together by disulphide bonds and includes small amounts of carbohydrates although their role in the function of fibrinogen are largely unknown (Kay, 1988).

Although the catabolism of fibrinogen is not fully understood it appears that the formation of fibrin from fibrinogen plays only a minor role. Fibrinogen is converted to fibrin in a 3 stage process. First, cleavage of 4 small peptides (2 fibrinopeptides A and 2 fibrinopeptides B) from the fibrinogen molecule by the proteolytic enzyme, thrombin. The release of the A peptide occurs more quickly than that of B peptides which are essential for fibrin monomer polymerisation in the formation of fibrin. Fibrin monomer is fibrinogen from which the peptides A and B have all been cleaved. The second stage is polymerisation of the fibrin monomer and the third stage involves the cross linking of soluble fibrin. The process requires factor XIII (thrombin stabilising factor) which is activated by thrombin and covalently links the polymer. Insolubility is conferred by the formation of disulphide bridges which are promoted by factor XIIIa (activated factor XIII).

3.7.2 Role of fibrinogen in ischaemic heart disease risk

Fibrinogen is involved both in blood clotting and in the determination of plasma and blood viscosities (Kay, 1988). A number of epidemiological studies which examined the role of fibrinogen and coronary disease have revealed links between plasma fibrinogen and the risk of IHD and stroke (for review see Ernst, 1990). The Northwick Park Heart study of 1510 men aged 40-64 years showed the first independent associations between fibrinogen and other clotting factors and coronary events. These associations were stronger than the associations between plasma cholesterol and coronary events (Meade *et*

al, 1986). The authors attributed a great deal of the increased fibrinogen concentrations to smoking, but concluded that a "hypercoagulable state" was at least as predictive of coronary events as total cholesterol. Another method by which an increase in fibrinogen was seen to increase IHD risk was by leading to rises in whole blood and plasma viscosity (Yarnell *et al*, 1991). These findings were from the Caerphilly and Speedwell study.

The Gothenburg prospective study followed a population of 792 men aged 54 years for 13.5 years. Ninety two cases of MI and 37 strokes occurred. Smoking, fibrinogen and cholesterol concentrations were once more identified as key risk factors for MI (Wilhelmson *et al,* 1984). The Framingham study results also confirmed the dose dependent relationship between smoking and fibrinogen (Kannel *et al,* 1987). Data from the Scottish MONICA study (Lowe *et al,* 1988) showed fibrinogen was increased in both male and female smokers. Blood viscosity, directly influenced by fibrinogen concentrations was strongly correlated with the principal risk factors for IHD. The results from these prospective studies showed strong and independent associations between high plasma fibrinogen concentrations and first episodes and incident coronary disease. All found elevated fibrinogen indirectly reflected the effect of established risk factors for IHD. A high plasma fibrinogen concentration was suggested as being thrombogenic (Ernst, 1991).

Fibrinogen concentrations have been reported as being increased in patients suffering from IHD, stroke and PVD when compared to subjects who were disease free (Ogston and Ogston, 1966). Plasma fibrinogen concentrations were increased with the number of affected coronary arteries, and were elevated in those diagnosed with angina pectoris (Rainer *et al,* 1987). Other accepted cardiovascular risk factors, smoking, hypertension, hyperlipidaemia and diabetes mellitus all elevate plasma fibrinogen concentrations.

A prospective study investigated fibrinogen concentrations in 120 patients with MI, and followed them to measure both the rate of re-infarction and fibrinogen concentration (Fulton *et al,* 1976). Re-infarction only occurred in cases where the initial fibrinogen level was elevated at above 750 mg dL^{-1}, which suggested lower plasma fibrinogen concentrations are tolerable and do not need a threshold.

3.7.3 Other influences on fibrinogen concentration

The factors which influence plasma fibrinogen are many and have been summarised (figure 3.4) Fibrinogen concentrations are positively correlated with age (Meade, 1981, Balliesen *et al,* 1985) and plasma viscosity (Ernst *et al,* 1986).

As previously stated, smoking has often described as the most influential factor in increased plasma fibrinogen (Belch *et al,* 1984, Balliesen *et al,* 1985) and these changes are known to be reversible on smoking cessation (Ernst *et al,* 1986, Meade *et al,* 1987). Essential hypertension is characterised by elevations in plasma fibrinogen concentrations, which is absent in those remaining normotensive (Fletcher *et al,* 1981).

Elevated plasma lipids, and in particular in type II hyperlipoproteinaemia, are accompanied by increased plasma fibrinogen concentrations (Lowe *et al,* 1979, 1982). However, in healthy men a positive relationship has also been observed between plasma cholesterol and plasma fibrinogen concentrations (Meade *et al,* 1977). Diabetes frequently is accompanied by raised plasma fibrinogen concentrations (Barnes *et al,* 1988). Considerable available evidence has supported the suggestion that increased plasma fibrinogen concentrations were present in both IDDM and NIDDM diabetes (Ostermann and van de Loo, 1986). Diabetic vascular disease in has been associated with increased plasma fibrinogen concentration, above that in those diabetics free of vascular complications (Lowe *et al,* 1980).

The effect of symptomatic and asymptomatic peripheral arterial disease on plasma fibrinogen concentrations and other risk factors was examined as part of the Edinburgh Artery Study (Fowkes *et al,* 1991, Lowe *et al,* 1993). A recent summary of all of the principal case control studies that have been completed in this area shows that all report significantly raised fibrinogen concentrations in those with peripheral arterial disease(Fowkes, 1995). The relationship between high plasma fibrinogen concentrations and peripheral arterial disease is stronger in men than in women (Fowkes *et al,* 1994) which has suggested that differences in fibrinogen concentration could account for some of the sex differences observed in disease susceptibility. Although the role of smoking in increasing peripheral arterial disease has been related to fibrinogen concentrations, an interaction was found between smoking, plasma fibrinogen and risk of peripheral arterial disease. This indicated the likelihood of more than one mechanism of developing peripheral arterial disease (Lowe *et al,* 1993).

Fibrinogen concentrations are known to be higher in women than men at all ages (Lee *et al,* 1990). The use of an oestrogen containing oral contraceptive agents, pregnancy, post menopausal state are all associated with increased plasma fibrinogen concentrations in women (Lowe *et al,* 1982, Lee *et al,* 1993).

Environmental factors account for less than 20% of the inter individual variability in fibrinogen concentrations in population samples. However, it has been estimated that the total genetic contribution to plasma fibrinogen concentrations can vary from 30-50% (Humphries, 1994).

Acute infections such as that which occur in dental disease can result in elevated plasma fibrinogen concentrations (Kweider *et al,* 1993). The findings from epidemiological studies have suggested that stress increases plasma fibrinogen concentrations (Meade, 1981, Rosengren *et al,* 1990). However, no evidence is available from the effect of

relaxation programmes, designed to alleviate stress, on plasma fibrinogen concentrations.

A seasonal variation in plasma fibrinogen concentrations has been established, with an increase in winter compared with summer, a finding which is also true for factor VII activity. It has been proposed that the increase of 0.13 g/L increase in plasma fibrinogen concentrations in winter can be related to the 15% elevation in IHD observed in this period (Woodhouse *et al,* 1994).

3.7.4 Influence of exercise on plasma fibrinogen concentrations

The influence of exercise on plasma fibrinogen has been examined within several epidemiological studies. The Whitehall study (Morris *et al,* 1990), the Gothenburg study (Rosengren *et al,* 1990) and the Northwick Park Heart study (Connelly *et al,* 1992) all showed an inverse relationship between physical activity and fibrinogen. In the Scottish Heart Health study individuals who were inactive in leisure time or at work had significantly higher fibrinogen concentrations, compared with the more physically active participants (Lee *et al,* 1990). When individuals with a history of either no, mild or strenuous exercise were compared, strenuous exercise was associated with lower fibrinogen concentrations even after age, smoking habits, alcohol consumption, BMI and occupation were accounted for (Connelly *et al,* 1992). The authors of the most comprehensive cross-sectional study, the Atherosclerosis in Communities study (Folsom *et al,* 1991), recorded reported exercise habit in units. Each unit was that which fulfilled the equivalent of 20 minutes vigorous exercise and was described as one unit of "sporting index". The authors extrapolated this information on exercise habit, to propose that one unit of sporting index would reduce plasma fibrinogen concentrations by 0.3 to 0.4 g/L for members of the general population. The effect was proposed as being effective as long as exercise habit is maintained.

Clinical research findings support the role of aerobic exercise in significantly reducing fibrinogen concentrations. Fifty five men, 12 months post coronary artery surgery, were randomised to 1 of 3 groups, a no exercise, regular aerobic exercise or regular power exercise (non aerobic) for a 6 month exercise intervention (Worsornu *et al*, 1992). Each session lasted for 12-60 minutes and was repeated 3 times weekly. Only the aerobic exercise was associated with a significant decrease in plasma fibrinogen concentrations. In healthy volunteers, fibrinogen concentrations were significantly decreased after a 9 week endurance exercise programme (Ernst *et al,* 1986). These workers concluded that regular endurance exercise decreased plasma fibrinogen concentrations by 0.4 g/L (Ernst and Resch 1995). The Northwick Park Heart study data indicated a change of 0.1 g/L in fibrinogen concentrations was related to a 15% fall in IHD risk (Meade *et al,* 1986). From these 2 studies it was possible to speculate that the potential effect of exercise, taken regularly and in the quantities described, on fibrinogen may be as great as a 60% reduction in IHD risk.

3.7.5 Plasma fibrinogen concentrations and body weight

The association between fibrinogen and the variables reflecting body weight have been demonstrated in several cross sectional studies, and been described as a close relationship (Ernst, 1993). In the Scottish Heart Health study (Lee *et al,* 1990) BMI and plasma fibrinogen concentrations were very closely correlated. In the Prospective Cardiovascular Munster study the Broca index, (ideal body weight: body length (cm) - 100 = ideal weight in kg) was also significantly correlated with fibrinogen concentrations (Balliesen *et al,* 1985). The Northwick Park Heart study showed a close relationship between skinfold thickness and plasma fibrinogen concentrations (Meade, 1981).

Plasma fibrinogen concentrations have been shown to be the major influence on plasma viscosity (Lowe *et al,* 1988) which has been shown to be significantly increased in overweight women (Ernst and Matrai, 1987). This is in agreement with data reported by the Atherosclerosis in Communities study (Folsom *et al,* 1991). Raised plasma fibrinogen concentrations were reported only in those subjects whose body weight was increased from that recorded at study baseline (Meade *et al,* 1987). The magnitude of this difference was measured as a change of 0.1g/L in fibrinogen per unit change in BMI. When related to IHD risk, this change in fibrinogen indicates a possible 15% reduction (Wosornu *et al,* 1992). The change was at least comparable with the potentially beneficial effects of exercise. A number of clinical studies also support the elevation of fibrinogen concentrations in overweight adults and children. Elevations in plasma fibrinogen in overweight children (Cacciari *et al,* 1988) and overweight adults have been reported (Ferlito *et al,* 1990, Le Devehat *et al,* 1992). The plasma fibrinogen concentrations of healthy weight overweight and obese subjects were compared (BMI 22.4, SD 0.8 kg/m^2, BMI 28.8, SD 0.4 kg/m^2, BMI 36.2, SD 2.5 kg/m^2) (Avellone *et al,* 1994). The overweight and obese group had 45% and 68% higher fibrinogen concentration than the healthy weight group.

3.7.6 Weight loss and plasma fibrinogen concentration

A number of clinical studies have examined whether the established increase in fibrinogen concentrations associated with increased BMI are reversible after weight loss. The studies are summarised (table 3.7). Only three of the dietary studies in adults have achieved a significant decrease in plasma fibrinogen concentrations (Hughes *et al,* 1984, Parenti *et al,* 1988, Kuyl *et al,* 1992) and one study in children (Fanari *et al,* 1993). Although the results of these studies are not consistent, the implication of the majority was that fibrinogen concentrations were lowered when body weight became normal in

previously obese individuals. Gastric bypass surgery and protein sparing fast (close to 400 kcal daily) were both used in a study of overweight diabetic patients (Hughes *et al,* 1984). Six subjects who received surgery achieved weight losses of 27% (117.0 to 86.0 kg). These losses were accompanied by a 13% fall in fibrinogen concentrations, from 3.8 to 3.3 g/L. The effects of weight loss in the six fasted subjects were similar, a 20% fall in body weight reduced plasma fibrinogen concentrations by close to 12%.

No correlation was observed between duration of the diet or the calorie intake. The surgical study reported the effects of a weight loss far in excess of anything achieved by conventional dietary interventions and was accompanied by a significant fall in fibrinogen (Primrose *et al,* 1992). No studies have specifically examined the effect of moderate weight loss on plasma fibrinogen concentrations.

3.7.7 Plasma fibrinogen concentrations and diet composition

Animal studies have suggested that high fatty acid concentrations stimulate fibrinogen production (Pickart *et al,* 1976). In humans, fibrinogen concentrations have been resistant to dietary manipulation, although fish oils have some beneficial effects (Hostmark *et al,* 1988, Meade, 1992). The reduction in fibrinogen concentrations appears to be dose dependent, a 2g daily dose of fish oils reducing fibrinogen by a mean 0.55g/L after a 20 week treatment period. The reductions were greatest with the higher baseline fibrinogen concentrations (Radack *et al,* 1989).

Fehily *et al* (1982) suggested that the influence of dietary composition on plasma fibrinogen was in the region of 5%, with cereal fibre intake showing an inverse relationship with plasma fibrinogen. The results of a smaller cross sectional study of Finnish men were in agreement. No relationship was observed between habitual dietary intake, measured by 7 day WI and fibrinogen concentrations (Rankinen *et al,* 1994). A

vegetarian diet has been reported as having no effect on fibrinogen concentrations (Ernst *et al,* 1986) although this finding has been contradicted by a 3 week study in which fibrinogen concentrations fell after a 3 week vegetarian diet in healthy weight subjects. However, in this later study fasted subjects had significantly reduced body weight (mean weight loss 6.0 kg). This change suggested weight loss rather than dietary change may have been influential (Hostmark *et al,* 1993). Polyunsaturated or monounsaturated fatty acid rich diets and their effects on plasma fibrinogen have also been studied. A 23 day diet which provided 16% energy from monounsaturated fatty acids showed no effect on fibrinogen in weight stable subjects (Heinrich *et al,* 1990). The influence of dietary composition on plasma fibrinogen composition appears small, only the fish oils showing any important influence.

3.7.8 Plasma fibrinogen concentrations and alcohol consumption

A negative relationship between self reported alcohol consumption and fibrinogen concentrations has recently been observed in both men and women (Lee *et al,* 1995). This finding supports the earlier findings of the Northwick Park Heart study (Meade *et al,* 1979) and the Scottish Heart Health study (Lee *et al,* 1990). A literature review confirmed that greater alcohol intakes were associated with lower plasma fibrinogen concentrations (Folsom, 1995).

3.7.9 Conclusions

Plasma fibrinogen concentrations are well established as a good index of IHD risk. They are raised in the overweight. Smoking has been established as the lifestyle factor most influential on plasma fibrinogen concentrations. Body weight losses in the overweight have significantly effected fibrinogen concentrations in only two studies in adults, one of which was using a VLCD approach. A number of studies have examined the effects of

changes in dietary composition on fibrinogen concentrations. Only the addition of fish oils to the diet proved effective in reducing fibrinogen concentrations.

3.8 General conclusions on haemostatic and rheological risk factors for ischaemic heart disease in relation to obesity

The haemostatic and rheological risk factors for IHD are known to be affected by the presence of diseases of particular relevance to this review, obesity, hyperlipidaemia and NIDDM. Epidemiological and limited clinical findings show a positive relationship between rheological factors, RCA and blood viscosity, coagulation factors, factor VII activity and fibrinogen and fibinolytic factors, PAI and t-PA with overweight and obesity. Their relationships with dietary composition, in particular habitual increased dietary fat intakes, are less strong than with raised body weight.

3.8.1 Important unanswered research questions

Moderate weight loss following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets (600 kcal/day energy deficit) would improve haemostatic and rheological risk factors for IHD.

Weight loss *per se* achieved by "healthy" overweight subjects, overweight subjects with angina and those close to healthy BMI would be equally effective in improving haemostatic and rheological risk factors for IHD.

Table 3-1 Weight loss and Factor VII activity

Study	n	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (%)	Change in factor VII activity (%)	p value
Primrose *et al,* 1992	19	154 (27) kg	jejuno ileal bypass	12	-41.5	-14	<0.005
Palareti *et al,* 1994	52	31 (range 32-55)	514	3	-17	-25	0.001
Folsom *et al,* 1993	148	31.0 (2.1)	low fat low E	6	-9.5	-11	0.003
Baron *et al,* 1989	135	77.8 (13.9)	1000-1200	3	-5.4	+ 4	ns
Gris *et al,* 1990	30	24 (2.2)	low fat, physical activity	3	-3.0	-13	*
Andersen *et al,* 1995	9	34 (range 29-41)	421	1	-8	-21	ns
Slabber *et al,* 1992	12	41.5 (7.4)	1000	12	+2.0	-31	<0.005

Data are presented as mean (SD) or range, E = energy, * missing value

Table 3-2 Weight loss and the fibrinolytic system: tissue plasminogen activity or antigen and plasminogen activator inhibitor

Study	(n)	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (kg or %)	t-PA % change	p value	PAI % change	p value
Primrose *et al,* 1992	19	154 (27)	jejuno ileal bypass	12	-41.5			-63 (total)	<0.0001
Folsom *et al,* 1993	148	31.0 (2.1)	Low fat low E	6	-9.5	-23 (ag)	0.001	-31 (ag)	0.004
Velthuis- te- Wierik *et al,* 1995	16	24.6 (1.8)	Low fat low E	2.5	-9.4	-36 (ag)	0.005	-28 (act)	0.01
Andersen *et al,* 1995	9	34 (range 29-41)	421	1	-8	+80 (act)	ns	-48 (act)	ns
Mehrabian *et al,* 1990	27	96.5 (25.2) kg	<10% fat E	<1	-4.7	-33 (act)	0.01	-33 (total)	0.0017

Data are presented as mean (SD) or range, IBW= ideal body weight, E = energy, ag = antigen, act = activity

Table 3-3 Weight loss and blood viscosity

Study	Population (n)	Initial BMI kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (kg)	Change in blood viscosity (mPas)	p value
Parenti *et al,* 1987	24	42.6 (6.7)	400 - 1800	↓20 kg weight	-20	-0.05	ns
Ernst & Matrai 1987	16	34 (31-39)	1000	3	-4 kg/m^2	-2.2 $^{\circ}s^{-1}$	<0.05
Fanari *et al,* 1993	20 children	36.0 (5.3)	1000	1	-8	-0.5	ns
Craveri *et al,* 1990	40	36.0 (6.20	1000-1400	12	-7	-0.02	ns
Tozzi *et al,* 1994	20	24.2 (3.8)	1300	12	-1.6 kg/m^2	-3.6	0.001
Ernst *et al,* 1989	7	106.2 (18)	300	15 days	not given	-0.5	ns

Data as mean (SD) or range, ↓ fall, # different measures of weight change

Table 3-4 Weight loss and plasma viscosity

Study	Population (n)	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (kg) #	Change in Plasma viscosity (mPas)	p value
Parenti *et al,* 1987	24	42.6 (6.7)	400 - 1800	↓20 kg weight	-20	-0.04	<0.025
Ernst & Matrai, 1987	16	34 (31-39)	1000	3	-4 kg/m^2	-0.6 $° s^{-1}$	<0.05
Poggi *et al,* 1994	23	38.1 (5)	524	3	-15.8	-0.05	0.001
Fanari *et al,* 1993	20 children	36.0 (5.3)	1000	1	-8	-0.06	<0.01
Craveri *et al,* 1990	40	36.0 (6.20	1000-1400	12	-7	-0.10	ns
Tozzi *et al,* 1994	20	24.2 (3.8)	1300	12	-1.6 kg/m^2	+0.7	ns
Ernst *et al,* 1989	7	106.2 (18.0)	300	15 days	not given	-0.2	ns

Data as mean (SD) or range, ↓ less, # different, measures of weight loss

Table 3-5 Weight loss and red cell aggregation

Study	Population (n)	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (kg)	Change in RCA (arb. units)	p value
Ernst & Matrai, 1987	16	34 (31-39)	1000	3	-4 kg/m^2	-2.9	<0.05
Poggi *et al,* 1994	23	38.1 (5)	524	3	-15.8	-5.5	0.001

Data as mean (SD) or range, ↓ fall, # different measures of weight change

Table 3-6 Weight loss and haematocrit

Study	Population (n)	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months)	Body weight change (kg) #	Change in blood haematocrit (%)	p value
Parenti *et al,* 1987	24	42.6 (6.7)	400 - 1800	↓20 kg weight	-20	+0.8	ns
Poggi *et al,* 1994	23	38.1 (5)	524	3	-15.8	-0.05	0.001
Ernst & Matrai, 1987	16	34 (31-39)	1000	3	-4 kg/m^2	-1.7	<0.05
Fanari *et al,* 1993	20 children	36.0 (5.3)	1000	1	-8	+0.7	ns
Craveri *et al,* 1990	40	36.0 (6.20	1000-1400	12	-7	+1.2	ns
Tozzi *et al,* 1994	20	24.2 (3.8)	1300	12	-1.6 kg/m^2	-1.6	ns

Data as mean (SD) or range, ↓ less, # different, measures of weight loss

Table 3-7 Weight loss and fibrinogen concentration

Study	Population (n)	Initial BMI (kg/m^2) or weight (kg)	Diet (kcal /day)	Duration (months) or other end point	Body # weight change (%)	Fibrinogen change (g/L)	p value
Primrose *et al,* 1992	19	154 (27) kg	jejuno ileal bypass	12	-41.5	-20.0	<0.05
Hughes *et al,* 1984	6	99.3 (11) kg	5.6 kcal/ kg IBW	reaching 120% IBW	-21	-13.0	0.08
Craveri *et al,* 1990	40	36.0 (6.20	1000-1400	12	-19.4	-4.0	ns
Palareti *et al,* 1994	52	31 (range 32-55)	514	3	-18	+0.3	ns
Parenti *et al,* 1987	24	42.6 (6.7)	400 - 1800	↓20 kg weight	-17	+5.0	ns
Rillaerts *et al,* 1989	20	36.2 (4.8)	1000	6	-16.9	-8.0	p<0.001
Ditschuneit *et al,* 1995	60	41.8 (8.6)	1200	9.5	-16.5	-5.0	ns
Craveri *et al,* 1992	children		1000-1400	2	-16	-6.0	ns
Poggi *et al,* 1992	23	38.1 (5)	524	3	-14.0	-2.0	ns
Fanari *et al,* 1993	20 children	36.0 (5.3)	1000	1	-12	-30.0	<0.01
Gelmini *et al,* 1989	29	34.4 (1.1)	18 kcal/kg IBW	3	-12	-7.0	<0.05
Folsom *et al,* 1993	148	31.0 (2.1)	Low fat low E	6	-9.5	-5.5	ns
Andersen *et al,* 1995	9	34 (range 29-41)	421	1	-8	+26.0	ns
Ernst *et al,* 1989	7	106.2 (18)	300	0.5	-6.5	None	ns
Mehrabian *et al,* 1990	27	96.5 (25.2) kg	<10% fat E	1	-4.6	-7.5	0.07

Data are presented as mean (SD) or range, IBW= ideal body weight, E = energy, ↓ less, # different, measures of weight loss

Figure 3-1 Coagulation cascade

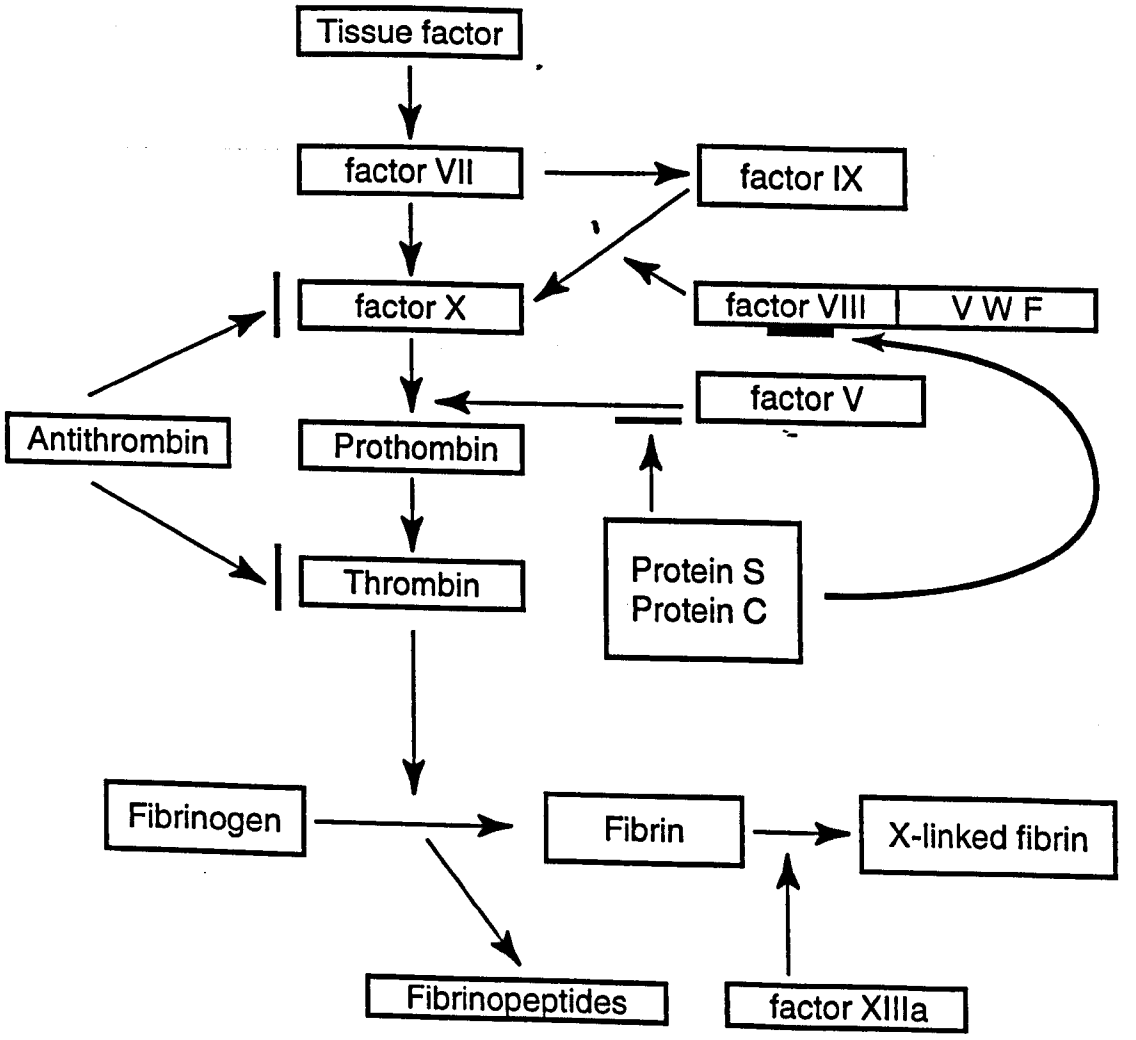


Figure 3-2 Fibrinolytic system

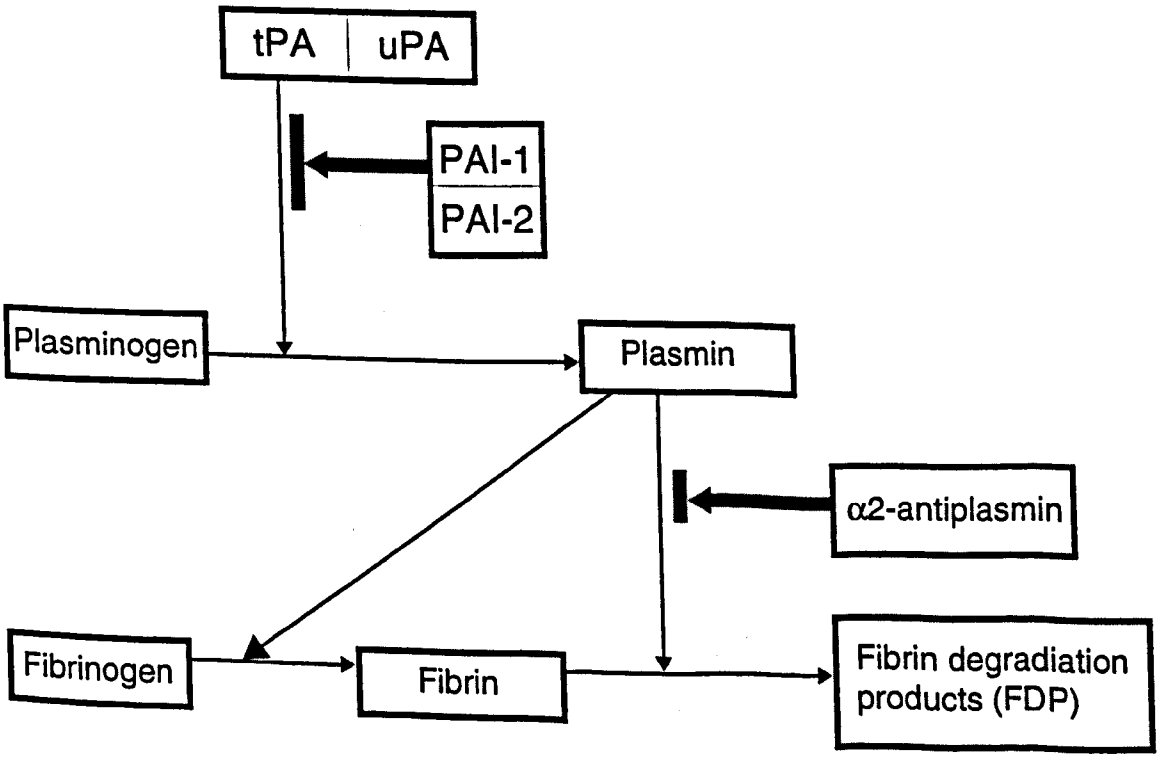


Figure 3-3 Laminar flow characteristics of blood

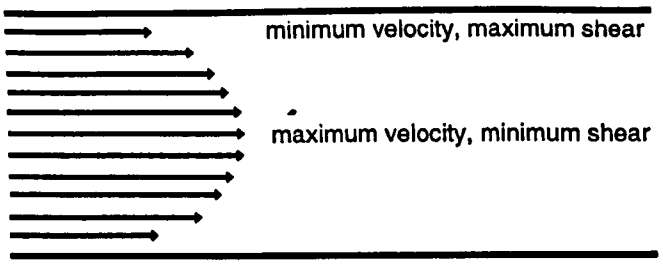
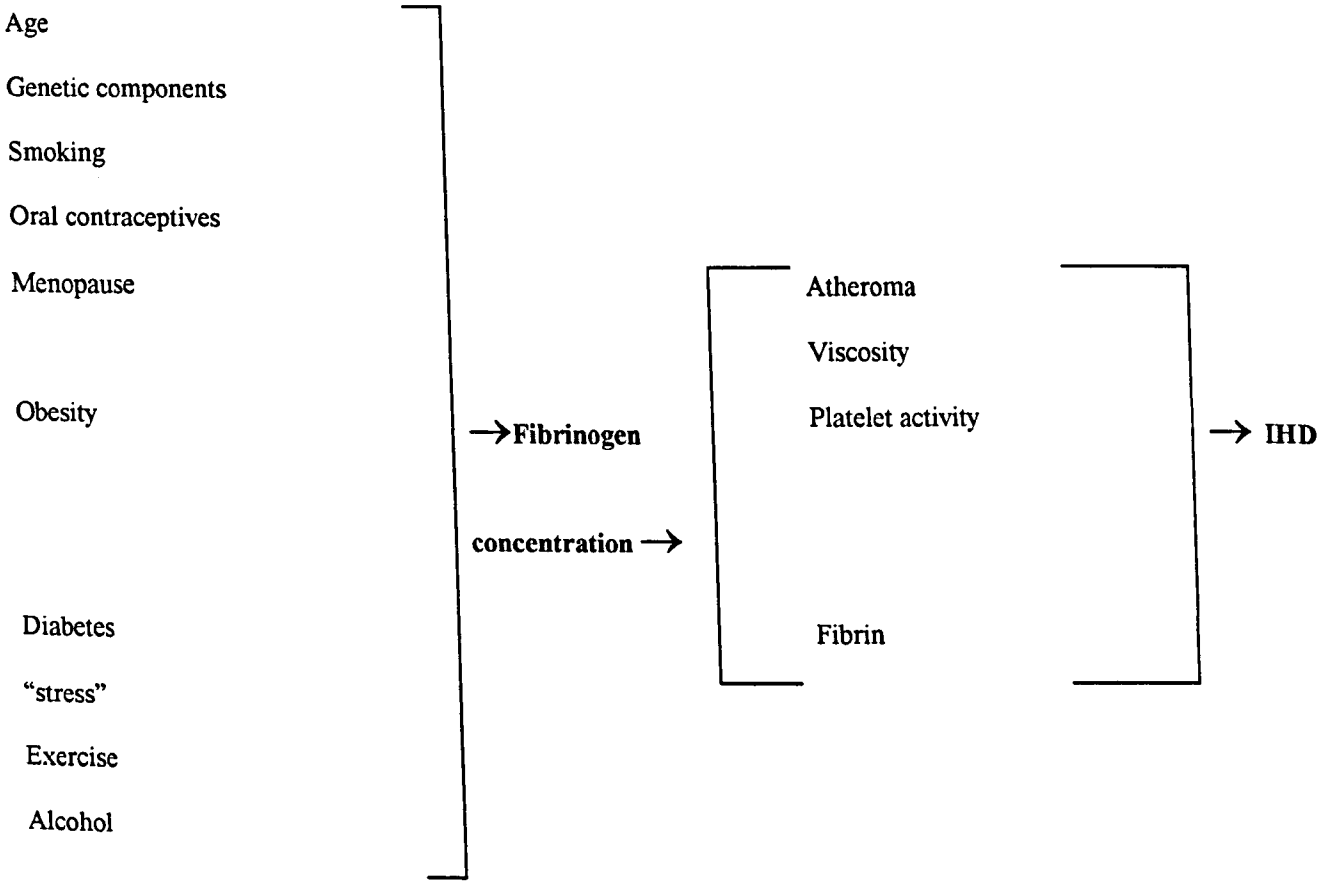


Figure 3-4 Fibrinogen: determinants and pathways



Adapted from Meade *et al*, 1994

Chapter 4 Methodological and statistical considerations for the work in this thesis

4.1 Introduction

The studies in this thesis evaluate dietary intervention to achieve "moderate weight loss", close to the theoretical 0.5 kg per week over 12 weeks, in three different groups of volunteers, using a standard individualised 600 kcal per day energy deficit approach. The volunteers varied in their body weight and health status, and comprised three groups. 1) uncomplicated overweight; 2) overweight with clinical evidence of IHD; 3) healthy weight, close to a BMI of 25 kg/m^2. The studies considered whether modest weight loss would differ between these groups, in particular, whether those subjects with IHD would show greater changes than the disease free subjects after dietary intervention, in terms of IHD risk factor measurements. The effects of the weight loss *per se* on IHD risk was also considered, adjusting for the three subject groups on the risk factors measured. It was accepted that the dietary intervention for weight loss would include effects from changes in dietary composition as well as changes in body weight. In free living subjects the separation of these two influences is impossible, and attempts at dietary assessment are invariably seriously confounded by under-reporting.

The key IHD risk factors chosen to investigate the effect of a dietary intervention to reduce body weight were haemostasis, rheology and plasma lipids. The specific measurements were RCA, whole blood and plasma viscosities, fibrinogen, factor VII activity, PAI activity, t-PA antigen and lipids.

Four research questions were addressed in this thesis after research in volunteers:

1. Does a dietary intervention incorporating an individualised energy deficit approach to weight loss (600 kcal per day) achieve body weight losses of close to 0.5 kg per week?

2. How does dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets affect haemostatic and rheological risk factors for IHD?

3. How does modest weight loss, following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets, affect plasma lipid concentrations?

4. Does the effectiveness in achieving intentional weight loss and changes IHD risk factors differ between the those with a diagnosed disease, the overweight and those close to healthy weight?

The planning of a study to examine the effects of weight loss on biological measurements requires careful consideration as to the most appropriate study design. Inadequate experimental conditions lead to inconclusive, unrepresentative results and the conduct of such research on patients or volunteers is unethical. Any effects that may weaken the interpretation of the results should be minimised at the design stage. The principal factors that confound measurements and analysis in medical research as a whole are those that give rise to errors and biases. These should be minimised by optimising recruitment, study design and measurement methods, and then by ensuring that the study has adequate power. Major influences on experimental design and analysis will be considered in relation to the studies within the present thesis.

4.2 Variance and error

Variance describes the distribution of results measurements in a sample or population. As a generalisation, distributions which are normal (i.e. symmetrical about the mean) are easier to handle statistically, describing variance in terms of standard deviation or standard error of the mean. Variance in non-normal distribution can be characterised

from median and inter-quartile range, or after normalisation by a procedure such as logging. Variance comprises the biological variation, which is the inherent differences between subjects, and error (Pocock, 1990). Biological variation can be minimised at the protocol design stage by the selection of as homogenous a sample as possible in terms of subject characteristics. This requires the use of closely defined inclusion and exclusion criteria for each study, and usually includes setting eligible age ranges. The selection of a specific sample group, and the reduction in biological variation, must be balanced against the need for validity in generalising the results to a given population. Practical issues like ease of recruitment often force issues like combining sexes in a single study, even though variance will be increased.

Error can either be random (chance) or systematic (bias). Random error leads to a wrong result due to chance, unknown and unpredictable sources of variation, and is equally likely to distort the sample in either direction. One source of random error is measurement error, although all measurements include a component of error. This can be estimated when the same measurement is repeated on a number of occasions. Improving the precision, reliability and consistency of any procedure reduces measurement error. In practice, the precision of measurements can be improved in a number of ways. These include ensuring the minimum number of trained investigators make measurements, frequent inclusions of standards in assays, calibrations of any machinery used and routinely carrying out all measurements in duplicate. Accuracy and precision differ in their meaning in a research setting. A very precise measurement is one that has nearly the same value each time it is measured. Precise measurements reflect the real or correct value for a given item. The more precise a measurement the greater the statistical power for a study at a given sample size. An error such as an incorrect dilution of a solution may make the measurement inaccurate, but it could still be precise.

Bias is an error effect that causes results either to depart systematically from the true values, or to fail to mirror the distribution in the normal population (Coggan, 1985). Bias can have the effect of either concealing a true relationship or introducing a spurious one. The application of results from a non-representative group of subjects to a wider population is an important source of bias. If the results from any study are to be applicable to a wider population, then the subjects recruited ought to be representative of that population. Bias can also result from the effects of seasonal variation as seasonality is known to influence a number of biological measurements. With regard to the IHD risk factor measurements any seasonality effects can be minimised most commonly by ensuring subject recruitment extends across all seasons.

Regression towards the mean can occur in clinical trials which are dependent on repeated measures of a variable which is also an entry or selection requirement. It is likely that the measurements in the highest and lowest part of the distribution within a given population will include a high proportion where biological variability and measurement error have played a part. A repeated measurement will be nearer the mean on purely statistical grounds i.e. high results from the first measurement will tend to be lowered when measured for a second time, whilst low results will tend to be higher. The large multiple risk factor intervention trial "MRFIT" examined the value of health promoting interventions, particularly diet, in samples selected on the basis of their high plasma cholesterol concentrations. Regression towards the mean led to confounded trial results (MRFIT, 1982). This effect depends mainly on random error, but there may also be biological influences, so it will be particularly true for diseases or dietary intakes that follow a cyclical pattern including body weight and plasma lipids. To minimise regression towards the mean in a clinical trial care must be taken when selecting subjects who are characterised by high or low values for any measurement (e.g. plasma cholesterol

concentrations or blood pressure). A simple solution is to make more than one measurement of the variable of concern, and disregard the first, defining one. For studies of weight loss, weight measurement is so accurate that a systematic tendency for the overweight to lose weight is more likely to be biological. Table 4.1 lists some potential sources of random error and of bias which may be met in studies of weight loss. The design of any studies of weight loss should consider possible sources of variation and error, including the selection and measurements of subjects, and statistical approaches used for data analysis. Many can be addressed by clear inclusion and exclusion criteria for subjects, by ensuring that the principals of good clinical practice are adhered to, and by incorporating statistical expertise.

4.3 Controls

Control subjects are required in clinical studies to be able to estimate, and correct for, the influence of confounding factors (e.g. age, social circumstances, secular influences not specific consequences of the intervention under study) that occur amongst subjects participating in a clinical trial.

"Study effects" are effects related to changes in lifestyle, dietary and behavioural patterns, which tend to occur simply as a result of participating in a research study. This is especially relevant to studies of weight loss because subjects volunteer with the expectation and intention of weight loss. They tend to modify diet and exercise irrespective of the planned treatment, and often lose weight even if not specifically requested to.

The differences between any measurements made in treatment and control groups can be considered to be the effects of the treatment. The use of a crossover design is a more efficient method with respect to sample size and power, with the subjects participating in

these studies being randomly allocated to the order in which they underwent each treatment period. A washout interval may be required between each of the periods to remove any carry over effects. The use of parallel controls may be preferable because they remove any need for washout periods, but obese subjects are often less willing to comply with non-intervention arms. The duration of treatment necessary for weight loss trials (a minimum of 3-4 months) usually precludes crossover designs.

4.4 Design of studies with weight loss as an outcome of treatment

Designing studies that examine the treatments for, and effects from, weight loss involve some rather unusual and complicated considerations. All studies that examine the effectiveness of a treatment in achieving weight loss must include a control or comparison group. However, for such studies whether of drugs, diet, lifestyle or other treatments the inclusion of a control group is problematic. It is not possible to have "placebo" lifestyle change for obese subjects recruited to a slimming study or a blind allocation, such as would be mandatory for a drug trial. Overweight recruits who receive no treatment and are described as a "control group" or "usual or routine treatment", may confuse the analysis because such subjects recruited as volunteers for weight loss inevitably do make extra changes. Comparison between different styles of management e.g. intensive versus usual, or between different behavioural or dietary approaches may be scientifically valuable, but results may not relate well to those in routine practice.

4.5 Design of studies with weight loss as a treatment

If the aim of a study is to achieve weight loss and then examine the effects of weight loss on a given biological measure (rather than evaluating the efficacy of a treatment in achieving weight loss) the issue of statistical control becomes complicated.

Firstly, as body weight is an unstable continuous variable it is impossible to offer "control subjects" "placebo" advice to remain weight stable. All participants in an ethical slimming study using a design with a dietary treatment and control will have been given information on the study aims and requirements. Thus outside of drug trials, participants will be aware as to which treatment they have been allocated to. Recruitment into a study using overweight as a selection criterion can result in weight loss even without specific advice, and control overweight subjects who receive no advice will frequently change weight (upwards or downwards), either intentionally or under secular influences (Blonk *et al*, 1994, Svedsen *et al*, 1994).

Interventions designed to produce weight loss do not, and cannot, introduce a fixed effect, but a range of individual responses, with some participants even gaining in body weight. This situation is very different to a trial where a fixed dose of medication allocated to all treatment subjects would have a more predictable effect, and the placebo no pharmacological effect. Weight loss as a "treatment" cannot be administered as "a preset dose" and therefore is never identical, such that a range of weight losses and gains occur in all groups. Treatment effectiveness can be assessed in terms of the number of subjects who achieve pre-determined degrees of weight loss e.g. 10%, and the main factor is usually acceptability or compliance. The division of subjects into "successes" and "failures" in terms of weight loss may have value in certain circumstances, but the cut-off between success and failure require careful definition, ideally at the design stage to avoid introducing bias from post hoc categorisation.

In a randomised controlled trial which is testing the effect of a particular "treatment" against a dummy or "placebo" treatment, the only difference between the two groups should be the treatment, with all other elements remaining the same. In the case of trials

of medication efficacy each group will include subjects whose compliance is poor. In dietary studies, there will be a range in the ability of subjects to comply with the advice, so some subjects in treatment and control groups may show rises and falls in outcome measures.

As an alternative to conventional placebo controlled trials employing two-sample treatment comparisons, the effects of variable weight loss on metabolic variables may be more appropriately analysed by correlation analysis, as a measure of linear association, and regression analysis to allow for the adjustment for interacting factors. This single sample approach is the most economical and more ecologically valid incorporating the range of weight losses and their consequences across a chosen population. In the context of the present studies where weight loss was a treatment and not an outcome, and where a range of weight changes (from + 2.0 kg to -10.0 kg) were present, the best use of the data was considered to be the application of correlation analysis between changes in weight and outcome measures. However, the study power estimation was based on the more conventional paired student's t-test.

An alternative approach to a "no treatment group" for comparison or reference is to relate measurements in subjects to data in historical controls considered to be have been exposed to the same secular and other influences. This may provide information about a given study sample compared with a larger representative one. This approach was chosen in chapter 6 and 7 with a subset from the Glasgow MONICA study population. Relationships for the subset of the MONICA population were shown between two physiological measurements of interest and BMI (Hankey *et al*, 1997). The same comparisons were made in the population of study participants, where the relationships between the measurements and BMI were also described. This comparison provided

information on the differences between the angina study subjects and a subset from the MONICA population at baseline. It also gave an indication of the expected influence of weight change on these specific measurements.

4.6 Subject selection

In practice, recruitment to clinical trials is always limited to willing subjects who are available to participate. The limited access a researcher has to subjects may lead to volunteer bias, with only those who are eager, health conscious and motivated, participating in and completing the experiment. Payments of expenses to volunteers may attract people who otherwise would not volunteer to participate in clinical studies so increasing numbers and the representative the sample were. On the other hand, paying fees as incentives could attract unsuitable participants, introduce unwanted effects, particularly to self-completed measurements. There is always a trade-off between the needs to obtain a representative sample, the need to reduce sources of variance and bias, and securing participants who will complete a study. Reducing uniformity could lead to other difficulties, as a dietary intervention could be effective in one sector but not another. An obvious issue is whether to study men, women, or both sexes together. Obesity is often more frequent in women, who are more likely to volunteer, but cardiac risk factors are worse and weight loss often better in men.

Recruiting participants to weight loss studies who have participated in many other attempts to reduce weight is another consideration which may introduce bias. The recruitment of subjects to a slimming study who have already intentionally begun to reduce their body weight immediately prior to participating would also introduce bias. This is likely as the participants may have already reached a plateau from where further weight loss may be problematic.

The use of a random selection procedure for recruitment to provide a representative sample of overweight individuals from the population would not have been a useful approach for the present studies. The requirements from study participants were onerous, and thus only certain subjects would have been prepared to participate in the study, at least much beyond attendance at a number of outpatient appointments. It is more reasonable to aim for a sample representative of those who seek help for weight loss.

For the present thesis, the recruitment of subjects used poster advertisements in and around Glasgow Royal Infirmary, (chapter 6), a press release (chapter 7), and e-mail (chapter 8) for each of the different studies. The selection of subjects was principally based on BMI, and being in good or stable health. Although it was impossible to recruit subjects who had not previously been engaged in slimming, the subjects had to report being weight stable within 3.0 kg over the previous three months to be eligible to participate.

4.7 Sample size and power calculations

After defining the smallest treatment effect which would be of clinical value, the required power must be decided, and then the size of any study can be calculated. The power of a study is the probability of detecting a clinically important difference, assuming it exists, and of excluding a difference where there is none (Gore and Altman, 1982, Rees, 1994). Statistical power to detect or exclude any treatment effects is defined by the variance and sample size. To decide on a sample size, it is therefore to know (or estimate) the treatment effect expected, the population variance for the main outcome measure and level of significance. Consideration should also be given to the ability to generalise results of any research. An increased range of subject selection criteria leads to an increase in variance, and the study numbers have to increase in order to maintain power.

Intervention studies with inadequate sample sizes are unethical wastes of patients or volunteers time. Too large a sample is as unethical as too small a sample, as additional subjects would be researched unnecessarily and resources wasted. Frequently, the data required to calculate study power are unavailable, especially if the planned research is the first study in a given area. In this case, a number of assumptions are made usually based on experience in related fields.

An appropriate equation for the calculation of power is:

$$n = \frac{\sigma^2}{(\mu_2 - \mu_1)^2} \times f(\alpha,\beta) \qquad \text{(Pocock, 1990)}$$

where σ = standard deviation of the difference of response, μ_2 and μ_1 are measurements at time 1 and 2. $f(\alpha,\beta)$ is a statistical function derived from the normal distribution which depends on the chosen power (i.e. β) and significance level for the study (i.e. α). α is commonly called the type I error, and is the probability of detecting a significant difference when there is none. This factor represents the risk of a false positive result. β is commonly called the type II error, and this factor represents the risk of a false negative result, failing to detect a difference when there is one. The values selected in this thesis were α =0.05, and β=0.1. This equation assumes a normal distribution of data, and a paired student's t-test comparison of means. Power becomes reduced when non-parametric comparisons are needed, which within this thesis was rare.

The study design, whether a parallel or single stranded study, affects the power of a study. The power of a single stranded study employing paired student's t-tests is substantially increased versus the two sample parallel study, as the biological variation is usually reduced with only one sample of subjects, each acting as his or her own control. Study numbers required are approximately halved. The following power calculations were carried out for the present studies, and the results used to decide on the required sample

size. The calculations used the results from any relevant studies that had examined the effects of weight loss on the principal end points, the haemostatic and rheological measures and DHEAS. It was considered unnecessary to carry out power estimations for plasma lipids, since these routine assays were included to provide additional information to interpret the results for the principal study measures.

Red cell aggregation

The RCA measurement is only used in research studies, and the size of the minimum clinically important difference or change is poorly defined. However, mean difference in RCA of -0.8 AU and standard deviation of the difference 1.2 AU after an intervention to reduce body weight have been estimated from experience (Lowe, 1996).

Baseline RCA 3.7, post-intervention value 4.5.

$$\frac{(1.2)^2}{(3.7-4.5)^2} \quad x \quad 10.5 \quad = 24 \text{ subjects}$$

The present studies were therefore powered sufficiently to exclude clinically relevant effects on RCA.

Factor VII activity

A change in factor VII activity which is considered clinically important is between 5-10% (Meade *et al*, 1991). A dietary intervention study which examined the effect of weight loss on plasma factor VII activity (Baron *et al,* 1988) after a mean weight loss of 4.2 kg found a change of -8% and standard deviation of the change of 12%.

Therefore, assuming a baseline factor VII activity 108%, post-intervention value 100%.

Sample size required: $\frac{(-12)^2}{(100-108)^2}$ x 10.5 = 25 subjects

The present studies were powered sufficiently to exclude clinically relevant effects on factor VII.

Plasma viscosity

A difference in plasma viscosity of 0.04 mPas can represent the difference between those who remain free of disease, and those with IHD (Koenig *et al*, 1994). After weight loss a mean change in plasma viscosity of -0.04 mPas (standard deviation 0.09 mPas) resulted (Poggi *et al*, 1994). Since the present studies used a modest energy deficit approach, it is estimated that the change in plasma viscosity would be only -0.04 mPas.

Baseline plasma viscosity 1.39 post-intervention value 1.37

$$\frac{(0.09)^2}{(1.34 - 1.39)^2} \quad \text{x } 10.5 \quad = 34 \text{ subjects}$$

The present study was powered sufficiently powered to exclude clinically relevant effects on plasma viscosity.

Whole blood viscosity

A clinically important difference in whole blood viscosity is 0.15 mPas, identified as separating subjects who experienced cardiovascular events from subjects who remained well (Lowe *et al*, 1997). No data exist on weight loss, but a difference of -0.2 and standard deviation of the difference 0.3 mPas in whole blood viscosity was seen between the measurements for an obese group of subjects (BMI 36 kg/m^2) and those of healthy BMI (BMI 22 kg/m^2) (Rillaerts *et al*, 1989). Since the present study expected weight loss was <6.0 kg, a treatment effect of -0.1 SD 0.3 mPas was proposed.

Whole blood viscosity in obese subjects 5.2, those of healthy BMI 5.0.

$$\frac{(0.3)^2}{(5.1 - 5.2)^2} \quad \text{x } 10.5 \quad = 95 \text{ subjects}$$

The present studies were expected to be under-powered for WBV.

Haematocrit

A clinically important difference in haematocrit has been shown as 1.0% representing an increased risk of IHD (Lowe *et al*, 1997). The difference between haematocrit values in obese and healthy weight subjects was 0.5% with a standard deviation of the difference 0.9% (Rillaerts *et al*, 1989). Therefore the estimated treatment effect in the present studies at 0.25 SD 0.9%.

$$\frac{(0.9)^2}{(41.6 - 41.9)^2} \quad \text{x } 10.5 \quad = 94 \text{ subjects}$$

The present studies were under powered with respect to haematocrit.

Fibrinogen

A difference in plasma fibrinogen of 0.25g/L is considered biologically important (Meade *et al*, 1986). The mean change in plasma fibrinogen after a mean weight loss of 16.5 kg was -0.2 g/L and the standard deviation of this change was 0.7 g/L (Ditschuneit *et al*, 1995). No CV was given for this assay.

Baseline fibrinogen 3.3, post-intervention value 3.1.

$$\frac{(0.7)^2}{(3.1 - 3.3)^2} \quad \text{x } 10.5 \quad = 129 \text{ subjects}$$

The present studies were under powered with respect to plasma fibrinogen concentrations.

Plasminogen activator inhibitor activity

A clinically important difference of 1.3 U/mL has been shown to divide those who have suffered a MI from those who remain well (ECAT, 1993). After dietary intervention for weight loss (8.0 kg) a mean difference in PAI activity was -2.9 U/mL with a standard deviation of 2.9 IU/mL (Velthuis-te Wierik *et al*, 1995). Baseline PAI activity was 13.2 U/mL with the post-intervention value 10.3 IU/mL. Assuming a 4.0 kg weight loss in the

present studies, half of this reduction (-1.5 U/mL) is assumed.

$$\frac{(2.9)^2}{(11.7-13.2)^2} \quad x\ 10.5 \quad = 39 \text{ subjects}$$

The present studies were just sufficiently powered to exclude clinically relevant effects on plasma PAI activity.

Tissue plasminogen activator antigen

Differences in t-PA antigen of 1.6 μg/mL have been found to be of clinical significance, and have reflected the difference between those who remain free of disease, and those with IHD (Jansson *et al*, 1994). After 8.0 kg weight loss, a mean difference in t-PA antigen of -3.1 and standard deviation 2.9 IU/mL resulted (Velthuis-te Wierik *et al*, 1995).

Assuming a 4.0 kg weight loss in the present studies, half of this reduction (-1.6 U/mL) is assumed.

$$\frac{(2.9)^2}{(4.8-6.3)^2} \quad x\ 10.5 \quad = \quad 40 \text{ subjects}$$

The present studies were just sufficiently powered to exclude clinically relevant effects on plasma t-PA antigen.

Dehydroepiandosterone sulphate

The sample size that was required to complete the studies examining the effects from weight loss on DHEAS was chosen depending on the minimum clinically important difference. This was estimated as close to 1.5 mol/L (Wallace, personal communication, 1996). The final power analysis was based on the results from Leenen *et al* (1994) who achieved a weight loss of 13.5 kg.

Mean difference in DHEAS concentrations (Leenen *et al*, 1994) 1.9 SD 3.3 μmol/L. Baseline DHEAS concentrations 5.3, post-intervention value 7.3 μmol/L, 5.9 μmol/L

based on a 4.0 kg weight loss.

$$\frac{(3.3)^2}{(5.9\text{-}5.3)^2} \quad \text{x } 10.5 = \quad 318 \text{ subjects}$$

The present studies were under powered with respect to DHEAS concentrations.

4.8 Application of statistical principles to the studies within this thesis

The studies in this thesis have all examined the effects of weight loss on established risk factors for IHD. These data also include the effects of a standard dietetic approach to weight loss. For the reasons already described, a single stranded study design was chosen which simply compared the measurements before and after weight loss.

Bias due to seasonal variation in plasma cholesterol and its fractions, factor VII activity, fibrinogen, and body weight was minimised with recruitment taking place throughout the year. Body weight measurements made in at different times of day, or days of the week are a possible sources of bias. This problem was minimised by ensuring as similar conditions as possible for weight measurements. The major sources of bias and error likely to be encountered in studies of weight loss are summarised in table 4.1.

The evaluation of the range of values and any patterns and relationships were examined using the statistical techniques of correlation and linear regression analyses. Data are presented as the mean and standard deviation for data that showed a normal distribution or as the median and inter-quartile ranges where data showed a skewed distribution. The effects of treatments, in different study groups, were examined for using the students t-test, where data were normally distributed, or the one sample Wilcoxon for skewed data.

The statistical methods employed in this thesis were completed with the aid of Minitab statistical software (Version 10, Minitab Inc., Philadelphia, USA), and the specific statistical methods are described in each chapter.

Figure 4-1 Possible sources of error and bias in studies of weight loss

Possible sources of error	Possible sources of bias
• Few entry criteria for participants	• Over restricting entry criteria
• Poor weighing scales, not regularly calibrated	• Completing weight measures at different of the day, or day of the week
• Poor training of investigators making measurements	• Seasonal variation in measurement of body weight and biochemical measures
• Failing to complete laboratory analyses in duplicate	• Regression to the mean of weight-related measurements in groups selected as overweight
• Pre-menstrual fluid accumulation (gender)	• Recruiting participants who have already participated in weight loss studies
• Changes in clothing between measures	• Recruiting participants who have already recently achieved intentional weight loss
• Combining sexes	• Subdividing subjects retrospectively on a weight loss basis to examine metabolic variable
• Statistical power may be insufficent to confirm negative conclusions	• To short study duration, effects of acute negative energy balance and of weight loss superimposed
• Failure to represent confidence intervals may fail to illustrate the range of the data	• Passage of time in long term studies
	• Poor matching for age, or unusual age distribution

Chapter 5 Methods

5.1. Statement of personal involvement and extent of collaboration

The overall plan and design of studies was completed by the author in collaboration with Professor Lean. The recruitment and consultation with all volunteers were arranged and completed by the author, as were all measurements of dietary intake and resting energy expenditure. The physical measurements including ABPI and sample collection, preparation and storage were also all completed by the author.

Of the biological assays: serum DHEAS were completed by the author as were the majority of measurements of RCA and haematocrit. The remaining assays: insulin, factor VII activity, fibrinogen, PAI activity, t-PA antigen, total and HDL cholesterol, triglyceride and plasma and whole blood viscosity measurements were performed by the staff of the Coagulation Laboratory and the Department of Pathological Biochemistry Glasgow, Royal Infirmary. The intra and inter-assay CV's for the samples collected in the studies of this thesis were unavailable. This was because the analyses were made by staff within other departments who included the samples from these studies within their larger batch assays. Some coefficients of variation for each assay are given, but these are the values recorded by the laboratory staff at the time when the majority of samples were analysed.

Data entry, checking and cleaning, as well as statistical analyses were carried out by the author, with guidance from Dr James Currall.

5.2. Anthropometric and physical measurements

5.2.1. Subjects and recruitment

Subjects were recruited for each of the dietary studies using a variety of approaches. Specific methodologies and subjects will be detailed in the relevant chapter.

5.2.2. Height, body weight and body mass index

Body weight was measured in duplicate using calibrated scales (Seca, Germany) to the nearest 100 g in light clothing and without shoes. Height was measured to the nearest 0.5 cm with subjects standing with their back to the stadiometer. The head was adjusted so that the Frankfort plane was horizontal (WHO, 1987). The subjects were all asked to breathe in deeply and reach up to a maximal height with their legs stretched, but feet flat on the ground (plate 5.1). The calculation of BMI weight (kg) / height (m^2) was made as an index of body weight, as it shows a reasonable correlation with body fatness as measured by densitometry (WHO, 1995, Han *et al*, 1996). The BMI has been used to classify obesity and a number of arbitrary cut offs have been established (WHO, 1995). A BMI below 18.5 kg/m^2 was considered to reflect under weight whereas a BMI between 20 - 25 kg/m^2 was considered to reflect a healthy body weight. A BMI between 25 - 30 kg/m^2 was defined as overweight and a BMI greater than 30 kg/m^2 was defined as obese.

5.2.3 Waist and hip circumferences

Waist circumference was measured mid way between the lower rib margin and iliac crest using a steel tape (Holtain Ltd, Dyfed, Wales). The hip circumference was measured at the level of the great trochanters with the measuring tape being held horizontal (WHO, 1989). Both measurements were made whilst individuals were standing with their legs slightly apart in line with their pelvis. All measurements were made in duplicate and a mean value established. If differences of greater than 5 mm were found for duplicate measurements, both measurements were repeated until a difference of 5 mm or below was found. The waist circumference was also used to estimate the percentage body fat (Lean *et al*, 1995).

5.2.4 Skin-fold thickness

Equations have been derived from which skin-fold thickness measured at pre-determined sites can be used to predict total body fat as determined by densitometry. Two main assumptions are associated with this method, firstly that subcutaneous fat is a constant proportion of body fat, and secondly that the chosen measurement sites also have a constant relationship to total body fat. The measurements were made at the 4 standard sites (triceps, biceps, subscapular and suprailiac) with skin-fold callipers (Holtain Ltd, Dyfed, Wales). The equations were designed for use in adults were used to derive values for total body fat (Durnin and Womersley, 1974). The measurements at each site were carried out using standard guidelines (WHO, 1989). For all sites the measurements were made in duplicate and the mean value taken. Each measurement was read to the nearest mm about 4 seconds after the pressure on the skinfold was fully applied from the calliper (Becque *et al,* 1986).

a) Triceps skin-fold

The triceps skin-fold measurement was made according to the standard methods of the World Health Organisation (WHO, 1987). The right arm was bent at right angles, and the length from the tip of the acromion process on the scapular to the olecranon process of the ulna was measured and the mid point marked. With the arm hanging limply by the side, the skin-fold at the mid-point level on the back of the arm was picked up between the thumb and forefinger of the left hand. The calliper was placed on the skin-fold just below the fingers. The fingers were then removed, and the first reading taken.

b) Biceps skin-fold

As for the triceps, but the measurement was made over the biceps muscle on the front of the arm.

c) Subscapular skin-fold

About 2.5 cm in and below the angle of the scapula towards the mid-line and at an angle of approximately 45° to the spine along the natural line of skin cleavage.

d) Suprailliac skin-fold

Midway between the anterior superior iliac crest and the lowest point of the ribs, horizontal to the floor, or just above the iliac crest in the mid-axillary line.

5.2.3 Ankle Brachial Pressure Index

The ABPI is the standard method for identifying peripheral vascular disease (PVD). It is the ratio between the ankle pressure and the pressure measured in the branchial artery. The ABPI correlates with the severity of ischaemia (Yao *et al,* 1969, Yao, 1973). This measurement was used in the study of angina patients to determine the presence or absence of PVD, a possible influence on coagulation and haemostatic measures (chapter 9). Subjects were rested supine for at least 5 minutes before the measurement was made. The branchial, anterior tibial and posterior tibial artery systolic blood pressures were measured after occlusion of the vein using a standard sphygmomanometer and cuff.

The anterior tibial and posterior tibial artery systolic blood pressures were measured using a Sonicaid Blood Velocimeter (Bognor Regis, UK) using an 8 MHz doppler probe as the blood pressure cuff was deflated and the pulse returned. The ABPI was calculated in both lower limbs using the highest tibial artery pressure divided by the branchial artery pressure. The limb with the lowest ABPI was used for the assessment of ischaemia in any individual subject.

5.3 Estimation and measurement of resting energy expenditure

5.3.1 Prediction equations for basal metabolic rate

A number of prediction equations have been devised for use in healthy subjects and the equations most commonly used in the UK are the Schofield equations (Schofield *et al*, 1985). Twelve equations by sex and age were derived to allow the calculation of BMR from weight for infants, children and adults. These equations were derived from indirect calorimetry measurements carried out in 1500 healthy adults aged below 60 years, and are most accurately used in comparable populations (Livesey and Elia, 1988). The use of the Schofield equations was less appropriate in our older population of subjects with angina. The predicted BMR was used in all studies except for that of angina patients.

Schofield equations to calculate BMR

Adults aged 18 - 30 years: Men: BMR = 0.063 (Wt) + 2.896

Women: BMR = 0.062 (Wt) + 2.036

Adults aged 30 - 60 years: Men: BMR = 0.048 (Wt) + 3.653

Women: BMR = 0.034 (Wt) + 3.538

5.3.2 Indirect calorimetry using ventilated hood method

The REE was measured in all subjects in the angina study because of their age range and poor health invalidated the predictive equations (chapter 7). The principles of indirect calorimetry measured using a ventilated hood method are based on measurements of respiratory gas exchange, the consumption of oxygen and the production of carbon dioxide (McLean and Tobin, 1990). These values were then used to calculate energy expenditure. The ventilated hood method (plate 5.2) has been compares favourably with

whole body calorimetery measurements of energy expenditure (Murgatroyd *et al,* 1993). The measurement of REE is the energy expenditure required to maintain physiological equilibrium whilst at rest in the fasted state. In ideal conditions it is measured as subjects are lying supine having just wakened, and before they have under taken any activities for the day.

Expired air was measured using the ventilated hood method, in this case the Deltatrac metabolic monitor (Merilainen, 1987) whilst the subjects laid at rest. The hood was ventilated with air at atmospheric pressure at a rate of 40 L/minute. The subject only inspired a small fraction of the air. The expired air was drawn over a 4 litre mixing chamber within the instrument and the sample passed through differential paramagnetic oxygen and infrared carbon dioxide sensors. The oxygen consumption and carbon dioxide production were then calculated from the ventilation rate and the differentials in gas concentration between the inspired and expired air. The instrument under micro-processor control provides minute-by-minute data on oxygen consumption, carbon dioxide production and energy expenditure. The REE was measured when the subject was awake, lying supine and having fasted and only consumed water for a minimum fasting period of 8 hours. Gas calibration was carried out before each measurement, using standard gas (95% oxygen and 5% carbon dioxide) and the barometric pressure measurement was adjusted to measured atmospheric pressure daily.

5.3.3 Factors affecting measurement of resting energy expenditure

The measurements of REE should be completed under conditions as strictly controlled and constant as possible. In practice, these factors include room temperature, time of day and resting time prior to measurement. The chosen measurement duration was 20 minutes, as beyond that time it was discovered that subjects found it difficult to remain

wakened. In order to allow for an acclimatising period when the subjects were adjusting to the environment beneath the hood, the initial 5 minutes of measurements were always discarded. During the measurements, care was taken to ensure subjects did not sleep and record artificially lowered REE values.

5.3.4 Dietary prescription methodology

Dietary intervention used an individual dietary prescription based on the Schofield equation (Schofield *et al,* 1985) to estimate BMR for the 2 healthy subject groups. This equation incorporated body weight, sex and age in its predictions. For the subjects with angina, REE was measured and was considered to be the equivalent of BMR. None of the subjects engaged in regular physical exercise so an activity factor of BMR x 1.3 was used to estimate daily energy requirements (COMA, 1991). A 600 kcal (2510 kJ) daily energy deficit was applied and the resulting dietary prescription was adjusted to the nearest 100 kcal with a minimum daily prescription of 1200 kcal (5021 kJ).

The prescribed dietary composition was designed to provide greater than 50% energy from carbohydrate, less than 35% energy from fat and under 20% from protein (COMA, 1991). Total fat energy targets were divided into approximately 10% energy from each of monounsaturated, polyunsaturated and saturated fatty acids respectively. Advice was given to restrict or avoid alcohol consumption.

5.4 Detailed dietary and behavioural advice

An eating plan employing standardised principles was devised and used for all three studies (appendix 3). The plan was based on an exchange system for three groups of foods "bread", "meat" and "fruit". The "bread" exchanges included foods rich in starchy carbohydrate such as bread, cereals, rice, pasta and potatoes. The "meat" exchanges

included lean meats, fish, eggs and cheese. The subjects were encouraged to increase their consumption towards the dietary compositional targets (COMA, 1991). The "fruit" exchanges were rich in carbohydrate which provided the main source of simple sugars, although some jam or marmalade was permitted in order to ensure the palatability of bread.

The portion sizes for each of the exchanges from each of the groups were described in household measures. The sizes were calculated using the guide to average portions (MAFF, 1993). A number of modifications had to be introduced in order to allow the overall eating plan to reach the dietary targets and the portion sizes are given (appendix 3).

Individual energy prescriptions from a minimum of 1200 to a maximum 2400 kcal daily were calculated in increments of 100 kcal per day using various combinations of these exchanges. For each of the exchange groups mean energy provision and macronutrient composition were calculated. The calculations were completed with the assumption that the subjects would chose to eat a range of different combinations of the foods in the exchange groups, and as such, their dietary intake would be as close as possible to the targets.

A minimum of 45 minutes were allowed for each of the dietetic consultations when the eating plan was given to each subject. A number of simple pieces of behavioural advice were included within each consultation (table 5.1). These were devised for the present studies, as the literature in this area has failed to detail the most effective components of behavioural treatments. In review consultations, subjects described their current dietary patterns before receiving their new eating plan. If subjects were failing to reach particular dietary targets within their present dietary intake their particular barriers to making these

changes were discussed together. Practical suggestions to incorporate the recommended lifestyle changes were considered.

5.4.1 Seven day weighed inventory

All studies reported within this thesis used the same method of dietary intake assessment, with only the duration of recording differing between studies. For the study of overweight but otherwise healthy subjects a 4 day WI diary was used, governed by the prior commitment of these particular subjects to a study of diet and medication. In the study of overweight subjects with angina, and healthy subjects whose BMI was close to 25 kg/m^2, 7 day WI diaries were used (Bingham *et al*, 1994). At least 30 minutes were taken to describe to the subjects how to make these recordings correctly. Subjects were advised to weigh (plate 5.3) and record each item of food or drink as it was consumed (plate 5.4). The food was weighed in the form in which it was to be eaten (i.e. after cleaning and cooking) and any left-overs were also recorded.

When possible, individual components or combined dishes should have been weighed. For example, the individual components of a sandwich such as the bread, spread, and filling should be weighed and recorded individually. For component dishes such as a casserole, the subject was usually asked to weigh the item whole, and to record detailed information about the quantities of ingredients used. A method, recipe and cooking time were requested as well as the number of portions served in order that its composition could be calculated later. Meals eaten outside the home and difficult to weigh were accorded standard portion weights or household measures were used (MAFF, 1993).

The subjects were asked to weigh everything that they consumed during the recording period, but in practice certain food items such as confectionery, crisps, commercially produced biscuits and cakes, drinks in public houses would be consumed in standard

weight portions and the subjects were advised of this. These items and their portion sizes were described. A number of food photographs which had previously been validated were included in each food diary (Edington *et al*, 1988). These were used to estimate portion sizes of commonly eaten foods which were difficult to estimate in terms of household measures, e.g. rice, potatoes, chips. When incomplete descriptions of foods were reported, or unaccounted gaps in the record appeared where food should have been consumed, dietary recall approaches were used to try to obtain any missing information and complete the record.

The 4 day WI diary included 2 consecutive weekend days and 2 weekdays. In order to make the best comparison with 7 day WI method, the 2 week day nutrient values were divided by 2 and multiplied by 5. These new totals were added to the sum of the 2 weekend day values. Division of the final totals by 7 provided a mean nutrient total with a reduced bias toward weekend food intakes. Food scales (Selectronic 2200, Salter, UK, plate 5.3) accurate to 2g were used to maximise the accuracy of dietary recording (Black, 1986). All subjects were asked to demonstrate use of scales, advised to use metric measurements and shown an example day from a completed food diary. All subjects were asked to complete food diaries at baseline, and week 12 (plate 5.4).

5.4.2 Dietary analysis

Dietary analysis was carried out using the Compeat 4.0 dietary analysis system, (Lifesystems, London) based on current food tables (Royal Society of Chemistry, 1991). Weights used for foods were estimated from either household measures or from packaging information. The chosen weight was recorded in the food intake diary being analysed. The accuracy of data entry and overall quality control was ensured by using a cross checking process. For each day from a food diary, a summary sheet with the totals

for all foods consumed was compiled. These totals were compared to the values for food intakes for each day within the dietary analysis programme. Any differences between each method were checked and any inaccuracies found were corrected. This method allowed the checking and entry of data to be completed by the same person.

5.4.3 Sample collection, handling and storage

Careful blood collection, sample handling and storage are essential for the accurate measurement of the variables reported in these studies. The reliability of these results ultimately reflected the care taken with these procedures. The withdrawal of blood is known to result in immediate changes in some of the haemostatic factors (Thomson, 1992) activating some, such as tissue factors and platelet release factors, whilst inactivating other factors such as t-PA and factor VII activity. To reduce the possibility of the pre-analytical phase affecting the results, protocols were generated based on the recommendations of ECAT (Thomson, 1992) and adherence to these protocols was strictly observed. The venepuncture procedures for the blood samples used for the work reported in this thesis thus used a standard procedure and were all carried out by the author. To minimise the acute effects eating or any diurnal variations in all studies, blood was sampled between the hours of 9-11 am following a 5 minute supine rest. Blood for viscosity and lipid measurements was anticoagulated with EDTA and used to generate plasma for viscosity after centrifugation for 15 minutes at 3000g. Aliquots of plasma were stored at 4 ^{o} C for later analysis. A maximum storage time of 6 months was allowed for all samples (Thomson, 1992).

Blood was added to tubes containing 0.109 mM trisodium citrate with a final ratio of 9 volumes of blood to 1 volume of citrate. These tubes were pre-cooled on crushed ice in order that the blood sample was cooled rapidly and the components were known to

deteriorate at higher temperatures were preserved. The tubes were then centrifuged within an hour at 2000g for 20 minutes in a refrigerated centrifuge at 4°C to produce platelet poor plasma. After the careful removal from the cells with a disposable Pasteur pipette the middle layer of the plasma was aliquoted and snap frozen at -70 °C.

5.5 Haemorhology:

5.5.1 Red cell aggregation

Measurement of RCA was performed in whole blood anticoagulated with EDTA (1.5 mg/ml). A photometric technique using an automated MAI aggregometer (Myrenne GMBH, Roetgen, Germany) was employed (Schmid-Shonbe *et al,* 1982, plate 5.5).

An aliquot of 25 μl of well mixed blood was placed on the aggregometer cone which contacted a glass slide on closing the machine. The sample was subjected to a 10 second period of high shear (around $600s^{-1}$) to disrupt any red cell aggregates. The change in light transmission over a 5 second period of stasis was then measured. The greater the RCA, the greater the transmission through the sample, and the higher the numerical value obtained in AU from the aggregometer. These measurements were carried out at ambient temperature (20 - 25 °C) and at native haematocrit, as the standardising of haematocrit at 45% has been shown to have no effect on RCA in the majority of clinical situations (MacRury, 1990). The mean of the duplicate readings was taken and the procedure was repeated if there was a difference of more than 0.3 units between the readings, until consistent readings were obtained.

5.5.2 Haematocrit

Haematocrit was measured by the Hawksley microhaematocrit method (Hawksley and Sons, Lancing, Sussex, UK) in accordance with the current international recommendations (International Committee for Standardisation in Haematology, 1986).

Whole blood anticoagulated with EDTA was well mixed, and duplicate samples were drawn up into glass capillary tubes of 1 mm in diameter which were then sealed at one end. The tubes were then placed in the Hawksley microhaematocrit centrifuge and spun at 13000 g for 5 minutes. The haematocrit was then read as a percentage of the sample which was packed cells, without any correction for plasma trapping. An average reading of the 2 samples was taken.

5.5.3 Whole blood viscosity

Whole blood viscosity was measured at 37^{o} C and at high shear rates (over 300 s^{-1}) with a Coulter Harkness semi-automatic capillary viscometer (Coulter Electronics, Luton, UK, plate 5.6). The instrument had a constant pressure head, which drew the sample through the capillary tube, with a CV close to 1% (Harkness, 1971).

The time taken for the blood to flow through the capillary tube was recorded electronically as the meniscus moved between 2 electrodes and the movement activated an electronic timer. The velocity of flow was determined by measuring the time for the mercury meniscus, which moved in a capillary tube parallel with the sample, to pass between the electrodes. This activated a electronic timer. The viscometer was calibrated daily with the standard viscosity solutions and the temperature of measurement checked. The inter-assay CV was <5% and the intra-assay CV was <2%.

5.5.4 Plasma viscosity

Plasma viscosity were measured at high shear rates (over 300 s^{-1}) at 37° C in a Coulter Harkness capillary viscometer (Coulter Electronics, Luton, UK, plate 5.6). The principles for plasma viscosity measurements were very similar to those described in whole blood viscosity measures. A 0.5 ml plasma sample was used for each measurement, and the procedures and sample preparation were in accordance with the International Committee for Standardisation in Haematology (1984). All measurements were made in duplicate and a mean value reported. The inter-assay CV for <5.0% and the intra-assay CV was <2.0%.

5.6 Coagulation measurements

5.6.1 Fibrinogen

Clottable fibrinogen was measured by the dilute thrombin clotting time method of Clauss (1957). This was carried out on a Coag-A-Mate X2 automated coagulometer (Organon Teknika, Cambridge, UK) using reagents and standards from the manufacturer. The international fibrinogen standard (Gaffney and Wong, 1992) was also used to check the manufacturers standard which was found to be satisfactory. An in-house plasma pool from 20 healthy donors was used as the internal quality control which was aliquotted and stored at -70 °C and used in each assay. Using the fibrinogen concentrations of the normal pool over 10 assays the CV for inter-assay variability was found to be 5.1%. The intra-assay variability was 3.2%, calculated from fibrinogen concentrations measured on the pooled plasma run 5 times in the same assay.

5.6.2 Factor VII activity

Factor VII activity was measured using a one stage clotting assay with human factor VII deficient plasma (Sigma Chemical Co., Poole, Dorset, UK) and rabbit thromboplastin. This was carried out on a Coag-A-Mate X2 automated coagulometer (Organon Teknika, Cambridge, UK) using reagents and standards from the manufacturer and the results were recorded as a percentage of a reference plasma pool. The inter-assay CV for <8.0% and the intra-assay CV was <5.0%.

5.7 Fibrinolytic measurements

5.7.1 Tissue plasminogen antigen

Plasma levels of t-PA were measured with a commercially available enzyme linked immunsorbent assay (ELISA) from Biopool AB, Umea, Sweden (Tintelize #101120). The assay quantifies human single chain and two chain t-PA antigen. No cross reaction with urokinase was observed. Maximal sensitivity of the assay, reported by the manufacturer, was 1.5 ng/ml.

In this assay some interference may occur from plasma levels of other antibodies such as anti-goat antibodies and rheumatoid factor. In order to exclude false positives, each sample is added to 2 wells, one containing normal goat IgG, and the other containing goat anti-human t-PA IgG. The difference in assay response between these two wells is highly t-PA specific. After initial binding of the sample to the pre-coated well, the second antibody which is conjugated to bound peroxidase is added to the wells. This will bind to free antigenic determinants on the t-PA molecules present. Unbound conjugate becomes washed away after a further incubation period, and the remaining peroxidase was then measured by the addition of the substrate orthophenylenediamine dichloride. The colour

development was proportional to the amount of t-PA bound to the well and the optical density was determined using an automatic MR700 microplate reader (Dynex, West Sussex, UK) at 492 nm. The results were compared to standard curve established using known amounts of t-PA.

Inter-assay CV was determined using a normal pool measured on 10 occasions and this was found to be 9.8%. Intra-assay CV calculated on 5 single assays was 9.0%. The assay was checked by testing the plasma from 6 healthy volunteers known to have a wide range of t-PA concentrations. These measurements were used to calculate the CVs and the duplicates were found to be very similar.

5.7.2 Plasminogen activator inhibitor

Plasma PAI activity concentrations were determined by a commercially available chromogenic substrate assay (Coatest PAI; Chromogenix, Epsom, UK). The assay involved the addition of a fixed amount of single-chain t-PA in excess to undiluted plasma, where most of it rapidly forms an inactive complex with the fast inhibitor PAI-1. The single chain t-PA was used in the assay as PAI-2 another inhibitor of t-PA found in plasma inhibits this single chain form very poorly. The residual t-PA then activated plasminogen to plasmin in the presence of a stimulator. The amount of plasmin formed was directly proportional to the PAI activity in the plasma sample (this was most often PAI-1). Plasmin levels are then determined by measuring the amidolytic activity of plasmin on a chromogenic substrate (S-2403) which releases *p*-nitroaniline, concentrations which are determined using an automatic microplate reader (MR700 from Dynex, West Sussex, UK) at 405 nm. The adsorbance of the sample was then compared with the standard curve generated on each test run, and a value for the concentration of PAI activity obtained. This is expressed in AU, one unit being defined as the amount

which inhibits one IU of t-PA/ml under the test conditions (Chmeilewska and Wiman, 1986, Gram *et al,* 1993).

The kit had a reported sensitivity of 5 AU /ml, and remained unaffected by the normal concentrations of alpha-2-antiplasmin. The pool or "control plasma" was produced by combining normal plasma from 20 healthy volunteers, 10 men and 10 women aged between 25 and 45 years, and freezing aliquots rapidly at -70 °C. The laboratory policy has since become to express the results as a percentage of the "normal pool" rather than as AU /ml. The value obtained for the normal pool was used to calculate the PAI activity values for the samples on that plate. Assay of the normal pool in each set of assays also gave information about the CV of the assay. The inter-assay CV performed on a normal pool on 10 occasions was found to be 8.1% and the intra-assay CV was 7.0%.

5.8 Endocrine measurements

5.8.1 Dehydroepiandosterone sulphate concentrations

Serum for DHEAS measurements was collected by centrifugation of clotted blood at 3000g for 20 minutes at room temperature. Aliquots were pipetted into glass tubes, capped and stored at -70° C for later analysis. Total serum concentrations of DHEAS were determined using a radioimmunoassay kit (ICN Biomedicals). The assay had a working range of 0.3 - 17.8 μmol/l DHEAS. Inter- and intra-assay CV's were 15% and 10% respectively. EDTA anticoagulated blood (10 ml) was used for lipoprotein and triglyceride analyses.

5.8.2 Insulin concentrations

Blood for insulin measurements was drawn from the anticubital vein with a minimum of stasis using a sterile butterfly needle and plastic syringe and anti-coagulated with lithium heparin. It was placed immediately on ice before centrifugation at 3000g for 20 minutes

at 4° C and frozen at -70° C. Insulin measurements were carried out using an "in house" immunradiometric assay with inter- and intra-assay CV's of 10% and 6%, respectively and a sensitivity of 0.5 mU/l.

5.9 Lipid measurements

Triglycerides and total cholesterol were measured on an automated analysis system (Boehringer Mannheim, Lewes, Sussex.). HDL cholesterol was measured using β-quantification and ultracentrifugation with the removal of low density lipoprotein cholesterol (LDL) by precipitation with heparin and manganese chloride. The Friedwald equation (Freidwald, 1972) was used to calculate LDL cholesterol. For total, HDL cholesterol and triglyceride measurements the inter-assay CV's were <5% and the inter-assay CV were <2%.

Table 5.1 Simple behaviour and eating guidelines

1. Regular meals
2. Fruit with each meal (3 daily)
3. Vegetables with both main meals
4. No butter or margarine
5. Eat only sitting down, off a plate, with a knife, fork and spoon
6. No eating in front of the television
7. 20 minutes daily exercise

Plate 5-1 Stadiometer and scales

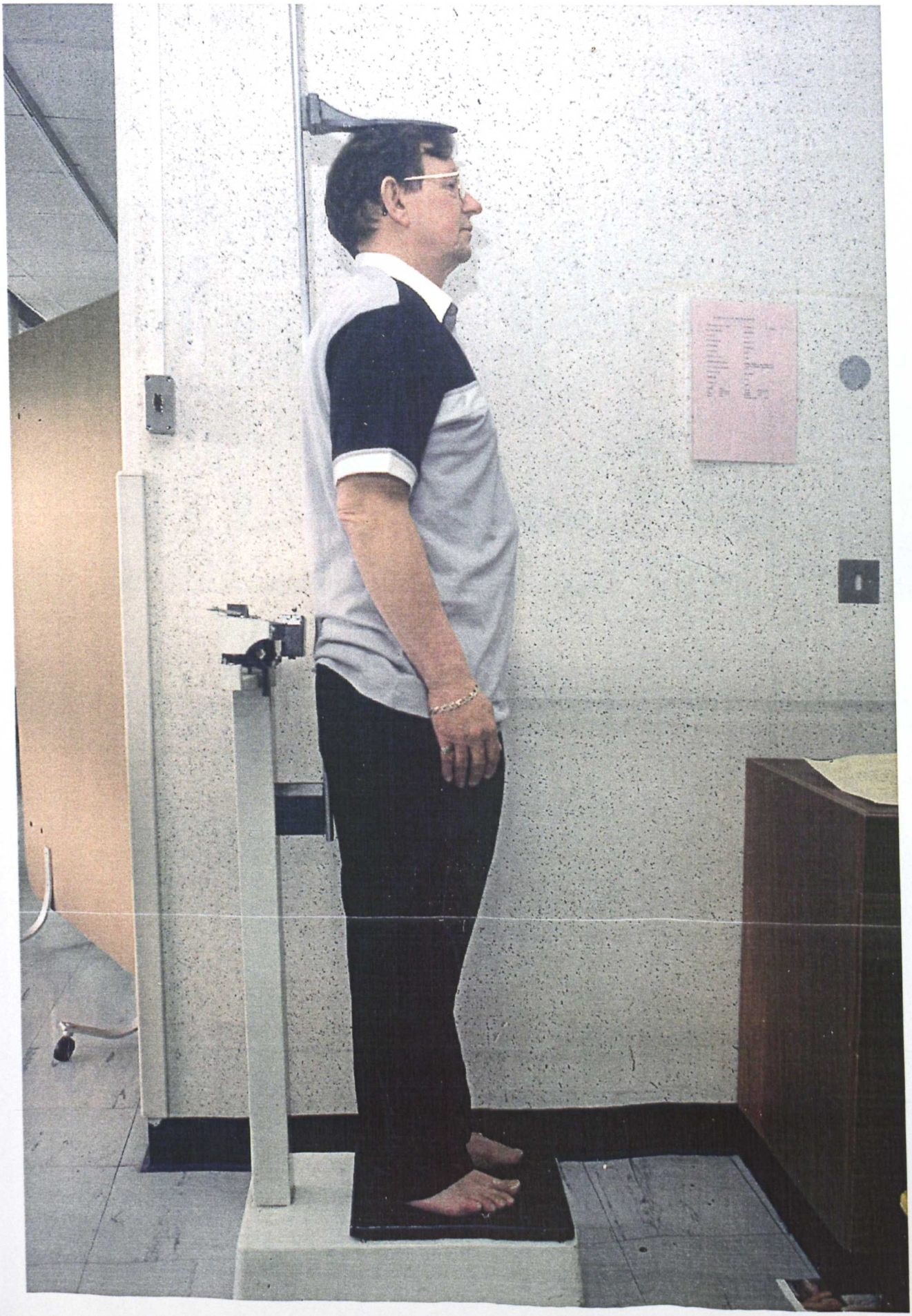


Plate 5-2 Delta trac metabolic monitor

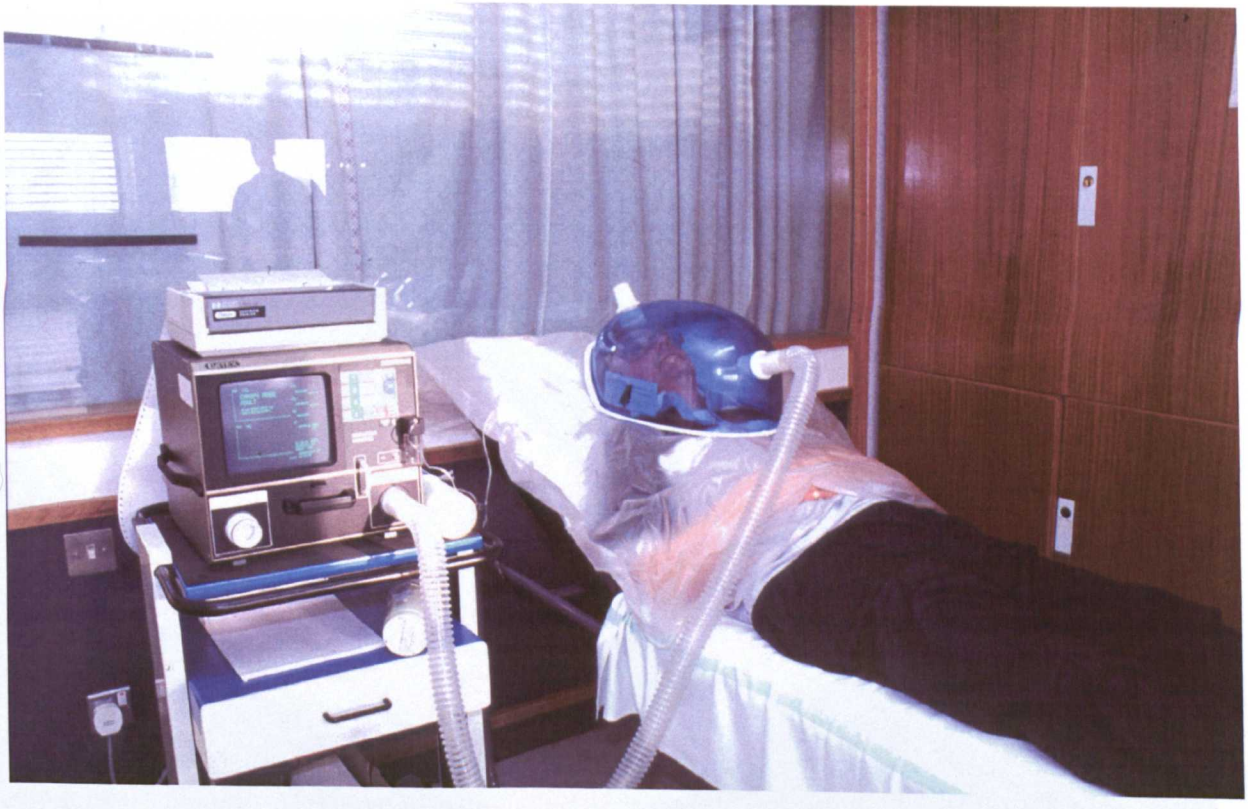


Plate 5-3 Food scales

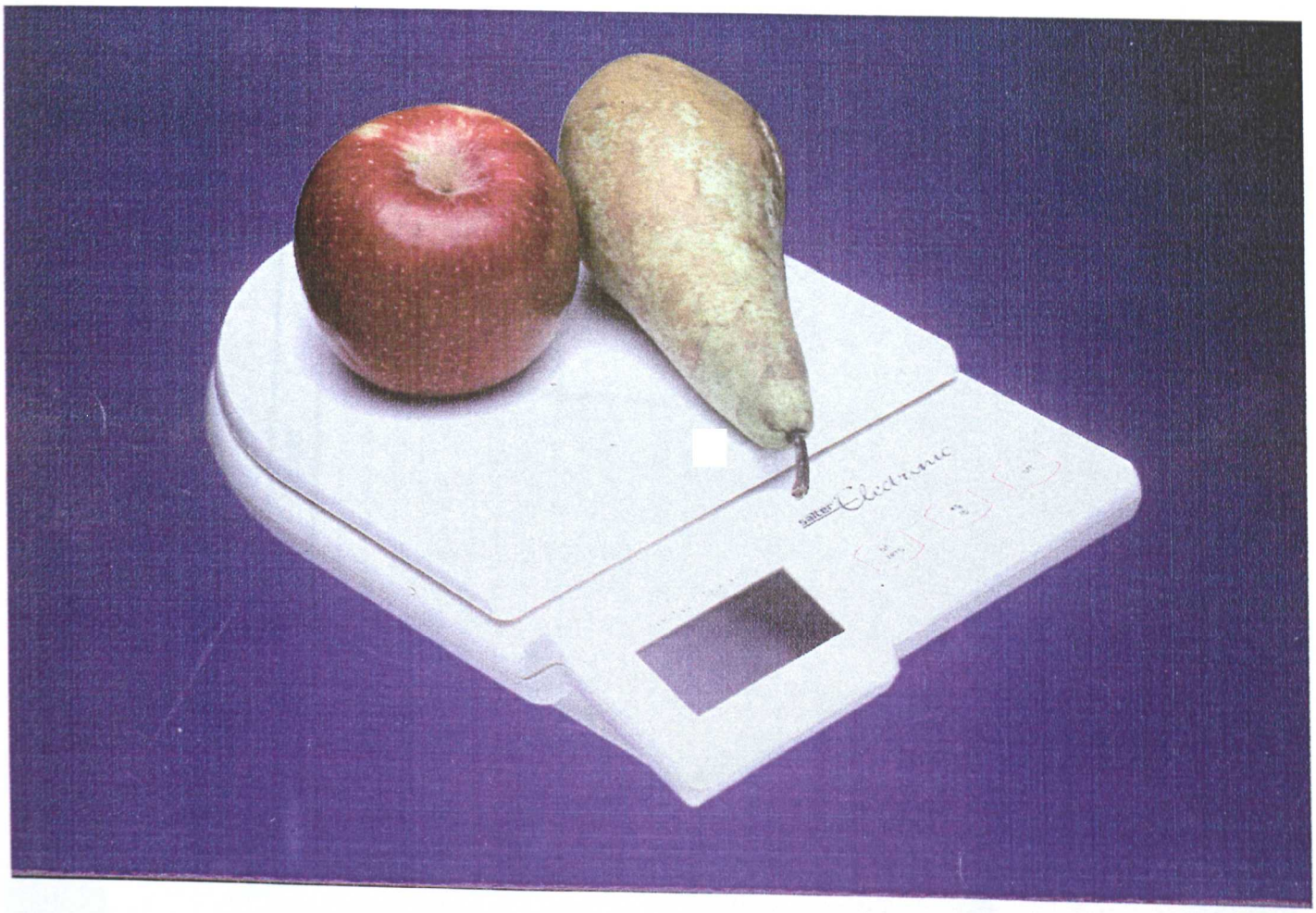


Plate 5-4 Food intake diary

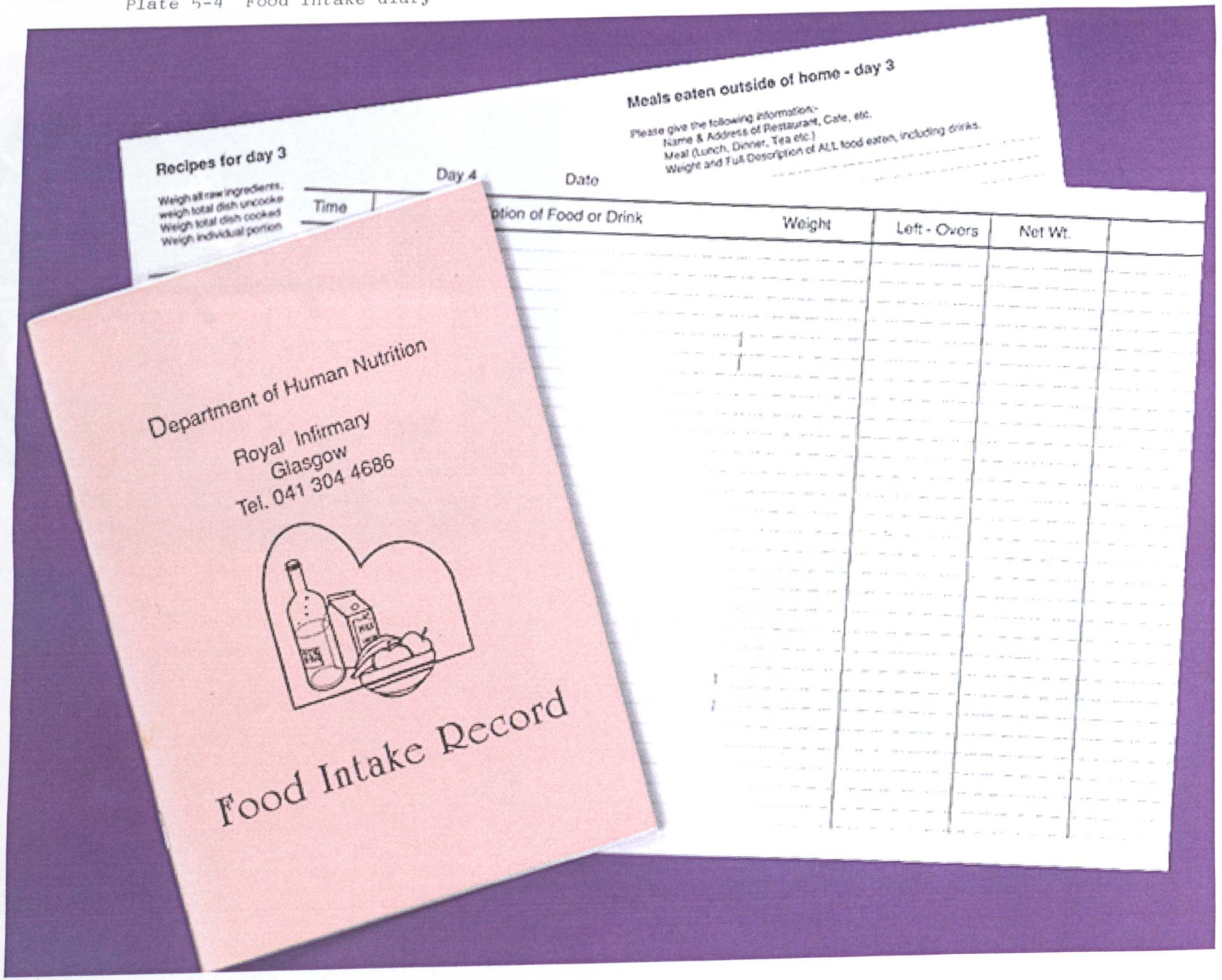


Plate 5-5 Myrenne cone-plate aggregometer

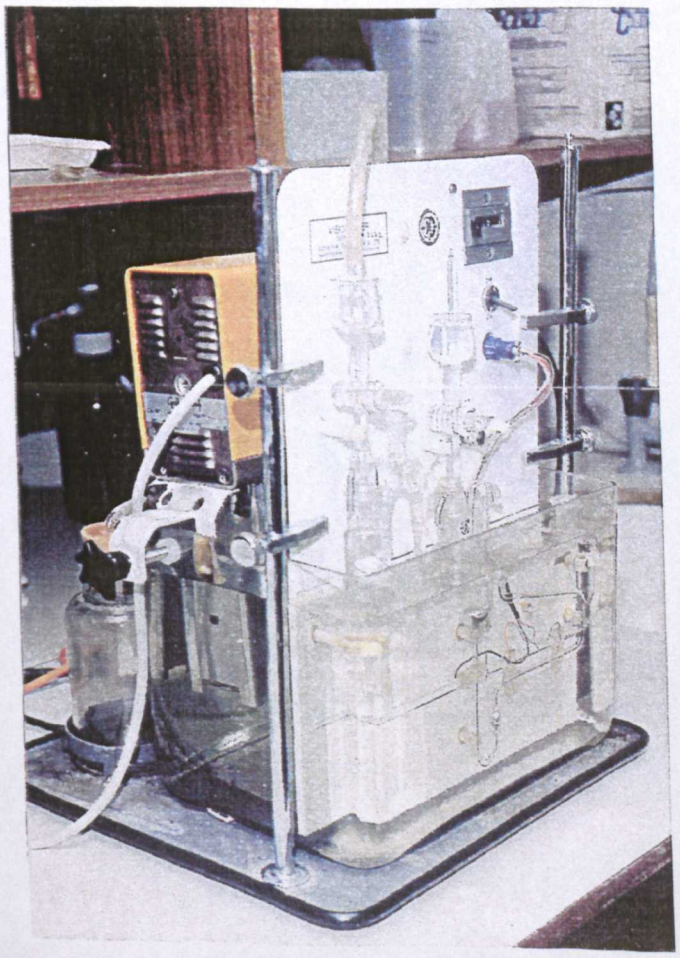


Plate 5-6
Harkness whole blood capillary viscometer

Chapter 6 The effects of moderate weight loss on haemostatic and rheological factors and plasma lipids in overweight subjects

6.1 Introduction and hypotheses

The introductory literature review chapters have described the considerable evidence which has supported overweight as being an important risk factor for IHD mortality. Elevations in blood pressure, blood lipids and insulin all encourage atheroma, and contribute to an increased tendency towards thrombosis in the overweight. Overweight individuals have elevated plasma coagulation factor VII activity, fibrinogen concentrations and impaired fibrinolysis with an increased PAI activity (Meade *et al,* 1986, Vague *et al,* 1986, Lee *et al,* 1990). Raised rheological indices of blood flow, haematocrit, whole blood and plasma viscosity and RCA have been shown to be related to IHD, stroke and peripheral disease (Lowe *et al,* 1990, 1991).

Clinical studies have shown altered coagulation and fibrinolytic measurements in the overweight (Landin *et al,* 1990, Poggi *et al,* 1994). These indices of coagulation and fibrinolysis can be improved with weight loss (Slabber *et al,* 1992, Ernst *et al,* 1993, Palareti *et al,* 1994). These studies used VLCD regimens, where effects from acute, severe energy restriction were likely to confound effects of weight loss *per se* (Henry and Gumbiner, 1991) and whose results are unlikely to persist in the long-term. The present study used currently recommended dietary advice (COMA, 1991) and a moderate daily energy restriction to investigate the effects of dietary intervention and weight loss on haemostatic and rheological variables.

This study aimed to investigate the effect of a dietary intervention to reduce body weight on RCA, factor VII activity, PAI activity, t-PA antigen, fibrinogen, whole blood viscosity and plasma viscosities and lipids.

There were three hypotheses to be tested in this study of subjects who were overweight but otherwise free of known disease:

1) if a dietary intervention incorporating an energy deficit approach to weight loss (600 kcal per day) would be achieve losses of close to the theoretical 0.5 kg per week in obese volunteers?

2) if weight loss following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets, would improve haemostatic and rheological risk factors for IHD?

3) if weight loss following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets, would be effective in improving plasma lipid concentrations?

6.2 Methods

6.2.1. Study design and statistical approaches

A 12 week study duration was chosen, to coincide with the period when maximum weight losses are achieved by most subjects using dietary methods (Cowburn *et al*, 1997). The experimental design, a single stranded study with biological measurements at baseline and week 12, was used in accordance with the methodological principles described in chapter 4. Power calculations to determine sample sizes are given in chapter 4.

In order to provide additional information about the study subjects, their results were compared with existing data from a West of Scotland population recruited from the Third North Glasgow MONICA survey (WHO, 1989b). This random sample was from the parent population of the study participants. Analysis of RCA and factor VII activity and

anthropometric measurements for the MONICA subset were made using the same methodology employed for the measurements of study subjects. The males and females from the subsets of the MONICA population were selected using criteria very similar to those used for the present study. These included being in good health, not following a special diet, never ever having been given a doctor's diagnosis of stroke, angina, or MI and having no ECG abnormality. A total of 521 males and 562 females fulfilled these criteria. Values were obtained for both RCA and BMI from 368 males and 261 females. Both factor VII activity and BMI results were obtained for and 431 males and 409 females.

A regression line was fitted for BMI, for both RCA and factor VII activity against BMI, and the 95% confidence intervals for the mean values were calculated by sex group.

6.2.2 Subject recruitment

Fifty-eight overweight subjects (49 women and 9 men) were recruited both from a local weight management clinic and from local advertisements. Recruitment was carried out throughout a whole year to minimise any seasonal influences. Twenty one subjects who were also participating in a double blind placebo controlled trial of the lipase inhibitor Orlistat (Roche Products Ltd, Welwyn Garden City) a weight reducing agent (Drent and van de Veen, 1993). These subjects received the same dietary intervention as other study participants who were not involved in the lipase inhibitor study. The degree of weight loss achieved by the "orlistat" group", and changes in haemostatic and rheological parameters were not different, so they were analysed alongside the other subjects as a single group. All participants with any significant cardiovascular abnormalities or diabetes (WHO, 1985) were excluded. All subjects were free of any medication known to influence haemostatic measures, such as oral contraceptives or diuretics (Lowe *et al*, 1980). All subjects described at least a period of slimming in the preceding 2 year period

before commencing the study, although no one had reduced their weight by greater than 3.0 kg within the 3 month period prior to recruitment. Ethical approval for the study was obtained from the Glasgow Royal Infirmary Joint Ethics Committee and signed informed consent was obtained from all study participants.

6.2.3. Physical measurements

Subjects attended as outpatients on 5 occasions over the 12 week study period. Anthropometric measurements of body weight, body fat distribution and percentage body fat estimated using skin-fold calliper recordings, were made.

6.2.4. Dietary intervention and monitoring

Dietary intervention used an individual dietary prescription based on the Schofield equation to predict BMR (Schofield *et al,* 1985). The dietary intake of each of the subjects was monitored by the completion of 4 day WI diaries during study week 1 and week 12, both recorded after dietary intervention.

6.2.5. Venesection and laboratory methods

Venesection was performed at baseline and after (week 12) dietary intervention. Whole blood and plasma viscosity, haematocrit and RCA were measured, as were fibrinogen, factor VII activity, PAI activity and t-PA. Plasma triglyceride, total and HDL cholesterol were measured, and LDL cholesterol was estimated using the Friedwald equation (Frieldwald, 1972).

6.3 Results

Thirteen subjects, 11 women and 2 men, failed to complete the study, and were excluded from all the analyses. The reasons included illness (2), and a lack of satisfaction with the

weight loss they achieved (4) and an inability to keep study appointments (7). There was no reason to think that these drop-outs introduced bias. The characteristics of the 45 subjects (36 women and 9 men) who completed the study are shown as one group and according to gender (table 6.1-6.3). The range of baseline body weight in the women subjects was 61.5-120.8 kg, and in the men was 86.6-153.2 kg. The mean weight loss of 4.5 (SD 2.9) kg represented a weekly weight loss of 0.38 kg, 76% of the target value, with no gender differences (p=0.18). Those subjects receiving Orlistat or placebo in addition to dietary advice showed reductions in BMI of -1.95 vs. -1.59 kg/m^2 respectively (p=0.75) and were not significantly different from the dietary advice only group.

Mean dietary energy prescription was 6729 (SD 1648) kJ/day: range 5439-11715 kJ/day (1608 SD 394) kcal/day, range 1300-2800 kcal/day). Recorded dietary intakes were 86% and 85% of total daily energy prescription for the whole group after dietary intervention at weeks 1 and week 12 respectively. Dietary analysis showed the reported intake of macronutrients differed from the diet prescription, with an elevated percentage energy from protein and a reduced percentage energy from carbohydrate. No significant differences were found between dietary records for either total macronutrients or the percentage energies for the whole group, or for either sex (table 6.4-6.6).

The haemostatic and fibrinolytic measurements are shown (table 6.7-6.9). Red cell aggregation was significantly reduced by 13.6% after intervention (P=0.022) in all subjects, in women (p=0.01) but not in men (p=0.73) (table 6.3). At baseline RCA was not associated with BMI, WHR, although it was at week 12 (table 6.10-11). However the changes in RCA were significantly associated with changes in WHR, but not BMI (table 6.12).

Mean factor VII activity was significantly reduced by 6% in all subjects (p=0.008), in women (p=0.0043) but not men (p=0.27). The relationships for factor VII activity against

BMI for the MONICA subset are shown (figure 6.2). The 95% confidence intervals represent the anticipated limits for the mean factor VII activity at individual fixed BMIs. For the study population the baseline and post-intervention values for factor VII activity are shown as the base and tips of arrows respectively. Factor VII activity at baseline was not significantly associated with anthropometric measures, but post-intervention was associated with WHR, although no relationships were seen with changes in the measures (table 6.10-6.12).

Amongst women (p=0.015) there were reductions in t-PA antigen concentrations of 22% after dietary intervention. The evidence was weaker in all subjects (p=0.06), in men (p=0.09).

Plasma cholesterol was reduced in all subjects by 7% (p=0.013) women (p=0.025) and, but not men (p=0.25). LDL cholesterol concentration was significantly reduced only in women (p=0.034). However, HDL cholesterol and triglyceride concentrations remained unchanged. Relationships between plasma lipid measurements, haemostatic and rheological risk factors are shown (table 6.14-6.16). At baseline significant relationships were shown between plasma viscosity and total and LDL cholesterol, these relationships were not shown after weight loss or between differences. Whole blood viscosity was significantly related to triglyceride concentrations at baseline. The concentrations of t-PA antigen were significantly related to plasma total cholesterol concentrations at baseline and week 12, although no relationships were seen with the changes after weight loss (table 6.14-6.16). Changes in PAI activity were significantly related with changes in total cholesterol concentrations (p=0.061).

Use of Pearson's correlation analysis revealed a significant relationship between the changes post-intervention in BMI and in factor VII activity (r=0.395, p=0.01) (figure 6.1). No significant relationships were found between changes in haemostatic and rheological

measures and serum lipid concentrations, or between changes in PAI activity, t-PA antigen, plasma and whole blood viscosity, haematocrit, fibrinogen and WHR or BMI (table 6.12).

The mean BMI for MONICA "healthy" men was 25.7 and the 25 and 95% percentiles were 23.0 and 28.0 kg/m^2 respectively. For "healthy" women these values were 25.7, 22.3 and 27.8 kg/m^2. The mean value for RCA for the subset of MONICA men was 3.8, with 3.0 and 4.6 the 25% and 95% percentiles. The equivalent women's values were 3.9, 3.1 and 4.7. For men the mean factor VII activity was 104.3 IU/dL and the 25% and 95% percentiles were 90.0 and 119.5. In women these values for factor VII activity were 109.3, 92.0 and 127.0 IU/dL. For the MONICA population figure 6.2 shows the anticipated limits for the mean RCA at individual fixed BMIs. For the study sample the baseline and post-intervention values for mean RCA against BMI are shown as lines.

6.4 Discussion

The principal aim of this study was to study the effect of weight loss on haemostatic and rheological risk factors for IHD. However, the first hypothesis for the present study concerned the effectiveness of energy deficit approach to weight loss in reducing body weight, the approach used to achieve weight loss. The mean weight losses of 5.9 kg in males and 4.1 kg in females after 12 weeks in this study are comparable with those achieved by the majority of other workers using conventional dietary approaches (Wadden, 1993). The results of Frost *et al* (1991), the only other published study to examine the energy deficit approach to weight loss in clinical practice, resulted in similar weight losses to this study. In males, the energy deficit approach resulted in weight losses almost identical to the 6.0 kg predicted by calculation. Mean weight losses for females

were 70% of the target. These differences could reflect an effect of gender in dietary compliance.

These short term results (12 weeks) support the use of the 600 kcal energy deficit approach to weight loss favoured in the SIGN guidelines (SIGN, 1996), in overweight but otherwise healthy subjects.

Reported nutrient intakes at baseline (after dietary change was advised) and week 12 were not significantly changed. These findings may be partly be explained by under reporting, a recognised feature in the reported dietary intakes completed by those who are overweight (Bingham, 1987). The results could suggest that the value of using weighed food records lies more with influences on improved dietary compliance, rather than providing accurate or meaningful information on food intakes. However, this assertion cannot be answered by the present findings.

The second hypothesis for this study concerned the effects of weight loss on haemostatic and rheological risk factors for IHD. Significant improvements in 2 measures of IHD risk: RCA and factor VII activity were found after moderate weight loss. For both RCA and factor VII activity the relationships between the study population and the representative MONICA subset were made with BMI. The subset was selected using the inclusion criteria for the current study population which were then applied to its' parent population, which was both random and representative of the West of Scotland. For men, the mean values for the study sample sat exactly on the MONICA data for both RCA and factor VII activity. Yet, for women, the study sample had lower factor VII activity and RCA in relation to BMI compared with the MONICA data, and no obvious explanation for this finding was apparent.

The MONICA comparison has provided information about the relationships between factor VII activity and BMI and RCA and BMI. The 95% confidence intervals only provide a guide as to what is anticipated about the relationships between mean RCA, mean factor VII activity and mean BMI because the confidence intervals are valid for the individual, rather than the mean BMI (Woodward, personal communication 1996). The finding that the baseline values for study subjects (represented by the point of the lines), were closer to the regression line than the week 12 values, with the exception of RCA in men, was unexpected. However, this probably reflected the acute effect of weight loss, although only a moderate energy deficit was applied. The effect of the change in dietary composition, to a lower percentage energy from fat may have also been influential. Nevertheless, the failure of any significant correlations between the haemostatic and rheological risk factors, or the changes are in agreement with these findings.

The lower RCA in the overweight women (figure 6.2) compared with a subset of the Third north Glasgow MONICA survey population was surprising, with no obvious explanation. Other studies of RCA values in overweight NIDDM, (MacRury *et al,* 1993) adults and children (Ernst *et al,* 1986, Wysocki *et al,* 1991, Poggi *et al,* 1994) have found RCA to be raised. In contrast, the trend towards increased RCA in the study population of males after weight loss when compared with the MONICA subset was unexpected. The 13% reduction in RCA achieved in the entire study group can be compared with similar findings in studies of healthy overweight subjects (Poggi *et al,* 1994). The association between reduced abdominal fat distribution and RCA could suggest a mechanism related to insulin resistance. However, other workers have found no clear evidence of a relationship between changes in haemorheological and WHR values (Poggi *et al,* 1994).

Elevated whole blood and plasma viscosities have been shown in overweight subjects compared with those within the range of 20-25 kg/m^2 BMI (Ernst *et al,* 1986) and in

overweight subjects with an elevated WHR compared with those with an acceptable ratio. A WHR of >0.8 for women, >1.0 for men has been shown to reflect low IHD risk (Cacciari *et al,* 1988). Raised plasma and whole blood viscosities have been reported in studies of the overweight (Ernst and Matrai, 1987, Fanari *et al,* 1993, Poggi *et al,* 1994). Poggi *et al* (1994) showed a significant reduction in plasma viscosity in subjects achieving a weight loss of 15.0 kg from a starting BMI exceeding 30 kg/m^2. Plasma viscosity in their study was 5% higher than in the present study (Poggi *et al,* 1994), however the values measured in this study were comparable with a representative West of Scotland population (WHO, 1987). An 11% weight loss in adolescents with BMI greater than 30 kg/m^2 reduced plasma viscosity significantly (Fanari *et al,* 1993) and in adults a 17 % weight loss from a BMI of 42.6 kg/m^2 significantly reduced plasma viscosity (Cacciari *et al,* 1988). The presence of elevated WHR influenced blood rheology, particularly whole blood viscosity, and haematocrit (Fanari *et al,* 1993), but not plasma viscosity. Blood viscosity showed significant reductions with a 17% weight loss when differences were corrected to a 45% haematocrit before measurement at a low shear rate (Fanari *et al,* 1993). No significant correlations were seen with BMI or WHR, excepting plasma viscosity, which suggested that these relationships were weak.

The amount of weight loss and the starting BMI appear to be key factors in achieving significant reductions in plasma and whole blood viscosities (Fanari *et al,* 1993). It seems likely that the subjects in this present study were both slimmer at baseline than those in others (Parenti *et al,* 1988) and also failed to lose sufficient weight to alter either plasma or blood viscosities.

An elevated haematocrit is associated with increased blood viscosity and has been shown as a significant risk factor for non fatal MI and coronary disease (Carter *et al,* 1983). The subjects' mean haematocrit was not elevated when compared with the non obese

population, and it remained unchanged after weight loss. These findings are in agreement with the results of some studies (Parenti *et al,* 1988, Fanari *et al,* 1993) but at variance with others (Poggi *et al,* 1994).

Plasma fibrinogen concentration is another important determinant of plasma viscosity (Koenig *et al,* 1994). However, the significant decrease in RCA was not, however, due to any decrease in plasma fibrinogen concentrations. These results are in agreement with those of other studies which suggest that the influence of both dietary intervention and weight loss to reduce plasma fibrinogen concentrations are small (Fehily *et al,* 1982, Ernst *et al,* 1986, Parenti *et al,* 1988, Ernst *et al,* 1989, Slabber *et al,* 1992, Palareti *et al,* 1994). Existing studies which have reported both the effects of weight loss and dietary change on plasma fibrinogen concentrations have been equivocal (Fehily *et al,* 1982, Ernst, 1993) and they have suggested that the influence of dietary intake on plasma fibrinogen was very small, accounting for around 5% of total variance. Interestingly, fibrinogen concentrations were significantly associated with BMI at baseline, which was in agreement with the evidence that increased body weight and increased plasma fibrinogen are associated (Ernst, 1991).

The influence of factor VII activity as an indicator of increased coronary risk has been demonstrated by epidemiological studies (Meade *et al,* 1986, Lee *et al,* 1990, Heinrich *et al,* 1994) and linked with increased body weight and dietary fat intake (Folsom *et al,* 1993). The 10% reduction in factor VII activity achieved in the present study is close to the results of others (Folsom *et al,* 1993, Palareti 1994). Similar differences in factor VII activity were demonstrated between groups of those at high and low heart disease risk within two large epidemiological surveys (Meade *et al,* 1986, Lee *et al,* 1990). In contrast, the results of a similar study which achieved weight loss using a 1200 kcal diet in contrast to that used in the present study, although factor VII activity remained

unchanged (Baron *et al,* 1989). A possible explanation for these results may be the effects of dietary composition, as half of the study population were consuming a diet with a percentage energy from fat of close to 50% after dietary intervention.

The influence of dietary fat intake on factor VII activity has also been considered (Miller *et al,* 1986). A significant association has been found between total dietary fat intake and factor VII activity. A significant decrease was seen in factor VII activity with reductions in dietary fat intake (31% energy from fat) without weight loss. A similar dietary intervention (32% energy from fat) with two different fat compositions, a low or high polyunsaturated to saturated fat ratio also significantly lowered factor VII activity (Marckmann *et al,* 1992). The present study did not find a relationship between dietary fat intake and factor VII activity at baseline, or between differences in these variables post-intervention.

The two components of the fibrinolytic system PAI activity and t-PA antigen measured in the present study are related to overweight and to increased IHD risk (Carlson *et al,* 1981, Vague *et al,* 1986, Landin *et al,* 1990). An elevated PAI activity has been associated with obesity and with raised WHR (Vague *et al,* 1986, Wysocki *et al,* 1991). Significant reductions in PAI-1 activity and t-PA antigen have been shown after weight losses (Vague *et al,* 1986, Landin *et al,* 1990, Mehrabian *et al,* 1990) double that observed in the present study (mean weight loss 10.0 kg). Once more the reason for the reduction in t-PA antigen in women subjects in the present study was probably the result of sufficient weight loss being achieved. Two other studies of weight loss, one using surgery (Sylvan *et al,* 1992) and the other using dietary intervention (Folsom *et al,* 1992) showed significant falls in t-PA antigen, 60% and 24% respectively. The actual weight losses were only available for the latter study (8.0 kg). The results of this present study found a 21% fall in t-PA antigen in women which is in line with these other findings.

The findings of the present study, that BMI was significantly correlated with factor VII activity at baseline, week 12, and with the differences in BMI after weight loss were in agreement with the majority of the literature. The relationships for PAI activity were in agreement with those for factor VII activity. These relationships are also encouraging as to what the effect of increased weight loss could possibly have been. Of the findings from the present study, these relationships between the changes in factor VII activity, PAI activity and BMI, WHR, fibrinogen with BMI and RCA with WHR were the only significant ones. Greater weight losses may have strengthed some of the other relationships. However, these relationships have supported other published results.

The third hypothesis for this study "that the dietary intervention used would be effective in lowering plasma lipids", was also proven. The degree of reduction in plasma cholesterol following weight loss is 1.3% per kg weight loss, in agreement with the majority of the literature (Dattilo and Kris-Etherton, 1992). However, the stability of HDL cholesterol and triglyceride found in this study following weight loss are in contrast with the majority of publications (Goldstein, 1992), and these results reflect the modest weight losses in the present study. The effects of dietary change and weight loss on plasma HDL cholesterol in the scientific literature are somewhat confused. The influences on plasma HDL cholesterol in this study are probably twofold. Firstly, the effect of reducing dietary fat intake would be to elevate the HDL cholesterol concentration, whilst the acute effect of weight loss would lower HDL (Leenen *et al*, 1994). These two opposing effects probably led to HDL cholesterol concentrations remaining unchanged. The few relationships between the lipids and haemostatic and rheological factors at baseline, week 12 and with weight loss, are probably a reflection of all factors remaining within the laboratory reference ranges and the limited weight loss observed.

6.5 Conclusion

The results of this study of dietary intervention for weight loss in healthy but overweight subjects suggest that moderate weight loss significantly improves two coagulation and rheological indices linked with increased IHD risk, (factor VII activity and RCA), and plasma cholesterol concentrations. Some less pronounced benefits on fibrinolytic measures were also seen. The 600 kcal energy deficit dietary approach to weight loss has been shown to be at least as effective as other more complex or invasive methods.

Table 6-1 Characteristics of women who completed the study at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	44.3 (10.5)			
Weight (kg)	87.6 (12.6)	83.5 (13.2)	-4.1 (2.9)	0.00001
BMI (kg/m^2)	34.1 (5.2)	32.4 (5.4)	-1.7 (1.1)	0.00001
Waist to hip ratio	0.97 (0.1)	0.97 (0.1)	0.0 (0.1)	0.20
Total cholesterol (mmol/l)	5.6 (0.9)	5.2 (0.95)	-0.4 (0.6)	0.025
LDL cholesterol (mmol/l)	3.6 (0.9)	3.4 (0.9)	-0.2 (0.5)	0.034
HDL cholesterol (mmol/l)	1.4 (0.3)	1.3 (0.4)	-0.1 (0.2)	0.22
Triglyceride (mmol/l)	1.2 (0.4)	1.3 (0.5)	+0.1 (0.3)	0.36

Data mean (SD), n=36

Table 6-2 Characteristics of men who completed the study at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	40.8 (9.8)			
Weight (kg)	116.8 (24.4)	110.9 (23.6)	-5.9 (3.3)	0.0007
BMI (kg/m^2)	38.5 (8.4)	36.5 (8.0)	-2.0 (0.9)	0.0003
Waist to hip ratio	1.1 (0.1)	1.0 (0.1)	-0.1 (0.1)	0.0045
Total cholesterol (mmol/l)	6.5 (1.1)	5.9 (0.8)	-0.6 (1.2)	0.25
LDL cholesterol (mmol/l)	4.4 (1.3)	4.0 (0.9)	-0.4 (1.6)	0.54
HDL cholesterol (mmol/l)	1.2 (0.9)	1.0 (0.2)	-0.2 (0.9)	0.60
Triglyceride (mmol/l)	1.8 (0.7)	1.8 (0.6)	0.0 (0.6)	0.96

Data mean (SD), n=9

Table 6-3 Characteristics of all subjects who completed the study at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	43.6 (10.4)			
Weight (kg)	93.5 (19.3)	88.9 (19.1)	-4.5 (2.9)	0.00001
BMI (kg/m^2)	34.9 (6.1)	33.2 (6.1)	-1.7 (1.1)	0.00001
Waist to hip ratio	0.99 (0.1)	0.98 (0.1)	-0.1 (0.1)	0.020
Total cholesterol (mmol/l)	5.8 (1.1)	5.4 (0.9)	-0.4 (0.8)	0.016
LDL cholesterol (mmol/l)	3.8 (1.1)	3.6 (0.9)	-0.2 (0.9)	0.11
HDL cholesterol (mmol/l)	1.3 (0.5)	1.2 (0.4)	-0.1 (0.5)	0.34
Triglyceride (mmol/l)	1.4 (0.6)	1.4 (0.6)	0.0 (0.4)	0.55

Data mean (SD), n=45

Table 6-4 Dietary information from study week one (record one) and study week twelve(record two) for women

	Week 1	Week 12	Difference	p
kJ	5299 (4869, 6167)	5015 (4511 6075)	-82 (-699, +817)	0.795
kcal	1266 (1164, 1474)	1198 (1078, 1452)	-19.5 (-167, +474)	0.795
P/S ratio	0.50 (0.40, 0.69)	0.73 (0.37, 0.60)	+0.07 (-0.19, +0.23)	0.483
% energy fat	32.1 (26.8, 38.4)	32.3 (28.5, 33.6)	+0.25 (-4.1, +4.4)	0.652
% energy saturated fat	9.8 (8.4, 12.0)	11.0 (9.5, 12.0)	+1.5 (-0.8, +2.8)	0.44
% energy polyunsaturated fat	5.5 (4.3, 7.0)	5.3 (4.6, 6.1)	-0.2 (-1.7. +1.7)	0.700
% energy protein	21.9 (19.2, 25.5)	21.6 (19.4, 23.9)	-0.4 (-2.7, +4.6)	0.718
% energy carbohydrate	43.3 (38.9, 48.3)	45.1 (41.5, 47.6)	-0.4 (-4.6, +3.1)	0.68
% energy alcohol *	0.0 (0.0, 0.1) (n=19)	0.8 (0.0, 3.1) (n=11)	+1.28 (-0.30, +9.7)	0.423

Data mean (SD) * n = number of alcohol consumers, n=45

Table 6-5 Dietary information from study week one (record one) and study week twelve (record two) for men

	Week 1	Week 12	Change	p
kJ	6489 (5567, 8537)	6934 (5925, 7786)	+255 (-1174, +1416)	0.906
kcal	1551 (1331, 2040)	1657 (1416, 1861)	+61 (-281, +338)	0.906
P/S ratio	0.46 (0.37, 0.60)	0.50 (0.29, 0.65)	-0.06 (-0.15, +0.09)	0.477
% energy fat	32.0(26.9 35.0)	36.0 (33.3-36.7)	+3.6 (-0.2, +7.1)	0.124
% energy saturated fat	10.8 (8.4, 12.5)	13.0 (10.5, 14.1)	+1.1 (+0.1, +2.8)	0.02
% energy polyunsaturated fat	5.6 (4.6, 7.0)	6.4 (4.3, 8.7)	+0.9 (-0.3, +3.1)	0.286
% energy protein	22.9 (19.1, 26.7)	24.7 (19.5, 25.2)	+0.3 (-3.0, +2.0)	0.834
% energy carbohydrate	41.3 (40.6, 48.1)	39.7 (37.8, 43.3)	+2.0 (-0.8, +8.4)	0.193
% energy alcohol *	4.7 (3.3, 4.9) (n=2 *)	5.9 (4.2, 5.7) (n=2 *)	+0.6 (-2.1, +4.8)	0.675

Data as median, 25 - 75% inter-quartile ranges,
n=9, *n = number of alcohol consumers

Table 6-6 Dietary information from study week one (record one) and study week twelve(record two) for all subjects

	Week 1	Week 12	Change	p
kJ	5573 (5021, 6228)	5602 (4604, 6812)	-79 (-743, +860)	0.928
kcal	1332 (1200, 1489)	1339 (1100, 1628)	-24 (-178, +206)	0.928
P/S ratio	0.53 (0.40, 0.60)	0.50 (0.36, 0.60)	+0.03 (-0.19, +0.21)	0.638
% energy fat	32.0 (27.2, 37.9)	32.4 (28.9, 35.3)	+0.10 (-4.8, +4.2)	0.778
% energy saturated fat	9.9 (8.4, 12.2)	11.3 (9.7, 12.9)	+1.4 (-0.5, +2.7)	0.007
% energy polyunsaturated fat	5.6 (4.4, 7.0)	5.4 (4.6, 6.2)	+0.2 (-1.5, +2.1)	0.813
% energy protein	21.9 (19.2, 25.4)	21.8 (19.7, 24.8)	0.0 (-2.6, +2.7)	0.811
% energy carbohydrate	43.3 (39.5, 47.9)	43.9 (40.2, 47.3)	+0.6 (-3.9, +3.6)	0.839
% energy alcohol	4.7 (2.1, 6.3)	3.8 (1.9, 5.4) (n=13)	+3.8 (+1.9, +5.4)	0.423

Data as median, 25 and 75 % inter-quartile ranges,
n=45, * n = number of alcohol consumers

Table 6-7 Haemostatic and fibrinolytic measurements at baseline and week twelve for women

	Baseline	Week 12	Difference	p
Whole blood viscosity (mPas)	2.9 (0.3)	2.9 (0.3)	0.0 (0.3)	0.73
Haematocrit (%)	43 (4)	43 (4)	0 (3)	1.00
Plasma viscosity (mPas)	1.31 (0.07)	1.29 (0.08)	-0.02 (0.07)	0.23
Red cell aggregation (AU)	4.4 (1.2)	3.8 (1.1)	-0.6 (1.2)	0.010
Fibrinogen (g/l)	3.3 (0.9)	3.2 (0.63)	-0.1 (0.7)	0.44
Factor VII activity (IU/dL)	115 (22)	108 (22)	-7 (18)	0.017
PAI activity (% pool)	147 (62)	137 (63)	-10 (54)	0.29
t-PA antigen (μg/mL)	9.2 (5.1)	7.2 (3.1)	-1.9 (3.5)	0.03

Data as mean (SD), n=36

Table 6-8 Haemostatic and fibrinolytic measurements at baseline and week twelve for men

	Baseline	Week 12	Difference	p
Whole blood viscosity (mPas)	3.5 (0.3)	3.4 (0.3)	-0.1 (0.2)	0.92
Haematocrit (%)	44 (2)	45 (3)	+1 (4)	0.18
Plasma viscosity (mPas)	1.36 (0.04)	1.33 (0.03)	-0.03 (0.06)	0.29
Red cell aggregation (AU)	4.7 (2.0)	4.8 (1.5)	+0.1 (1.8)	0.85
Fibrinogen (g/l)	2.9 (0.7)	3.5 (0.8)	+0.6 (0.7)	0.06
Factor VII activity (IU/dL)	114 (10)	107 (10)	-7 (11)	0.14
PAI activity (% pool)	194 (58)	178 (74)	-16 (85)	0.61
t-PA antigen (μg/mL)	9.8 (2.4)	11.1 (0.9)	+1.3 (2.1)	0.16

Data as mean (SD), n=9

Table 6-9 Haemostatic and fibrinolytic measurements at baseline and week twelve for all subjects

	Baseline	Week 12	Difference	p
Whole blood viscosity (mPas)	3.05 (0.38)	3.04 (0.39)	-0.01 (0.40)	0.82
Haematocrit (%)	43 (4)	43 (3)	0 (4)	0.45
Plasma viscosity (mPas)	1.32 (0.06)	1.30 (0.08)	-0.02 (0.07)	0.48
Red cell aggregation (AU)	4.4 (1.3)	4.0 (1.2)	-0.4 (1.4)	0.04
Fibrinogen (g/l)	3.2 (0.8)	3.4 (0.7)	+0.2 (0.7)	0.11
Factor VII activity (IU/dL)	114 (21)	107 (20)	-7 (17)	0.006
PAI activity (% pool)	157 (62)	150 (69)	-7 (59)	0.23
t-PA antigen (μg/mL)	8.7 (4.5)	8.3 (3.1)	-0.4 (3.4)	0.12

Data as mean (SD), n=45

Table 6-10 Relationships between body mass index and waist to hip ratio with haemostatic and rheological factors at baseline

Haemostatic factors	BMI kg/m^2	WHR
Whole blood viscosity (mPas)	r= 0.111 p= 0.474	r= 0.168 p= 0.282
Haematocrit (%)	r= -0.123 p= 0.431	r= -0.125 p= 0.431
Plasma viscosity (mPas)	r= 0.369 p= 0.016	r= 0.381 p= 0.014
Red cell aggregation (AU)	r= 0.245 p= 0.110	r= 0.171 p= 0.274
Fibrinogen (g/L)	r= 0.471 p= 0.002	r= 0.221 p= 0.170
Factor VII activity (IU/dL)	r= 0.020 p= 0.902	r= -0.253 p= 0.111
PAI activity (% pool)	r= 0.410 p= 0.006	r= 0.347 p= 0.026
t-PA antigen (μg/mL)	r= 0.252 p= 0.150	r= -0.072 p= 0.685

n=45

Table 6-11 Relationships between body mass index and waist to hip ratio with haemostatic and rheological factors at week twelve

Haemostatic factors	BMI kg/m^2	WHR
Whole blood viscosity (mPas)	r= 0.262 p= 0.102	r= 0.184 p= 0.299
Haematocrit (%)	r= 0.044 p= 0.783	r= 0.072 p= 0.683
Plasma viscosity (mPas)	r= 0.209 p= 0.207	r= 0.142 p= 0.430
Red cell aggregation (AU)	r= 0.400 p= 0.009	r= 0.683 p= 0.835
Fibrinogen (g/l)	r= 0.460 p= 0.175	r= 0.175 p= 0.323
Factor VII activity (IU/dL)	r= 0.021 p= 0.892	r= 0.419 p= 0.009
PAI activity (% pool)	r= 0.508 p= 0.001	r= 0.508 p= 0.014
t-PA antigen (μg/mL)	r= 0.207 p= 0.290	r= 0.207 p= 0.182

n=45

Table 6-12 Relationships between changes in body mass index and waist to hip ratio with changes in haemostatic and rheological risk factors for all subjects

Change in haemostatic factors	Change in BMI kg/m^2	Change in WHR
Change in whole blood viscosity (mPas)	r= -0.13 p= 0.421	r= -0.01 p=0.946
Change in haematocrit (%)	r= 0.11 p= 0.491	r= -0.16 p= 0.373
Change in plasma viscosity (mPas)	r= -0.20 p= 0.244	r= 0.10 p= 0.614
Change in red cell aggregation (AU)	r= -0.09 p= 0.551	r= 0.34 p= 0.057
Change in fibrinogen (g/l)	r= 0.01 p= 0.946	r= -0.04 p= 0.841
Change in factor VII activity (IU/dL)	r= 0.39 p=0.014	r= -0.18 p= 0.337
Change in PAI activity (% pool)	r= 0.43 p= 0.005	r= -0.30 p= 0.101
Change in t-PA antigen (μg/mL)	r= 0.21 p= 0.305	r= -0.15 p= 0.509

n=45

Table 6-13 Relationships between haemostatic and rheological factors and plasma lipids at baseline

Haemostatic factors	Cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
Whole blood viscosity (mPas)	r = 0.106 p = 0.577	r = 0.166 p = 0.409	r = 0.279 p=0.142	r = 0.427 p = 0.021
Haematocrit (%)	r = 0.054 p = 0.775	r = 0.102 p = 0.614	r = -0.144 p=0.457	r = 0.250 p = 0.457
Plasma viscosity (mPas)	r = 0.418 p = 0.027	r = 0.454 p = 0.023	r = -0.214 p=0.285	r = 0.384 p = 0.285
Fibrinogen (g/L)	r = 0.040 p = 0.843	r = -0.023 p = 0.913	r = 0.083 p = 0.686	r = 0.020 p = 0.686
Factor VII activity (IU/dL)	r = 0.099 p = 0.610	r = -0.015 p = 0.944	r = 0.019 p = 0.922	r = 0.181 p = 0.922
PAI activity (% pool)	r = 0.356 p = 0.063	r = 0.228 p = 0.273	r = -0.037 p = 0.856	r = 0.354 p = 0.856
t-PA antigen (µg/mL)	r = 0.448 p = 0.037	r = 0.270 p = 0.263	r = 0.07 p = 0.758	r = 0.288 p = 0.758

n=45

Table 6-14 Relationships between haemostatic and rheological factors and plasma lipids at week twelve

Haemostatic factors	Cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
Whole blood viscosity (mPas)	r = 0.066 p = 0.279	r = -0.123 p = 0.568	r = -0.296 p=0.143	r = 0.218 p = 0.284
Haematocrit (%)	r = 0.112 p =0.571	r = 0.106 p = 0.615	r = -0.150 p=0.359	r = 0.337 p = 0.085
Plasma viscosity (mPas)	r = 0.450 p = 0.021	r = 0.448 p = 0.032	r = -0.224 p=0.318	r = 0.327 p = 0.111
Fibrinogen (g/L)	r = 0.119 p = 0.563	r = 0.089 p = 0.694	r = -0.183 p = 0.450	r = 0.294 p = 0.153
Factor VII activity (IU/dL)	r = 0.338 p = 0.068	r = 0.145 p = 0.471	r = 0.305 p = 0.287	r = 0.131 p = 0.497
PAI activity (% pool)	r = 0.225 p = 0.250	r = 0.140 p = 0.505	r = -0.056 p = 0.956	r = 0.347 p = 0.076
t-PA antigen (µg/mL)	r = 0.490 p = 0.024	r = 0.574 p = 0.010	r = -0.401 p = 0.378	r = 0.572 p = 0.007

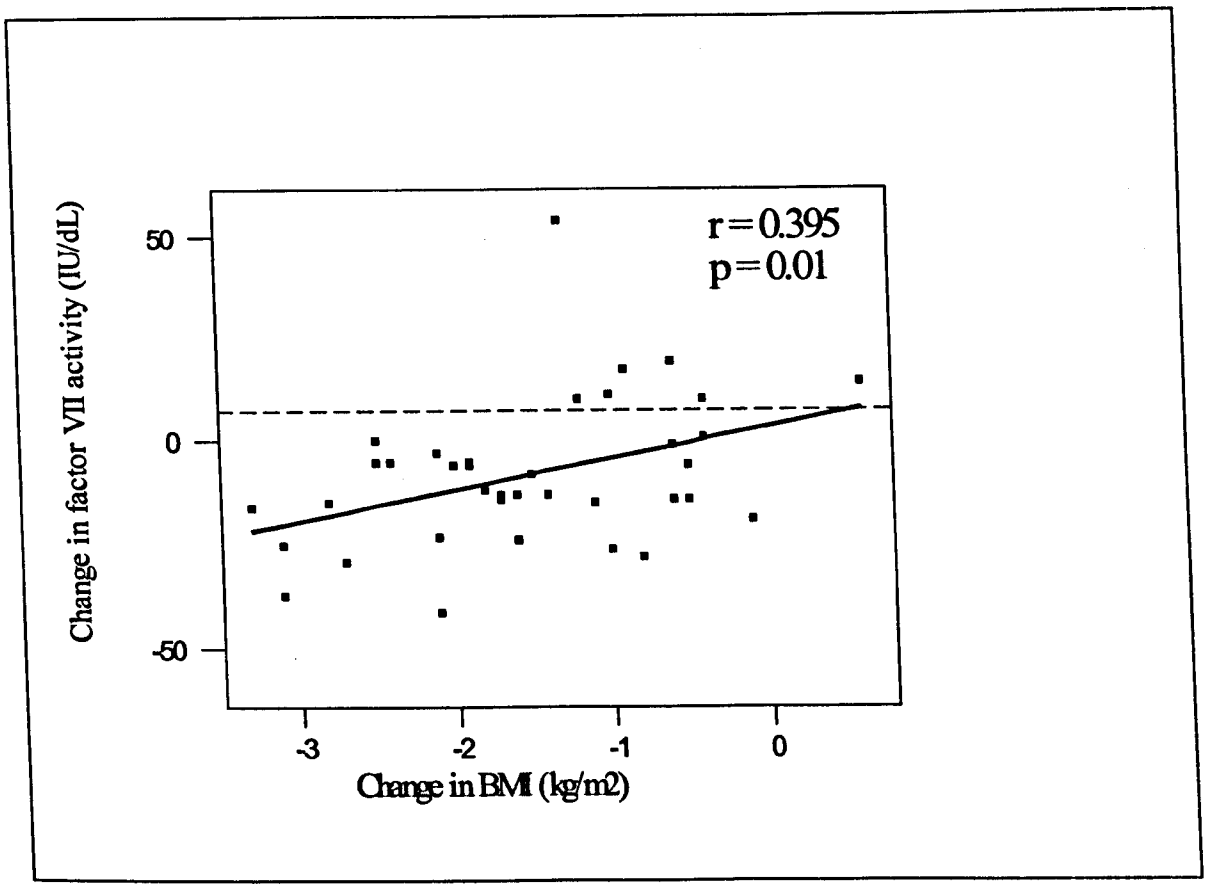
n=45

Table 6-15 Relationships between changes in haemostatic and rheological factors and plasma lipids

Change in haemostatic factors	Change in cholesterol mmol/L	Change in LDL cholesterol mmol/L	Change in HDL cholesterol mmol/L	Change Triglyceride mmol/L
Whole blood viscosity (mPas)	r=-0.238 p=0.242	r= 0.154 p= 0.484	r= -0.180 p= 0.390	r= -0.002 p= 0.994
Haematocrit (%)	r=0.021 p=0.917	r= -0.016 p= 0.942	r= -0.189 p= 0.356	r= -0.424 p= 0.031
Plasma viscosity (mPas)	r= 0.262 p=0.228	r= -0.336 p= 0.148	r= -0.212 p= 0.331	r= -0.191 p= 0.394
Red cell aggregation (AU)	r= 0.259 p= 0.183	r= -269 p= 0.194	r= -0.194 p= 0.616	r= -0.064 p= 0.750
Change in fibrinogen (g/l)	r= -0.028 p= 0.896	r= 0.012 p= 0.957	r=0.111 p=0.652	r= 0.191 p= 0.372
Factor VII activity (IU/dL)	r= 0.098 p= 0.620	r= 0.015 p= 0.942	r= 0.200 p= 0.316	r= -0.072 p= 0.721
PAI activity (% pool)	r= 0.373 p= 0.061	r= -0.226 p= 0.300	r= 0.224 p= 0.281	r= 0.083 p= 0.693
t-PA antigen (μg/mL)	r= 0.141 p= 0.576	r= 0.091 p= 0.737	r= 0.230 p= 0.374	r= 0.065 p= 0.798

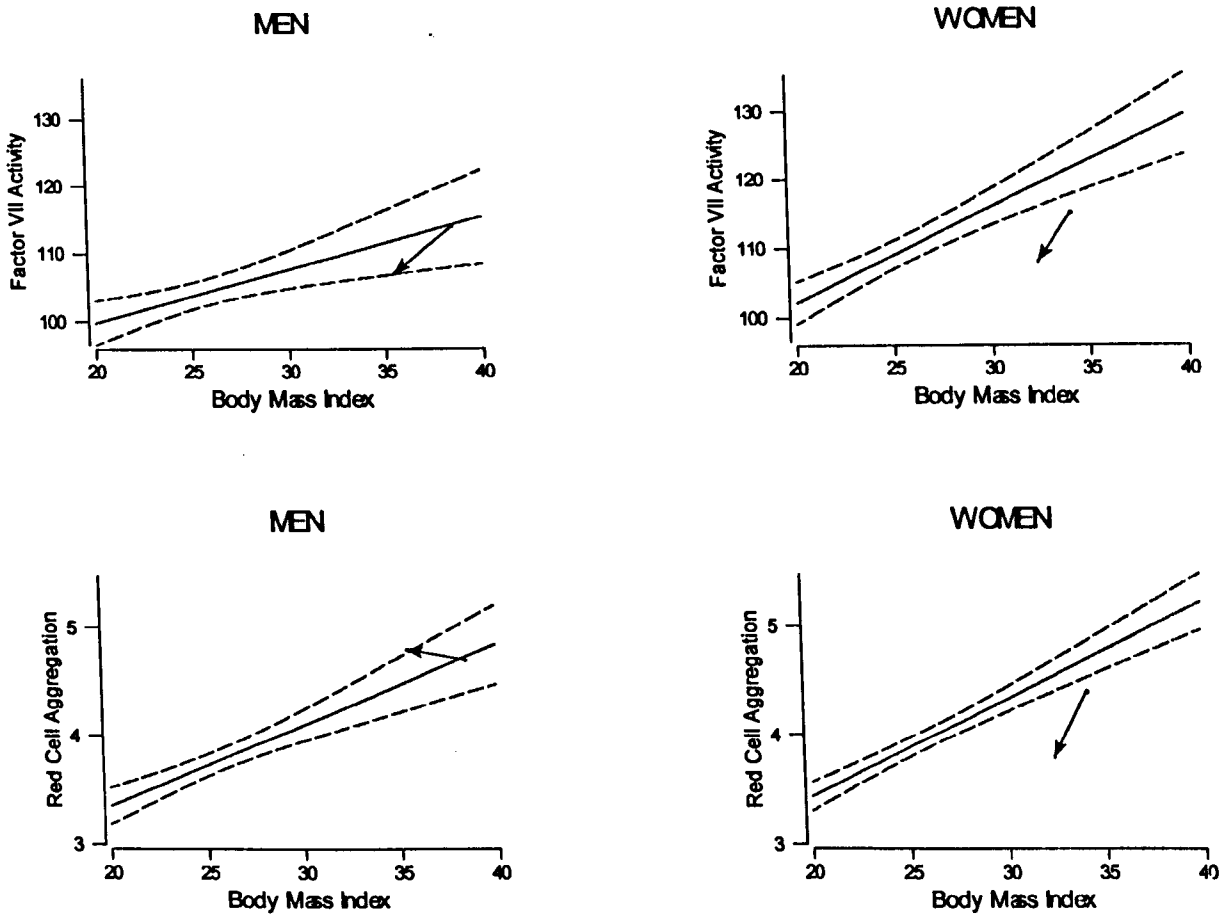
n=45

Figure 6-1 Relationship between changes in factor VII activity and BMI



n = 37 (20 women, 7 men)

Figure 4-2 Fitted regression lines of red cell aggregation and factor VII activity against body mass index for the MONICA subsets of men and women



Dashed lines show the 95% confidence interval for the mean. The lines indicate the changes achieved, on average, before and after intervention in the study women (n=36) and men (n=9)

Chapter 7 The effects of moderate weight loss on haemostatic and rheological factors and plasma lipids in overweight subjects with angina pectoris

7.1 Introduction and hypotheses

A number of clinical studies support the epidemiological observations that haemostatic and fibrinolytic measurements are altered in the overweight (Le Devehat *et al,* 1992). In addition, some of these haemostatic and fibrinolytic factors have shown greater alterations in those with known IHD and angina (Aznar *et al,* 1988). The present study used recommended dietary composition (COMA, 1991) and a moderate daily energy restriction to investigate the effects of weight loss in angina subjects.

The main aim of this study was to investigate the effect of moderate weight loss on haemostatic and rheological factors in older overweight subjects with established IHD, in this case angina pectoris, who might show greater changes than healthy subjects. The effects of weight loss on plasma lipid concentrations and the frequency of anginal pain were also examined.

A link between plasma lipid concentrations and haemostatic and rheological factors has been observed (Miller *et al*, 1986). The hypothesis that improvements in plasma lipid concentrations are associated with improvements in haemostatic and rheological factors has been proposed (Mitropoulos *et al*, 1991). The present study examined relationships between the triglyceride and total, HDL and LDL cholesterol concentrations at baseline and between changes in the measurements after dietary intervention for weight loss.

There were four hypotheses to be tested in this study of overweight subjects diagnosed with angina pectoris. It was hypothesised that the effects of dietary intervention on the principal outcome measures may be increased in subjects with established IHD.

1) A dietary intervention incorporating an energy deficit approach to weight loss (600 kcal per day) would be effective in achieving losses of body weight close to 0.5 kg per week in subjects with angina.

2) Weight loss after a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets would improve haemostatic and rheological risk factors for IHD in overweight subjects with angina.

3) Weight loss after a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets would improve plasma lipids in overweight subjects with angina.

4) Dietary intervention and weight loss would reduce anginal symptoms.

7.2 Methods

7.2.1 Study design and statistical approaches

A single stranded design was used for the study. This method was chosen according to the principles described in chapter 5. The same power analyses calculated for the previous study (chapter 4) was used to determine the required sample size of 48 based on factor VII as the principle outcome measure. Parametric statistical methods were applied as the differences between the haemostatic, rheological and lipid measurements were normally distributed, when determined by a distribution plot. The relationships between the changes in haemostatic and rheological measurements and plasma lipids were examined using Pearson's correlation analyses.

7.2.2 Subject recruitment

Fifty-four overweight subjects with angina (31 men, 23 women) with a BMI > 26 kg/m^2

who had been given a medical diagnosis of angina pectoris but who had not had dietetic advice were recruited. Several West of Scotland newspapers included the recruitment appeal as feature stories (appendix 4). All respondents made telephone contact with the department and were posted an initial screening questionnaire which requested them to provide personal details including their age, anthropometric measurements and medical history. Angina was confirmed with a positive modified Rose questionnaire completed when the subjects attended the Department of Human Nutrition (Rose, 1962). All subjects described at least one period of unsuccessful slimming in the preceding 2 year period before commencing the study, though no one had reduced their weight by greater than 3.0 kg during the 3 month period prior to recruitment. Ethical approval for the study was obtained from the Glasgow Royal Infirmary Joint Ethics Committee and signed informed consent was obtained from all participants.

Coagulation and haemostatic measures from study subjects were compared with existing data from a representative West of Scotland population: the North Glasgow Third MONICA survey database (WHO, 1987). For men and women a representative subset of the MONICA population was selected on the criteria of having a diagnosis of angina given by a doctor, no history of stroke, a positive Rose questionnaire, no Q\QS interval on the ECG and no history of MI. A total of 77 men and 74 women fulfilled these criteria. Values were available for both RCA and BMI for 52 men and 41 women and for factor VII activity and BMI for 60 men and 51 women.

7.2.3 Physical measurements

Each subject attended the University of Glasgow Department of Human Nutrition on 5 occasions over a 12 week study period. Body weight, waist and hip circumferences were measured and BMI, body fat composition and WHR were calculated.

In order to characterise health of the subjects, 2 further measures were made. Any cardiac changes reflecting coronary disease which may have also been present in addition to the angina, were examined using an ECG (Cardiofax V, Nihon Kohdon). In addition, the presence or absence of PVD was assessed using the brachial and ankle systolic blood pressures. The pressure measurements were made using an ultra-sonic flow detector and ABPI was calculated (Yao *et al*, 1969). The ABPI measurement reflects long term vascular disease and does not change acutely over a short period such as 12 weeks. For these reasons the measurement was only made at baseline (Yao, 1973). Subjects reported medication use and anginal frequency at baseline and study week 12. The subjects reported the frequency of anginal pain alongside the food intake information in the 7 day WI diaries.

7.2.4 Dietary intervention and monitoring

Dietary intervention used an individualised dietary prescription based on the indirect measurement of REE, because prediction equations for BMR are only valid for healthy subjects. This was measured using an indirect ventilated hood calorimeter (Delta-trac) which was calibrated at 4 weekly intervals by alcohol burning (Merilainen *et al* 1987). The predicted BMR was also calculated using the Schofield equation (Schofield *et al*, 1986) and the two methods were compared. This methodology has been described in greater detail in chapter 5.

Dietary intake was monitored by the subjects completion of 7 day WI food diaries during the week before dietary intervention, (baseline), and at week 5, 7 and 12 of the intervention period. The first (week 0) and last (week 12) monitoring periods were considered the most important and only their values and the differences between the two measurements are given in the results. The final recording day on each occasion was the

day before the last study visit. The dietary records were completed using the standardised methodology already described (chapter 5).

7.2.5 Venesection and laboratory methods

Venesection was performed at baseline, (pre-intervention) and week 12 (post-intervention). The measurements made included haemostatic and rheological factors, factor VII activity, fibrinogen, PAI activity, t-PA antigen, plasma viscosity, RCA, haematocrit and plasma lipids. The lipid measurements comprised total and HDL cholesterol and triglyceride. The LDL cholesterol concentration was calculated using the Friedwald equation (Friedwald, 1972). The measurements were all carried out according to the established methods described earlier (chapter 5).

7.3 Results

The characteristics of the subjects who completed the study are described (table 7.1-7.3). The prescribed medications for these subjects relevant to their cardiovascular disease were glyceryl trinitrate spray (27), aspirin (20), diuretics (12), β blocking agents (29) and lipid lowering medications (1). There was no change in any prescribed medications after the dietary intervention was complete.

There were no significant differences between the sexes for age ($p=0.76$) however the mean BMI in men was greater than that in women 30.9 (SD 4.5) kg/m^2 (range 25.6 to 40.9) vs. 27.8 (SD 2.5) kg/m^2 (range 25.0 to 32.7) ($p=0.04$). Mean weight change over the 12 study weeks was similar in men and women at 3.5 kg (table 7.1-7.3). The frequency of anginal pain in all subjects between baseline and week 5 was unchanged, but was significantly reduced below baseline at weeks 7 and 12 (table 7.4, figure 7.1).

Mean energy prescription was 6217 (SD 1498), range 4184-1172 kJ, and 1485 (SD 358),

range 1000-2670 kcal, per day for all subjects. For men this was 6941 (SD 1292), range 5020-10460 kJ, and 1658 (SD 309), range 1199-2500 kcal, and for women 5322 (SD 1292), range 4184-7949 kJ, and 1271 (SD 309), range 1000-1899 kcal. The measurements made with the Delta-trac metabolic monitor are shown (table 7.3). Comparison of the estimated BMR calculated using the Schofield equations 6703 (SD 1025) kJ and 1602 (SD 245) kcal, and the measured values, 6113 (SD 1058) kJ and 1461 (SD 253) kcal showed the estimated values significantly exceeded the actual values ($p=0.0062$). This comparison illustrated the value of measuring REE in subjects who did not match the criteria of healthy subjects aged below sixty years of age, from whom the equations were derived.

Recorded dietary intakes were 106% of total daily energy prescription for all subjects at week twelve, and 105% and 107% for men and women respectively. Dietary analysis showed elevated protein and decreased carbohydrate in comparison with the dietary prescription (table 7.5-7.7). After dietary intervention, reported dietary intakes fell significantly for energy and percentage energy provided by fat and alcohol, and dietary protein and carbohydrate increased (except for alcohol in men and women) for both sexes (table 7.5-7.7).

The biochemical measurements are reported at baseline and week 12 together with the differences for all subjects. Factor VII activity at baseline was higher in women than men, 130 (SD 20) vs. 114 (SD 26) IU/dL ($p=0.04$) but no sex difference was observed in the other measures (table 7.1-7.3).

Of all the haemostatic and fibrinolytic indices measured, significant changes were only observed in RCA and factor VII activity (table 7.1 - 7.3). The RCA and factor VII values were compared with a subset of the North Glasgow Third MONICA survey population. These plots show men and women separately because there was an effect of sex on factor

VII activity (p=0.0002) and a sex by BMI interaction on RCA (p=0.02) in the MONICA data. This showed baseline values well below the regression line for all measures excepting factor VII activity in men. RCA and factor VII activity in the present study showed similar falls with 4.2% weight loss (figure 7.2). No significant changes were found in the other rheological or fibrinolytic measurements. There was some suggestion of a trend towards reductions in PAI activity in men with weight loss (p=0.09).

Waist circumferences were significantly reduced in all subjects (p=0.0005), in men (p=0.0007), but not women alone (p=0.11). A significant correlation was found between the changes in factor VII activity and changes in BMI for all subjects and in the group of men (table 7.8-7.10). Changes in HDL cholesterol were significantly related with changes in waist in all subjects and men and in WHR for all subjects (table 7.8-7.10). No other significant relationships were found between changes in body weight or alterations in waist circumference and changes in biochemical measures throughout the course of the study.

To examine whether a normal or altered ECG measurement or the presence of PVD would influence haemostatic and rheological factors the study sample was divided according to ECG or ABPI measurements. ABPI measurements were only made at baseline, as the measurement reflects long term changes in the peripheral vasculature which take place over years, not weeks (Yao, 1973). Eleven subjects (2 women) showed signs of occlusive PVD with ABPI below 0.9. No differences in plasma measures could be related to ABPI. There was no effect of lowered ABPI measurements on baseline measures or changes in biochemical measures.

Twenty-seven subjects had a normal ECG (14 women), whereas 13 subjects (4 women) showed sinus bradycardia and 9 (4 women) showed significant signs of myocardial ischaemia. There were no differences in biochemical measures according to ECG at

baseline, or in the changes in biological measures after intervention. The exception was factor VII activity. The group with a normal ECG showed a significant fall in mean factor VII activity 121 (SD 21) to 115 (SD 19) IU/dL, mean difference 6 (SD 16) IU/dL (p=0.037) but this fall was not observed in the other two groups after dietary intervention.

Plasma cholesterol was reduced by 6% in all subjects (p=0.0001), in men (p=0.019), and in women (p=0.011), HDL cholesterol was unaffected by body weight change. The LDL cholesterol fraction was significantly lowered in all subjects (p=0.008), in men (p=0.010) and women (p=0.021) (table 7.1-7.3).

At baseline, HDL cholesterol was significantly correlated with factor VII activity in the group of men and in all subjects (table 7.12 – 7.13). Factor VII activity was also related to total cholesterol concentrations at baseline in women and all subjects (table 7.11, 7.13). For women baseline t-PA antigen was significantly correlated with total and LDL cholesterol concentrations (table 7.11). For men, triglyceride concentration was correlated with PAI activity (table 7.12). For all subjects factor VII activity was significantly correlated with total and HDL cholesterol concentrations (table 7.13).

After intervention, some significant relationships between the changes in the LDL cholesterol fraction and in the haemostatic and fibrinolytic factors were seen (table 7.14-7.16). For all subjects there were no significant correlations between changes in total cholesterol, HDL cholesterol and triglyceride concentrations and the fibrinolytic indices t-PA antigen and PAI activity and the coagulation indices, fibrinogen and factor VII activity. However, significant relationships between changes in LDL cholesterol and haemostatic factors were found in all subjects. Figure 7.3 shows the correlation between changes in PAI activity and LDL cholesterol (p=0.002). Figure 7.4 shows a similar relationship between changes in t-PA antigen and LDL cholesterol (p=0.010). Significant correlations were found between the changes LDL cholesterol concentrations and factor

VII activity (p=0.07) (figure 7.5) and between changes in fibrinogen and LDL cholesterol concentrations (p=0.033) (figure 7.6). Figures 7.3 - 7.6 show that a number of potential outlying values can be observed, a re-analysis with the removal of apparent outliers showed no effects on the significance of any final relationships. These analyses suggest a number of relationships between changes in haemostatic and rheological measures and changes in plasma lipids.

7.4 Discussion

This study has demonstrated improvements, from quite modest weight losses, in four indices related to IHD risk. These were RCA, factor VII activity, plasma total and LDL cholesterol concentrations and the frequency of anginal pain.

However, the first hypothesis for this study concerned the approach used to achieve weight loss. This was that a dietary intervention incorporating an energy deficit approach to weight loss would be effective in achieving weight losses of close to 0.5 kg per week in subjects with IHD. The 3.5 kg (4%) weight loss was only 58% of what should have been achieved with 100% compliance to the dietary advice. A comparison with the weight loss achieved by other workers after a 3 month study, suggests it is a little below the average 4.5 kg achieved using similar approaches (Goldstein, 1992, Wadden, 1993). It has been proposed that increasing age may decrease the effectiveness with which dietary changes can be adopted (Seidell, 1996, Schaefer *et al*, 1997). This hypothesis may provide a partial explanation as to why these older subjects lost less weight than their younger counterparts given the same dietary intervention (chapter 6). However, research findings either supporting or refuting a negative relationship between increasing age and decreased intentional weight loss are scarce. The lack of evidence may be related to the majority of studies comprising of small and usually unrepresentative volunteer populations

(Williamson *et al*, 1992).

The condition of angina may lead patients to become less active in order to reduce the number of acute anginal episodes. This change may favour gradual weight gain, or oppose weight loss. However, moderate physical activity should usually be recommended for angina patients to strengthen their heart muscles, and possibly encourage re-vascularisation of the myocardium. Although the subjects with angina who completed this study were not inactive, they anecdotally reported that they had become less active than prior to the onset and diagnosis of angina pectoris.

The extent of compliance with dietary advice directly effects the amount of weight loss achieved. It has been shown that the presence of a medical diagnosis often leads to improved compliance with any treatment programme particularly a diagnosis of IHD (Barnes and Terry, 1991). The subjects in the present study were not newly diagnosed with angina although all had all volunteered to participate in this dietary study. This perhaps indicated a personal interest or concern, which could have already led to their achieving as much weight loss as possible, alone. The reported dietary intakes completed by all study participants at baseline showed a lower fat intake than estimated to be usually consumed by the Scottish population (Gregory *et al*, 1990). Once more this finding may indicate a greater personal interest in this group.

Improved nutritional intakes, such as the lower fat intake may reflect selective under-reporting of food intakes. A recent a Finnish study has examined the effect of under reporting on the macronutrient composition of a group. The presence or absence of subjects known to be under-reporting their dietary intakes within different statistical analyses failed to significantly affect the percentage energy values for macro-nutrients (Hirvonen *et al*, 1997). These results are particularly encouraging for the validity of the present findings.

The second hypothesis was whether weight loss after a dietary intervention would improve haemostatic and rheological risk factors for IHD. The improvement in RCA after weight loss is in agreement with studies of NIDDM subjects (MacRury *et al*, 1993) and healthy adults given a VLCD (Poggi *et al*, 1994). The stability of plasma viscosity following weight loss is also in accord with previous studies (Fanari *et al*, 1993) and suggests that substantial weight losses are required to change this measure. The acute effects of a VLCD and weight loss have both been seen to reduce plasma viscosity (6.9 % higher at baseline than in our study) (Poggi *et al*, 1994). An 11% weight loss in adolescents with BMI greater than 30 kg/m^2 significantly reduced plasma viscosity (Fanari *et al*, 1993). Our findings of an unchanged haematocrit following dietary intervention and weight loss concur with the majority of studies (Le Devehat *et al*, 1992, Palareti *et al*, 1994) but are at variance with others (Poggi *et al*, 1994). It seems likely that much greater weight loss would be necessary to achieve significant changes. Additionally, the mean haematocrit for both men and women were not elevated but remained within the laboratory reference ranges at baseline and study week 12. It was unfortunate that in the present study equipment limitations meant that only RCA measures on women could be made.

In order to examine the relationships between BMI and RCA in a larger West of Scotland population, a comparison with a representative subset from the MONICA population was made. For men and women the mean values for RCA were below the regression line for the MONICA data.

As described in chapter 6, the 95% confidence intervals only provide a guide as to what is anticipated about the relationships between mean RCA and mean BMI in the MONICA comparison because the intervals are valid for the individual rather than the mean BMI.

The reasons why the study population had a lower RCA than the MONICA population could include an effect of a small sample size and reflect the sample including men and women with low mean RCA. A possible role for laboratory influences exists, although identical equipment was used to measure RCA in this study to that reported in chapter 6, where the mean values for RCA in chapter 6 were much higher. In addition, the subset of the MONICA population with angina were much smaller than the subset compared with the study sample for chapter 6.

Other studies have suggested only a small influence from a combination of changed dietary composition and weight loss on plasma fibrinogen concentrations but they have all lacked power (Le Devehat *et al*, 1992, Palareti *et al*, 1994). The influence of dietary composition on plasma fibrinogen concentrations has been shown to be small (Fehily *et al*, 1982). Although the power of the present study was low with respect to plasma fibrinogen concentrations, the values for the study population allowed the sample to be classified according to the quintile of cardiovascular risk. The present study population had mean plasma fibrinogen concentrations in the highest quintile of cardiovascular risk, 3.25 g/L and above (Lowe *et al*, 1987, Fowkes *et al*, 1994), a finding which was in agreement with the presence of IHD.

Factor VII activity has been linked with increased body weight and dietary fat intake and the significant decrease in factor VII activity achieved in this study concurs with others (Marckmann *et al*, 1991, Palareti *et al*, 1994). In contrast, other workers using a 1200 kcal weight reducing diet in overweight subjects failed to achieve a significant change in factor VII activity despite a similar weight loss (Baron *et al*, 1989). This fall in factor VII activity was less than that observed in a previous study with healthy overweight subjects (Hankey *et al*, 1997, chapter 6) whose factor VII activity was 5% lower than that of the angina subjects in this present study. This may suggest dietary modifications and weight

loss in those with IHD are less effective in reducing IHD risk factors than in those in good health.

The observed effects on factor VII activity in this present study and that reported in chapter 6, are most likely to reflect actual weight loss, and not the effects of starvation seen in VLCD studies (Palareti *et al*, 1994). Factor VII activity has been related to a high fat diet, and the study sample reported consuming a fat intake 5% below the figure shown for the Scottish population by the Survey of British Adults (Gregory *et al*, 1990). Thus, the effects of dietary intervention in the angina subjects may not have led to marked changes in dietary composition. The lack of effect of altered ECG on factor VII activity was also surprising, as it was the group with the normal ECG who showed the improvement in plasma factor VII activity. It may have been expected that the presence of ischaemia (shown on ECG trace) would have been related with increased factor VII activity (Meade *et al*, 1991).

Once more a comparison was made between the mean factor VII activity of the study sample and with the values in a subset of the MONICA population. This provided information about what could be anticipated about the relationship between mean factor VII activity and mean BMI. The reasons why men had a mean factor VII activity above the MONICA subset with angina, and females were below, is open to speculation. It could be that the smaller MONICA subset with angina was not as representative as the larger subset included in chapter 6. Most importantly the data suggest that the study sample were at lower IHD risk than the MONICA subset, and this may confirm the theory that they had, prior to enrolling in the study, already been trying to improve the composition of their diets and control their body weight.

A number of studies have succeeded in lowering PAI activity and t-PA antigen following large weight losses in subjects whose fibrinolytic activity was impaired (Landin *et al*,

1990, Folsom *et al*, 1993, Palareti *et al*, 1994). The PAI activity at baseline of the IHD subjects was 9% below the values for the overweight but healthy subjects (Hankey *et al*, 1997, chapter 6) which suggested that fibrinolytic activity was not raised. The stability of the fibrinolytic variables may also reflect insufficient weight loss, and confirm the stability of the subjects' angina.

The third hypothesis, that a reduction in plasma cholesterol and its fractions would improve with moderate weight loss was proven according to the findings of this study. The value of weight loss in improving plasma lipid concentrations has been shown previously (Wolf and Grundy, 1983, Folsom *et al*, 1993, Svedsen *et al*, 1995). However, the reductions in plasma lipids achieved in this study are encouraging for the value of moderate weight loss. The significant fall in plasma LDL concentrations was of particular interest, given LDL cholesterol concentrations of greater than 4.14 mmol/L are an independent risk factor for coronary artery disease (Expert Panel, 1988). The falls in LDL cholesterol of 4% in women, 8% in men and 6.5% overall are comparable with free living dietary modification studies (Hjerman *et al*, 1981). Recently, the main determinants of LDL have been identified as baseline LDL concentrations and age (Schaefer *et al* 1997), both of which were raised in this study. In contrast, the stability of the unchanged HDL cholesterol and triglyceride concentrations support the finding that the weight loss achieved was insufficient to affect these measures (Goldstein, 1992), or in the case of triglyceride, that the plasma concentrations were already low. The stability of HDL may reflect two contrasting effects, a fall in body weight and a decrease in dietary fat intake (Leenen *et al*, 1994).

The expected positive relationship between factor VII activity and triglyceride was not found in this study (Miller *et al*, 1986, Mitropoulos *et al*, 1989, Mitropoulos, 1994) nor was any relationship between the changes in other haemostatic factors and triglyceride

concentrations. The explanation probably lies in the very low mean triglyceride concentrations in the angina subjects (1.7 mmol/L), well below the upper reference range of 2.3 mmol/L. This observation possibly indicates previous dietary improvement in these subjects. Raised triglyceride concentrations are usually associated with increased body size and low HDL cholesterol concentrations (Seidell *et al*, 1992). Other workers have identified a positive relationship between triglyceride concentrations and factor VII activity (Mitropoulos *et al*, 1989). However, this subject group had wider ranging triglyceride concentrations, the mean 2.0 (SD 1.6) and range of 0.4 - 10.1 mmol/L far wider than in the present study (0.6 - 4.45 mmol/L).

The positive relationships between the changes in LDL cholesterol after weight loss and plasma fibrinogen concentrations, factor VII activity, PAI activity and t-PA antigen provide more evidence to support the many benefits from moderate weight loss on IHD risk factors.

The finding that weight loss and dietary change improve both haemostatic factors and plasma lipids after weight loss has been reported in a middle aged population (Folsom *et al*, 1992). These findings could not prove whether the improvements were causally linked. One possible explanation for the relationship between the benefits of moderate weight loss and dietary change could be simultaneous improvement in both the LDL cholesterol concentrations and the levels of haemostatic factors. However, the hepatic synthesis of haemostatic factors, particularly factor VII activity, and the lipid fractions may be related (Mitropoulos, 1994). The relationship between factors noted here has been known to be open to confounding by factors including age and cigarette smoking (Krauss *et al*, 1991).

The fourth hypothesis concerned the effect of dietary intervention for weight loss on the

symptoms of anginal disease. The finding of an improvement in anginal pain frequency with weight loss is in accordance with clinical impressions. Surprisingly, there are no reports of evidence from controlled trials for angina reduction with weight loss. It has recently been reported that close to 40% of obese "angina patients" are in fact free from significant ischaemic disease (Bahadori *et al*, 1995). In addition, it has been reported that the perception of angina deteriorates with advancing age (Umachandran *et al*, 1991), and the present study population were in the age group identified as least able to recognise anginal symptoms (Umachandran *et al*, 1991).

After 5 weeks of dietary intervention it was surprising that no change in the reported frequency of anginal pain was seen, despite the achievement of significant weight loss. However, the significant fall in the frequency of anginal pain at week twelve was most encouraging. Perhaps a minimum of 12 weeks of dietary intervention is required before dietary changes can significantly influence the frequency of anginal pain.

7.5 Conclusions

A 4% weight loss as part of a dietary intervention to change both the composition and quantity in overweight subjects with angina improves some, but not all, risk factors for IHD, the principal hypothesis of this study. The results also suggest that the changes after 12 weeks dietary intervention and moderate weight loss may be further increased with greater weight loss. This may lead to more valuable benefits on rheological and fibrinolytic measures. The improvements in plasma lipids, haemostatic and rheological measurements and plasma lipids support the broad benefits of moderate weight loss in those who are overweight with IHD.

Table 7-1 Characteristics and plasma biochemistry at baseline and week twelve for women

	Baseline	Week 12	Difference	p
Age (years)	61.2 (5.7)			
Weight (kg)	76.2 (8.2)	72.7 (8.1)	-3.5 (2.6)	0.00001
BMI (kg/m^2)	27.5 (2.7)	26.3 (2.8)	-1.2 (0.9)	0.00001
Body fat (%)	40.5 (1.9)	38.9 (1.9)	-1.6 (1.6)	0.0001
Waist circumference (cm)	106.3 (9.4)	104.3 (10.8)	-2.0 (5.8)	0.11
Waist to hip ratio	0.99 (0.06)	1.00 (0.08)	+0.01 (0.07)	0.48
Systolic blood pressure (mm Hg)	143 (16)	141 (17)	-2 (19)	0.61
Diastolic blood pressure (mm Hg)	89 (12)	86 (11)	-3 (15)	0.39
ABPI	0.9 (0.2)			
Plasma insulin (mU/L)	13.1 (7.6)	14.2 (11.6)	+1.1 (7.7)	0.54
Total cholesterol (mmol/L)	6.7 (1.2)	6.4 (0.9)	-0.3 (0.6)	0.011
HDL cholesterol (mmol/L)	1.4 (0.3)	1.3 (0.3)	-0.1 (0.1)	0.11
LDL cholesterol (mmol/L)	5.6 (1.1)	5.4 (0.9)	-0.2 (0.5)	0.021
Triglyceride (mmol/L)	1.7 (0.8)	1.6 (0.6)	-0.1 (0.6)	0.68
Haematocrit (%)	43 (6)	44 (5)	+1 (5)	0.67
Plasma viscosity (mPas)	1.33 (0.07)	1.30 (0.07)	-0.03 (0.07)	0.17
Red cell aggregation (AU)	3.8 (1.6)	2.8 (0.5)	-1.0 (1.3)	0.019
Fibrinogen (g/L)	3.5 (0.8)	3.4 (0.7)	-0.1 (0.8)	0.40
Factor VII activity(IU/dL)	130 (20)	121 (16)	-9 (22)	0.08
PAI activity (% pool)	124 (62)	125 (57)	+1 (41)	0.88
t-PA antigen (μg/mL)	10.4 (2.8)	9.9 (2.8)	-0.5 (2.0)	0.11

Data as mean (SD), n=22

Table 7-2 Characteristics and plasma biochemistry at baseline and week twelve for men

	Baseline	Week 12	Difference	p
Age (years)	59.5 (7.1)			
Weight (kg)	85.9 (12.4)	82.4 (11.9)	-3.5 (2.6)	0.00001
BMI (kg/m^2)	30.8 (4.5)	29.5 (4.3)	-1.3 (0.9)	0.00001
Body fat (%)	30.1 (3.7)	28.6 (4.7)	-1.5 (5.9)	0.22
Waist circumference (cm)	101.9 (8.9)	99.6 (9.2)	-2.3 (3.3)	0.0007
Waist to hip ratio	1.00 (0.05)	0.98 (0.06)	-0.02 (0.04)	0.0038
Systolic blood pressure (mm Hg)	144 (15)	143 (16)	-1 (15)	0.70
Diastolic blood pressure (mm Hg)	83 (6)	85 (23)	+2.0 (23)	0.58
ABPI	1.00 (0.23)			
Plasma insulin (mU/L)	12.9 (8.7)	9.7 (5.1)	-3.2 (7.4)	0.047
Total cholesterol (mmol/L)	5.9 (0.8)	5.5 (0.9)	-0.4 (0.8)	0.0019
HDL cholesterol (mmol/L)	1.2 (0.3)	1.1 (0.3)	-0.1 (0.1)	0.48
LDL cholesterol (mmol/L)	4.9 (0.8)	4.5 (0.8)	-0.4 (0.7)	0.010
Triglyceride (mmol/L)	1.8 (0.9)	1.7 (1.3)	-0.1 (0.6)	0.34
Haematocrit (%)	47 (5)	46 (4)	-1 (6)	0.62
Plasma viscosity (mPas)	1.27 (0.07)	1.28 (0.08)	+0.01 (0.09)	0.74
Red cell aggregation (AU)	*	*	*	*
Fibrinogen (g/L)	3.4 (1.1)	3.5 (0.8)	+0.1 (0.8)	0.51
Factor VII activity(IU/dL)	114 (26)	111 (21)	-3 (19)	0.53
PAI activity (% pool)	135 (79)	117 (65)	-18 (66)	0.094
t-PA antigen(μg/mL)	11.2 (3.5)	11.3 (3.9)	+0.1 (3.4)	0.77

Data mean (SD), n=27, * missing values

Table 7-3 Characteristics and plasma biochemistry at baseline and week twelve for all subjects

	Baseline	Week 12	Difference	p
Age (years)	60.3 (6.5)			
Weight (kg)	81.6 (11.7)	78.1 (11.3)	-3.5 (2.6)	0.0001
BMI (kg/m^2)	29.3 (4.3)	28.1 (4.0)	-1.2 (0.9)	0.0001
Body fat (%)	33.9 (5.9)	33.2 (6.3)	-0.7 (4.0)	0.087
Waist circumference (cm)	103.9 (9.3)	101.6 (10.2)	-2.3 (4.5)	0.0005
Waist to hip ratio	1.00 (0.06)	0.99 (0.07)	-0.01 (0.06)	0.40
Systolic blood pressure (mm Hg)	144 (16)	142 (17)	-2 (17)	0.99
Diastolic blood pressure (mm Hg)	86 (10)	85 (18)	-1 (19)	0.14
ABPI	0.96 (0.22)			
Plasma insulin (mU/L)	13.1 (8.1)	11.8 (8.9)	-1.3 (7.8)	0.29
Total cholesterol (mmol/L)	6.3 (1.1)	5.9 (1.0)	-0.4 (0.7)	0.0001
HDL cholesterol (mmol/L)	1.3 (0.3)	1.2 (0.3)	-0.1 (0.6)	0.092
LDL cholesterol (mmol/L)	4.6 (0.9)	4.3 (0.8)	-0.3 (0.6)	0.008
Triglyceride (mmol/L)	1.8 (0.8)	1.7 (1.0)	-0.1 (0.6)	0.31
Haematocrit (%)	45.5 (5.4)	45.0 (4.2)	-0.5 (5.6)	0.32
Plasma viscosity (mPas)	1.30 (0.08)	1.29 (0.08)	-0.01 (0.08)	0.33
Red cell aggregation (AU)	*	*	*	*
Fibrinogen (g/L)	3.4 (1.0)	3.6 (0.8)	+0.2 (0.1)	0.15
Factor VII activity(IU/dL)	120 (14)	115 (20)	-5 (20)	0.04
PAI activity (% pool)	134 (75)	123 (59)	-11 (57)	0.10
t-PA antigen (μg/mL)	10.9 (3.2)	10.6 (3.6)	-0.3 (2.8)	0.21

n=49

Data as mean (SD)

* missing values

Table 7-4 Angina frequency at baseline and weeks five, seven and twelve

	Baseline	Week 5	p	Week 7	p	Week 12	p
Angina frequency	3.2 (4.5)	3.4 (10.6)	0.28	1.6 (4.6)	0.027	1.4 (2.5)	0.009
Frequency of use of glyceryl trinitrate	5.3 (7.1)	*		*		4.3 (3.2)	0.17

Data as mean (SD), p values show the differences between baseline and weeks 5, 7 and 12,
n=49, * indicates failure to record glyceryl trinitrate and anginal pain measurements at weeks 5 and 7

Table 7-5 Measured resting energy expenditure and reported dietary intake before and after weight loss in women

	Baseline REE	Dietary intake Baseline	Week 12	Change	p
Energy kJ	6768 (1347)	6932	5611	-1033	0.003
		(6159, 7870)	(4562, 6247)	(-1887, +50)	
Energy kcal	1618 (322)	1657	1341	-247	0.003
		(1472, 1881)	(1112, 1493)	(-451, +12)	
Fat % energy		34.9	31.2	-2.5	0.006
		(29.8, 38.6)	(24.2, 35.3)	(-6.6, -0.4)	
Protein % energy		17.9	19.2	+1.5	0.015
		(16.2, 20.1)	(17.9, 20.6)	(-0.5, +3.4)	
Carbohydrate % energy		46.2	48.0	+2.4	0.092
		(42.3, 49.3)	(44.4, 51.6)	(-56.7, +14.9)	
Alcohol % energy		1.1	0.3	-0.2	0.485
		(0.0, 2.8)	(0.0, 2.3)	(-1.7, +0.6)	

Data as median and inter-quartile range except for REE measure mean (SD), n= 22

Table 7-6 Measured resting energy expenditure and reported dietary intake before and after weight loss in men

	Baseline REE	Dietary intake Baseline	Week 12	Change	p
Energy kJ	6506	8707	7201	-5665	0.0001
	(1075)	(7778, 10259)	(5732, 8594)	(-6518, -4527)	
Energy kcal	1555 (257)	2081	1721	-1354	0.0001
		(1864, 2452)	(1370, 2054)	(-1557, -108)	
Fat % energy		32.2	29.9	-2.8	0.001
		(29.5, 35.4)	(26.7, 32.8)	(-8.0, -0.2)	
Protein % energy		16.9	19.4	+1.3	0.002
		(14.7, 19.8)	(17.1, 20.8)	(0.0, +4.1)	
Carbohydrate % energy		44.4	46.5	+2.7	0.015
		(41.0, 48.7)	(43.3, 53.5)	(-1.5, +7.7)	
Alcohol % energy		2.8	0.75	0.0	0.478
		(0.2, 6.6)	(0.0, 5.9)	(-1.9, +0.5)	

Data as median and inter-quartile range except for REE measure mean (SD), n=27

Table 7-7 Measured resting energy expenditure and reported dietary intake before and after weight loss in all subjects

	Baseline REE	Dietary intake Baseline	Week 12	Change	p
Energy kJ	6106 (1057)	7929	6108	-1439	0.00001
		(6564, 8865)	(5213, 8075)	(-2247, -88)	
Energy kcal	1461 (253)	1895	1460	-344	0.0001
		(1569, 2119)	(1246, 1930)	(-537, -21)	
Fat % energy		33.4	30.0	-2.7	0.00001
		(29.5, 38.2)	(25.9, 33.5)	(-6.8, - 0.3)	
Protein % energy		17.4	19.3	-0.7	0.00001
		(15.6, 19.9)	(17.6, 20.7)	(-2.3, +0.8)	
Carbohydrate % energy		45.5	48.0	-1.3	0.004
		(40.9, 48.9)	(44.0, 53.4)	(-3.8, +2.3)	
Alcohol % energy		1.4	0.2	-0.5	0.0019
		(0.0, 5.3)	(0.0, 3.9)	(-2.7, +1.9)	

Data as median and inter-quartile range except for REE measure mean (SD), n=49

Table 7-8 Relationships between changes in body mass index, waist circumference and waist hip ratio and changes in haemostatic and rheological risk factors after weight loss for women

Change in haemostatic factors	Change in BMI (kg/m^2)	Change in waist (cm)	Change in WHR
Haematocrit (%)	r = 0.288 p = 0.247	r = 0.198 p = 0.431	r = 0.198 p = 0.431
Plasma viscosity (mPas)	r = 0.025 p = 0.931	r =0.059 p = 0.841	r = 0.112 p = 0.703
Red cell aggregation (AU)	r = -0.202 p = 0.632	r = 0.192 p = 0.649	r = 0.192 p = 0.649
Fibrinogen (g/L)	r = 0.016 p = 0.947	r = -0.289 p = 0.216	r = -0.289 p = 0.216
Factor VII activity (IU/dL)	r = 0.200 p = 0.427	r = 0.081 p = 0.74	r = 0.081 p = 0.74
PAI activity (% pool)	r = 0.123 p = 0.605	r = 0.279 p = 0.234	r = 0.279 p = 0.234
t-PA antigen (μg/mL)	r = 0.016 p = 0.947	r = -0.289 p = 0.216	r = -0.291 p = 0.219
Cholesterol (mmol/L)	r = 0.088 p = 0.713	r = 0.162 p = 0.494	r = 0.175 p = 0.460
HDL cholesterol (mmol/L)	r = 0.073 p = 0.813	r = 0.261 p = 0.389	r = 0.448 p = 0.124
LDL cholesterol (mmol/L)	r = 0.052 p =0.865	r = -0.045 p = 0.833	r = 0.008 p = 0.980
Triglyceride (mmol/L)	r =0.324 p =0.176	r = -0.171 p = 0.485	r = -0.426 p = 0.069

n=22

Table 7-9 Relationships between changes in body mass index, waist circumference and waist to hip ratio and changes in haemostatic and rheological risk factors after weight loss for men

Change in haemostatic factors	Change in BMI (kg/m^2)	Change in waist (cm)	Change in WHR
Haematocrit (%)	r = -0.024 p = 0.913	r = -0.005 p = 0.983	r = 0.113 p = 0.598
Plasma blood viscosity (mPas)	r = 0.377 p = 0.076	r = 0.172 p = 0.645	r = -0.101 p = 0.645
Red cell aggregation (AU)	*	*	*
Fibrinogen (g/L)	r = 0.235 p = 0.248	r = 0.559 p = 0.003	r = 0.123 p = 0.551
Factor VII activity (IU/dL)	r = 0.665 p = 0.0001	r = 0.012 p = 0.953	r = 0.214 p = 0.285
PAI activity (% pool)	r = 0.073 p = 0.767	r = -0.028 p = 0.767	r = 0.073 p = 0.766
t-PA antigen (μg/mL)	r = 0.288 p = 0.153	r = 0.003 p = 0.596	r = 0.109 p = 0.566
Cholesterol (mmol/L)	r = -0.105 p = 0.609	r = 0.274 p = 0.175	r = -0.229 p = 0.260
HDL cholesterol (mmol/L)	r = 0.003 p = 0.991	r = 0.532 p = 0.019	r = 0.337 p = 0.158
LDL cholesterol (mmol/L)	r = 0.091 p = 0.710	r = 0.328 p = 0.170	r = -0.033 p = 0.894
Triglyceride (mmol/L)	r = 0.162 p = 0.430	r = 0.048 p= 0.816	r = -0.207 p = 0.309

n=27, * missing values

Table 7-10 Relationships between changes in body mass index, waist circumference and waist to hip ratio and changes in haemostatic and rheological risk factors after weight loss for all subjects

Change in haemostatic factor	Change in BMI (kg/m^2)	Change in waist (cm)	Change in WHR
Plasma blood viscosity (mPas)	r = 0.243 p = 0.147	r = 0.109 p = 0.522	r = -0.035 p = 0.836
Haematocrit (%)	r = 0.147 p = 0.352	r = -0.105 p = 0.507	r = 0.086 p = 0.589
Red cell aggregation (AU)	*	*	*
Fibrinogen (g/L)	r = 0.147 p = 0.328	r = 0.082 p = 0.589	r = -0.054 p = 0.724
Factor VII activity (IU/dL)	r = 0.434 p = 0.006	r = 0.104 p = 0.528	r = -0.084 p = 0.611
PAI activity (% pool)	r = 0.238 p = 0.145	r = 0.125 p = 0.448	r = 0.226 p = 0.145
t-PA antigen (μg/mL)	r = 0.167 p = 0.262	r = 0.169 p = 0.256	r = 0.134 p = 0.371
Cholesterol (mmol/L)	r = -0.022 p = 0.883	r = 0.201 p = 0.180	r = 0.024 p = 0.872
HDL cholesterol (mmol/L)	r = 0.008 p = 0.967	r = 0.384 p = 0.030	r = 0.348 p = 0.051
LDL cholesterol (mmol/L)	r = 0.050 p =0.787	r = 0.143 p = 0.435	r = -0.006 p = 0.976
Triglyceride (mmol/L)	r = 0.231 p =0.126	r = -0.068 p = 0.656	r = -0.286 p = 0.057

n=49

Table 7-11 Relationships between lipid fractions and haemostatic factors at baseline in women

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.558 p=0.009	r=0.131 p=0.570	r=0.587 p=0.008	r=0.168 p=0.454
HDL cholesterol (mmol/L)	r=0.438 p=0.135	r=-0.008 p=0.979	r=0.558 p=0.074	r=-0.186 p=0.542
LDL cholesterol (mmol/L)	r=0.586 p=0.035	r=-0.204 p=0.504	r=0.450 p=0.165	r=-0.274 p=0.365
Triglyceride (mmol/L)	r=0.272 p=0.233	r=0.356 p=0.113	r=0.253 p=0.296	r=-0.096 p=0.296

n=22

Table 7-12 Relationships between lipid fractions and haemostatic factors at baseline in men

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.056 p=0.791	r=0.073 p=0.739	r=0.275 p=0.174	r=-0.259 p=0.202
HDL cholesterol (mmol/L)	r=-0.276 p=0.254	r=-0.200 p=0.427	r=0.587 p=0.008	r=-0.349 p=0.143
LDL cholesterol (mmol/L)	r=0.179 p=0.462	r=-0.078 p=0.759	r=-0.157 p=0.520	r=-0.175 p=0.552
Triglyceride (mmol/L)	r=0.144 p=0.408	r=0.610 p=0.002	r=0.195 p=0.339	r=-0.169 p=0.408

n=27

Table 7-13 Relationships between lipid fractions and haemostatic factors at baseline in all subjects

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.229 p=0.126	r=0.064 p=0.678	r=0.468 p=0.001	r=-0.042 p=0.7777
HDL cholesterol (mmol/L)	r=-0.082 p=0.654	r=-0.096 p=0.608	r=0.625 p=0.0001	r=-0.120 p=0.512
LDL cholesterol (mmol/L)	r=0.236 p=0.194	r=-0.014 p=0.939	r=0.119 p=0.293	r=0.055 p=0.764
Triglyceride (mmol/L)	r=0.198 p=0.187	r=-0.513 p=0.016	r=0.182 p=0.231	r=-0.137 p=0.352

n=49

Table 7-14 Relationships between changes in haemostatic factors and changes in plasma lipid fractions after weight loss for women

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.558 p=0.009	r=0.089 p=0.710	r=0.586 p=0.011	r=0.168 p=0.454
HDL cholesterol (mmol/L)	r=0.438 p=0.135	r=-0.097 p=0.764	r=0.558 p=0.074	r=0.186 p=0.542
LDL cholesterol (mmol/L)	r= 0.259 p= 0.393	r= 0.298 p= 0.347	r= 0.213 p= 0.530	r= 0.526 p= 0.063
Triglyceride (mmol/L)	r=0.272 p=0.135	r=0.349 p=0.131	r=0.257 p=0.304	r= 0.096 p=0.670

n=22

Table 7-15 Relationships between changes in haemostatic factors and changes in plasma lipid fractions after weight loss for men

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.056 p=0.791	r=0.073 p=0.056	r=0.275 p=0.174	r=-0.259 p=0.202
HDL cholesterol (mmol/L)	r=-0.276 p=0.254	r=-0.200 p=0.427	r=0.587 p=0.008	r=-0.349 p=0.143
LDL cholesterol (mmol/L)	r= 0.510 p= 0.026	r= 0.638 p= 0.014	r= 0.366 p= 0.123	r= 0.321 p= 0.194
Triglyceride (mmol/L)	r=0.144 p=0.492	r=0.610 p=0.002	r=0.195 p=0.339	r=-0.169 p=0.408

n=27

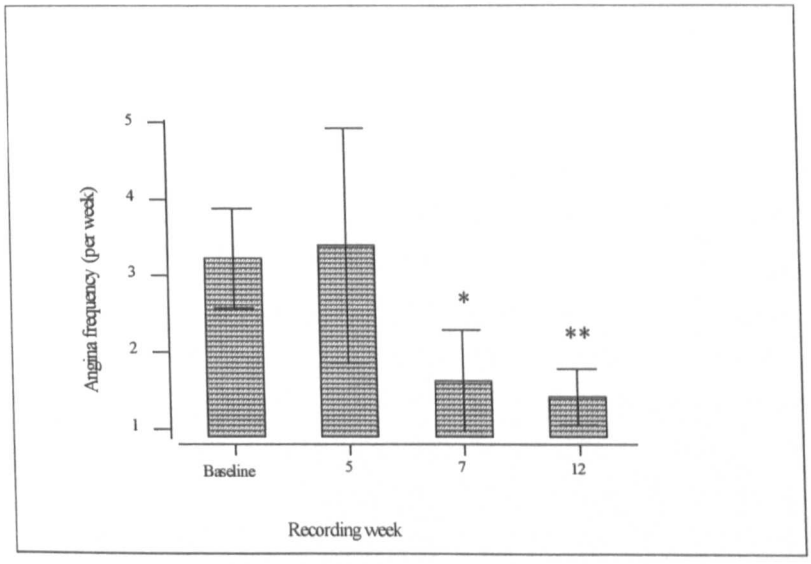
Table 7-16 Relationships between changes in haemostatic factors and changes in plasma lipid fractions after weight loss for all subjects

	t-PA ag (ug/mL)	PAI activity (% pool)	FVII activity (IU/dL)	Fibrinogen (g/L)
Cholesterol (mmol/L)	r=0.166 p=0.264	r=0.238 p=0.144	r=0.223 p=0.150	r=0.147 p=0.330
HDL cholesterol (mmol/L)	r=0.159 p=0.385	r=0.343 p=0.071	r=0.159 p=0.385	r=0.342 p=0.059
LDL cholesterol (mmol/L)	r= 0.451 p= 0.010	r= 0.574 p= 0.002	r= 0.345 p= 0.07	r= 0.383 p= 0.037
Triglyceride (mmol/L)	r=0.093 p=0.533	r=-0.161 p=0.326	r=0.040 p=0.792	r=0.067 p=0.658

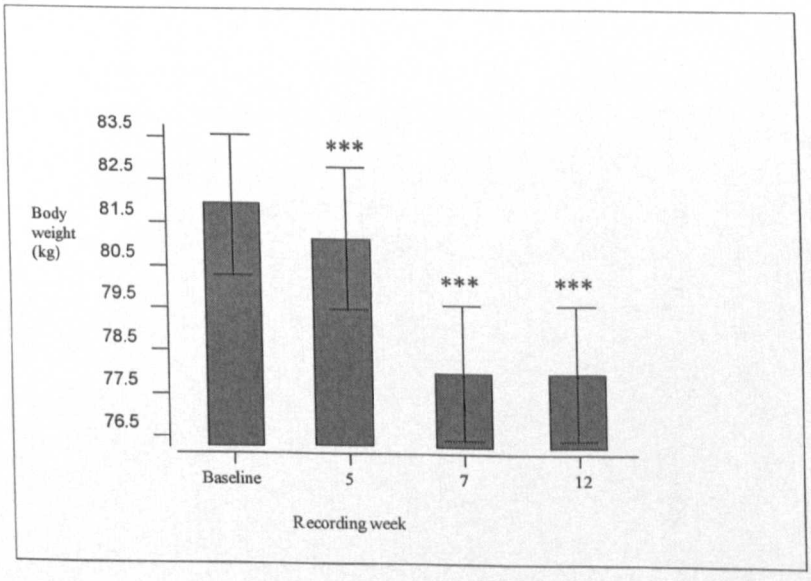
n=49

Figure 7-1 (a) Angina frequency and (b) changes in body weight before during and after intervention for all subjects

a)

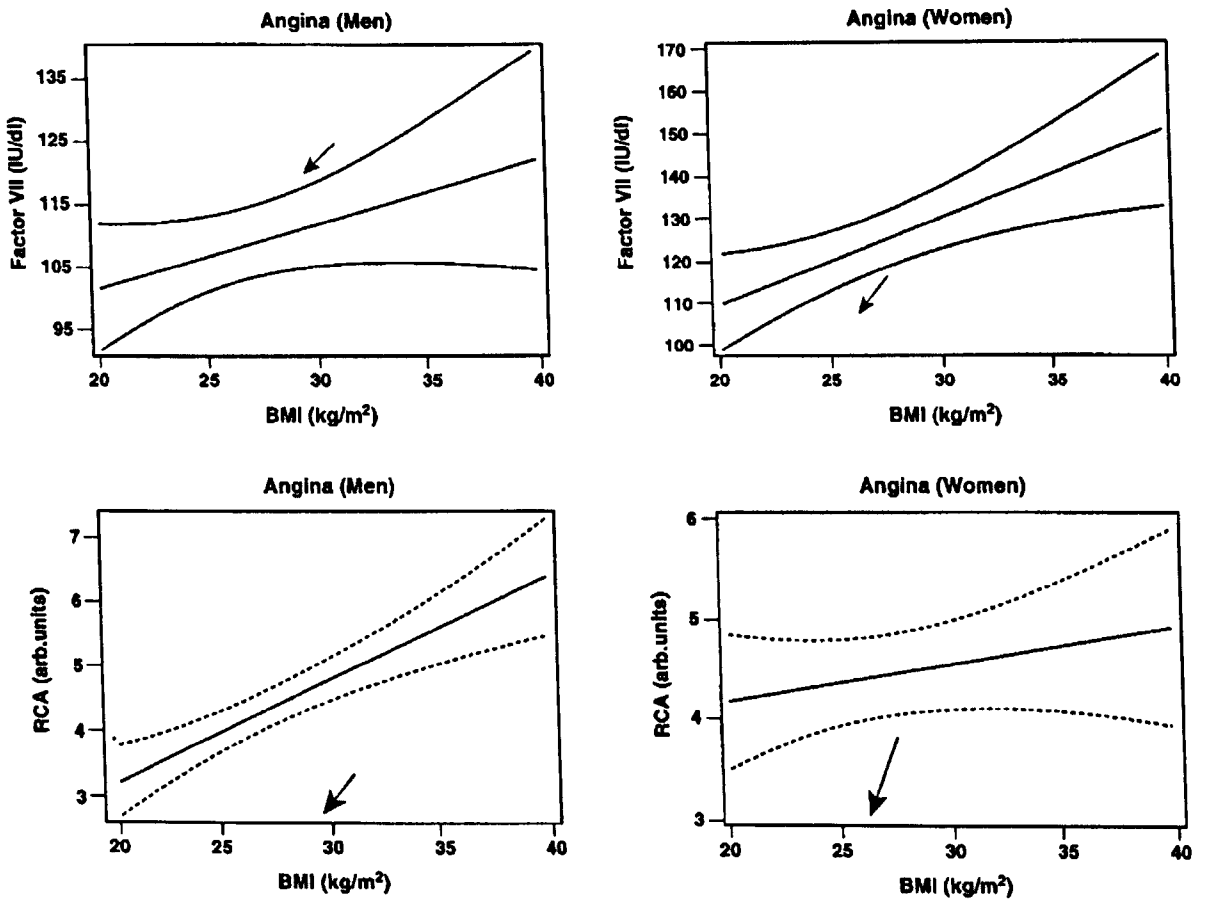


b)



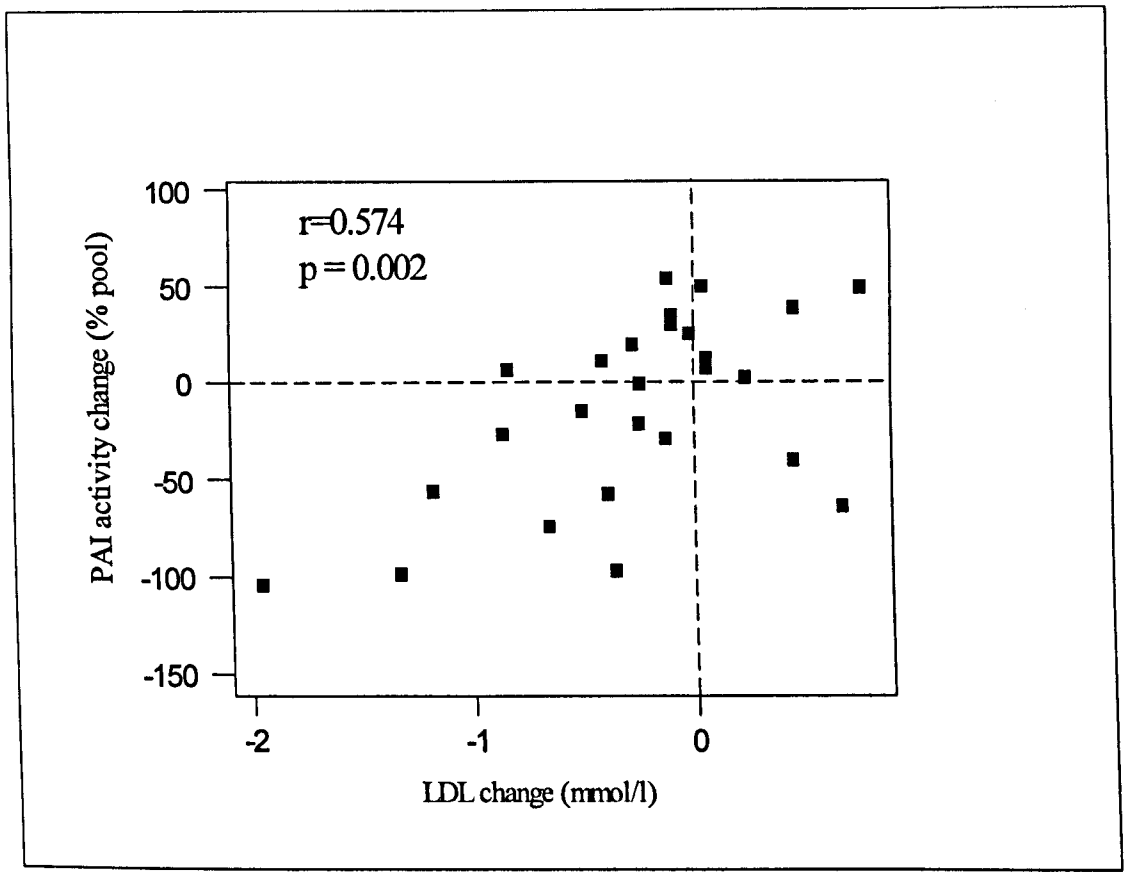
Data are mean (SEM).
* $p<0.05$ * * $p<0.01$* * * $p<0.001$ when compared with baseline

Figure 7-2 Fitted regression lines of red cell aggregation (RCA) and factor VII activity against body mass index (BMI) for the MONICA subsets of men and women



Dashed lines show the 95% confidence interval for the mean. The arrow indicates the changes achieved, on average, before and after intervention in the study populations of men and women

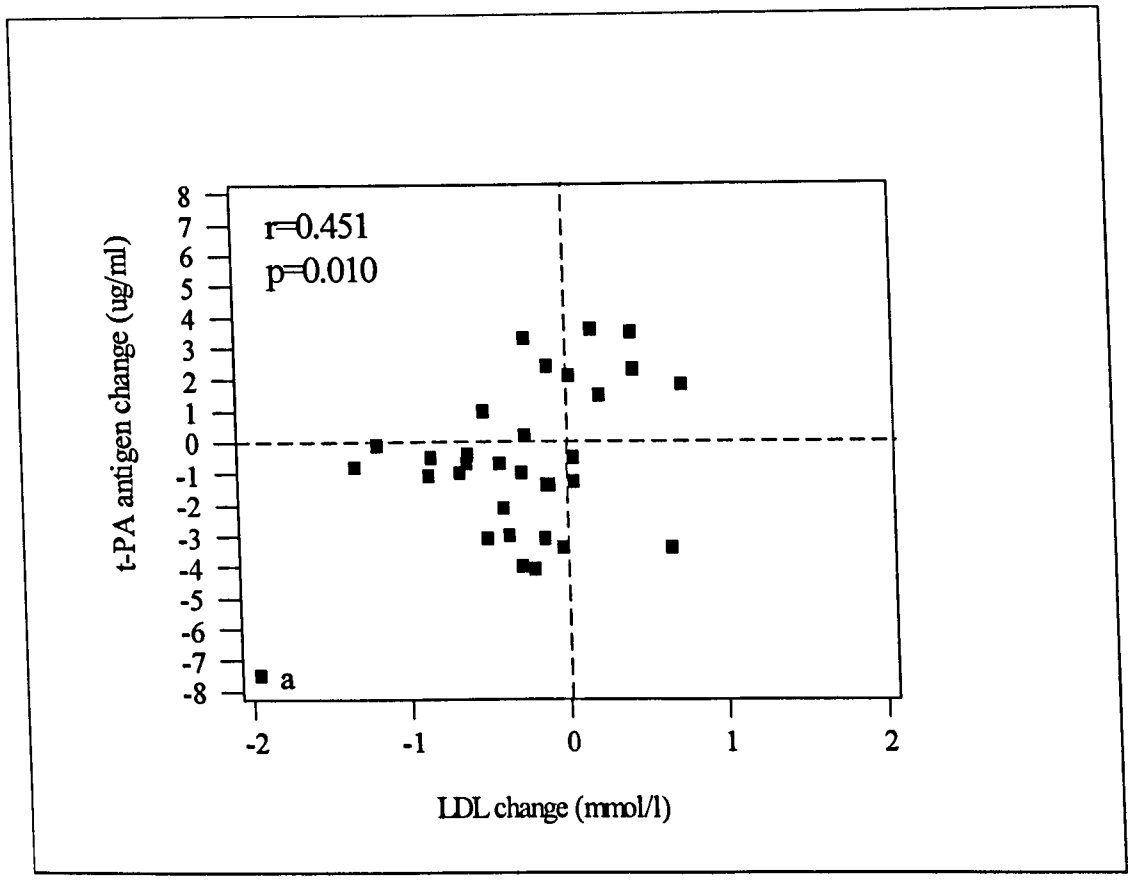
Figure 7-3 Relationship between changes in PAI activity and LDL cholesterol after weight loss



n=25 (11 women, 14 men)

The relationship between changes in PAI activity and LDL cholesterol after weight loss was reanalysed with a possible outlier (a) removed but this did not affect the significance of the relationship (r=0.470 p=0.018).

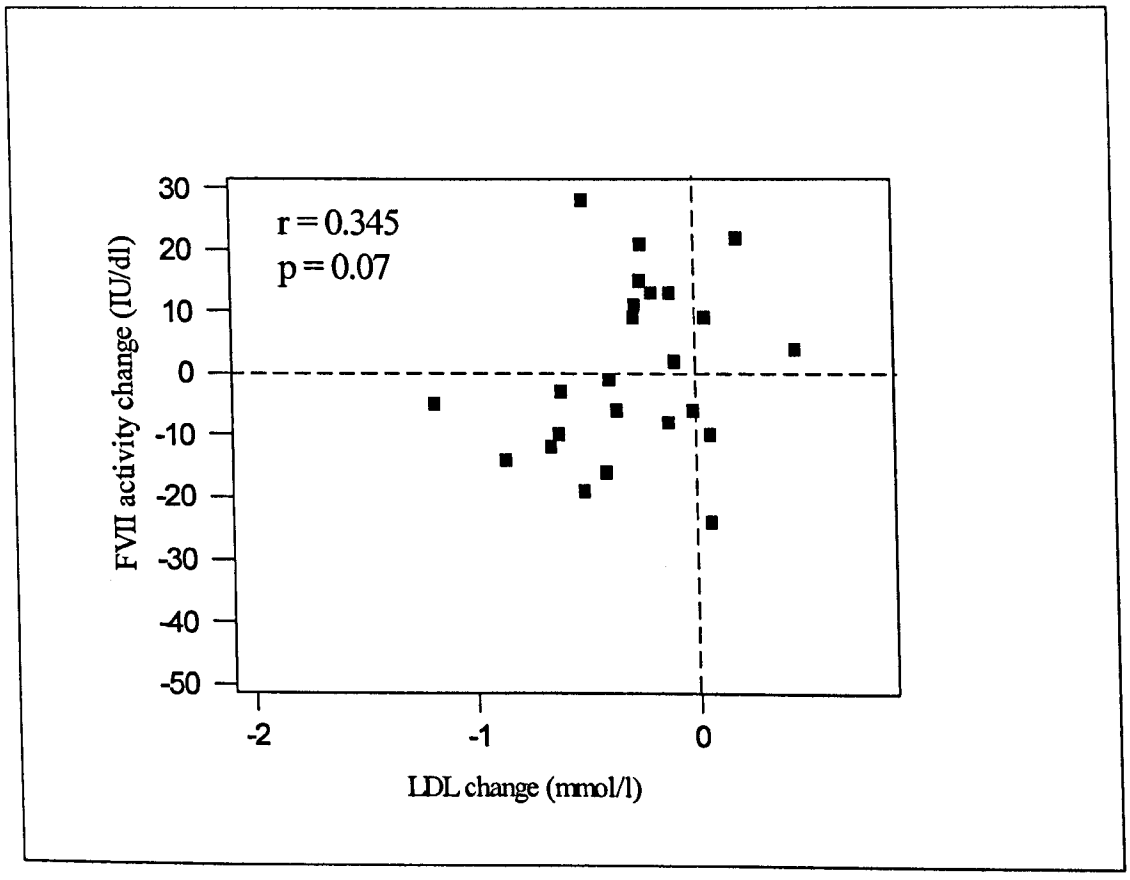
Figure 7-4 Relationship between changes in t-PA antigen and LDL cholesterol after weight loss



n = 32 (13 women, 19 men)

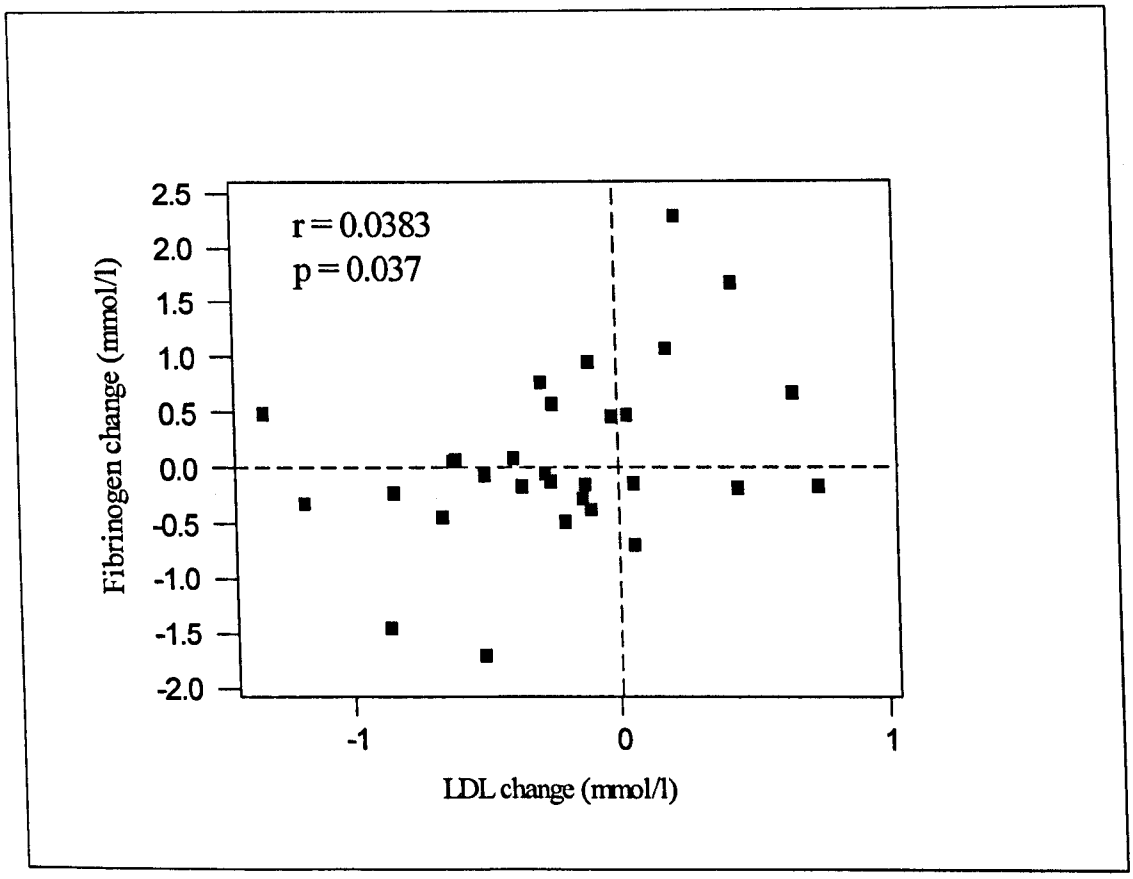
The relationship between changes in t-PA antigen and LDL cholesterol after weight loss was reanalysed with a possible outlier (a) removed but this did not affect the significance of the relationship (r=0.253 p=0.017).

Figure 7-5 Relationship between changes in factor VII activity and LDL cholesterol at week twelve



n = 24 (10 women, 14 men)

Figure 7-6 Relationship between changes in fibrinogen and LDL cholesterol at week twelve



n=29 (17 men, 11 women)

Chapter 8 The effect of moderate weight loss on haemostatic and rheological factors and plasma lipids in subjects of body mass index close to 25 kg/m^2

8.1 Introduction and hypotheses

Risk of coronary death is known to be doubled at BMI 25 kg/m^2 compared to BMI 22 kg/m^2 (Colditz *et al*, 1995). The benefits of moderate weight loss have been established in those whose BMI exceeds the "healthy weight" category (BMI > 25 kg/m^2). Some of the benefits of weight loss in terms of haemostatic and rheological factors have already been described within this thesis, and other benefits of moderate weight loss have already been established with reference to other recognised IHD risk factors (Goldstein, 1992). Many people, especially women, who are not obese (BMI > 30 kg/m^2) or even overweight (BMI 25 - 30 kg/m^2) seek weight loss for other reasons. The medical value of reducing body weight below a BMI of 25 kg/m^2 remains largely unproven, although the suggestion has been made that moderate weight loss could offer health benefits (Chan *et al,* 1994).

The main aim of this study was to investigate the effect of moderate weight loss on haemostatic and rheological factors and plasma lipids in healthy subjects with BMI close to 25 kg/m^2. Weight loss was targeted by using the same dietetic approach, a 600 kcal (2510 kJ) daily energy deficit, already used for the two earlier studies, chapters 6 and 7. The effectiveness of achieving weight loss in a group of subjects close to healthy weight was also examined.

There were three hypotheses to be tested in this study of healthy weight subjects who had expressed a desire to achieve weight loss.

1) A dietary intervention incorporating an energy deficit approach to weight loss (600 kcal per day) would be effective in achieving losses of close to 0.5 kg per week. This

approach would be effective in subjects (with an expressed desire to reduce body weight) whose BMI was close to 25 kg/m^2.

2) Weight loss after a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets would improve haemostatic and rheological risk factors for IHD in subjects whose BMI was close to 25 kg/m^2.

3) Weight loss after a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets would be effective in improving plasma lipid concentrations in subjects whose BMI was close to 25 kg/m^2.

8.2 Methods

8.2.1. Study design and statistical approaches

A 12 week study duration was chosen, to coincide with the period when maximum weight loss using dietary methods was achieved, and to ensure comparisons could be made with the results of the other studies in this thesis. The chosen experimental design was the single stranded study, with the biological and physical measurements being made at baseline and post-intervention (week 12). The study design was chosen following the statistical principles already described (chapter 4), and the sample size with sufficient power to determine the differences that the study was designed to seek, at 80% with a 5% level of significance. The power analysis calculations are applicable to those given in chapter 4.

Data for the differences in biological measurements before and after dietary intervention were approximately normally distributed, as determined from a distribution plot, and thus were analysed using a paired Student's t test. The differences between the two dietary measurements showed a skewed distribution and were analysed using a paired Wilcoxon

test. Pearson's correlation analysis was carried out to investigate any relationships between changes in body weight or BMI, and changes in haematological, fibrinolytic and plasma lipid measurements.

8.2.2 Subjects

In order to recruit "healthy" subjects for weight loss without clinical disease and a BMI close to 25 kg/m^2 a different approach was required to the two earlier studies. In particular, it was considered to be impractical both in terms of travel and time constraints to expect subjects, likely to be in current employment, to attend study appointments in a hospital setting. The use of financial incentives could have attracted inappropriate subjects, or led to alterations in methods used by the subjects. The start of this study coincided with the launch by the Health Education Board for Scotland for their "Healthy Work-sites" initiative (HEBS, 1996), to draw attention to the work setting as a location for health promotion. At the time the occupational health personnel were contacted, no implementation of the Health Education Board for Scotland's initiative had taken place, although those company employees whose responsibility was Health and Safety had been informed of the aims. The use of the work-site allowed a new sector of the Scottish population to be specifically targeted for health promotion. Eighteen work-sites employing greater than 200 employees in the West Lothian area were identified from the West Lothian Business Directory (West Lothian District Council, 1994). Either the Occupational Health, Human Resource or Health and Safety leaders within each company were personally contacted by a letter describing the aims and practical requirements of the study. Four companies indicated that they were uninterested in hosting the study and 8 companies requested further information. No reply was received from the remaining 6 companies.

The BP Oil refinery was the work-site chosen to host the study (plate 8.1). The staff of the Occupational Health Department were delighted to host the study and their Occupational Health Centre was large, with sufficient clinical space to accommodate the study. The Occupational Health Leader regarded the study as another opportunity to target the employee population with a health promotion initiative not yet addressed at the refinery site. Other reasons for the choice of the refinery site included the large work force (1200 people) and the existence of an individual electronic mail system with individual and confidential mail boxes for each employee.

The BP work-force was 90% male of which 43% were shift workers. Initial recruitment and advertising for the study used the work-site electronic mail system. A refinery-wide message advertising the study and detailing selection criteria was sent to all employees. The message requested healthy volunteers to take part in a study which offered them the opportunity to find out about the composition of their present diet and to receive specialised advice about how to adjust their diet in line with current dietary targets (COMA, 1991, Scottish Office, 1996). Physical measurements of body composition and plasma lipid concentrations were also offered. One hundred and ninety requests for further information were received. A screening questionnaire was sent to all these employees who had registered an interest in the study and each was to asked detail their anthropometric information (body weight, height) and their recent medical history. A guarantee concerning the confidentiality of this information was made. From the original sample of 190 volunteers, 51 subjects were approached who fulfilled the principal selection criteria. The criteria included a BMI between 22.5-28.5 kg/m^2, being in good health, and a desire to lose body weight. Those with any significant cardiovascular abnormalities or diabetes (WHO, 1985) were excluded. All subjects were free of any medication known to influence haemostatic measures, such as oral contraceptives or

diuretics (Lowe *et al,* 1980). No subject had reduced their weight by greater than 3.0 kg within the 3 month period prior to recruitment. Recruitment was carried out from May through to September to minimise any seasonal influences. The 51 volunteers, 13 women and 38 men, who fulfilled the inclusion criteria were recruited to the study. Subjects were requested to attend the Occupational Health Centre on 3 occasions over the 12 week study period (study week -1, baseline and week 12). In order to reinforce the requirements of the study, a further 2 telephone contacts were planned at weeks 4 and 8. Any subjects who expressed a preference to attend the Occupational Health Centre in person for these contacts were offered this option.

Ethical approval for the study was obtained from the Forth Valley Health Board joint ethics committee and signed informed consent was obtained from all study participants.

8.2.3 Physical measurements

Body weight, body fat distribution, waist and hip circumferences, waist to hip ratio and percentage body fat were measured according to the methods of Lean *et al* (1995).

8.2.4 Dietary intervention and monitoring

Dietary intervention used an individual dietary prescription based on the Schofield equation to predict BMR (Schofield *et al,* 1985). Dietary intake was monitored by the completion of 7 day WI food diaries during study week 1 and week 12 (Bingham, 1987). Dietary analysis was carried out using the Compeat dietary analysis programme (Nutrition Systems, London).

8.2.5 Venesection and laboratory methods

Venesection was performed before (week 0) and after (week 12) dietary intervention. Blood was centrifuged on site within 10 minutes of venepuncture. Plasma viscosity,

haematocrit, fibrinogen and factor VII activity were measured. PAI activity and t-PA antigen concentrations were measured as indices of the fibrinolytic system. Plasma triglyceride, total cholesterol and HDL cholesterol were also measured and LDL cholesterol estimated using established calculations (Friedwald *et al*, 1972).

8.3 Results

Of the 51 subjects recruited to the study, 13 subjects, 5 female and 8 male, failed to complete the study, and were excluded from all analyses. The reasons were a lack of motivation to fulfil the study requirements (6), a desire to remain weight stable (4) and relocation from Grangemouth (3). The characteristics of the 38 subjects (31 male), who completed the study are shown (table 8.1-8.3). For all subjects BMI range was 57.8-91.0 kg and 22.5-28.2 kg/m^2. The weight loss and changes in BMI after intervention are shown (table 8.1). No gender subdivisions were made due to the small number of females completing the study. The results were analysed for the study population as one group. The mean weight loss for the entire group was 3.3% which represented a weekly weight loss of 0.2 (SD 0.2) kg ($p<0.0001$). The weekly weight loss was 40% of the target value.

At baseline the individual daily energy requirement was calculated for all subjects. The Schofield equations were used to estimate the individual's resting metabolic rate and a PAL of 1.4 was applied (COMA, 1991). The daily energy requirement was then calculated. Median energy requirements were 10255, range 7753 - 11397 kJ, 2451, range 1853 - 2724 kcal. A one sample Wilcoxon test was used to compare the differences between the values for energy requirements and reported energy intakes measured before intervention. No significant differences were observed ($p=0.29$). Reported energy intakes and dietary composition measured both at baseline and week 12 are shown (table 8.2). After dietary intervention a significant fall in reported energy intake was observed ($p=0.0001$). Total energy from fat and saturated fat also fell significantly after

intervention. Percentage energy from polyunsaturated fat remained unchanged. These changes were in accordance with the dietary advice given to the study participants. The percentage dietary energy from carbohydrate was significantly increased, although that from protein was increased post-intervention. Dietary energy from alcohol was unchanged post-intervention.

Median dietary energy prescription for the group was 7531 kJ: range 4602 - 8368 kJ/day (1800 kcal/day, range 1100-2000 kcal/day). Recorded dietary intakes were 115% of the mean total daily energy prescription after dietary intervention at week 12.

The subjects' mean haemostatic and fibrinolytic measurements are shown (table 8.3). No significant changes were seen in any of the haemostatic and rheological factors with weight loss. The mean value for plasma viscosity at baseline of 1.24 m Pas was significantly below the mean values for those healthy overweight subjects already studied, (chapter 6) and not significantly changed by weight loss. Haematocrit was very close to the current reference values in healthy subjects (45.7%) and was significantly reduced with weight loss ($p=0.04$). Fibrinogen concentrations were unchanged with weight loss. The mean values for fibrinogen concentrations in the earlier study of healthy overweight subjects were closely comparable with those of the present study group (chapter 6). Factor VII activity also remained unchanged with weight loss, although a positive relationship between reductions in factor VII activity and weight loss was indicated (figure 8.1). The fibrinolytic factor t-PA antigen showed no effects due to weight loss, however PAI activity was lowered and the change was very close to the 5% significance level.

Plasma total cholesterol and HDL cholesterol were reduced ($p=0.037$, 0.033) while triglyceride and LDL cholesterol concentrations remained unchanged.

At baseline, there were few significant relationships between plasma lipids and haemostatic factors, the exceptions being plasma viscosity and t-PA antigen with total cholesterol and LDL concentrations and triglyceride (table 8.5). After dietary intervention total and LDL cholesterol concentrations were significantly correlated with fibrinogen and t-PA antigen, as were HDL cholesterol concentrations with plasma viscosity and haematocrit (table 8.5).

After intervention, some trends towards significant relationships between the changes in BMI and t-PA antigen, factor VII activity and LDL cholesterol were seen ($p=0.016$, 0.058, 0.053) (table 8.6). However, no significant relationships between changes in waist circumference, WHR, haemostatic, rheological factors or plasma lipids were found (table 8.4, table 8.7).

8.4 Discussion

The initial process of choosing a location from which to run the study was an encouraging one as it has identified new settings and target groups for health promotion. The enthusiastic responses received from the individual companies who responded to the initial letter discussing the possibility of hosting the study suggested that the study was carried out in an optimal climate. The reasons for this may have included influence of the HEBS healthy work-site initiative. This targeted the staff responsible for the health of employees to increase their use of health promotion in these work-site populations. The positive responses from companies to the request to host the study suggested future dietary intervention or health promotion initiatives may be well received.

The results of this study have shown a significant improvement in haematocrit but in none of the other haemostatic and rheological risk factors for IHD, after a mean body weight loss close to 3%. These findings are in contrast to the results from the two earlier studies

(chapter 6 and 7) when factor VII activity and RCA were significantly reduced. The baseline values for a number of the IHD were lower than the other two groups. This will be discussed fully in chapter 9. Aside from haematocrit, these findings do not support the first hypothesis of the study, that moderate weight loss in those whose BMI was close to the healthy range (BMI 25 kg/m^2) would improve haemostatic, rheological and lipid risk factors for IHD. The findings were in keeping with the comparison of the laboratory reference ranges for all the indices measured with the mean study values. For all of the indices measured, none of the mean values for study subjects were outside the reference ranges. In addition, it was unfortunate that due to practical limitations, it was not possible to make RCA measurements in this study.

Although a number of patients failed to lose weight, when regression analysis was carried out significant relationships between changes in BMI and factor VII activity were shown (table 8.4). Other significant relationships with changes in BMI were t-PA antigen and LDL cholesterol concentrations. These findings all suggested a greater weight loss could have significantly effected the plasma indices examined. The scientific data available has suggested that the reduction of body weight in the overweight could be valuable in the improvement of the haemostatic and rheological risk factors for IHD.

In the present study, the values for plasma viscosity were well below the cut-off of 1.33 m Pas (1.25 mPas at baseline) which has been identified with low risk of stroke or coronary disease (Lowe *et al*, 1997). However, the amount of weight loss and the starting BMI appear to be key factors in achieving significant reductions in plasma viscosity (Fanari *et al*, 1993, Wadden, 1993). It seems likely that our subjects, slimmer at baseline than those in other studies and in good health (Parenti *et al*, 1988) did not lose sufficient weight to alter plasma viscosity, which was not elevated.

Mean haematocrit fell significantly with weight loss, but was not elevated at baseline when compared with the values in an obese population or a population with increased IHD risk (Lowe *et al*, 1997). Other studies of weight loss in the overweight have shown that haematocrit remained unchanged with weight loss (Parenti *et al,* 1988, Fanari *et al,* 1993). Only the results of a large weight loss / VLCD programme were in agreement with our study results of a significant fall in haematocrit with weight loss (Poggi *et al,* 1994). The possible reasons why a mean weight loss of 3% was effective in reducing haematocrit are unclear and require further study.

The failure of weight loss to reduce fibrinogen concentrations in this present study is in agreement with the following studies, all of which lack power to make definitive statements (Fehily *et al,* 1982, Ernst *et al,* 1986, Parenti *et al,* 1988, Ernst *et al,* 1989, Slabber *et al,* 1992, Ernst, 1993, Palareti *et al,* 1994, Ditschuneit *et al*, 1995) and earlier chapters of this thesis.

A fall in factor VII activity of close to 10 % appears to be the extent of change achieved in the majority of studies of dietary change and weight loss (Folsom *et al,* 1993, Palareti 1994). In agreement with the findings of this present study, but in contrast with the majority of the literature, are the findings of Baron *et al* (1989). They examined the effect of intentional weight loss in factor VII activity. A mean weight loss of close to 4.0 kg using a 1200 kcal diet was achieved after 12 weeks and factor VII activity remained unchanged. This could have reflected a number of influences including the type of dietary intervention offered, a 1200 kcal energy prescription was advised for all subjects, of which half of the subjects were advised to consume 1200 kcal in the form of a high fat diet (50% energy from fat), insufficient weight loss, or the effects of seasonal variation. Seasonal variation has been identified as altering factor VII activity, which is known to rise in winter along with plasma fibrinogen concentrations (Woodhouse *et al*, 1994).

Practical constraints governed when the recruitment for this present study commenced, but it may have coincided with the greatest variation in factor VII, between autumn and the onset of winter. However, the failure of weight loss to reduce factor VII activity was probably due to two factors. Firstly, the insufficient weight loss, and a mean plasma factor VII activity that was not elevated. These ideas are confirmed by the relationship between reducing body weight and factor VII activity shown (figure 8.1). This suggests that further weight loss may have strengthened the relationship.

As in any study which was aimed to achieve weight loss in a free living population, there are changes in both energy intake and dietary composition. The influence of dietary fat intake on factor VII activity has been shown to be significant (Miller *et al,* 1986). A significant decrease was seen in factor VII activity with reductions in dietary fat intake (31% energy from fat) without weight loss. A similar dietary intervention (32% energy from fat) with two different fat compositions, a low or high P/S ratio also significantly lowered factor VII activity (Marckmann *et al,* 1992). The present study did not find a relationship between dietary fat intake and factor VII activity at baseline, or between changes in these variables after intervention. The plasma triglyceride concentration itself has been identified as a possible mediator of the relationship between dietary fat intake and factor VII activity (Miller *et al*, 1990). However, the subjects in this study had low mean plasma triglyceride concentrations, with only a small range. In addition, they also reported a percentage energy from dietary fat of 35%, close to 5% below the estimated dietary fat intake of a sample of the Scottish population. These two factors, together with factor VII activities, which were not elevated and with only a small range of values, may explain the failure of a significant relationship to become apparent. Alternatively, it may be that dietary intake assessment is not possible in these subjects.

The fibrinolytic system enzyme PAI activity and t-PA antigen are related to overweight and to increased IHD risk (Carlson *et al,* 1981, Vague *et al,* 1986, Landin *et al,* 1990). Significant reductions in PAI-1 activity and t-PA antigen have been shown after weight loss at least three times greater than that observed in this present study (Vague *et al,* 1986, Landin *et al,* 1990, Mehrabian *et al,* 1990). It was encouraging that the relatively small amount of weight loss achieved in this present study appeared to affect PAI activity, although no influence on t-PA antigen was apparent. However, regression analysis revealed a significant relationship between BMI change and t-PA antigen. This may be a suggestion that greater weight losses could have resulted in a greater reduction in t-PA concentrations. These results are in contrast to those of chapter 6, where t-PA antigen fell significantly with weight loss in overweight women. Once more, the baseline values for these subjects were very close to the reference range, which may explain why the weight loss achieved was not influential.

The second hypothesis concerned the effects of dietary intervention and weight loss on plasma lipids. The significant reduction in total and HDL cholesterol concentrations following weight loss is in agreement with the majority of the literature (Dattilo and Kris-Etherton, 1992, Goldstein, 1992). The mean plasma cholesterol concentrations in those who reduced body weight were similar to the current European Atherosclerosis Society recommendations of a plasma cholesterol of 5.2 mmol/L, or below (European Atherosclerosis Society, 1987).

The examination of relationships between the changes in haemostatic and rheological risk factors and the changes in plasma lipids provided an opportunity to examine whether the dietary intervention led to similar effects on the two areas of measurement. The significant relationship of changes in PAI activity with total and LDL cholesterol was of interest, and provided more evidence of the many benefits from moderate weight loss on

IHD risk factors. This evidence supports the finding of Folsom *et al* (1992) of a link between haemostatic factors and plasma lipids after weight loss. However, no evidence exists as to whether this relationship is causal, and further research is required in this area.

The final hypothesis examined the effects on body weight of a 600 kcal daily energy deficit in a group of close to healthy weight subjects. The target weight loss was 0.5 kg per week. The mean weight loss for the whole group was 0.2 kg per week, 40% of the target value. Anecdotally expressed opinions from fellow dietetic professionals have suggested that a structured approach towards weight loss, such as the 600 kcal energy deficit, would be impractical and ineffective in a group of subjects with a close to healthy BMI. Studies which have examined the effectiveness of weight reduction using this approach in close to healthy weight subjects group are few.

Velthuis-te Wierik *et al* (1995) used a similar daily energy deficit approach to this present study and achieved a far greater weight loss (7.0 kg over ten weeks) in men whose mean BMI was 24.6 kg/m^2 at baseline. It is likely that their success was due largely to differences in the design of the intervention and in the approach to energy prescription and food provision. In order to calculate an appropriate energy prescription, their subjects were asked to complete a 7 day WI food diary. The prescription was calculated to be only 80% of the individuals estimated energy requirements as measured by the completion of a 7 day WI. Significant under-reporting of food intakes, 13%, was identified using the doubly labelled water method. Thus, subjects had a mean daily energy deficit of 33% (close to 3810 kJ / 911 kcal) below requirements, which exceeded the daily 600 kcal deficit used in this present study. The second reason why the mean weight loss achieved was much closer to the target of 0.5-1.0 kg per week was the approach used in the management of subjects (Velthuis-te Wierik *et al*, 1995). Food comprising the complete diet and which achieved each subjects' individual energy prescription was provided in the

form of pre-prepared meals and snacks, so all the foods consumed for the ten week study were effectively a gift to the subjects. Measured by weight lost, this approach resulted in substantially improved dietary compliance. The mean weight loss of 2.6 kg after 12 weeks in this study was considerably below that achieved by the majority of other workers using a conventional dietary approach in overweight and obese subjects (Wadden, 1993).

It was encouraging to find that no significant difference was found between the reported energy intakes at baseline and the estimated energy requirements (using the WHO/Schofield equations). This would suggest the group as a whole was not significantly under-reporting food intake at baseline. However, under-reporting has been well recognised as a feature of self reported dietary intakes completed by overweight and close to normal weight subjects (Velthuis-te Wierik *et al*, 1995). The majority of the significant changes between reported nutrient intakes at baseline (before dietary intervention) and week 12 were in agreement with the principle targets for dietary change. These included a reduction in total and saturated fat and an increase in percentage energy from carbohydrate (COMA, 1991). The exception remained the significant increase in the percentage energy from dietary protein post-intervention, which exceeded its dietary target. This most likely reflected the difficulty that subjects found in adjusting their dietary protein intake within the context of a lowered energy intake. The failure of subjects to achieve at least 50% dietary energy from carbohydrate whilst lowering their energy intakes was probably related. Subjects often reduce the quantity of food consumed to fulfil the requirement to lose body weight, but often fail to reduce the quantity of protein in their diet. Personal dietetic experience suggests that subjects often fail to reduce their protein intakes as the "protein rich" component this part of their meals are often pre-portioned, e.g. chops, steak, fish fillets etc. This change requires considerable planning to adjust to and subjects often fail to achieve reductions in their protein intakes.

The apparent reluctance of subjects to increase their consumption of bread and potatoes exacerbated this problem, and probably led to the lower than optimal carbohydrate consumption. Both of these findings are in agreement with the reported dietary intakes in the two earlier studies. However, at present there is insufficient scientific evidence to support these possible explanations.

8.5 Conclusions

Dietary intervention and moderate weight loss of 2.6 kg in subjects of near normal weight achieved significant reductions in haematocrit and total cholesterol concentrations. In contrast, the failure of the weight loss to influence the haemostatic and rheological indices, particularly factor VII activity, linked with increased IHD risk was not unexpected. The haemostatic and rheological risk factors for IHD were within their laboratory reference ranges at baseline, i.e. the values associated with a minimal incidence of chronic disease. However, the significant relationships shown by correlation analyses, suggest strongly that a greater amount of weight loss may have shown significant falls in a number of IHD measures. These relationships suggest weight loss of between to 5-10% body weight may be of value in reducing IHD risk. The present results also suggest that moderate weight loss can be advocated to improve cholesterol concentrations, haematocrit and possibly PAI activity, all established risk factors for IHD. Weight losses in this subject similar to the present group of can be recommended following this study. Furthermore other subjects with a close to healthy BMI, but the presence of greater IHD risk, e.g. diabetes, would be even more likely to show greater benefits, and possibly reductions in IHD risk following moderate weight loss.

Table 8-1 Characteristics of all subjects who completed the study at baseline and week twelve

	Baseline	Week 12	Difference	p
Age	41.6 (8.5)			
Weight (kg)	77.9 (8.1)	75.3 (7.7)	-2.6 (2.6)	0.00001
BMI (kg/m2)	25.3 (1.4)	24.4 (1.5)	-0.9 (0.8)	0.00001
WHR	0.88 (0.06)	0.87 (0.08)	-0.01 (0.06)	0.31
Total cholesterol (mmol/L)	5.5 (0.9)	5.2 (1.1)	-0.3 (0.8)	0.037
LDL cholesterol (mmol/L)	3.4 (0.8)	3.2 (1.0)	-0.2 (0.3)	0.033
HDL cholesterol (mmol/L)	1.5 (0.4)	1.3 (0.4)	-0.2 (0.3)	0.18
Triglyceride (mmol/L)	1.3 (0.8)	1.4 (1.1)	+0.1 (0.9)	0.50
Systolic blood pressure (mmHg)	121 (11)	116 (10)	-5 (10)	0.021
Diastolic blood pressure (mm Hg)	80 (9)	80 (8)	0 (3)	0.84

Data mean (SD), n= 38

Table 8-2 Dietary information from baseline (record one) and week twelve (record two).

Nutrient value	Baseline	Week 12	Difference	p
kJ	9980 (8833, 11283)	8109 (7206, 9267)	-1660 (-3465, -209)	0.0001
kcal	2380 (2107, 2688)	1929 (1710, 2200)	-408 (-833, -39)	0.0001
P/S ratio	0.54 (0.38, 0.65)	0.58 (0.47, 0.69)	+0.08 (-0.08, +0.20)	0.09
Fat % energy	35.6 (33.2, 41.4)	28.6 (26.7, 32.4)	-6.1 (-9.8, -2.4)	0.0001
Saturated fat % energy	12.3 (10.9, 14.6)	9.4 (7.9, 11.4)	-2.7 (-5.8, -1.3)	0.0001
Polyunsaturated fat % energy	6.3 (5.3, 7.7)	5.0 (4.0, 6.3)	-0.8 (-2.1, 0.0)	0.004
Protein % energy	14.4 (13.3, 16.2)	16.7 (15.0, 18.1)	+2.3 (0.2, 3.6)	0.0001
Carbohydrate % energy	43.5 (37.9, 48.0)	49.2 (44.3, 52.8)	+3.7 (-1.1, +10.0)	0.001
Alcohol % energy	4.2 (2.4, 10.1) (n = 35)	4.6 (3.6, 8.6) (n = 35)	0.0 (-2.0, +2.2) (n = 35)	0.950

n=38

Data as median, 25 and 75 % inter-quartile ranges

Table 8-3 Haemostatic and fibrinolytic measurements at baseline and week twelve

	Baseline	Week 12	Difference	p
Haematocrit (%)	46 (3)	45 (3)	-1 (3)	0.04
Plasma viscosity (m Pas)	1.25 (0.07)	1.24 (0.07)	-0.01 (0.03)	0.44
Fibrinogen (g/L)	2.54 (0.59)	2.76 (0.74)	+0.22 (0.82)	0.12
Factor VII activity (IU/dL)	108 (17)	105 (17)	-3 (10)	0.19
PAI activity (% pool)	86 (39)	68 (30)	-18 (16)	0.06
t-PA antigen (μg/mL)	8.4 (3.8)	8.1 (3.5)	-0.3 (2.9)	0.53

Data as mean (SD), n=38

Table 8-4 Relationships between changes in body mass index, waist circumference and waist to hip ratio with changes in haemostatic and rheological measures

Change in haemostatic factors	Change in BMI (kg/m2)	Change in waist (cm)	Change in WHR
Haematocrit (%)	r = -0.006 p = 0.979	r =0.051 p = 0.809	r = -0.311 p = 0.130
Plasma viscosity (mPas)	r = 0.083 p = 0.698	r = 0.350 p = 0.094	r = -0.174 p = 0.416
Fibrinogen (g/L)	r = -0.039 p = 0.835	r = 0.242 p = 0.182	r = 0.130 p = 0.477
Factor VII activity (IU/dL)	r = 0.335 p = 0.053	r = -0.120 p = 0.543	r = -0.166 p = 0.231
PAI activity (% pool)	r = -0.277 p = 0.161	r = 0.122 p = 0.553	r = -0.244 p = 0.231
t-PA antigen (μg/mL)	r = -0.460 p = 0.016	r = -0.019 p = 0.924	r = -0.192 p = 0.338
Cholesterol (mmol/L)	r = 0.344 p = 0.040	r = 0.085 p = 0.616	r = -0.172 p = 0.308
HDL cholesterol (mmol/L)	r = -0.046 p =0.801	r = -0.072 p = 0.687	r = 0.019 p = 0.917
LDL cholesterol (mmol/L)	r = 0.333 p = 0.058	r = 0.132 p = 0.457	r = -0.129 p = 0.468
Triglyceride (mmol/L)	r =0.217 p =0.203	r = -0.114 p = 0.501	r = -0.125 p = 0.461

n=38

Table 8-5 Relationships between plasma lipids and haemostatic and rheological measures at baseline

Haemostatic factors	Cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
Haematocrit (%)	r = 0.142 p = 0.381	r = 0.313 p = 0.072	r = 0.130 p=0.445	r = -0.085 p = 0.604
Plasma viscosity (mPas)	r = 0.445 p = 0.004	r = 0.436 p = 0.012	r = 0.313 p=0.059	r = 0.529 p = 0.0001
Fibrinogen (g/L)	r = 0.046 p = 0.806	r = 0.020 p = 0.918	r = -0.073 p = 0.706	r = 0.252 p = 0.172
Factor VII activity (IU/dL)	r = 0.219 p = 0.254	r = 0.107 p = 0.611	r = 0.056 p = 0.780	r = 0.322 p = 0.088
PAI activity (% pool)	r = -0.286 p = 0.166	r = -0.218 p = 0.318	r = -0.244 p = 0.250	r = 0.117 p = 0.577
t-PA antigen (μg/mL)	r = 0.127 p = 0.529	r = -0.201 p = 0.325	r = -0.203 p = 0.320	r = 0.088 p = 0.662

n=38

Table 8-6 Relationships between plasma lipids and haemostatic and rheological measures at week twelve

Haemostatic factors	Cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
Haematocrit (%)	r = 0.029 p = 0.890	r =0.158 p = 0.471	r = -0.664 p = 0.0001	r = 0. 128 p = 0. 543
Plasma viscosity (mPas)	r = 0.249 p = 0.241	r = 0.295 p = 0.183	r = -0.430 p = 0.04	r = 0.469 p = 0.021
Fibrinogen (g/L)	r = 0.356 p = 0.046	r = 0.387 p = 0.038	r = -0.243 p = 0.188	r = 0.154 p = 0.401
Factor VII activity (IU/dL)	r = 0.100 p = 0.607	r = 0.059 p = 0.776	r = 0.050 p = 0.800	r = -0.041 p = 0.832
PAI activity (% pool)	r = 0.218 p = 0.284	r = -0.323 p = 0.123	r = -0.050 p = 0.814	r = 0.288 p = 0.154
t-PA antigen (μg/mL)	r = 0.454 p = 0.017	r = 0.459 p = 0.016	r = -0.223 p = 0.264	r = 0.330 p = 0.092

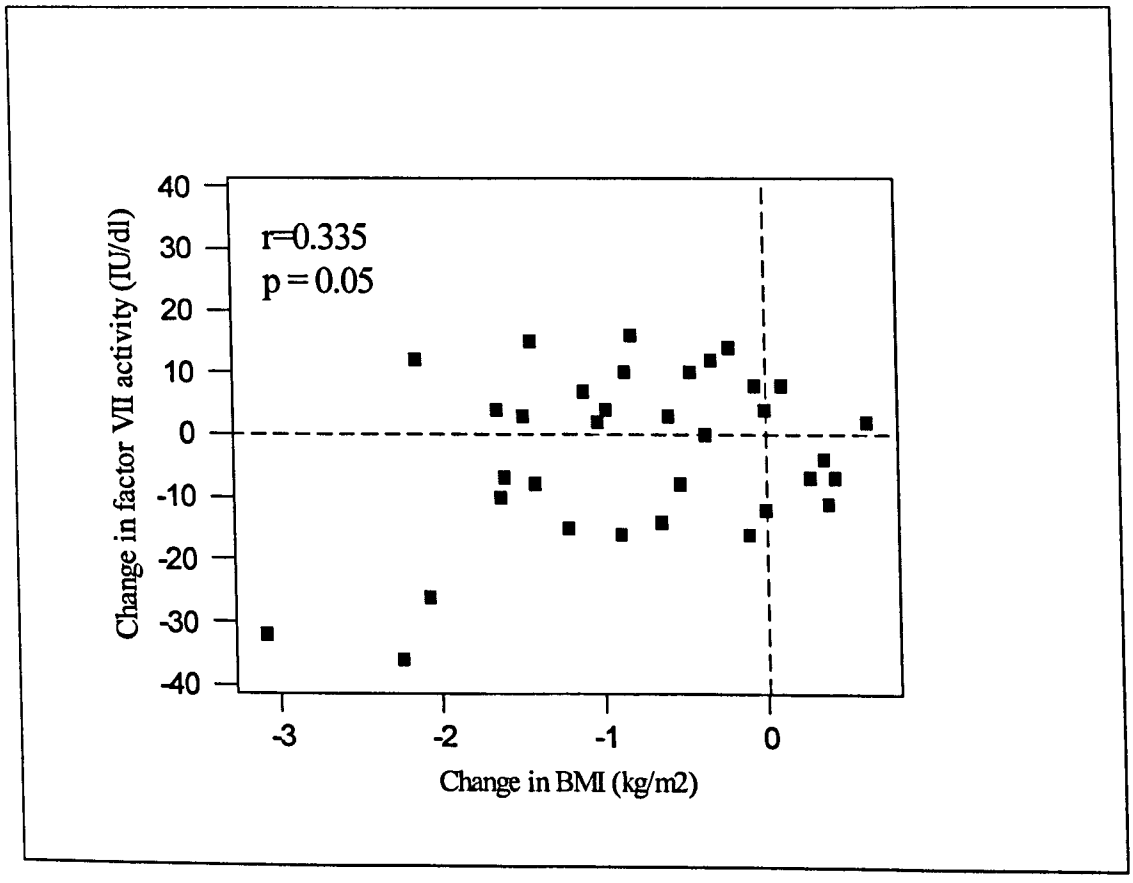
n=38

Table 8-7 Relationships between changes in plasma lipids and changes in haemostatic and rheological measures

Change in haemostatic factors	Change in cholesterol (mmol/L)	Change in LDL cholesterol (mmol/L)	Change in HDL cholesterol (mmol/L)	Change in triglyceride (mmol/L)
Haematocrit (%)	r = 0.209 p = 0.328	r =0.237 p = 0.288	r = 0.016 p = 0.945	r = -0.237 p = 0.265
Plasma viscosity (mPas)	r = 0.129 p = 0.556	r = 0.144 p = 0.533	r = -0.035 p=0.881	r = 0.207 p = 0.342
Fibrinogen (g/L)	r = 0.016 p = 0.932	r = -0.015 p = 0.939	r = -0.189 p = 0.335	r = 0.225 p = 0.224
Factor VII activity (IU/dL)	r = 0.041 p = 0.838	r = -0.049 p = 0.819	r = 0.141 p = 0.512	r = -0.008 p = 0.968
PAI activity (% pool)	r = -0.534 p = 0.006	r = -0.549 p = 0.007	r = -0.215 p = 0.325	r = 0.234 p = 0.260
t-PA antigen (μg/mL)	r = -0.230 p = 0.248	r = -0.287 p = 0.155	r = -0.157 p = 0.443	r = -0.232 p = 0.244

n=38

Figure 8-1 Relationship between changes in factor VII activity and body mass index



n = 36, (4 women, 32 men)

Plate 8-1 British Petroleum Refinery, Grangemouth

Chapter 9 The effects of moderate weight loss on haemostatic and rheological factors and plasma lipids in all subjects who participated in the present studies

9.1 Introduction and hypotheses

A BMI 25.0-30.0 kg/m^2, BMI > 30.0 kg/m^2, obesity and a BMI at the top of the healthy range (BMI 22.5-25.0 kg/m^2) are all established as increasing IHD risk (Lew and Garfinkel, 1979, Manson *et al*, 1990, Colditz *et al*, 1995). The earlier chapters have described the effects from the same dietary intervention for weight loss in these three different weight catagories. The outcome measures were established IHD risk factors relating to haemostasis, rheology and plasma lipids. This chapter firstly examined the effects from dietary intervention and weight change on IHD risk factors for all subjects considered as a single group. Secondly, it sought any differences in IHD risk factors and success in achieving weight loss between the groups.

There were five research questions to be answered:

1) Does the subject group diagnosed with angina show raised IHD risk factors in comparison to those of the other subject groups at baseline?

2) Does the angina group achieve greater weight losses and changes in IHD risk factors, haemostatic and rheological measures and plasma lipids, compared with the other subject groups after intervention?

The remaining questions concern the three subject groups considered as one.

3) Does a dietary intervention incorporating an energy deficit approach to weight loss (600 kcal per day) achieve losses of close to the theoretical 0.5 kg per week?

4) Does weight loss following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets, improve haemostatic and rheological risk factors for IHD across a wide BMI range?

5) Does weight loss following a dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets, improve plasma lipid concentrations across a wide BMI range?

9.2 Methods

The data concerning the subjects from each group (chapter 6, 7 and 8) were merged and analysed in two ways. Firstly, one way analysis of variance was used to determine any differences between the groups in their body weight and IHD risk factors at baseline. Mann Whitney or Student's t-tests were used to examine any differences between the groups. These same approaches were used to examine if any changes achieved in body weight and IHD measurements after dietary intervention were significantly different according to group. The second analysis in all subjects used Students t-test to test for ay changes between body weight and BMI and IHD risk factors after dietary intervention. Any relationships between changes in body weight or BMI, and IHD risk factors were examined by correlation analysis and linear regression analysis. The regression analyses were completed to evaluate the effects of age, sex, study group and presence of disease on any changes in the outcome measures of interest.

9.3 Results

A total of 132 subjects (69 women, 63 men) completed the weight loss interventions. The overweight/obese subjects numbered 45, 36 women and 9 men with a body weight range of 86.6 -153.2 kg. The overweight/obese subjects with angina group numbered 49, 27

men and 22 women, with a body weight range of 68.8 - 114.0 kg. The close to healthy weight group numbered 38, 31 men and 7 women, with a body weight range of 57.8 - 91.0 kg.

The laboratory data were incomplete for all 132 subjects, due either to insufficient assay sample, or lost samples. The number of analyses for each IHD risk factor are given in the relevant table.

The characteristics of each study group at baseline are given in table 9.1. There were significant differences in age, with the angina group significantly older than the other two groups ($p<0.05$). The overweight group had a higher mean body weight and BMI than the angina or close to healthy weight groups ($p<0.05$), with the latter group having the a lower WHR ($p<0.01$). Plasma total and LDL cholesterol concentrations were highest in the angina subjects, and lowest in the close to healthy BMI subjects ($p<0.01$). Surprisingly, the haematocrit was highest in the close to healthy BMI subjects and lowest in the overweight group ($p<0.01$). Plasma viscosity, fibrinogen, PAI activity, t-PA antigen and were lowest in the close to healthy weight group. Factor VII activity, HDL cholesterol and triglyceride concentrations did not differ significantly according to group.

The changes in body weight, BMI and the IHD risk factors according to group are shown in table 9.2. Weight loss was significantly greater in the overweight (4.8%) than the healthy weight subjects (3.3%) $p=0.0026$. The angina subjects were not significantly different in their weight loss compared to either group. There was no change according to group in WHR or plasma lipids, total, HDL, LDL or HDL cholesterol or triglyceride concentrations. There were no significant differences in the changes in the IHD risk factors after intervention according to study group (table 9.2).

Since there were no differences between the groups in changes in haemostatic and rheological factors, there were examined as a single group. The characteristics at baseline, post-intervention and changes in anthropometric and IHD measurements for all subjects are given in table 9.3. The RCA is not included as it was not measured in all of the angina group or in any of the close to healthy weight subjects. The range of baseline body weight was 57.8 to 150.2 kg. There was a significant (4.2%) fall in body weight and BMI (p=0.00001). This represented an average weekly weight loss of 0.3 kg, which represented 60% of the target value. Total cholesterol (6.7%, p=0.00001), LDL (5.0%, p=0.015) and HDL cholesterol (7.1%, p=0.028), fell significantly, whilst triglyceride remained unchanged. Mean factor VII activity was significantly reduced (-5.1%) in all subjects (p=0.0011), and fibrinogen concentrations rose (+3.2%, p=0.042).

The relationships between the changes in body weight and IHD risk factors examined by Pearson's correlation analyses are shown in table 9.4. There was a significant relationships between changes in body weight and factor VII activity (p=0.001, figure 9.1), total cholesterol, (p=0.026, figure 9.2), and LDL cholesterol (p=0.015, figure 9.3). Regression analysis showed no influence of age, sex, study group or BMI on these changes. The change in BMI as a covariate in the regression analysis was a significant influence on the changes in factor VII activity (p=0.003), total (p=0.03) and LDL cholesterol (p=0.004) after dietary intervention.

9.4 Discussion

The three subject groups recruited to this series of studies were compared in terms of their IHD risk factors. The first research question was whether the subject group with angina, would show higher IHD risk factors compared to the other two groups at baseline. The

results for IHD risk factors were highest in those with angina, which proved the research question. However, the question whether the angina subjects would achieve the greatest weight losses and in turn the greatest falls in IHD risk factors was unproven. The failure of the presence of IHD to improve dietary compliance of the angina group and exceed that in the other groups, without a diagnosis of IHD, could reflect many factors. It seemed important that the diagnoses of angina were not new, and therefore the impetus often associated with a new diagnosis was not apparent. A future study examining newly diagnosed angina patients and their compliance with dietary advice would be interesting to try and evaluate this effect. The greater age of the angina group was likely to have contributed both to their increased IHD risk and possibly, their difficulty in achieving good dietary compliance.

The overall weight loss of all subjects was 60% of the target suggesting that the individualised energy deficit approach to weight loss has promise for the wider application in weight management. It was interesting to note that the overweight subjects with the highest mean BMI lost the greatest amount of body weight whereas the healthy weight subjects lost the least. This latter group has been rarely exposed to prescriptive dietary advice and therefore the results were the most difficult to predict. However, the fact that these subjects, with a desire to lower their body weight, were the leanest at baseline, made it less easy to predict their compliance with prescriptive dietary advice. The results in terms of weight loss support the theory that, in the short term, the greater body weight ensures the greatest weight loss.

As all participants were volunteers and therefore motivated to take part in the study, it was expected that the weight loss would have been closer to the calculated 6.0 kg. Considering the effect of dietary advice in all subjects as one group, the 60% target weight loss was only moderately encouraging. In a routine clinical setting the weight loss results

following this approach may be less, with routine patients rather than volunteers being advised, and perhaps being insufficient to result in any measurable health benefits.

The differences in body weight loss between study groups in terms of target weight, 75% (overweight), 58% (angina) and 43% (close to healthy weight), provide some support in these subject groups for the use of the 600 kcal energy deficit approach to weight loss.

The results from the present studies have proven research questions four and five with significant reductions noted in IHD risk factors after weight loss. These findings supported the role of moderate weight loss in improving some plasma lipids, haemostatic and rheological risk factors.

9.5 Conclusion

The 600 kcal energy deficit dietary approach to weight loss, currently favoured in the SIGN guidelines (SIGN, 1996), resulted in 60% of target weight loss being achieved. This appears, from available clinical data, to be at least as effective as other dietary approaches to weight loss currently used in clinical practice. The present results showed that close to 5% weight loss reduced IHD risk. However, it was surprising that the presence of IHD did not improve dietary compliance in comparison to healthy subjects. These studies support the further testing of this approach to weight management in clinical practice.

Table 9-1 Anthropometric and IHD risk factor measures at baseline according to study group

	Overweight	Angina	Healthy weight	p
Age (years)	43.6 (10.4)[a, b]	60.3 (6.5)[a]	41.6 (8.5)[b]	0.0001
BMI (kg/m^2) *	34.9 (6.1)[a]	29.3 (4.1)[b]	25.3 (1.4)[c]	0.0001
Weight (kg) *	93.5 (19.3)[a]	81.6 (11.7)[b]	77.9 (8.1)[c]	0.0001
Waist to hip ratio	0.99 (0.05)[a, b]	1.00 (0.06)[a]	0.88 (0.06)[b]	0.0001
Total cholesterol (mmol/l)	5.8 (1.1)[a, b]	6.3 (1.1)[a]	5.5 (0.9)[b]	0.005
LDL cholesterol (mmol/l)	3.8 (1.1)[a, b]	4.6 (0.9)[a]	3.4 (0.8)[b]	0.001
HDL cholesterol (mmol/l)	1.3 (0.5)[a]	1.3 (0.3)[a]	1.5 (0.4)[a]	0.232
Triglyceride (mmol/l)	1.4 (0.6)[a]	1.8 (0.8)[a]	1.3 (0.8)[c]	0.089
Haematocrit (%) *	43 (4)[a]	45 (5)[b]	46 (3)[c]	0.008
Plasma viscosity (mPas)	1.31 (0.07)[a]	1.30 (0.08)[a, b]	1.25 (0.07)[b]	0.0001
Fibrinogen (g/L) *	3.3 (0.9)[a]	3.3 (0.9)[b]	2.5 (0.5)[c]	0.001
Factor VII activity (IU/dL)	115 (2)[a]	120 (23)[a]	108 (17)[a]	0.092
PAI activity (% pool) *	147 (62)[a]	134 (75)[b]	86 (39)[c]	0.0001
t-PA antigen (μg/mL) *	9.2 (5.1)[a]	10.9 (3.2)[b]	8.4 (3.8)[c]	0.006

Significantly different from each other when superscripts differ (t-test, or * indicates Mann Whitney test), Data as mean (SD), n= 132

Table 9-2 Changes in anthropometric and IHD risk factors measures after intervention according to study group

	Overweight	Angina	Healthy weight	p
Weight (kg)	-4.5 (2.9)[a]	-3.5 (2.6)[a, b]	-2.6 (2.6)[b]	0.008
BMI (kg/m^2)	-1.7 (1.1)[a]	-1.2 (0.9)[a, b]	-0.9 (0.8)[b]	0.008
Waist to hip ratio	-0.1 (0.1)[a]	-0.01 (0.06)[a]	-0.01 (0.06)[a]	0.458
Total cholesterol (mmol/l)	-0.4 (0.8)[a]	-0.4 (0.8)[a]	-0.3 (0.8)[a]	0.713
LDL cholesterol (mmol/l)	-0.2 (0.9)[a]	-0.3 (0.6)[a]	-0.2 (0.3)[a]	0.490
HDL cholesterol (mmol/l)	-0.1 (0.5)[a]	-0.1 (0.6)[a]	-0.1 (0.9)[a]	0.714
Triglyceride (mmol/l)	0.0 (0.4)[a]	-0.1 (0.9)[a]	0.0 (0.1)[a]	0.722
Haematocrit (%)	0 (4)[a]	-0.5 (6)[a]	-1 (3)[a]	0.332
Plasma viscosity (mPas)	-0.02 (0.07)[a]	-0.01 (0.2)[a]	-0.01 (0.03)[a]	0.455
Fibrinogen (g/l)	-0.4 (1.4)[a]	+0.2 (0.1)[a]	+0.2 (0.8)[a]	0.334
Factor VII activity (IU/dL)	-7 (17)[a]	-5 (20)[a]	-3 (10)[a]	0.334
PAI activity (% pool)	-7 (59)[a]	-11 (72)[a]	-18 (16)[a]	0.500
t-PA antigen (μg/mL)	-0.4 (3.4)[a]	-0.3 (2.8)[a]	-0.3 (2.9)[a]	0.669

Significantly different from each other when superscripts differ (t-test)

Data as mean (SD), n= 132

Table 9-3 Characteristics of all subjects who completed the study at baseline and week twelve

	n	Baseline	Week 12	Change	p
Weight (kg)	132	84.5 (15.4)	80.9 (14.8)	-3.6 (2.8)	0.00001
BMI (kg/m^2)	132	30.0 (5.9)	28.8 (5.6)	-1.2 (1.0)	0.00001
Waist to hip ratio	132	0.93 (0.07)	0.92 (0.09)	-0.01 (0.06)	0.12
Total cholesterol (mmol/l)	109	5.9 (1.1)	5.5 (1.1)	-0.4 (0.8)	0.00001
LDL cholesterol (mmol/l)	84	4.0 (1.0)	3.8 (0.9)	-0.2 (0.8)	0.015
HDL cholesterol (mmol/l)	86	1.4 (0.4)	1.3 (0.4)	-0.1 (0.4)	0.028
Triglyceride (mmol/l)	105	1.4 (0.7)	1.5 (1.0)	+0.1 (0.9)	0.37
Haematocrit (%)	110	45 (4)	44 (3)	-1 (4)	0.46
Plasma viscosity (mPas)	103	1.28 (0.08)	1.27 (0.08)	-0.01 (0.07)	0.56
Fibrinogen (g/l)	114	3.1 (0.9)	3.2 (0.8)	+0.1 (0.8)	0.042
Factor VII activity (IU/dL)	109	116 (21)	110 (20)	-6 (20)	0.011
PAI activity (% pool)	106	122 (65)	115 (63)	-7 (49)	0.18
t-PA antigen (μg/mL)	99	9.5 (3.9)	8.8 (3.5)	-0.7 (3.3)	0.056

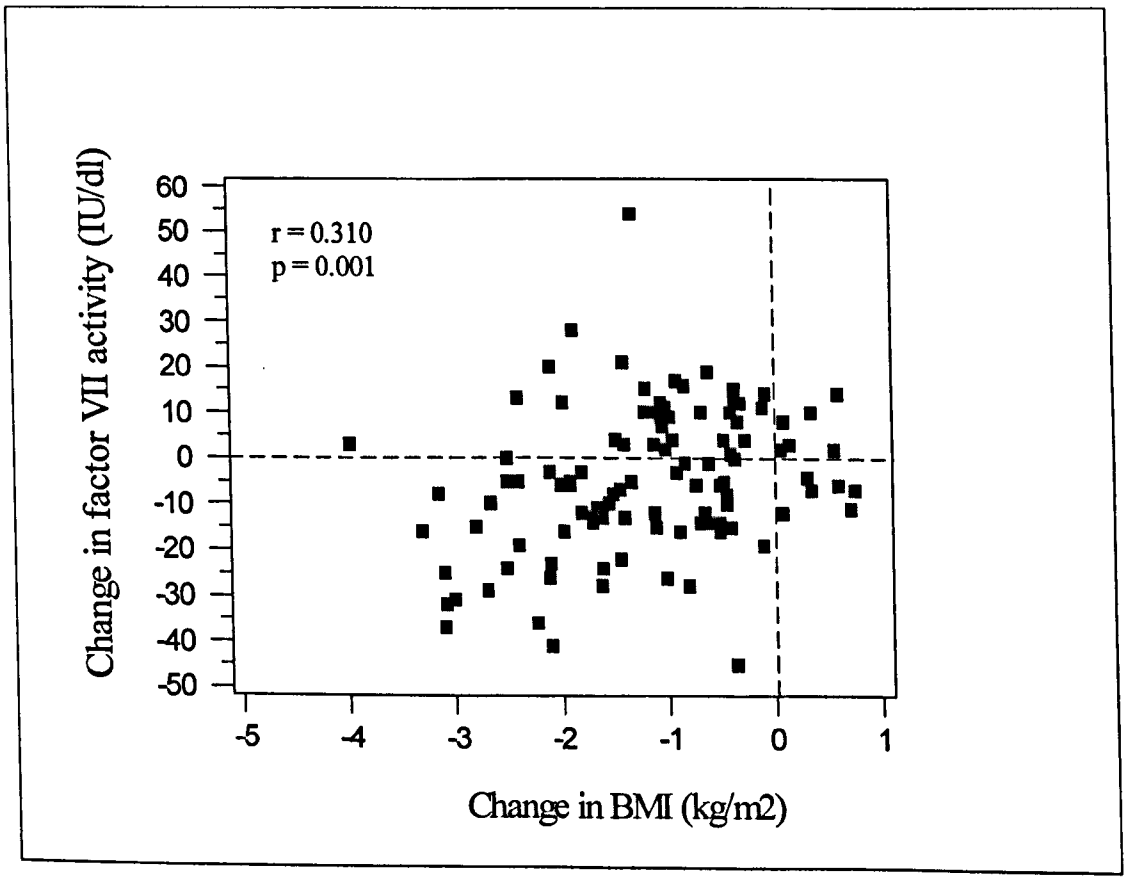
n=132

Table 9-4 Relationships between changes in haemostatic and rheological factors plasma lipids and weight change

Change in risk factor	Correlation coefficient
Total cholesterol (mmol/L)	r=0.213 p=0.026
HDL cholesterol (mmol/L)	r=0.111 p=0.307
LDL cholesterol (mmol/L)	r=0.264 p=0.015
Triglyceride (mmol/L)	r=-0.027 p=0.784
Haematocrit (%)	r=-0.059 p=0.539
Plasma viscosity (mPas)	r= -0.038 p=0.705
Change in fibrinogen (g/l)	r= 0.005 p= 0.960
Factor VII activity (IU/dL)	r= 0.310 p= 0.001
PAI activity (% pool)	r= 0.078 p= 0.430
t-PA antigen (μg/mL)	r= 0.134 p= 0.185

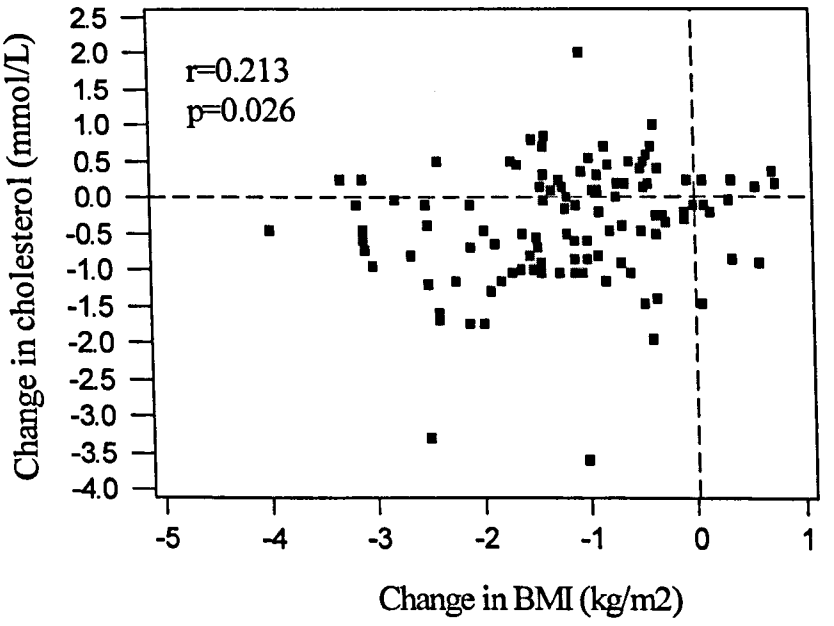
n=132

Figure 9-1 Changes in body weight and factor VII activity



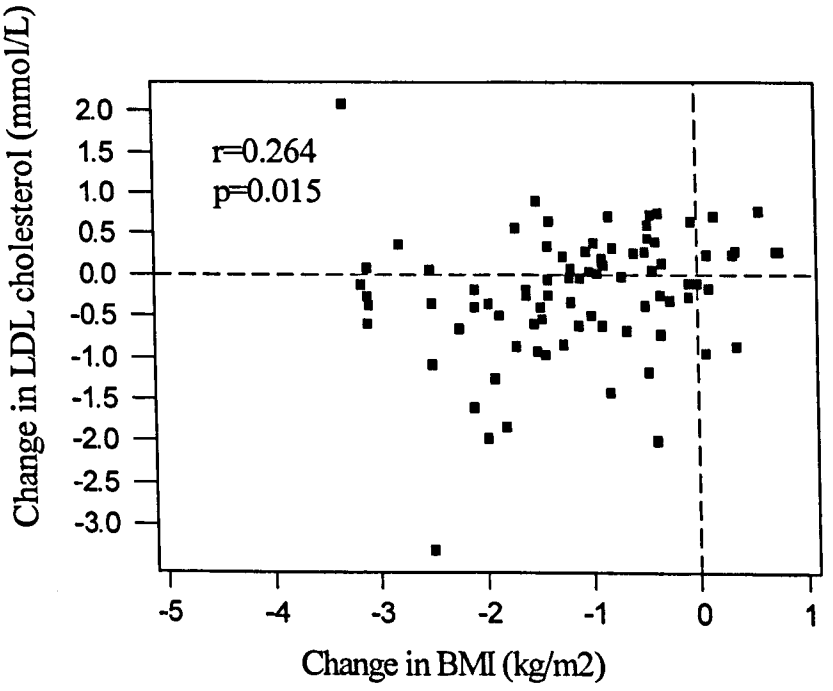
n = 107

Figure 9-2 Changes in body weight and total cholesterol concentrations



n = 109

Figure 9-3 Changes in body weight and LDL cholesterol concentrations



n=48

Chapter 10 The effects of moderate weight loss on serum dehydroepiandosterone sulphate concentrations in overweight subjects with angina pectoris and in those with body mass index close to 25 kg/m^2

10.1 Introduction and hypotheses

Intentional weight loss in the overweight offers real benefits in terms of mortality (Williamson *et al,* 1995) and morbidity (Goldstein, 1992). The effects of weight loss in older subjects with established ischaemic disease and elevated coronary risk have been less rigorously examined, although Williamson *et al* (1995) suggested a benefit in women with existing obesity related disorders.

Androgens are increasingly identified as key components of the insulin resistance "metabolic syndrome", with particular reference to PCOS (Nestler *et al,* 1988). A role has been proposed for DHEAS, the major adrenal androgen, in the prevention of atherosclerosis. DHEAS has been postulated as the possible link between hyperinsulinaemia and obesity (Nestler *et al,* 1992). Recent evidence suggests that DHEAS concentrations exert multiple anti-atherogenic effects and that hyperinsulinaemia may reduce serum DHEAS concentrations by decreasing production and enhancing clearance. Lower DHEAS concentrations are found in those with elevated coronary risk and the overweight (Barrett-Connor *et al,* 1986, Nestler *et al,* 1989, Nestler *et al,* 1992). Men who survived MI have also been shown to have reduced serum DHEAS concentrations in comparison with healthy matched controls (Slowinska-Srzednicka *et al,* 1989, Mitchell *et al,* 1994). In women, a prospective study failed to find any relationship between DHEAS concentrations and IHD (Barrett-Connor and Goodman-Gruen, 1995).

Some improvements have been reported in DHEAS concentrations with weight loss, in subjects with a mean age of 40 years of both sexes (Leenen *et al,* 1994, Jakubowicz *et al,*

1995) but not older subjects with known IHD. The present studies used dietary advice which reflected the present recommended dietary composition (COMA, 1991) together with a moderate daily energy restriction to investigate the effects of weight loss in angina subjects on serum DHEAS concentrations, urinary steroid metabolites and insulin concentrations. Although the effect of considerable amounts of weight loss on circulating DHEAS concentrations in healthy but overweight subjects has now been studied (Leenen *et al*, 1994), no information is available as to the effect of moderate weight loss alone. In the present study a dietetic led approach was used to reduce body weight in healthy subjects who were above their own preferred body weight, but with a BMI close to the reference range (18.5-25.0 kg/m^2).

The hypotheses for this work was that weight loss would increase plasma DHEAS concentrations, thus reducing IHD risk. There were three research questions related to DHEAS and urinary steroid metabolites for these studies.

1) What is the effect of moderate weight loss on circulating DHEAS concentrations in overweight elderly subject with angina pectoris?

2) What is the effect of moderate weight loss on the principal urinary steroid metabolites?

3) What is the effect of weight loss on circulating DHEAS concentrations in a group of subjects close to healthy weight?

It was proposed that moderate weight loss may be beneficial in terms of both DHEAS concentrations and IHD risk in younger subjects, prior to the onset of the age related reductions in serum DHEAS.

10.2 Methods

10.2.1 Study design and statistical approaches

The study design was a single stranded study, according to the reasons given in chapter 4. The sample size that was required to complete these studies was 57 subjects. The biological data were normally distributed as determined from a distribution plot and were analysed using paired Student's t tests. The dietary data showed a skewed distribution and were analysed using a paired Wilcoxon test. Pearson's correlation analysis was carried out to investigate any relationships between changes in body weight, or BMI and percentage body fat and DHEAS and insulin concentrations and plasma lipid measurements.

10.2.2 Subjects

Fifty four overweight angina subjects (31 men) with a BMI > 26 kg/m^2 who had received a medical diagnosis of angina pectoris were recruited (chapter 7). Fifty one healthy subjects were recruited to a worksite intervention (chapter 8). The details of the physical measurements made and the dietary interventions given to all subjects have already been described (chapters 5, 7-8).

10.2.3 Venesection and laboratory methods

Fasting blood was sampled at baseline and post-intervention (week 12) for all subjects. Total serum concentrations of DHEAS concentrations were determined using a radioimmunoassay kit (ICN Biomedicals, High Wycombe, Bucks., UK). Insulin assays were determined using an "in house" immunoradiometric assay. Both of these methods have already been described (chapter 5). For the angina subjects, complete volume 24 hour urine collections were made and urinary steroid profiles were conducted on 10 mL aliquots. The gas chromatography/mass spectrometry method used was based on that of

Shackleton (Shackleton, 1985) using a Fisons MD 800 G C mass spectrometer. The inter- and intra-assay CV's were < 15% and <10%, respectively for all of the major urinary steroid metabolites measured. The androgen (aetiocholanolone and androsterone) and glucocorticoid metabolites, tetrahydrocortisone, tetrahydrocortisol, and allo-tetrahydrocortisone, were quantified. Each set of pre- and post-treatment measurements were assayed in the same batch.

10.3 Results

10.3.1 Angina subjects

Thirteen subjects failed to complete the protocol (9 men, 4 women). Data describing the characteristics of the 41 subjects (21 men) who completed the study are described (table 10.1). There were no significant differences between the sexes for age (p=0.76) although men were significantly heavier than women with mean BMI's of 30.5 (SD 4.7) vs. 27.4 (SD 2.9) kg/m^2 (p=0.013). Percentage body fat was significantly higher in women than men (p=0.0001). At baseline no significant differences were seen in the biochemical measurements according to gender, and therefore the subjects were analysed as a single group. A significant relationship was observed between age and DHEAS concentrations (p=0.009, figure 10.1). No significant associations between either BMI or percentage body fat were observed for baseline DHEAS concentrations, principle urinary steroid metabolites or plasma insulin. No significant relationship between DHEAS and BMI was noted (p=0.279, figure 10.2). The urinary steroid metabolites did not significantly change after intervention (table 10.2).

10.3.2 Effects of weight loss in angina subjects

Mean weight change over 12 weeks was similar at -3.3 in men and women, range -11.1 to +1.7 kg in the angina study. There was a significant fall in plasma insulin in women (13.1

to 9.7 mU/L, p=0.023) (table 10.1). At baseline, there were few significant associations from correlations between biochemical measures, BMI, waist circumference or WHR. The exceptions were the associations between BMI and the urinary metabolites tetrahydrocortisone and the total of allo-tetrahydrocortisol, tetrahydrocortisol, tetrahydrocortisone and tetrahydrocortisol (table 10.3). At week 12 these same relationships were seen, and additional relationships between BMI and urinary andosterone and 5 alpha tetrahydrocortisol (table 10.4). There were no associations between the measures of greatest interest in this study, plasma insulin and serum DHEAS concentrations, at baseline (r=-0.055, p=0.769), week 12 (r=0.073, p=0.696) or the changes (r=-0.062, p=0.702).

After intervention there were no associations between changes in BMI and changes in serum DHEAS and urinary steroid metabolites.

10.3.3 Healthy subjects

Amongst the healthy subjects 13 subjects failed to complete the protocol. The characteristics of the remaining 31 subjects are shown (table 10.6). Women had substantially lower serum DHEAS concentrations, which were substantially lower than those of men, mean 4.4 (SD 3.5) vs. 8.7 (SD 4.6) μmol/L. Due to their small numbers, women were discarded from the analysis. The mean weight loss for the male subjects was 2.9 (SD 2.7) range -9.8 - +2.10 kg (table 10.6). There were no significant relationships between age or baseline BMI and serum DHEAS concentrations, except between BMI and plasma insulin concentrations post-intervention (r=0.030, p=0.873, figure 10.3, r=0.013, p=0.947, figure 10.4).

10.3.4 Effects of weight loss in healthy subjects

The characteristics at baseline and week 12 and the changes in physical and biochemical measures are shown (table 10.6). Post-intervention there was no change in serum DHEAS or plasma insulin after intervention. Percentage body fat, BMI or serum DHEAS concentrations showed no significant associations at baseline or post-intervention (table 10.7-10.9). Insulin was the exception and BMI at baseline and post-intervention. At week 12 plasma insulin concentration was significantly related to waist circumference ($r=0.323$, $p=0.045$, table 10.8). There were no associations with BMI, waist and WHR and changes in serum DHEAS and plasma insulin concentrations post-intervention (table 10.9). In particular, there were no significant associations between changes in insulin and serum DHEAS concentrations.

10.3.5 Healthy subjects and angina subjects

In order to examine the relationships between body weight, weight change, percentage body fat and serum DHEAS and insulin the healthy subjects and those with angina were combined into a single group of 72 subjects with a wider ranges of age, BMI and weight losses.

10.3.6 Effects of weight loss in healthy subjects and angina subjects

The physical and biochemical measurements for all subjects are shown (table 10.10). No significant changes in serum DHEAS or plasma insulin were observed. At baseline significant correlations were found between age, BMI, WHR and serum DHEAS and plasma insulin concentrations (table 10.11). Post-intervention these relationships were maintained, with the exception of WHR (table 10.12). At each measurement occasion, no relationship between serum DHEAS and plasma insulin was observed, and no significant relationships were seen between the changes pre- and post-intervention. After

intervention there were no significant associations between changes in BMI, body weight or percentage body fat and changes in serum DHEAS or insulin concentrations.

The data were examined for correlations between BMI, and WHR, at baseline and week 12 and significant relationships were found (table 10.11-10.13). At baseline plasma insulin and serum DHEAS concentrations were significantly correlated with BMI, and WHR, and there were similar relationships at week 12. However, no significant relationships were seen between the changes in BMI, WHR and serum DHEAS and plasma insulin (table 10.13).

The relationships between age and serum DHEAS are shown (r=-0.578, p=0.0001, figure 10.5, and between serum DHEAS and BMI before and after intervention (r=0.264, p=0.026, figure 10.6,) and r=-0.313, p=0.008, figure 10.7). The positive relationships between insulin and BMI before and after intervention are shown (r=0.285, p=0.016, figure 9.8, r=0.310, p=0.011, figure 9.9).

10.4 Discussion

DHEAS has been proposed as a potential anti-obesity agent (Berdanier *et al*, 1993) based mainly on studies confounded by age. The theoretical benefits of increasing serum DHEAS concentrations in terms of combating the effects of ageing (Morales *et al*, 1994), reducing the risk for IHD and the possible role as an anti-obesity agent has led to DHEAS supplementation studies (Herbert, 1995). However, the results from these studies are equivocal. Supplementation of DHEAS has led to the development of a highly androgenic state and insulin resistance in post-menopausal women when given at 1600 mg per day for 28 days (Mortola and Yen, 1990). In contrast, DHEAS supplementation in men at 50 mg per day for 6 months lowered LDL cholesterol concentrations and reduced body fat (Nestler *et al*, 1988). Another study found no anti-obesity effects with DHEAS supplementation (Usiskin *et al*, 1990). The side effects of supplementation remain

unknown and therefore any supplementation with steroid hormones must be approached with caution. Therefore the act of losing weight as a potential mechanism of raising endogenous DHEAS appears attractive.

The hypotheses for the present studies was that body weight loss would increase plasma DHEAS concentrations, thus reducing IHD risk. The first research question for these studies was to examine whether moderate weight loss would effect serum DHEAS concentrations in overweight, elderly subjects with angina pectoris. Three reports have suggested an increase in serum DHEAS concentrations with substantial weight loss in the overweight, (Leenen *et al*, 1994, Crave *et al*, 1995, Jakubowicz *et al*, 1995). All three studies recruited subjects aged close to a mean age of forty years who were free of cardiovascular disease. A 1000 kcal dietary plan was used in a study of overweight men and pre-menopausal women and aged below 51 years (Leenen *et al*, 1994). These subjects had a higher BMI than those in the present study (range 28-38 kg/m^2) and a 14% weight loss (12-13 kg) resulted in acute increases in serum DHEAS concentrations of 36% and 32% in women and men and a corresponding 45% and a 35% reduction in insulin concentrations in women and men respectively. Another study achieved 4.9 kg (5.8%) weight loss in hyperinsulinaemic pre-menopausal hirsute women using a conventional dietary approach of 1500 kcal daily (Crave *et al*, 1995). This increased subjects serum DHEAS concentrations by 15-20% and decreased their plasma insulin concentrations by 15-20 %. The third study recruited both men and pre-menopausal women and achieved a 10% weight loss using a 1000-1400 kcal per day conventional dietary regimen. Men showed significant elevations (125%) in serum DHEAS concentrations although no such changes were observed in women. The authors were unable to explain these sex specific effects on serum DHEAS as both men and women showed significant falls in insulin concentrations after weight loss (Jakubowicz *et al*, 1995). The results from these three studies were promising as to the potential benefits of

moderate weight loss on increasing serum DHEAS and lowering plasma insulin concentrations. However, the older subjects in the present study were probably approaching their lowest adrenal production rate (DePretti *et al*, 1978). The ability of older persons to increase or alter their steroid hormone production rate or their metabolic clearance rate is uncertain (Kurtz *et al*, 1987).

In men high serum DHEAS concentrations have been related to a central fat distribution, while low serum DHEAS concentrations have been related to hyperinsulinaemia (Herranz *et al*, 1995). This finding was also shown in pre-menopausal women (Williams *et al*, 1993). A review has suggested that insulin had helped to lower serum DHEAS by inhibiting the production, rather than the clearance of serum DHEAS and related steroids (Nestler *et al*, 1991). A significant inverse correlation was found between BMI and DHEA (not DHEAS) concentrations in a study of morbidly obese pre-menopausal women (mean BMI 41.8 (SD 10.3) kg/m^2 (De Pergola *et al*, 1991) and between increasing visceral fat and increasing serum DHEAS concentrations (De Pergola *et al*, 1994). The results of the present study failed to confirm either a relationships between serum DHEAS and insulin, or between plasma insulin and serum DHEAS concentrations and body fat percentage pre-intervention. The lack of association between changes in insulin and changes in serum DHEAS are contrary to other results (Leenen *et al*, 1994, Crave *et al*, 1995, Jakubowicz *et al*, 1995) and may reflect the rather modest degree of overweight in our subjects, and the fact that they were not selected on the basis of hyperinsulinaemia. This is despite the care taken in providing an accurate dietary prescription derived using measured REE. The use of hyperinsulinaemia as a selection criteria was most pertinent to the comparison with the findings of the Crave study (Crave *et al*, 1995). Their population was both obese and hirsute indicating the presence of insulin resistance unlike that in the study population. The weight loss of 4.9 kg (5.9% body weight) was very close to that achieved in this present study. However, the subjects in the present study were not

selected on the criteria of hyperinsulinaemia. The other study of weight loss (Jakubowicz *et al*, 1995) included subjects who were not insulin resistant but who had achieved an 11% body weight loss, greater than that achieved in this study.

From theoretical considerations a lower fat intake could enhance insulin sensitivity and therefore in older subjects increase serum DHEAS (Ward *et al*, 1994). However, a mixed omnivorous diet has been shown to have no important influence on serum DHEAS in comparison with a vegetarian diet (Hill and Wynder, 1979). In view of this finding seems unlikely that the relatively low fat intake achieved in the present study (30% v 34% energy) would have had any major effects. Another study in pre-menopausal women which sought to examine the relationship between dietary lipid and adrenal hormones found a significant positive relationship between the level of dietary fat and serum DHEAS concentrations. An inverse relationship was seen with serum DHEAS and plasma insulin concentrations (Bhathena *et al*, 1989).

To gain a greater insight into how any change in body weight may have influenced the metabolism of steroids, urinary steroid metabolites were measured, in accordance with the second research question. Would moderate weight loss effect the principle urinary steroid metabolites? The principal urinary excretion of steroid metabolites (androsterone, aetiocholanolone, tetrahydrocortisol, tetrahydrocortisone and allo- tetrahydrocortisol) were unaffected by dietary intervention and weight loss within this study. The other studies which have examined the effect of weight loss, mentioned in this chapter did not include these urinary measurements (Leenen *et al*, 1994, Crave *et al*, 1995, Jakubowicz *et al*, 1995). An early study (Hendrikx *et al*, 1968) reported a fall in the excretion of serum DHEAS and metabolites with slimming over a 14 day period. Plasma insulin concentrations are known to increase both conjugation and excretion of steroids (Nestler *et al*, 1993). However, there are difficulties with requesting volunteers to make 24 hour

urinary collections. The value of any laboratory measurements depend on the completeness of the 24 hour urine collections as the results are expressed by urine volume. It has been established using para-amino benzoic acid that twenty four hour collections are often incomplete (Bingham and Cummings, 1985). Primarily, as urine collections require a large amount of subject co-operation, and are awkward to carry out and ensure no urine has been discarded. The angina subjects were an elderly group, and their age may have compounded these difficulties. The finding that there was no change in urinary steroids after weight loss was not unexpected. Difficulties in obtaining complete 24 hour urine collections may have masked any small differences before and after intervention, given the moderate degree of weight loss which had failed to affect either serum DHEAS or plasma insulin concentrations.

The third research question for this study was whether moderate weight loss in subjects who were not "elderly", (prior the onset of major age related falls), would increase serum DHEAS. As the healthy subjects had both close to healthy BMI and serum DHEAS concentrations within the reference ranges (Orentreich *et al*, 1984) the failure of moderate weight loss to affect both serum DHEAS and plasma insulin was perhaps not surprising. Once more, the subjects were primarily chosen on BMI criteria and not because of the presence of insulin resistance or any particular other metabolic criteria. The findings for the study on healthy weight subjects found serum DHEAS values within the accepted reference ranges 2-9 μmol/L. The increase in the serum DHEAS values in the healthy subject group, 42.4 (SD 8.0) years compared with the angina group given their greater age 61.3 (SD 6.5) years was expected.

The dietary change in the healthy weight subjects was similar to that reported by the angina group, with a reduction in dietary fat intake from 36.4 to 28.6%. It was expected that a lower dietary fat intake would influence and even decrease insulin sensitivity, and

that as a consequence of this serum DHEAS concentrations would increase. However the moderate weight loss achieved in this study was insufficient to affect changes in plasma insulin or serum DHEAS concentrations. Even considering these weight losses, the subjects did not seem to be insulin resistant, given the relatively crude assessment method, fasting insulin, that was used. The more precise methodology to assess insulin sensitivity involving euglycaemic clamps (Bonora *et al*, 1989) was outside the practical limitations of this work. The results from both subjects groups (healthy and angina subjects) were consistent and showed that plasma insulin concentrations were unaltered by moderate weight loss.

This study confirmed several relationships. These included BMI, body weight and percentage body fat inversely with DHEAS and positively with insulin concentrations (Leenen *et al*, 1994). It was of interest to consider all subjects as one group for the second analyses. This was valid given the almost identical approaches in terms of recruitment and dietary intervention. These analyses shed light on the failure of the healthy group to show any significant negative relationship with age and serum DHEAS concentrations. Considerable evidence exists to support the fall in adrenal steroid production with increasing age (DePretti *et al*, 1978). It seems most likely that this established relationship of age and serum DHEAS did not become apparent in the healthy group, as the group only represented a narrow age range where all participants were of employable age. The relationship between age and serum DHEAS concentrations was very significant when all subjects were considered as one group. These findings were in agreement with the majority of the literature (Orentreich *et al*, 1984). The significant relationship between serum DHEAS and BMI could have been due to the confounding influence of age. The subjects were older in the angina group and they were also more overweight. The healthy subjects were also younger and slimmer. As age has been established as the principle factor that affects the production of adrenal steroids, the influence of age but not

BMI, was probably the most influential factor. In addition, the other established positive relationship, that of age with plasma insulin, was also seen.

10.5 Conclusions

This study indicates no important effects of moderate weight loss on serum DHEAS and plasma insulin concentrations in older overweight subjects in younger healthy and non overweight, subjects of employable age. Expected relationships between age and DHEAS and between the three measures of body composition and these biochemical measures were seen when the two groups were considered as one.

Table 10-1 Characteristics of angina subjects at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	61.3 (6.5)			
Weight (kg)	80.8 (12.1)	77.5 (11.7)	-3.3 (2.3)	0.00001
BMI (kg/m^2)	29.1 (4.2)	27.9 (4.1)	-1.2 (0.8)	0.00001
% Body Fat *	34.5 (6.2)	33.7 (5.5)	-0.8 (3.5)	0.11
Waist circumference (cm)	104 (10)	101 (11)	-3.0 (4.3)	0.0004
WHR	1.00 (0.05)	0.99 (0.08)	-0.01 (0.06)	0.97
Serum DHEAS (μmol/L)	2.6 (1.9)	2.5 (1.6)	-0.1 (0.9)	0.35
Plasma insulin (mU/L)	13.3 (8.2)	12.0 (9.0)	-1.3 (7.9)	0.15

Data as mean (SD), n=41

* assessed by the method of Lean *et al* (1996)

Table 10-2 Serum and urinary hormonal concentrations at baseline and week twelve for angina subjects

	n	Baseline	Week 12	Difference	p
Urinary androsterone (ug/24 hours)	22	773 (510)	858 (456)	+85 (357)	0.86
Urinary aetiocholanolone (μg/24 hours)	22	678 (401)	729 (383)	+51 (387)	0.73
Urinary androsterone / aetiocholanolone ratio (μg/24 hours)	22	0.95 (0.35)	1.03 (0.48)	+0.08 (0.35)	0.64
Urinary THF (μg/24 hours)	21	2075 (864)	2261 (802)	+186 (950)	0.81
Urinary Allo- THF (μg/24 hours)	21	2105 (1076)	2260 (1178)	+155 (876)	0.79
Urinary tetrahydrocortisone (μg/24 hours)	21	3563 (1120)	3922 (1399)	+359 (1514)	0.85
Total urinary androsterone aetiocholanolone (μg/24 hours)	22	1451 (857)	1587 (788)	+136 (715)	0.28
Total urinary THF Allo-THF and THE (μg/24 hours)	20	7710 (2597)	8552 (2941)	+842 (3146)	0.31

Data as mean (SD). Numbers vary for the values for urinary metabolites and reflect the number of urinary collections made by subjects at baseline and week 12
THF = tetrahydrocortisol, THE = tetrahydrocortisone

Table 10-3 Relationships between baseline dehydroepiandosterone sulphate, insulin, urinary steroid metabolites, body mass index, waist circumference and waist to hip ratio for angina subjects

	n	BMI (kg/m^2)	Waist circumference (cm)	WHR
Age (years)	41	r =-0.260 p =0.109	r = -0.141 p = 0.386	r = 0.150 p = 0.355
Serum DHEAS (μmol/L)	41	r = 0.162 p =0.328	r = -0.072 p = 0.659	r = -0.153 p = 0.346
Plasma insulin (mU/L)	41	r = 0.192 p = 0.247	r = 0.158 p = 0.329	r = 0.284 p = 0.070
Urinary androsterone (μg/24 hours)	22	r = 0.252 p = 0.226	r = -0.068 p = 0.753	r = 0.059 p = 0.785
Urinary aetiocholanolone (μg/24 hours)	22	r = 0.232 p = 0.263	r = -0.007 p = 0.975	r = 0.269 p = 0.204
Urinary androsterone / aetiocholanolone ratio (μg/24 hours)	22	r = 0.171 p = 0.440	r = -0.114 p = 0.605	r = -0.229 p = 0.293
Urinary THF (μg/24 hours)	21	r = 0.212 p = 0.329	r = 0.165 p = 0.441	r = 0.312 p = 0.138
Urinary Allo-THF (μg/24 hours)	21	r = 0.373 p =0.079	r = 0.048 p = 0.823	r = 0.066 p = 0.759
Urinary THE (μg/24 hours)	21	r = 0.492 p = 0.016	r = 0.245 p = 0.259	r = 0.339 p = 0.113
Total urinary aetiocholanolone and androsterone (μg/24 hours)	22	r = 0.260 p = 0.214	r = -0.044 p = 0.840	r = 0.160 p = 0.456
Total urinary THF Allo-THF and THE (μg/24 hours)	20	r = 0.430 p = 0.042	r = 0.182 p = 0.406	r = 0.265 p = 0.221

Numbers vary for the values for urinary metabolites and reflect the number of urinary collections made by subjects at baseline
THF = tetrahydrocortisol, THE = tetrahydrocortisone

Table 10-4 Relationships at week twelve between dehydroepiandosterone sulphate, insulin, urinary steroid metabolites, body mass index, waist circumference and waist to hip ratio for angina subjects

	n	BMI (kg/m^2)	Waist circumference (cm)	WHR
Serum DHEAS (μmol/L)	41	r = 0.082 p = 0.614	r = -0.027 p = 0.614	r = -0.022 p = 0.891
Plasma insulin (mU/L)	41	r = 0.107 p = 0.512	r = 0.145 p = 0.512	r = 0.139 p = 0.391
Urinary androsterone (μg/24 hours)	22	r = 0.475 p = 0.025	r = 0.177 p = 0.430	r = -0.005 p = 0.983
Urinary aetiocholanolone (μg/24 hours)	22	r = 0.300 p = 0.175	r = 0.262 p = 0.238	r = 0.091 p = 0.687
Urinary androsterone / aetiocholanolone ratio (μg/24 hours)	22	r = 0.316 p = 0.188	r = -0.027 p = 0.914	r = -0.132 p = 0.591
Urinary THF (μg/24 hours)	21	r = 0.335 p = 0.137	r = 0.111 p = 0.632	r = 0.123 p = 0.597
Urinary Allo-THF (μg/24 hours)	21	r = 0.448 p = 0.04	r = 0.028 p = 0.903	r = -0.073 p = 0.755
Urinary THE (μg/24 hours)	21	r = 0.549 p = 0.0008	r = 0.239 p = 0.284	r = -0.034 p = 0.882
Total urinary aetiocholanolone and androsterone (μg/24 hours)	22	r = 0.421 p = 0.051	r = 0.230 p = 0.303	r = 0.041 p = 0.855
Total urinary THF Allo-THF and THE (μg/24 hours)	20	r = 0.520 p = 0.016	r = 0.150 p = 0.516	r = 0.009 p = 0.967

Numbers vary for the values for urinary metabolites and reflect the number of urinary collections made by subjects
THF = tetrahydrocortisol, THE = tetrahydrocortisone

Table 10-5 Relationships after weight loss between changes in dehydroepiandosterone sulphate, insulin, urinary steroid metabolites, body mass index, waist circumference and waist to hip ratio for angina subjects

	n	BMI (kg/m^2)	Waist circumference (cm)	WHR
Serum DHEAS (μmol/L)	41	r = 0.149 p = 0.358	r = 0.082 p = 0.616	r = 0.029 p = 0.859
Plasma insulin (mU/L)	41	r = 0.116 p = 0.478	r = -0.055 p = 0.736	r = 0.003 p = 0.702
Urinary androsterone (μg/24 hours)	22	r = -0.087 p = 0.722	r = 0.128 p = 0.603	r = 0.021 p = 0.931
Urinary aetiocholanolone (μg/24 hours)	22	r = -0.098 p = 0.690	r = 0.092 p = 0.707	r = 0.045 p = 0.853
Urinary androsterone / aetiocholanolone ratio (μg/24 hours)	22	r = 0.180 p = 0.461	r = -0.032 p = 0.895	r = -0.041 p = 0.869
Urinary THF (μg/24 hours)	21	r = 0.125 p = 0.621	r = -0.062 p = 0.808	r = -0.059 p = 0.815
Urinary Allo-THF (μg/24 hours)	21	r = 0.088 p = 0.704	r = 0.062 p = 0.788	r = 0.037 p = 0.874
Urinary THE (μg/24 hours)	21	r = -0.092 p = 0.717	r = -0.086 p = 0.735	r = -0.090 p = 0.721
Total urinary aetiocholanolone and androsterone (μg/24 hours)	22	r = -0.064 p = 0.778	r = 0.153 p = 0.100	r = 0.069 p = 0.760
Total urinary THF Allo-THF and THE (μg/24 hours)	20	r = 0.072 p = 0.764	r = 0.007 p = 0.850	r = 0.001 p = 0.996

Numbers vary for the values for urinary metabolites and reflect the number of urinary collections made by subjects
THF = tetrahydrocortisol, THE = tetrahydrocortisone

Table 10-6 Characteristics of healthy subjects at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	42.4 (8.0)			
Weight (kg)	80.6 (5.5)	77.7 (5.9)	-2.9 (2.7)	0.00001
BMI (kg/m^2)	25.4 (1.4)	24.5 (1.4)	-0.9 (0.8)	0.00001
% body fat	26.5 (7.0)	23.1 (4.8)	-3.56 (3.2)	0.0003
Waist	88.6 (7.6)	85.5 (7.6)	-3.1 (4.5)	0.0003
WHR	0.90 (0.05)	0.88 (0.07)	-0.02 (0.07)	0.38
Serum DHEAS (μmol/L)	8.7 (4.6)	8.3 (4.2)	-0.4 (4.4)	0.65
Plasma insulin (mU/L)	8.6 (7.6)	8.1 (7.9)	-0.5 (9.4)	0.78

Data mean (SD), n=31

Table 10-7 Relationships between baseline dehydroepiandosterone sulphate, insulin, body mass index, waist circumference and waist to hip ratio for healthy subjects

	BMI (kg/m^2)	Waist circumference (cm)	WHR	Serum DHEAS (μmol/L)
Serum DHEAS (μmol/L)	r=0.013 p=0.947	r = 0.209 p = 0.267	r = 0.108 p = 0.569	
Plasma insulin (mU/L)	r=0.311 p=0.048	r = 0.246 p = 0.121	r = 0.205 p = 0.198	r = -0.055 p = 0.769

n=31

Table 10-8 Relationships at week twelve between dehydroepiandosterone sulphate, insulin, body mass index, waist circumference and waist hip ratio for healthy subjects

	BMI (kg/m^2)	Waist circumference (cm)	WHR	Serum DHEAS (μmol/L)
Serum DHEAS (μmol/L)	r = -0.193 p = 0.306	r = 0.173 p = 0.370	r = 0.307 p = 0.105	
Plasma insulin (mU/L)	r = 0.415 p = 0.007	r = 0.323 p = 0.045	r = 0.234 p = 0.151	r = -0.055 p = 0.769

n=31

Table 10-9 Relationships between changes in dehydroepiandosterone sulphate, insulin, body mass index and waist circumference in healthy subjects

	BMI (kg/m^2)	Waist circumference (cm)	WHR	Serum DHEAS (μmol/L)
Serum DHEAS (μmol/L)	r = -0.097 p = 0.542	r = -0.036 p = 0.824	r = 0.215 p = 0.183	
Plasma insulin (mU/L)	r = 0.137 p = 0.426	r = 0.033 p = 0.853	r = 0.118 p = 0.505	r = -0.107 p = 0.566

n=31

Table 10-10 Characteristics of healthy subjects and angina subjects combined at baseline and week twelve

	Baseline	Week 12	Difference	p
Age (years)	51.0 (11.9)			
BMI (kg/m^2)	27.7 (3.8)	26.6 (3.6)	-1.1 (0.9)	0.00001
Body weight (kg)	81.2 (9.6)	77.9 (9.4)	-3.3 (2.6)	0.00001
Body fat (%)	30.6 (7.2)	28.6 (7.8)	-2.0 (3.8)	0.00001
Waist (cm)	96.9 (11.6)	94.5 (12.1)	2.4 (4.4)	0.00001
WHR	0.94 (0.1)	0.93 (0.1)	0.01 (0.06)	0.19
DHEAS (μmol/L)	4.9 (4.3)	4.8 (3.9)	-0.1 (2.7)	0.65
Insulin (mU/L)	11. (8.1)	10.1 (8.6)	-0.9 (8.5)	0.35

Data mean (SD), n=72

Table 10-11 Relationships at baseline between dehydroepiandosterone sulphate, insulin, body mass index, waist and waist hip ratio in healthy subjects and angina subjects combined

	Age (years)	BMI (kg/m^2)	Waist (cm)	WHR	DHEAS (μmol/L)
DHEAS (μmol/L)	r=-0.578 p=0.0001	r=-0.264 p=0.026	r=-0.371 p=0.001	r=-0.434 p=0.001	
Plasma insulin (mU/L)	r=0.228 p=0.046	r=0.285 p=0.016	r=0.328 p=0.002	r=0.374 p=0.0001	r=-0.180 p=0.132

n=72

Table 10-12 Relationships at week twelve between dehydroepiandosterone sulphate, insulin, body mass index, waist and waist hip ratio in healthy subjects and angina subjects combined

	BMI	Waist	WHR	DHEAS (μmol/L)
DHEAS (μmol/L)	r=-0.306 p=0.007	r=-0.419 p=0.0001	r=0.029 p=0.805	
Plasma insulin (mU/L)	r=0.252 p=0.021	r=0.390 p=0.0001	r=0.396 p=0.0001	r=-0.180 p=0.132

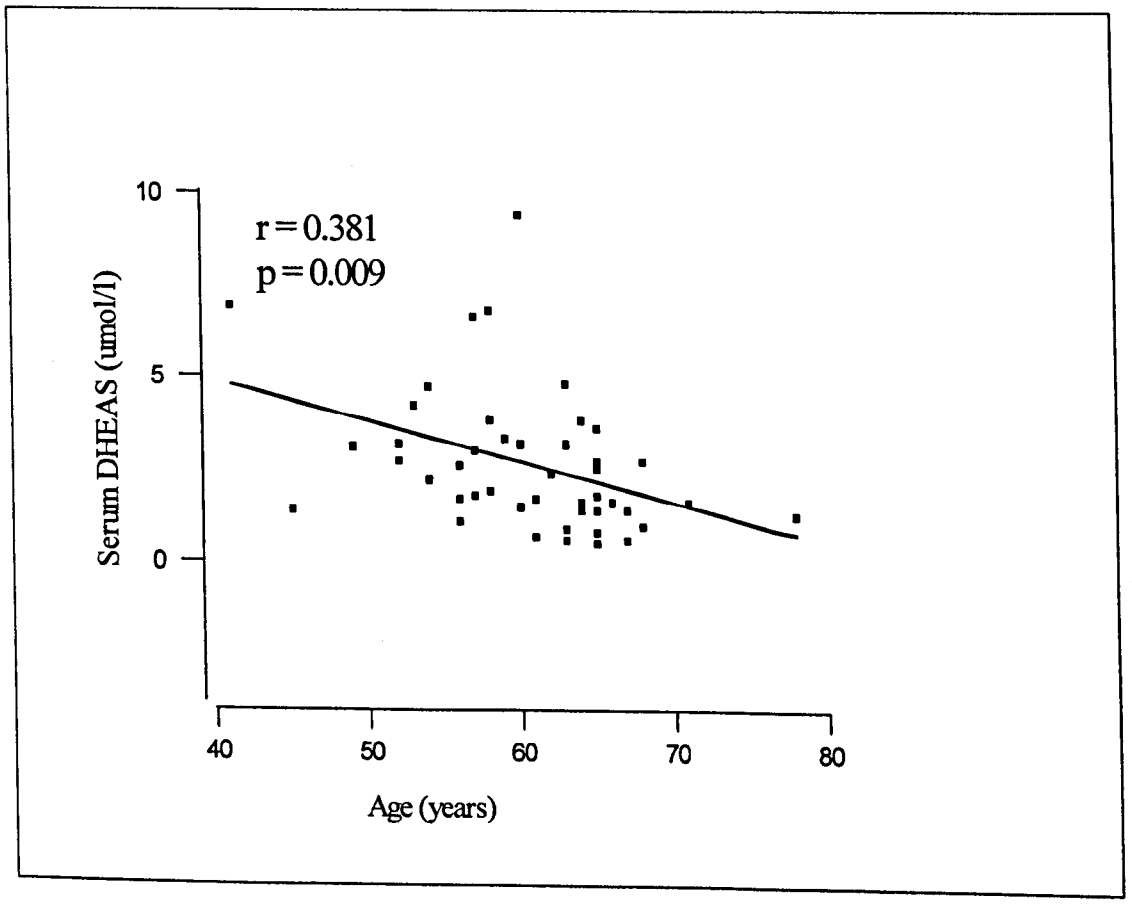
n=72

Table 10-13 Relationships between changes in dehydroepiandosterone sulphate, insulin, body mass index, waist and waist to hip ratio in healthy subjects and angina subjects combined

	BMI	Waist	WHR	DHEAS (μmol/L)
DHEAS (μmol/L)	r=-0.046 p=0.698	r=0.046 p=0.699	r=0.056 p=0.636	
Plasma insulin (mU/L)	r=0.155 p=0.161	r=0.086 p=0.444	r=0.172 p=0.124	r=-0.090 p=0.456

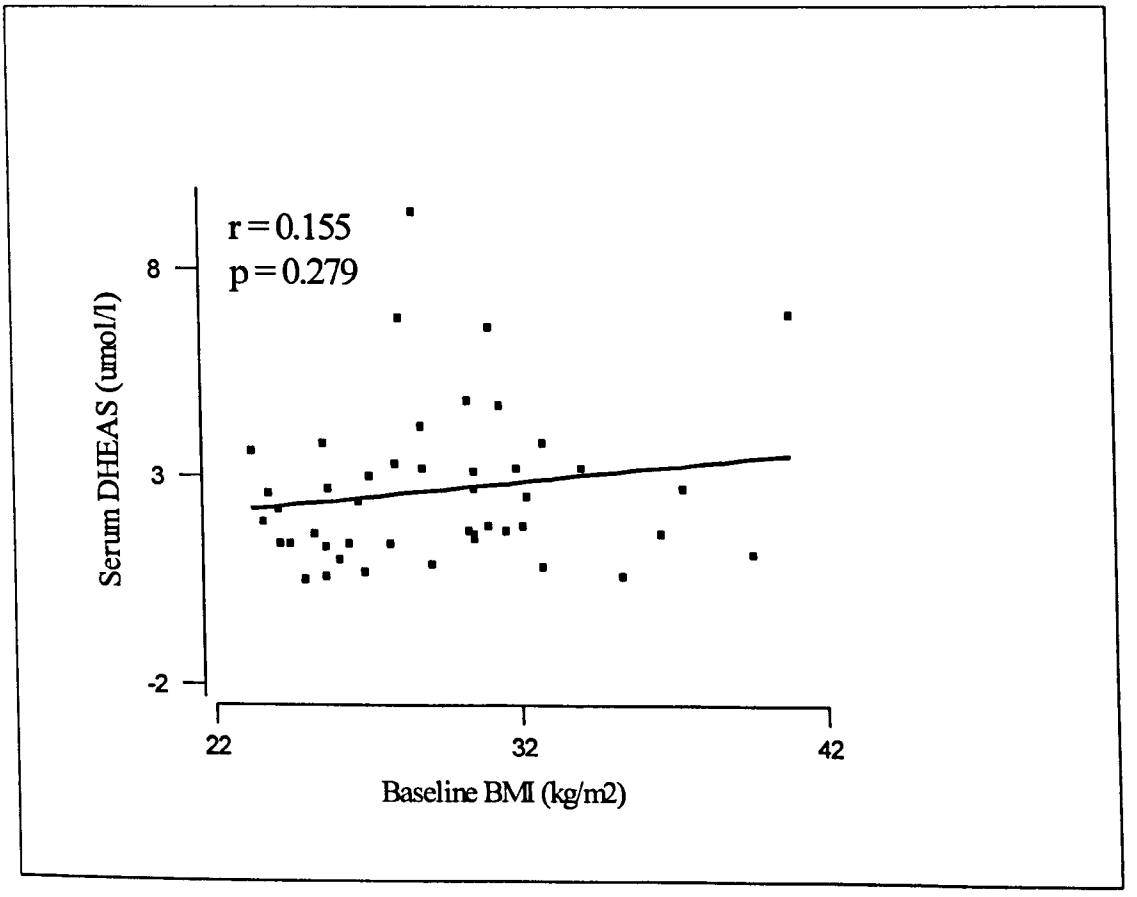
n=72

Figure 10-1 Relationship between dehydroepiandosterone sulphate concentrations and age in angina subjects



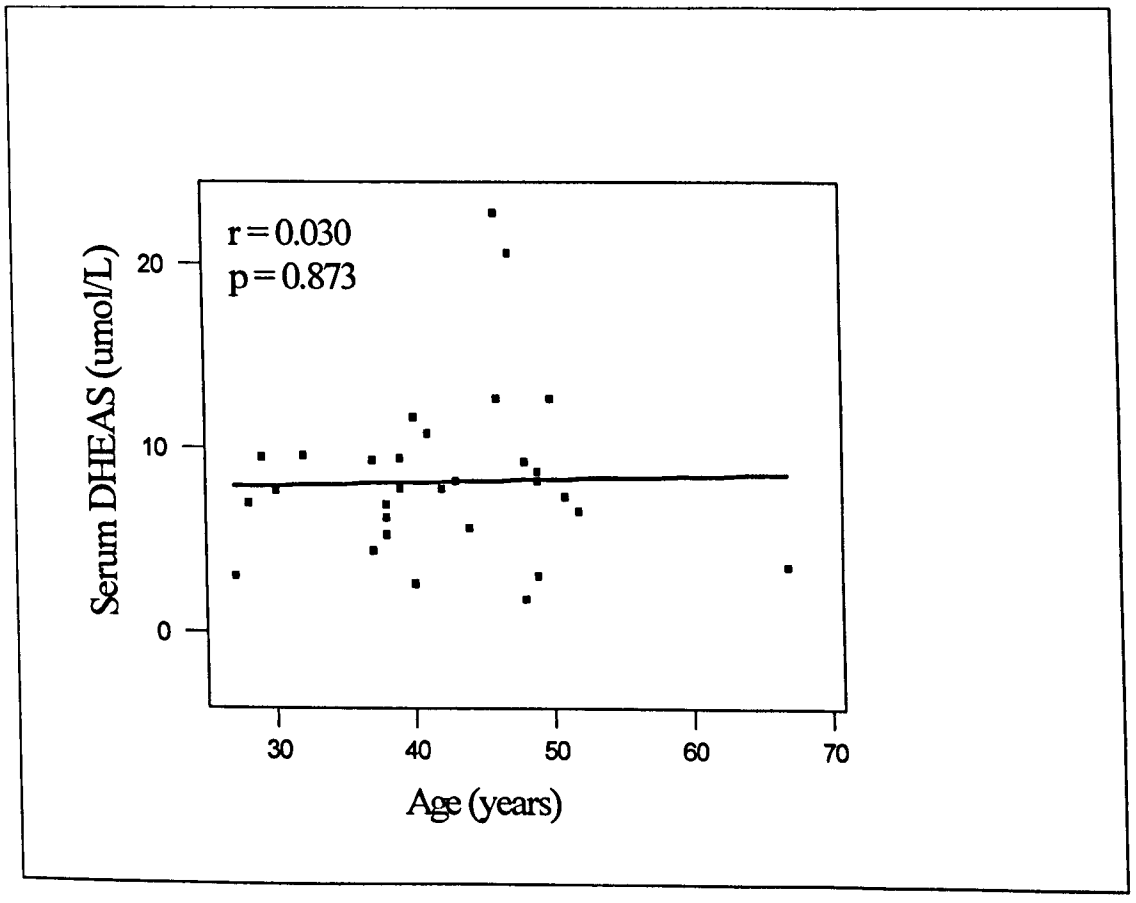
n=41

Figure 10-2 Relationships at baseline between dehydroepiandosterone sulphate concentrations and body mass index in angina subjects



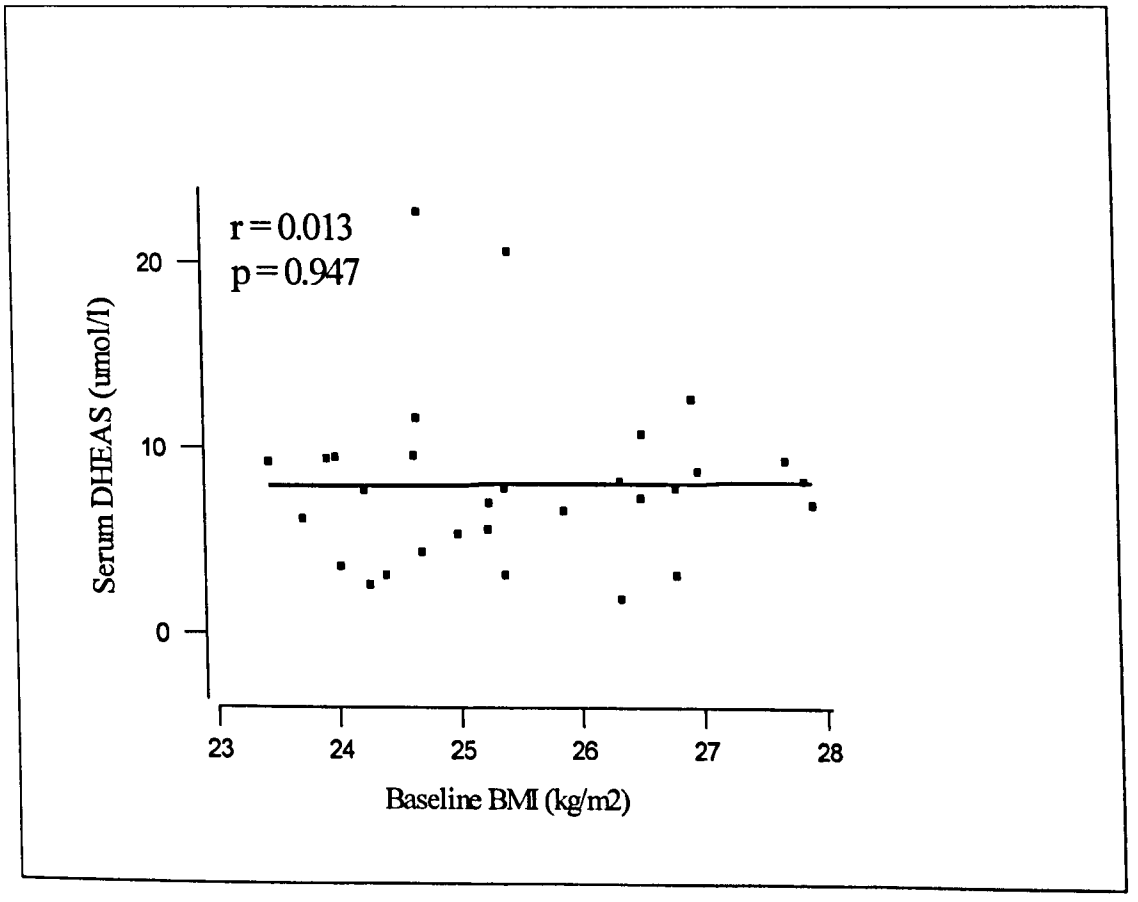
n=41

Figure 10-3 Relationship between dehydroepiandosterone sulphate concentrations and age in healthy subjects



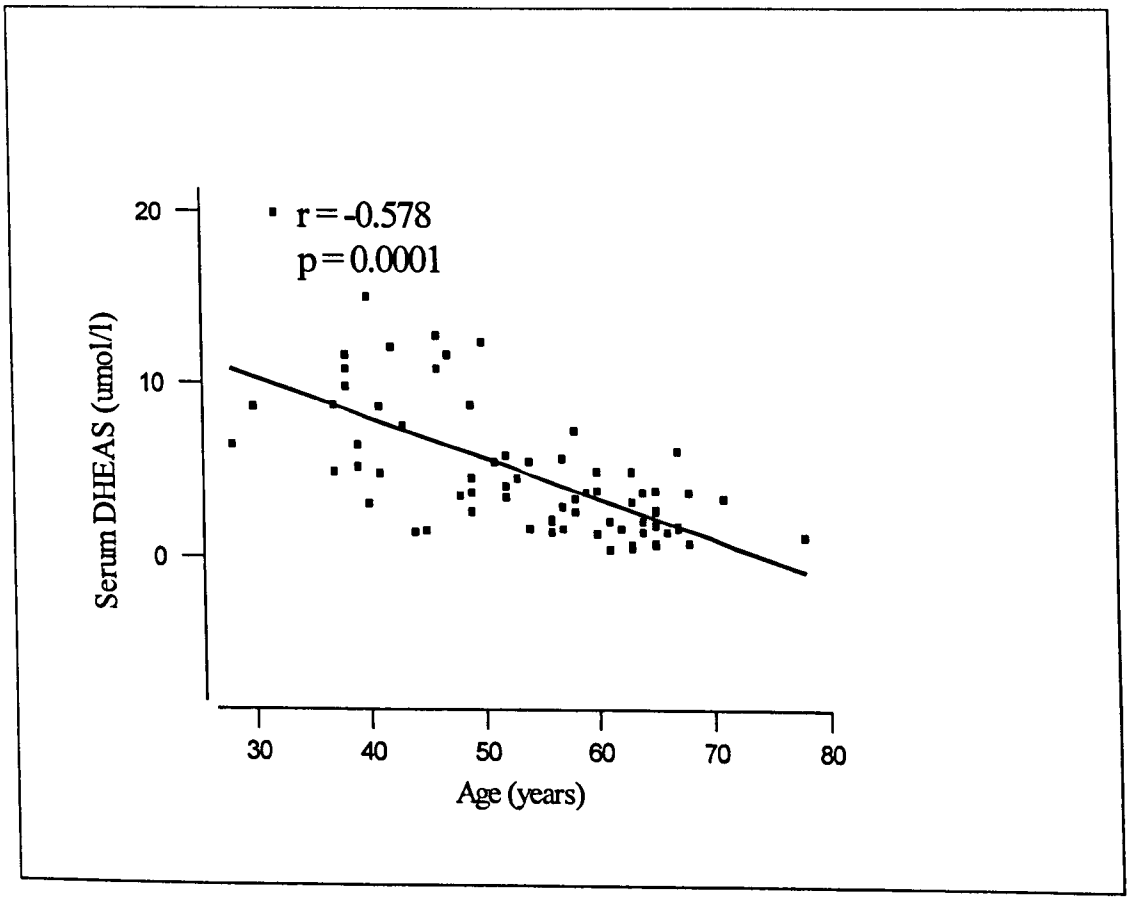
n=31

Figure 10-4 Relationships at baseline between dehydroepiandosterone sulphate concentrations and body mass index in healthy subjects



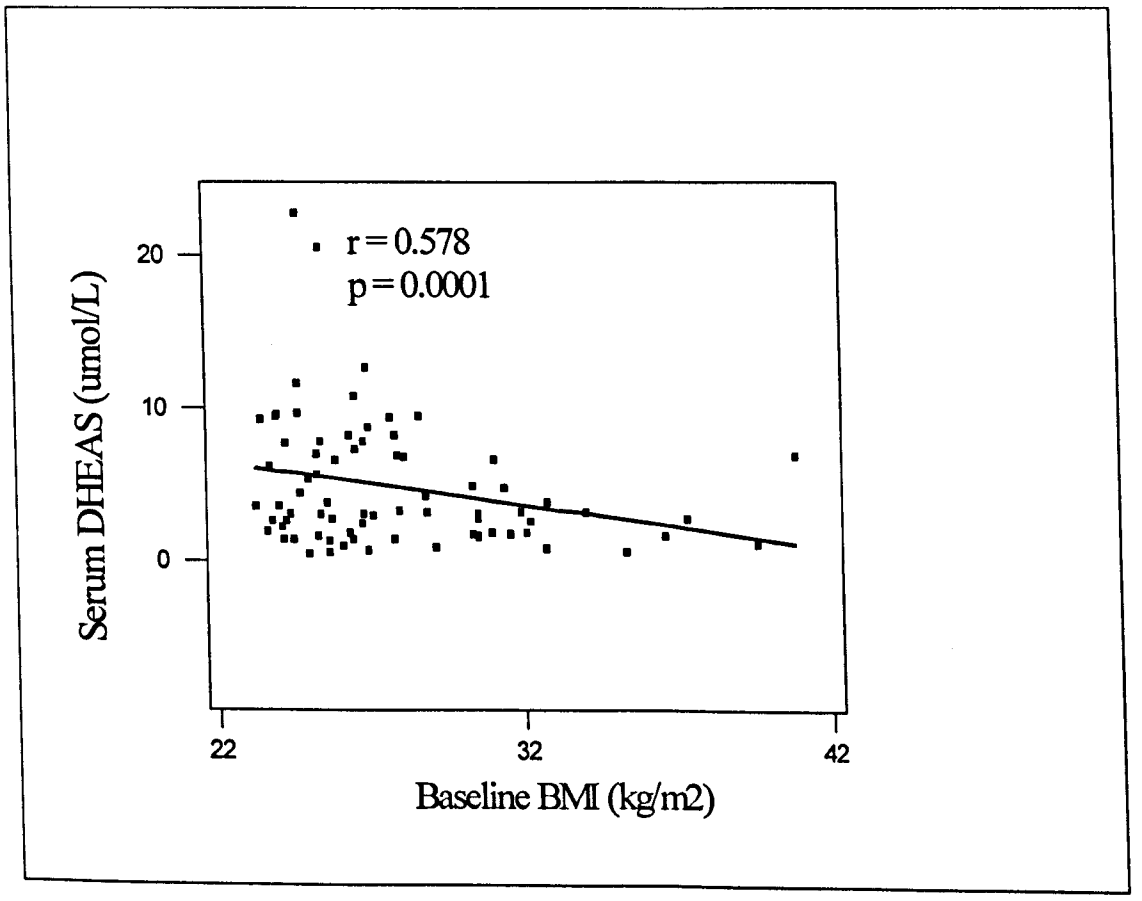
n=31

Figure 10-5 Relationships between dehydroepiandosterone sulphate concentrations and age in healthy subjects and angina subjects combined



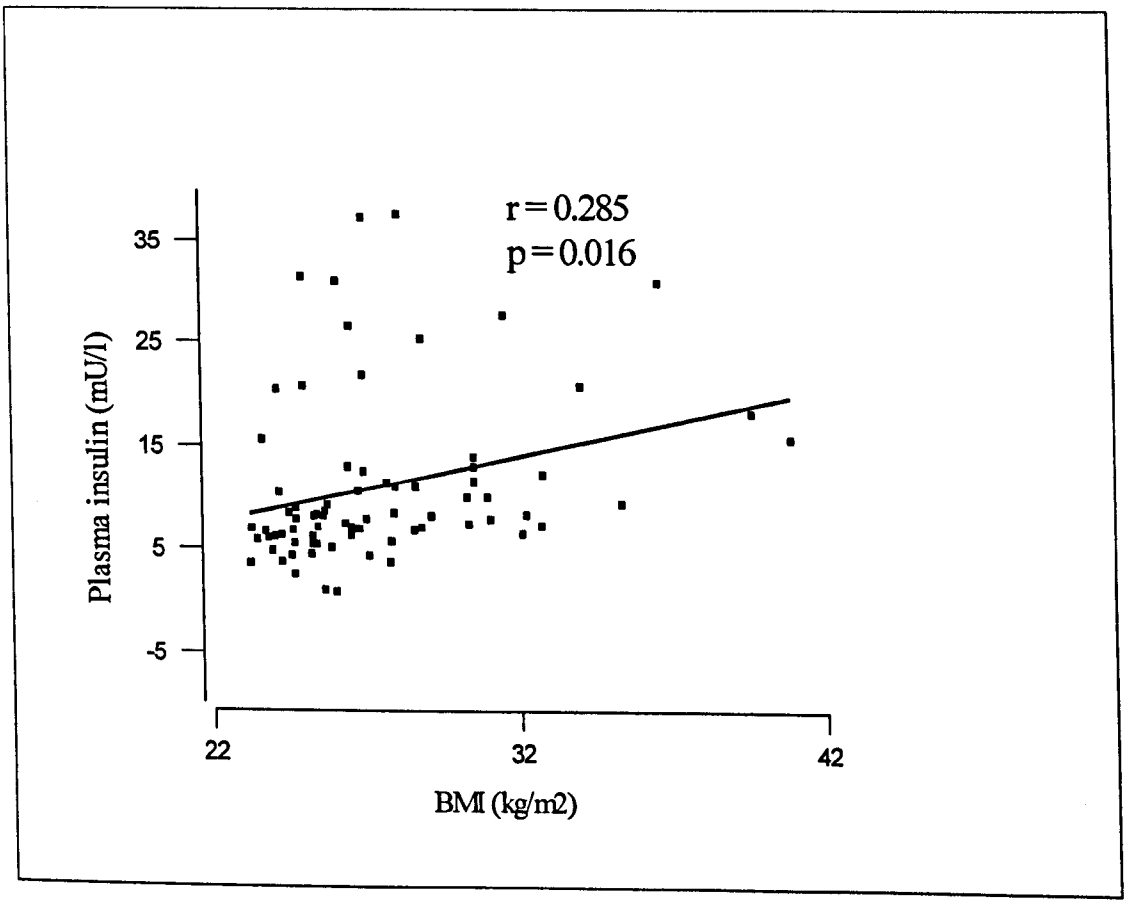
n=72

Figure 10-6 Relationships at baseline between dehydroepiandosterone sulphate concentrations and body mass index in healthy subjects and angina subjects combined



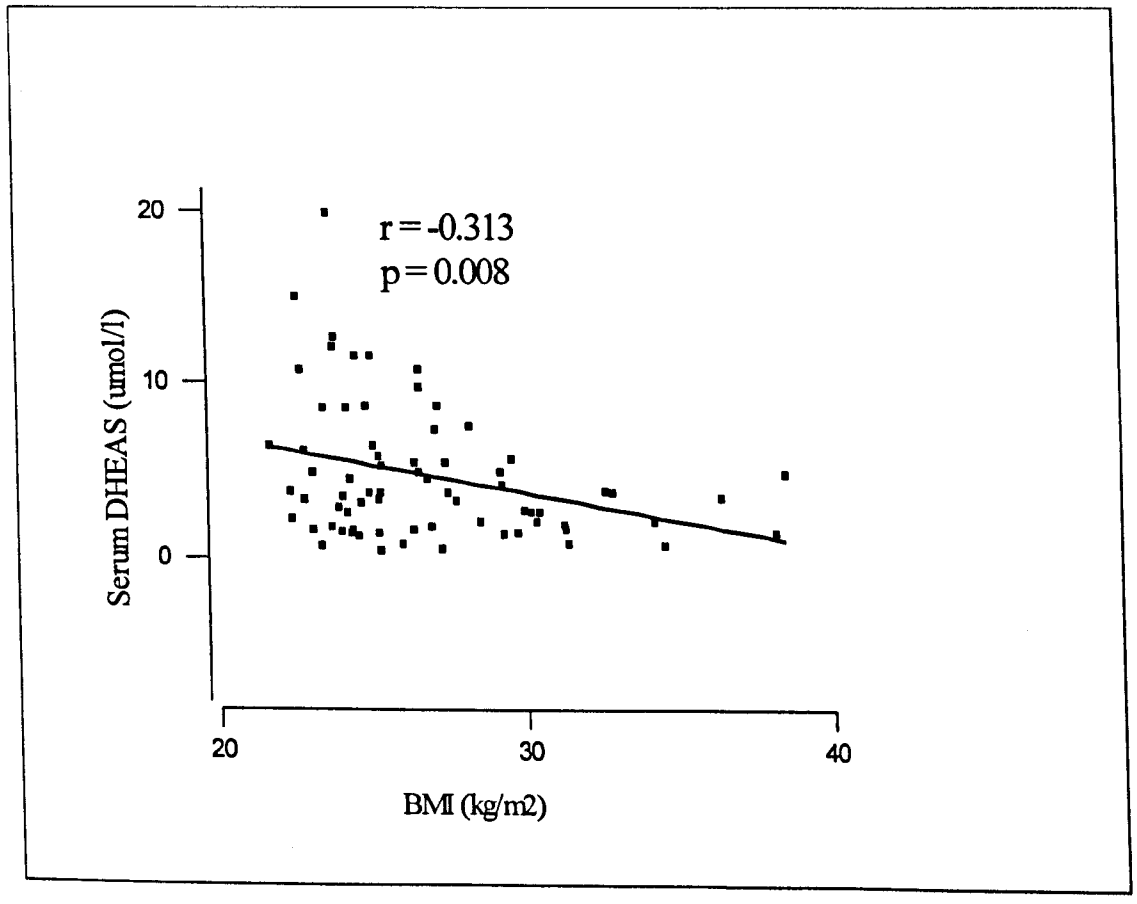
n=72

Figure 10-7 Relationships at baseline between insulin concentrations and body mass index in healthy subjects and angina subjects combined



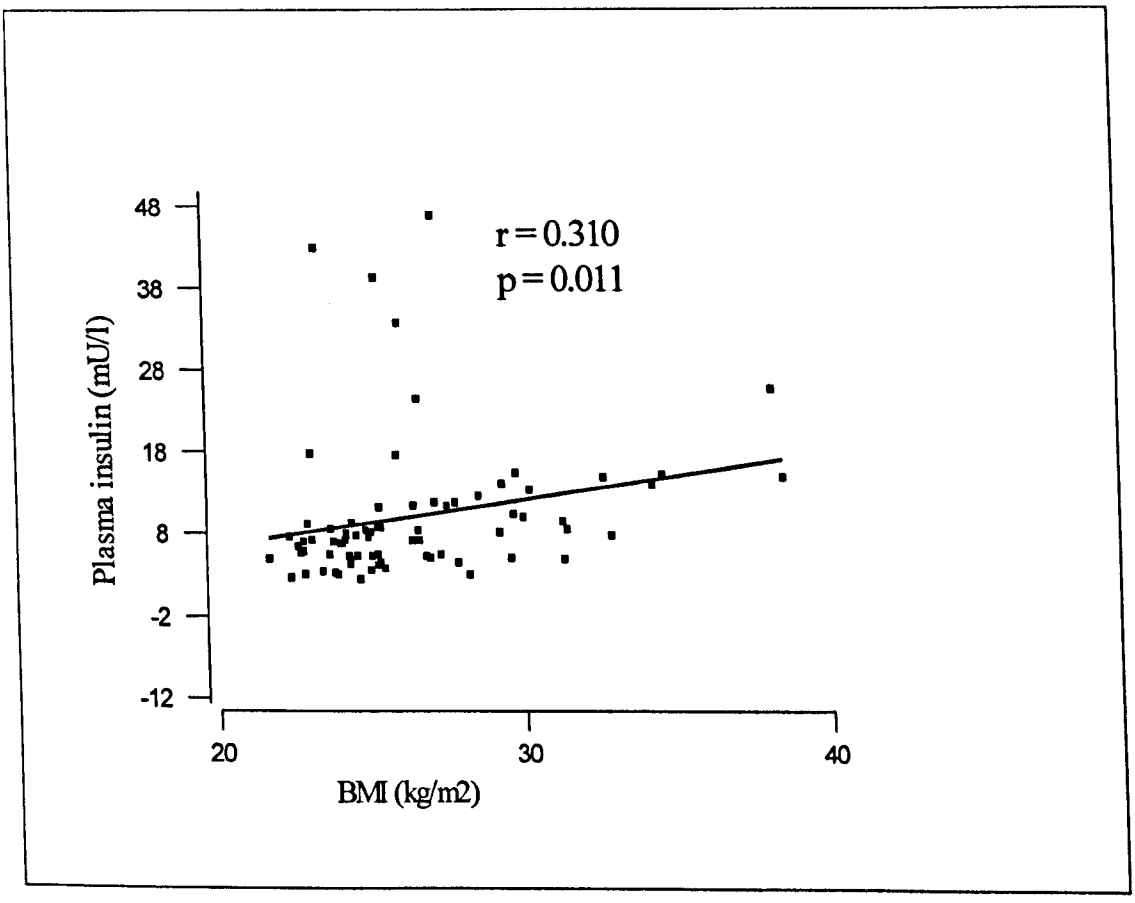
n=72

Figure 10-8 Relationships at week twelve between dehydroepiandosterone sulphate concentrations and body mass index in healthy subjects and angina subjects combined



n=72

Figure 10-9 Relationships at week twelve between plasma insulin concentrations and body mass index in healthy subjects and angina subjects combined



n=72

Chapter 11 Final discussion and recommendations for further work

11.1 Introduction and review

This programme of PhD research was initiated in September 1992. Since then, the publication of a number of studies, by workers outside this Glasgow laboratory have addressed several of the original issues considered in this thesis. These include the effects of dietary change and weight loss on haematological and rheological factors. Hence, the findings of the present studies within this thesis can be discussed in the context of more recent research.

The present studies have compared the effect of similar individualised dietary interventions, in three different subject groups, with the IHD risk factor measurements the principle end points. The study design aimed to evaluate the effects of a practical dietetic intervention for weight loss in free living subjects. Despite the different settings from which subjects were sought, the protocols and nutrition targets remained the same, namely dietary change and weight loss, and meaningful inter-study comparisons were possible. This design did not allow the examination of the effects of specific nutrient consumption on the chosen IHD risk factors, because of the difficulties inherent in attempts to quantify nutrient intakes in the context of obesity and weight loss.

In general, all volunteers were "healthier" than the general population and included very few smokers. All subjects' risk factors were within the laboratory reference ranges, with the exception of hyperlipidaemia. This is frequently a feature of health concerned individuals who tend to volunteer for research.

The results of all three studies have shown that the prescription of a 600 kcal daily energy deficit diet and the resulting weight loss were important in reducing the measured indices

of blood coagulation. In answering the research question, "does dietary intervention to lower total energy intake and change dietary composition in line with current dietary targets improve haemostatic and rheological risk factors for IHD?" the results showed some risk factor improvements although there were few differences between studies on different BMI categories.

In comparison with a conventional hospital base for clinical research, the work-site setting (chapter 8) illustrated a large number of benefits both to the study investigators and subjects. The advent of e-mail allowed easy contact, on an individual basis, to a large employee population. Details of the recruitment requirements were sent to each employee's mailbox, and the enthusiastic response may have reflected this individualised approach. Secondly, the work-site population was fresh to the notion of volunteering to become part of a study, whereas this was a much more common occurrence in the hospital setting. By granting one hour weekly to attend the study appointments, without loss of salary, the employer actively encouraged staff participation as part of health promotion, and further work has been initiated.

11.1.1 Whole blood and plasma viscosity

After weight loss, plasma and whole blood viscosities remained unchanged in all studies. These findings, that moderate weight loss does not lower plasma viscosity, were in agreement with those present in the literature (Ernst *et al*, 1989, Craverie *et al*, 1990, Tozzi *et al*, 1994). However, the literature reported that only large amounts of weight loss (15-20 kg) significantly influenced viscosity (Parenti *et al*, 1987) and moderate weight loss of around 7.0 kg was insufficient (Craveri *et al*, 1990). Little evidence remains available for the effect of weight loss on WBV, which remained unchanged in the present

studies. The present studies were under-powered for WBV, and therefore the influence of β errors remained a possibility.

11.1.2 Red cell aggregation

The results of the overweight and angina studies showed RCA was significantly reduced with weight loss. These results, which concur with Ernst and Matrai, 1987, Poggi *et al*, 1994, are amongst the few reports showing that moderate weight loss can improve RCA. The RCA measures were not made in the close to healthy weight subjects due to practical limitations, but the comparison with the two other subject groups would have been of interest. Inter-study comparisons with published data were also impossible (Ernst and Matrai, 1987, Poggi *et al*, 1994) as the method of RCA measurement differed between studies. The available data suggest that RCA becomes elevated in the overweight, but that moderate weight loss can be beneficial in improving measures.

11.1.3 Fibrinogen

The stability of plasma fibrinogen despite weight loss in each study did not support the hypothesis that moderate weight loss could lower plasma fibrinogen. Although these present findings were in agreement with the majority of the literature (Hughes *et al*, 1984, Craveri *et al*, 1990, Palareti *et al*, 1994, Ditschuneit *et al*, 1995, Craveri *et al*, 1992, Gelmini *et al*, 1989, Folsom *et al*, 1993, Anderson *et al*, 1995, Ernst *et al*, 1989 and Mehrabian *et al*, 1990), the studies were under powered with respect to fibrinogen and β errors may have been present. However, the present results and those from the literature support the interpretations of the review article by Ernst (1993). This article reported that that weight loss, in any amount failed to significantly lower plasma fibrinogen concentrations. The reasons why weight loss has such small effects on plasma fibrinogen are unclear, given the strong epidemiological relationships between increasing BMI and

plasma fibrinogen. In conclusion, moderate weight loss appears to have no significant effect on plasma fibrinogen concentrations.

11.1.4 Factor VII activity

The healthy overweight and angina studies both showed a significant fall in factor VII activity after moderate weight loss. In contrast, the close to healthy weight subjects showed no significant change in factor VII activity after weight loss. Two influential factors for the latter result are likely to have been initial plasma concentrations within the healthy range, and insufficient weight loss. However, greater weight losses than achieved in the present studies may have improved factor VII activity, even in this healthy group. In conclusion, the present findings support the value of weight management in reducing IHD risk in overweight and angina subjects.

11.1.5 Plasminogen activator inhibitor activity and tissue plasminogen activator antigen

In the present studies, moderate weight loss failed to change the fibrinolytic indices significantly. Research elsewhere has confirmed the effect of greater weight loss in reducing both PAI activity and t-PA antigen. The present studies were just sufficiently powered with respect to both PAI activity and t-PA antigen, with an associated risk of β errors, particularly in the study of healthy weight subjects. However, sub-group analysis found lowered PAI activity after moderate weight loss in overweight women, overall findings from all the present studies confirm that moderate weight loss does not improve fibrinolytic indices. Once more the lower than expected overall weight loss may have offered a partial explanation for these findings.

11.1.6 Lipids

Lower plasma total and LDL and an unchanged HDL cholesterol concentrations were

consistent results of moderate weight loss in the present studies. The failure of weight loss to lower LDL cholesterol in this study of overweight subjects was surprising, with no obvious explanation, other than insufficient weight loss. However, when all subjects from the three studies were considered as a group, LDL concentrations significantly fell. This reduction is in agreement with the majority of the literature (Leighton *et al*, 1990, Bremner *et al*, 1994, Litchenstein *et al*, 1994).

The failure of HDL cholesterol concentration to increase after weight loss was in contrast with expectations, and other findings from the literature (MacMahon *et al*, 1985, Bremner *et al*, 1994). However, shorter term studies, e.g. sixteen weeks duration, showed falls in HDL cholesterol, which reflect the acute effects of weight loss (Litchenstein *et al*, 1994). This is likely to have been either a significant fall in HDL concentrations, or them remaining the same, as was the case in the present studies. Often weight loss confounds the results of short term studies (less than 4 months), and can even leads of an elevation in plasma cholesterol known as the "transient hypercholesterolaemia of weight loss" (Phinney *et al*, 1991). However, when weight losses are maintained an improvement in plasma cholesterol fractions, usually to below the pre-weight loss concentrations, is frequently seen (Phinney *et al*, 1991).

Research studies have shown that weight loss favours reductions in plasma triglyceride concentrations, in contrast to the present findings (Stewart *et al*, 1990). The stability of plasma triglyceride and the lack of any positive correlations with factor VII activity were in contrast to the evidence to support such a relationship in hypertriglyceridaemic subjects (Miller *et al*, 1991, Meade *et al*, 1993). The present results probably reflected the relatively low concentrations of all indices, within their reference ranges. The finding that changes in haemostatic, rheological factors and plasma lipids were unrelated to moderate weight loss has supported the current theory concerning the two systems relationship.

11.1.7 Dehydroepiandosterone sulphate and insulin

The concentrations of DHEAS and insulin are both known to be affected by BMI. A rise in insulin concentrations and a fall in DHEAS concentrations have been observed with increasing BMI. The failure of moderate weight loss to change either of these measures in the present studies has rejected the study hypothesis that reducing body weight in overweight and close to healthy weight subjects would lead to increases in DHEAS. These results may be explained by having insufficient weight loss in both of these groups. The greater age of the angina patients reflected their lowered DHEAS concentrations (DePretti *et al*, 1978). These lowered concentrations were probably influential in the failure of weight loss to effect changes in DHEAS, possibly increasing the susceptibility of angina subjects to further development of ischaemic disease.

11.2 Effectiveness of methods of dietary assessment and intervention

11.2.1 The energy deficit approach to weight loss

The present studies provided an opportunity to implement the 600 kcal daily energy deficit approach in three different BMI categories. These comparisons are new, the only other evidence being an audit where the energy deficit approach was shown to be more effective than a 1200 kcal diet (Frost *et al*, 1991). Other evidence from the placebo arm of anti-obesity medication studies support the findings of Frost *et al*, 1991, (Drent and van der Veen, 1993).

It was proposed that the presence of a diagnosis of angina would improve the compliance of subjects with a dietary regimen and to favour increased weight loss. However, the results did not support the value of an established diagnosis in improving dietary compliance. A possible explanation was that these health-conscious volunteers had already made appropriate dietary modifications. This is similarly postulated to explain

poor results in NIDDM patients (Ha and Lean, unpublished). However, the similar weight loss between different study groups challenges this hypothesis. The study of weight loss in healthy subjects was perhaps the most interesting and novel aspect of this work with a less predictable outcome. The similar weight losses observed probably reflected a similar degree of dietary compliance between groups. These results following a quantitative approach in volunteers with a desire to reduce body weight appeared not to be significantly influenced by BMI.

11.3 Overall conclusions: practical lessons and ideas for further work

The results of these studies suggest that moderate weight loss has value in the management of the risk of IHD by reducing factor VII activity, RCA, total and LDL cholesterol. The approach that was used in this present work has now been adopted into the current guidelines for the management of overweight in Scotland (SIGN, 1996).

Epidemiological studies have described some elevated haemostatic indices as important risk factors for IHD. Overweight and obesity have been associated with raised levels of these measurements. In the present work the moderate weight loss that was achieved was below that planned for, and this may explain why only factor VII activity, RCA, total and LDL cholesterol showed significant falls. However, the fact that the majority of the measures of IHD risk factors at baseline were within their laboratory reference ranges may also have contributed to their stability after weight loss.

Longer-term studies would allow greater insight into the effectiveness of the 600 kcal daily energy deficit approach. The effect of increasing and maintaining these reductions in body weight on the specific IHD indices would also be of great interest.

A number of practical methodological insights have resulted from the completion of this work. With the advantage of hindsight, these would have modified the way the present

work was carried out. In general, all dietary intervention studies are very reliant on the good will and commitment of participants. In addition, although dietary studies appear time consuming, in practice, completion time seems to exceed all conservative predictions. Although three different methods were used, recruitment was a major issue always taking longer to complete than envisaged. The use of the popular press for recruitment in the angina study was a quick, and almost too effective, method, which met with an excellent telephone response. On the other hand these were a highly health conscious group, looking with hope to benefit from any advances in the care of those with angina. In contrast, e-mail recruitment in the work-site setting was a more effective method, not reliant on the help of others. The work-site setting using e-mail recruitment would have been ideally suited as an approach for the overweight study.

A number of possible research projects have resulted from the studies presented in this thesis, which are summarised in the following four questions.

1. A controlled study of the effects of weight loss on the reported frequency of chest pain observed in angina subjects.

2. A comparison and longer term follow-up of a 600 kcal daily energy deficit diet versus a low energy diet plan using a randomised, controlled experimental design, together with a long term follow-up.

3. The investigation of the influence of increased age on the ability of volunteers to achieve intentional weight loss.

4. The effect of weight loss and weight maintenance on the stability of haemostatic, rheological and lipid risk factors for IHD.

In conclusion, the work in this thesis confirms the value of moderate weight loss in

achieving improvements in risk factors for IHD. The value of a 600 kcal deficit diet, which was effective in achieving these moderate weight losses, was demonstrated. Good counter arguments could be made to support the use of a VLCD approach together with a weight maintenance programme (Wing *et al*, 1994). However, the results from a comprehensive follow up of the participants over time would have been of interest.

The potential value of the 600 kcal daily energy deficit approach has been illustrated, especially given the encouraging comparison across three different study groups. Further studies over a longer period of time would be of interest to try to increase weight loss and examine any effects of measures of health status, and IHD risk factors. However, as already discussed in chapter 9, these results represent findings in health conscious volunteers. Frost *et al* (1991) used the routine clinical setting for their encouraging weight loss results for the 600 kcal energy deficit approach. A positive viewpoint on the broadly similar weight loss response in all groups to the 600 kcal energy deficit diet suggests a wide application across populations.

Appendices

Appendix 1 Publications

1.1 Abstracts

1995 Hankey, C.R., Rumley, A., Lowe, G.D.O. and Lean, M.E.J. Weight loss improves thrombotic and rheological risk factors for ischaemic heart disease. *Proceedings of the Nutrition Society,* **54**, 2, 94A.

1995 Hankey, C.R., Wallace, A.M., Lean, M.E.J. Changes in plasma dehydroepiandosterone sulphate (DHEAS) in overweight elderly angina patients during weight loss. *International Journal of Obesity,* **19**, (suppl. 2), 376.

1995 Hankey, C.R., Rumley, A., Lowe, G.D.O, Lean, M.E.J. Weight loss and factor VIIc activity in overweight subjects. *Proceedings of the Nutrition Society,* **55**, 1A, 137A.

1995 Hankey, C.R., Lean, M.E.J., Wallace, A.M. Influence of weight loss on plasma DHEAS in overweight postmenopausal women with angina pectoris. *International Symposium on DHEA Transformation into Androgens and Estrogens in Target Tissues.*

1996 Hankey, C.R., Lean, M.E.J. Estimation of energy requirements and reported dietary intakes in overweight subjects with ischaemic disease. *International Journal of Obesity,* **20**, (suppl.4), 29.

1997 Hankey, C.R., Rumley, A., Lowe, G.D.O., Lean, M.E.J. Reductions in LDL concentrations after weight loss are associated with improvements in haemostatic measures in overweight subjects with angina. *Atherosclerosis*, **128**, 2, 261.

1997 Hankey, C.R. and Lean M.E.J. Patterns of reported energy intakes and weight loss between repeated measurement periods during a slimming study in overweight subjects with ischaemic heart disease. *Proceedings of the Nutrition Society,* **56,** 119A.

1997 Hankey, C.R., Lean, M.E.J. Individualised energy prescription for weight management in overweight, overweight with angina and healthy weight subjects. 16th International Congress of Nutrition, Montreal.

1997 Hankey, C.R., O'Donohugue, D, Lean, M.E.J. (1996) Dietary management in a worksite setting. Health Promotion in Worksites. Health Education Board for Scotland.

1997 Hankey, C.R., O'Donohugue, D, Lean, M.E.J. (1996) Dietary management in a worksite setting. Good health is good business. FONEU, Brussels.

1998 Hankey, C.R. and Lean, M.E.J. Results of an energy deficit approach to weight loss and a weight maintenance programme on the anthropometric and plasma lipid measurements of healthy subjects. A work-site study. *European Society for Clinical Investigation,* 32 nd Annual Meeting, Cracow, Poland.

1998 Hankey, C.R. and Lean, M.E.J. An energy deficit approach to weight loss plus weight maintenance. The effects on anthropometric and lipid measurements in healthy subjects. *International Journal of Obesity, (in press).*

1998 Hankey, C.R., O'Donohugue, D., Lean, M.E.J. Dietary management in a work-site setting. *Proceedings of the Nutrition Society, (in press).*

1.2 Peer reviewed publications

1996 Hankey, C.R, Wallace, A.M., Lean, M.E.J. Plasma lipids, dehydroepiandosterone sulphate and insulin concentrations in elderly overweight angina patients, and effect of weight loss. *International Journal of Obesity*. **21**, 1, 72-77.

1997 Hankey, C.R, Rumley, A., Lowe, G.D.O., Lean, M.E.J. Moderate weight reduction improves red cell aggregation and factor VII activity in overweight subjects. *International Journal of Obesity*, **21**, 644-650

1.3 Manuscripts in preparation

Hankey, C.R, Rumley, A., Lowe, G.D.O., Lean, M.E.J. Indices of coagulation, fibrinolysis and disease symptoms in overweight angina patients before and after weight loss.

Hankey, C.R, Lean, M.E.J and Currall, J.E.P. Experimental design and statistical analysis of clinical trials to examine the benefits of weight loss.

Hankey, C.R, and Lean, M.E.J. The effects of a weight loss dietary intervention on respiratory quotient and resting energy expenditure.

Hankey, C.R., O'Donohue, D. and Lean, M.E.J. The use of electronic mail to implement a weight management programme in a work-site setting.

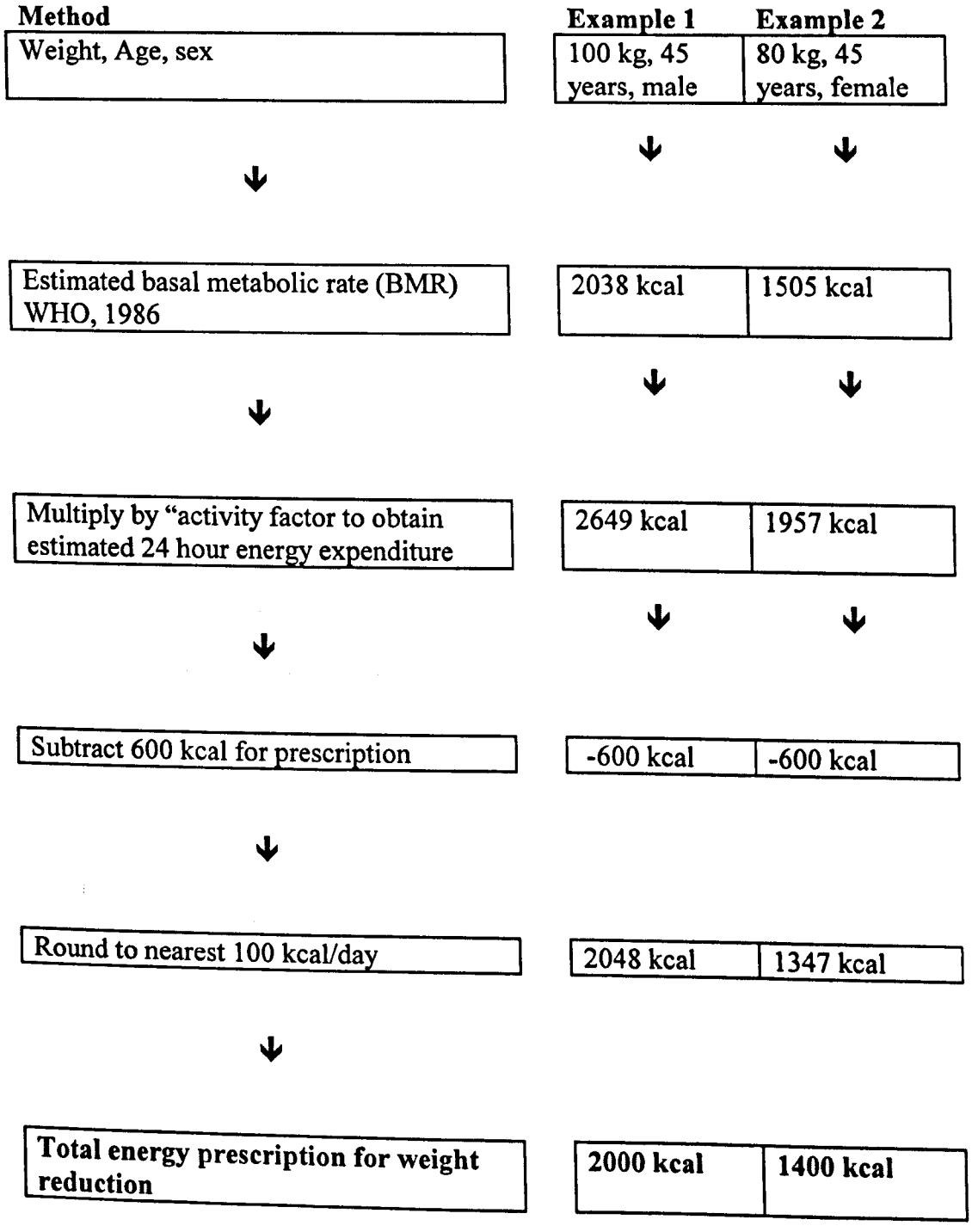
Hankey, C.R, and Lean, M.E.J. The comparison of two methods of dietary assessment in overweight angina patients pre and post dietary intervention.

1.4 Review publications

1997 Cowburn, G., Hillsdon, M., Hankey, C.R. Obesity management by life-style strategies. In: Obesity. *British Medical Bulletin*, **53**, 2, 389-408.

1998 Lean, M.E.J. and Hankey, C.R. Benefits and risks of weight loss, obesity and weight cycling. In: *Clinical Obesity*, Blackwells, Edited Kopelman, P and Stock, M.

Appendix 2 Flow chart showing the daily 600 kcal energy deficit approach to weight loss: designing the energy content of a diet for weight loss at 0.5 - 1.0 kg per week



Appendix 3 Healthy eating dietary advice plan

The Healthy Eating Plan

Designed by

Department of Human Nutrition
University of Glasgow

Food intake plans

This eating plan has been designed for you and the amounts of foods are based on your needs related to your age, sex and level of activity. Do try not to eat less than the healthy eating plan for you. When you are hungry, do not just give up, but fill up on foods from the bread or fruit exchange lists. Try to plan your meals ahead and use a shopping list when buying food to make your shopping in line with the healthy eating plan.

Good ideas

- Eat fish three times weekly, tuna, sardines in sandwiches, or fish such as cod or kippers.
- Choose skimmed or semi- skimmed milk
- Eat at least three fresh fruit, one pure fruit juice and two large helpings of vegetables daily
- Grill or bake instead of frying foods
- Avoid eating pies, sausages or pates
- Use polyunsaturated margarine or low fat spreads, or no spread

Bread exchanges

All amounts shown contain approximately 100 kcal, with valuable protein, carbohydrate, vitamins and minerals as well as being low in saturated fat.

Bread	**One bread exchange**
White Bread	1 slice
Wholemeal bread	1 slice
Wholemeal / white soft roll	1 slice
Pitta bread	1/2
Crisp breads	2
Cereal	
Weetabix	1 biscuit
Cooked porridge made with water	5-6 tablespoons
Whole grain or other cereal (e.g. frosties, Cornflakes, Rice Krispies, Ricicles, Sugar Puffs, Special K)	5 tablespoons
Unsweetened Muesli	3 tablespoons
Other foods	
Mushy peas	5 tablespoons
Baked beans	5 tablespoons
Potatoes boiled or baked	2 egg sized
Pasta (Cooked)	5 tablespoons
Brown rice cooked	3 tablespoons
White rice cooked	3 tablespoons
Vegetable soup	3/4 large can
Biscuits (rich tea, osbourne)	2
Oven chips (low fat)	10 chips

Meat exchanges

All contain around 120 kcal, with important minerals such as iron and calcium and a range of vitamins. Use the exchanges to choose alternatives to red meat as a focus to your meals.

Meat	Corned beef	2 thin slices
	Cooked lean ham	3 slices
	Cooked lean mince (turkey)	3 tablespoons
	Cooked lean stewing steak	3 tablespoons
	Cooked lean pork	3 tablespoons
	Grilled lean bacon (back)	2 -3 rashers
	Bolognese sauce (turkey mince)	4 tablespoons
	Chilli (turkey mince,	4 tablespoons
	or vegetable, or soya)	5 tablespoons
	Shepherds pie	5 tablespoons
Chicken	Cooked chicken/turkey breast	4-5 slices
	Chicken casserole	1 cupful
Fish	Choose fish at least three times weekly.	
	Tuna fish (drained) in brine	1 small tin
	Salmon tinned	3/4 small tin
	Sardines in tomato sauce/brine	3 sardines
	Mackerel, kippers herring	1/2 medium fillet
	Grilled/poached haddock/cod	1/2 medium fillet
	Grilled/poached plaice/sole	1/2 medium fillet
	Fish fingers grilled	3
	Fish pie	5-6 tbs.
Eggs	Eggs	1
Cheese	Cheddar cheese	30g (1 oz)
	Reduced fat cheddar	60g (2 oz)
	Low fat cheese spread	40g (1 1/2 oz)
	Edam/brie	30g (1 oz)
	Cottage cheese	100g (3 1/2 oz)

Fruit exchanges

All contain around 70 kcal, little fat, some fibre and plenty of vitamins. Regularly eating fruit is especially important if you smoke. You should try to take only 1 fruit exchange as pure fruit juice, and the remainder as the whole fruit, which contain many other important protective dietary factors.

Eat fruit at least three times daily.

Fruit juice (unsweetened)	1 wine glass
Apple	1
Orange	1
Peach	1
Tangerine	2
Pear	1
Pineapple	1 large slice
Grapes	small bunch (12)
Plums	2
Banana	1
Small bowl fresh fruit salad	
Tinned fruit in natural juice e.g.: Mandarin oranges Pears Peaches Fruit cocktail	1 small tin of fruit canned in fruit juice

Convenience food exchanges: **Many represent the use of bread plus meat exchanges**
Only use these items occasionally, they are expensive and not good value.

Birds Eye		**Bread exchange**	**Meat Exchange**
Gravy and roast chicken.	200g		1
Beef curry and rice	1 pkt	1 plus	1
Chicken and mushroom casserole	1 pkt		1
Mince beef with vegetables and gravy	1 pkt		1
Chicken and mushroom pancake	one	1 plus	1
Cheese and ham pancake	one	1 plus	1
Potato waffle, grilled		1	
Minced beef pancake	one	1 plus	1
Heinz			
Baked beans with pork sausages	100g	1 plus	1
Macaroni cheese	100g	1 plus	1
Spaghetti in tomato sauce	100g		1
Ravioli in tomato sauce	100g	1 plus	1
Spaghetti bolognese	100g	1 plus	1
Marks & Spencer			
Lasagne	100g	1 plus	1
Chilli con carne	100g	1 plus	1
Beef stew with dumplings	100g	1 plus	1
Braised beef with vegetables	100g	1	
Braised steak	100g	1	
Haddock mornay	100g	1	
Fish lasagne	100g	1 plus	1
Fish pie	100g	1 plus	1
Cod in parsley sauce	100g	1	
Ocean pie	100g	1 plus	1
Seafood pasta	100g	1 plus	1
Chicken supreme	100g	1 plus	1
Chicken and potato bake	100g	1 plus	1
Chicken and chilli sauce	100g	1	
Chicken chasseur	100g	1	
Sweet and sour chicken	100g	1	
Findus French bread pizza cheese and tomato	one	1 plus	1 plus

IDEAS FOR MEALS

Breakfasts: these are often different at weekends to weekdays, and here are some ideas for alternatives to a fried breakfast.

- 4 tbs. cereal, 125 mL semi- skim milk, 1 slice bread toasted, 1 tsp. jam or marmalade

= 2 Bread exchanges and milk from allowance

- 2 Slices bread toasted, 30g Cheddar type cheese

= 2 Bread exchanges 1 Meat exchange

- 1 soft roll, 2-3 rashers grilled lean bacon

= 2 Bread exchanges, 1 Meat exchange

- 4 -5 tbs. cooked porridge, 2 crisp-breads, 1 tsp. jam or marmalade

= 2 Bread exchanges, 1 Meat exchange

- 1 small tub cottage cheese, 1 toasted roll

= 2 Bread exchanges, 1 Meat exchange

- 2 slices bread toasted, 1 boiled egg

= 2 Bread exchanges 1 Meat exchange

- 1 small tin baked beans, 2 slices of toast

= 3 Bread exchanges

IDEAS FOR MEALS

Lunches

- Healthy option!: 100g cottage cheese with a filling if preferred, 4 crisp breads, 1 Orange

= 2 Bread exchange, 1 Meat exchange, and 1 Fruit exchange

- 100g cottage cheese, 1 medium baked potato, 1 tablespoon pasta sauce

= 2 Bread exchanges 1 Meat exchange

• 1 tbs. pasta sauce, 100g cottage cheese, 100g baked potato, 1 banana

= 1 Meat exchange, 2 Bread exchanges, 1 Fruit exchange

• 100g tuna in brine (drained), jacket potato (100g), mixed salad

= 1 Meat exchange, 2 Bread exchanges

• 3 sardines in tomato sauce, 2 slices white bread, 1 apple

= 3 Bread exchanges, 1 Meat exchange

• 2 -3 rashers lean grilled bacon, 2 slices wholemeal bread, 1 diet yoghurt

= 1 Meat exchange, 2 Bread exchange and milk from allowance

• 1 bowl Scotch Broth, 2 crisp breads, 100g tinned mandarin oranges

= 1 Meat exchange, 2 Bread exchanges, 1 Fruit exchange

IDEAS FOR MEALS

Evening meals

• 5 tbs. spaghetti in tomato sauce, 1 grilled haddock fillet, assorted veg, 1 slice bread

= 1 Meat exchange, 2 Bread exchange

• 2 egg sized boiled potatoes, 3 grilled fish fingers, fresh carrots

= 1 Meat exchange, 1 Bread exchange

• 1 small tin baked beans, 1 slice toast, 1 poached egg, 1 apple

= 2 Bread exchange, 1 Meat exchange, 1 Fruit exchange

• 5 tbs. Shepherds pie, assorted veg (free foods), 1 wholemeal roll, 4 tablespoons fruit jelly

with milk from allowance

= 1 Meat exchange, 3 Bread exchange, from milk allowance

- 4 tbs. Chilli, 6 tbs. cooked white rice, 1 diet fromage frais

= 1 Meat exchange, 2 Bread exchanges, 1 Fruit exchange

- 1 bowl vegetable broth, 3 tbs./50g roast lean pork, 2 egg sized potatoes, assorted vegetables, 1 small banana

= 2 Bread exchanges, 1 Meat exchange, 1 Fruit exchange

- 2-3 lean bacon rashers, 2 tbs. mashed potato, 5 tbs. mushy peas, 1 fresh fruit

=1 Meat exchange, 2 Bread exchanges, 1 Fruit exchange

FOODS TO AVOID FOR WEIGHT GAIN

Sugary Foods -Syrup treacle, lemon curd, sweets, chocolates, cakes, pastries. Most puddings and ice creams, except those included in the any of the exchange lists.

Fatty Foods - Dripping, lard, cooking fat, cooking oils and all fried foods. Potatoes cooked in fat e.g. chips, crisps, sauté, roast and stovies.

Cream, cream cheese, pate, fatty meats, salted or coated nuts. Rich sauces and thick gravies.

Salad cream and mayonnaise.

Buttery rolls (rowies), sausage rolls, pies and bridies.

Drinks - Drinking chocolate, cocoa, Horlicks, Ovaltine and other malted drinks.

Fruit drinks, squashes and fizzy drinks containing sugar.

ALCOHOL

Most alcoholic drinks are high in calories. Also, it is easy to drink quite a lot, without realising how much alcohol you are having. To help to control your weight it is important to limit your alcohol intake.

You are allowed a maximum of 6 units of alcohol per week.

1 unit of alcohol is equivalent to:

1 pub measure of spirits - whisky, gin, vodka with a mixer, 1 $^{1}/_{2}$ pint beer, lager, cider.
1 small glass red/white wine, liqueurs, excepting creamy ones

UNRESTRICTED FOODS

Remember to try and avoid nibbling between meals. But if you feel you are going to, try to choose something from the snack or free foods list.

Drinks

Black coffee, tea, herb tea, Bovril, Oxo, Marmite, tomato juice, soda water, Perrier water and "Low calorie" drinks, e.g. Diet Cola, Diet Lilt, Reduced Cal, Sugar free squashes.

Vegetables and salads

Dress with vinegar, herbs, or oil free dressing. Avoid oil and mayonnaise. Cucumber, cress, lettuce, radish, tomato, pickled onion, asparagus, broccoli, cabbage, carrot, cauliflower, celery, courgette, endive, kale, leeks, marrow, mushrooms (boiled not fried), onion, spinach, Swede, sprouts, green beans, turnip, beetroot, parsley, peppers.

Fresh fruit

Fresh grapefruit, rhubarb, gooseberries, strawberries, raspberries, black currants (sweetened with artificial sweetener), lemon, lime, melon.

Seasoning

Make your meals more interesting!

Spices, herbs, pepper, mustard, lemon juice, vinegar, mint, Worcestershire sauce, gravy browning, tomato puree.

HELPFUL HINTS: EATING OUT

1. Extend your pre meal drink with low calorie drinks e.g. lemonade, tonic or soda water.
2. Mineral water is very popular, drink it neat or mix it with wine to make it go further.
3. Save calories by choosing a starter such as tomato juice, a slice of melon or soup.

4. Have a fair sized portion for you main course, but avoid chips, rich sauces, pastry and fried foods. Ask for extra vegetables.

5. Have second helpings of vegetables or potatoes if you are still hungry.

6. Move straight to coffee, with milk or black. Send back cream and ask for milk.

7. Eat slowly and enjoy your food.

TEMPTATION

All people following a healthy diet have good days when eating healthily seems easy. There are also days when it may seems almost impossible to follow this eating plan.

1. Have a hot drink - tea, coffee, beef or vegetable extract.

2. Always have on hand some vegetable nibbles, carrots, celery or pickled onions.

3. Occupy yourself with hobbies, sewing, knitting, gardening or just go out for walks.

4. Put health at the top of your agenda, - it's just not worth sacrificing your long term goal for a few minutes enjoyment.

CHECKLIST

1. Have managed to eat all the exchanges on your diet

2. Have managed to eat all the five portions of fruit and vegetables?

NAME
ADDRESS
SUBJECT NUMBER.....

BASELINE WEIGHT **DESIRED WEIGHT**

DATE	WEIGHT Metric	Imperial

3.1 1200 kcal diet prescription

The Healthy Diet 12

Daily allowances

250 mL semi skimmed milk
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
6 x bread exchange
2 x meat exchange
3 x fruit exchange
1 x wine glass fruit juice (125 mL)

Polyunsaturated spread: 20g or 2 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
10g sunflower margarine

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 1 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	2 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	1 x meat exchange 2 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
1 Fruit juice	45	188	/	/	/	/	11.0	0.6
6 Bread	563	2355	9.6	3.6	2.4	3.0	102.0	
2 Meat	240	1004	12.6	1.6	4.5	5.1	5.0	13.6
10g pufa Margarine	71	297	7.8	4.1	2.6	1.6	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1190	4979	34.0	9.3	10.8	12.1	170	60.4
% energy			25.7	7.0	8.1	9.1	53.6	20.3

3.2 1300 kcal diet prescription

The Healthy Diet 13

Daily allowances

250 mL pint semi skimmed milk
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
7 x bread exchange
2 x meat exchange
3 x fruit exchange
1 x wine glass fruit juice (125 mL)

Polyunsaturated spread: 20g or 2 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
sunflower margarine 10g e.g. flora, vitalite or sunflower margarine

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	2 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	1 x meat exchange 2 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
1 Fruit juice	45	188	/	/	/	/	11.0	0.6
7 Bread	657	2747	14.6	4.2	2.8	3.5	119.3	27.0
2 Meat	240	1004	12.6	1.6	4.5	5.1	5.0	13.6
10g pufa Margarine	71	297	7.8	4.1	2.6	1.6	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1284	5369	39.0	9.9	11.2	12.6	187.4	64.3
% energy			27.3	6.9	7.8	8.8	54.7	20.0

3.3 1400 kcal diet prescription

The Healthy Diet 14

Daily allowances

250 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
7 x bread exchange
2 x meat exchange
3 x fruit exchange
2 x wine glass fruit juice (125 mL)

Polyunsaturated spread: 40g or 3 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or** 20g sunflower margarine e.g. flora, vitalite **or** 16g
(11/2 tablespoons sunflower oil)

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	2 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	1 x meat exchange 2 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
2 Fruit juice	90	376	/	/	/	/	22.0	1.2
7 Bread	657	2747	14.6	4.2	2.8	3.5	119.3	27.0
2 Meat	240	1004	12.6	1.6	4.5	5.1	5.0	13.6
20g pufa Margarine	142	594	15.6	8.1	2.6	3.2	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1400	5857	46.8	13.9	11.2	14.2	198.4	64.9
% energy			30.0	8.9	7.2	9.1	53.0	18.5

3.4 1500 kcal diet prescription

The Healthy Diet 15

Daily allowances

250 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
7 x Bread exchange
3 x meat exchange
3 x fruit exchange
2 x wine glass fruit juice (125 mL)

Polyunsaturated spread: 30g or 2 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
15 g sunflower margarine, e.g. flora, vitalite. **or** 12g sunflower oil (1 tablespoon)

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	2 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 2 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
2 Fruit juice	90	376	/	/	/	/	22.0	1.2
7 Bread	657	2747	14.6	4.2	2.8	3.5	119.3	27.0
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
15g pufa Margarine	106.5	445.6	11.7	6.1	1.9	2.4	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1484	6211	49.1	12.7	12.8	16.0	201.0	78.6
% energy			29.7	7.7	7.7	9.7	51.0	21.1

3.5 1600 kcal diet prescription

The Healthy Diet 16

Daily allowances

250 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
8 x Bread exchange
3 x meat exchange
3 x fruit exchange
2 x wine glass fruit juice (125 mL)
Polyunsaturated spread: 40g or 4 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
20g sunflower margarine, vitalite or flora **or** 16g (1 1/2 tablespoons) sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	2 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 3 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
2 Fruit juice	90	376	/	/	/	/	22.0	1.2
8 Bread	750	3138	12.8	4.8	3.2	4.0	136.4	30.4
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
20g pufa Margarine	142	594	15.6	8.1	2.6	3.2	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1613	6748	51.2	15.3	13.8	17.3	218	82.0
% energy			28.5	8.5	7.6	9.6	50.6	20.3

3.6 1700 kcal diet prescription

The Healthy Diet 17

Daily allowances

250 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
9 x Bread exchange
3 x meat exchange
3 x fruit exchange
2 x glass fruit juice
Polyunsaturated spread: 40g or 2 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
20g sunflower margarine, flora, vitalite, **or** 16g 11/2 tablespoons sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	1 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 3 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
2 Fruit juice	90	376	/	/	/	/	22.0	1.2
9 Bread	844	3253	14.4	5.4	3.6	4.5	153.4	34.2
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
20g pufa Margarine	142	594	15.6	8.1	2.6	3.2	/	/
250 mL SSkim milk	115	481	4.0	/	1.2	2.5	12.5	8.25
Total nut.	1707	7142	52.8	15.9	14.2	17.8	235	85.8
% energy			27.8	8.3	7.4	9.3	51.6	20.1

3.7 1800 kcal diet prescription

The Healthy Diet 18

Daily allowances

300 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
9 x Bread exchange
3 x meat exchange
3 x fruit exchange
3 x wine glass pure unsweetened fruit juice
Polyunsaturated spread: 50g or 5 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
25g sunflower margarine, flora or vitalite, **or** 20g sunflower oil (2 tablespoons).

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 2 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 3 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
9 Bread	844	3253	14.4	5.4	3.6	4.5	153.4	34.2
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
25g pufa Margarine	177	741	19.5	10.1	3.3	4.0	/	/
300 mL SSkim milk	138	577	4.8	/	1.4	3.0	15.0	9.9
Total nut.	1810	7573	57.5	17.9	15.1	19.1	248.5	88.1
% energy			28.5	8.9	7.5	9.4	51.0	18.2

The Healthy Diet 19

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
10 x Bread exchange
3 x meat exchange
3 x fruit exchange
3 x glass fruit juice

Polyunsaturated spread: 50g, 5 scrapes (e.g. sunflower spread, flora lite, vitalite lite) **or** 25g sunflower margarine **or** 20g 2 tablespoons sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 3 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 3 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x glass fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
10 Bread	938	3924	16.1	6.0	4.0	5.0	170.5	38.0
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
25 g pufa Margarine	177	741	19.5	10.1	3.3	4.0	/	/
325 mL SSkim milk	149	623	5.2	/	1.4	3.25	16.25	10.6
Total nut.	1915	8012	59.6	18.5	15.5	19.9	266.8	92.6
% energy			28.0	8.6	7.2	9.3	52.0	19.3

3.9 2000 kcal diet prescription

The Healthy Diet 20

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
11 x Bread exchange
3 x meat exchange
3 x fruit exchange
3 x glass fruit juice

Polyunsaturated spread: 50g, 5 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
25 g sunflower margarine e.g. flora, vitalite **or** 20 g sunflower oil. (2 tablespoons)

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 3 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 1 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
11 Bread	1032	4317	17.5	6.6	4.4	5.5	187.6	41.8
3 Meat	360	1506	18.8	2.4	6.8	7.6	7.5	40.7
25g pufa Margarine	177	741	19.5	10.1	3.3	4.0	/	/
325 mL SSkim milk	149	623	5.2	/	1.4	3.25	16.25	10.6
Total nut.	2009	8406	61.3	19.1	15.9	20.4	283.9	96.4
% energy			27.0	8.5	7.1	9.1	52.9	19.1

The Healthy Diet 21

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
11 x Bread exchange
4 x meat exchange
3 x fruit exchange
3 x glass fruit juice
Polyunsaturated spread: 50g or 5 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) **or**
25 g sunflower margarine e.g. flora, vitalite **or** 20 g sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 3 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 2 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange
Supper	1 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
11 Bread	1032	4317	17.5	6.6	4.4	5.5	187.6	41.8
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
25 g pufa Margarine	177	741	19.5	10.1	3.3	4.0	/	/
300 mL SSkim milk	138	577	4.8	/	1.4	3.0	15.0	9.9
Total nut.	2120	8869	67.1	19.9	31.4	22.6	285.0	109.2
% energy			28.4	8.4	13.3	9.5	50.4	20.6

3.11 2200 kcal diet prescription

The Healthy Diet 22

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
12 x Bread exchange
4 x meat exchange
3 x fruit exchange
3 x wine glass fruit juice
Polyunsaturated spread: 50g or 5 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) or 25g sunflower margarine e.g. flora or vitalite or 20g sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 3 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 2 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange 1 x fruit juice
Supper	2 x bread exchange 1 x fruit exchange 1 x fruit juice

]Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
12 Bread	1126	4711	19.3	7.2	4.8	6.0	204.6	45.6
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
25g pufa Margarine	177	741	19.5	10.1	3.3	4.0	/	/
325 mL SSkim milk	149	623	5.2	/	1.4	3.25	16.25	10.6
Total nut.	2225	9309	69.0	20.5	18.6	23.4	303.4	113.7
% energy			27.9	8.2	7.5	9.4	51.0	20.4

3.12 2300 kcal diet prescription

The Healthy Diet 23

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
12 x Bread exchange
4 x meat exchange
3 x fruit exchange
3 x wine glass fruit juice

Polyunsaturated spread: 60g or 6 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) or 30g sunflower margarine e.g. flora, sunflower margarine, vitalite or 25g sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 3 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 2 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange 1 x fruit juice
Supper	2 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
12 Bread	1126	4711	19.3	7.2	4.8	6.0	204.6	45.6
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
30 g pufa Margarine	212	887	23.4	12.1	3.9	4.8	/	/
375 mL SSkim milk	172.5	721	6.0	/	1.8	3.8	18.8	12.4
Total nut.	2283	9553	73.7	22.5	19.6	24.7	306	126.1
% energy			29.0	8.8	7.7	9.7	50.2	22.0

3.13 2400 kcal diet prescription

The Healthy Diet 24

Daily allowances

350 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
13 x Bread exchange
4 x meat exchange
4 x fruit exchange
3 x wine glass fruit juice
Polyunsaturated spread: 60g or 6 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) or 30g sunflower margarine e.g. flora or vitalite or 25g sunflower oil.

Suggested meal plan. Remember try to take all the exchanges

Breakfast	2 x glass of fresh fruit juice 4 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	3 x bread exchange 2 x meat exchange Salad / vegetable 2 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange 1 x fruit juice
Supper	2 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
3 Fruits	156	652	/	/	/	/	39.6	1.5
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
13 Bread	1219	5100	20.9	7.8	5.2	6.5	221.6	49.4
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
30 g pufa Margarine	212	887	23.4	12.1	3.9	4.8	/	/
350 mL SSkim milk	161	673	5.6	/	1.6	3.5	17.5	11.55
Total nut.	2365	9894	74.9	23.1	19.8	24.9	334.9	118.9
% energy			27.8	8.6	7.3	9.2	51.9	19.6

3.14 2500 kcal diet prescription

The Healthy Diet 25

Daily allowances

325 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
14 x Bread exchange
4 x meat exchange
4 x fruit exchange
3 x wine glass fruit juice
Polyunsaturated spread: 60g or 6 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) or 30g sunflower margarine e.g. flora or vitalite or 25g sunflower oil.

Suggested meal plan.	Remember try to take all the exchanges
Breakfast	2 x glass of fresh fruit juice 4 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	4 x bread exchange 2 x meat exchange Salad / vegetable 2 x fruit exchange 1 x fruit juice Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange 1 x fruit juice
Supper	2 x bread exchange 2 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
4 Fruits	208	870	/	/	/	/	52.8	2.0
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
14 Bread	1312	5489	22.5	8.4	5.6	7.0	238.6	53.2
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
30g pufa Margarine	212	887	23.4	12.1	3.9	4.8	/	/
325 mL SSkim milk	149	623	5.2	/	1.4	3.25	16.25	10.6
Total nut.	2498	10451	76.1	23.7	20.0	25.2	350.6	121.6
% energy			27.4	8.5	7.2	9.0	52.6	19.4

3.15 2600 kcal diet prescription

The Healthy Diet 26

Daily allowances

500 mL semi skimmed milk.
(100 mL of milk can be taken as a diet, natural yoghurt or as a diet fromage frais)
14 x Bread exchange
4 x meat exchange
4 x fruit exchange
3 x wine glass fruit juice

Polyunsaturated spread: 60g or 6 scrapes
(e.g. sunflower spread, flora lite, vitalite lite) or 30 sunflower margarine e.g. flora or vitalite or 25g sunflower oil.

Suggested meal plan.	Remember try to take all the exchanges
Breakfast	1 x glass of fresh fruit juice 4 x bread exchange Spread jam or marmalade Tea / coffee
Mid-Morning	Tea / Coffee or low calorie drink
Lunch	4 x bread exchange 2 x meat exchange Salad / vegetable 1 x fruit exchange Tea / Coffee or low calorie drink
Mid afternoon	Tea / Coffee or low calorie drink
Evening meal	2 x meat exchange 4 x bread exchange Salad / vegetable 1 x fruit exchange 1 x fruit juice
Supper	2 x bread exchange 1 x fruit exchange 1 x fruit juice

Exchanges	kcal	kJ	fat g	pufa g	mufa g	sfa g	cho g	prot g
4 Fruits	208	870	/	/	/	/	52.8	2.0
3 Fruit juice	135	565	/	/	/	/	33.0	1.8
14 Bread	1312	5489	22.5	8.4	5.6	7.0	238.6	53.2
4 Meat	482	2017	25.0	3.2	9.1	10.1	10.0	54.2
30g pufa Margarine	212	887	23.4	12.1	3.9	4.8	/	/
500 mL SSkim milk	230	962	78.9	23.7	20.9	26.9	25.0	16.5
Total nut.	2283	10790	78.9	23.7	20.9	26.9	359.4	127.8
% energy			31.1?	9.3	8.2	10.6	59.0?	22.3?

University wants angina volunteers

PEOPLE who suffer from angina are being asked to volunteer for a Glasgow University project to find out if a healthy diet would make them feel better. Research scientist Catherine Hankey said yesterday: "We are looking for all shapes and sizes, so both slim and overweight people will be welcome. Whatever their size, we'll devise a diet for them. Our volunteers will also find out how much of their weight is muscle and how much is fat." After evaluating their diet by computer, Ms Hankey will provide the volunteers with individually designed diets for a 12-week period. Angina sufferers who can help should contact Ms Hankey on 0141 304 4686

THE HERALD, September 15 1993

Volunteers sought for heart study

GLASGOW University wants to hear from angina sufferers.

They are all needed for a special project into diet being carried out in the Department of Human Nutrition. Researcher Catherine Hankey and Professor Mike Lean say a lot of research has been done on foods which increase the risk of heart disease.

But they add that little is known about the benefits of a healthy diet and any effect it may have on clotting or thrombosis.

Catherine believes a healthy diet would help angina sufferers but she needs the facts to back her up.

She will evaluate volunteers' current diet and provide them with an individually designed diet.

During that time they will have to visit Glasgow Royal Infirmary.

Anyone who can help should contact Catherine on 304 4686.

EVENING TIMES
September 14 1993

Appeal for angina study volunteers

A DIET specialist at Glasgow University is looking for angina sufferers to help her research the complaint.

Catherine Hankey, of the Department of Human Nutrition, wants 50-70 volunteers for the 12-week project.

"There has been a lot of research done on foods which might increase the risk of heart disease - by narrowing the arteries for example," she said. "But little is known about the benefits of a healthy diet and any effect it may have on the rate of blood clotting or thrombosis."

Ms Hankey will ask the volunteers to record everything they eat for four days and will then draw up individual diets for them.

"We are looking for people of all shapes and sizes," said Ms Hankey, who can be contacted on 0141-304 4686.

THE SCOTSMAN
September 15 1993

Diet for medical research

ANGINA sufferers in Linlithgow have been asked to reveal their eating habits in a bid to help a local woman's research.

For Catherine Hankey of Linlithgow is a researcher at Glasgow University. She is studying the benefits of a healthy diet and its effect it has on the rate of blood clotting and thrombosis.

Volunteers will have their diet evaluated by computer and given individually designed diets for a 12-week period.

During that time, five visits to Glasgow Royal Infirmary will be made for tests, including two blood checks.

Catherine said: "We're looking for all shapes and sizes, so both slim and overweight people will be welcome. Whatever their size we'll devise a diet for them."

Volunteers will also have the opportunity to find out just how much of their weight is muscle and how much is fat.

It does not matter if volunteers are already on drug treatment for angina but the programme is not suitable for people who are already on a special diet or involved in othr research.

If you are an angina sufferer and can help, please contact Catherine Hankey on 0141-304-4686.

LINLITHGOW ADVERTISER
September 22 1993

Fancy a real hearty diet?

By MIKE RITCHIE
Health Correspondent

UNIVERSITY researcher Catherine Hankey is on the look-out for angina sufferers.

Because she wants to know what they eat!

Angina is a chest pain sparked off by the heart not getting enough blood or oxygen.

It's a major contributor to Scotland's grim annual heart death toll.

There's been a lot of research on foods which might increase the risk of heart disease - by narrowing the arteries, for example.

But little is known about the benefits of a healthy diet or the effect it could have on the rate of blood-clotting or thrombosis.

Angina can be sparked off by factors such as smoking and obesity. Common sense suggests that a healthy diet would improve matters.

But Catherine wants to look closer at what angina sufferers actually tuck into.

After evaluating their diet by computer, she will provide them with individually-designed diets for a 12-week period.

This diet will be calculated on the basis of age, weight and sex.

The research team is hoping to find up to 70 volunteers.

"We're loking for all shapes and sizes. Whatever their size, we'll devise a diet for them," said Catherine.

The volunteers will also have the chance to find out just how much of their weight is muscle and how much is actually fat.

It doesn't matter if volunteers are already on drug treatment for angina.

IMPROVE

The programme is **NOT** suitable for people already on a special diet or involved in other research.

"I'm sure that many sufferers would like to do their bit to help scientists know more about angina," said Catherine.

"If the new diets improve their condition, so much the better."

Catherine can be contacted on 041-304 4686.

DAILY RECORD
September 16 1993

Bibliography

Alexander, R.W. (1994) Inflammation and coronary artery disease. *New England Journal of Medicine,* **331**, 468-469.

Alkjaersig, N., Fletcher, A.P., de Ziegler, D., Steingold, K.A., Meldrum, D.R. and Judd, H.I. (1980) Blood coagulation in post-menopausal women given oestrogen treatment: comparison of transdermal and oral administration. *Journal of Laboratory and Clinical Medicine,* **111**, 224-228.

Almer, L. and Kjellstrom, T. (1986) The fibrinolytic system and coagulation during benzafibrate treatment of hyperlipidaemia. *Atherosclerosis* **61**, 81-85.

Altman, D.G. (1991) Statistical analysis of comparisons between laboratory methods. *Journal of Clinical Pathology,* **44**, 700-702.

Andersen, P., Nilsen, D.W.T., Beckmann, S.L., Holme, I. and Hjermann, I. (1988) Increased fibrinolytic potential after dietary intervention in healthy high coronary risk individuals. *Acta Medica Scandinavia,* **223**, 499-506.

Andersen, P., Seljeflot, I., Abdelnoor, M., Arnesen, H., Dale, P.O., Lovik, A. and Birkeland, K. (1995) Increased insulin sensitivity and fibrinolytic capacity after dietary intervention in obese women with polycystic ovary syndrome. *Metabolism* **44**, 611-616.

Anderson, T., Stokholm, K.H. and Backer, O.G. (1988) Long term results after horizontal gastroplasty or very low calorie diet for morbid obesity. *International Journal of Obesity,* **12**, 277-284.

Anderson, J.W., Brinkman-Kaplan, V., Hamilton, C.C., Logan, J.E., Collins, R.W., Gustafson, N.J. (1994) Food containing hypocaloric diets are as a effective as liquid-supplement diets for obese individuals with NIDDM. *Diabetes Care*, **17**, 6, 602-604.

Anderson, R.E., Wadden, T.A., Bartlett, S.J., Vogt, R.A., Weinstock, R.S. (1995) Relation of weight loss to changes in serum lipids and lipoproteins in obese women. *American Journal of Clinical Nutrition*, **62**, 350-357.

Andersson, I. and Rossner, S. (1997) Weight development, drop-out pattern and changes in obesity-related risk factors after two years treatment of obese men. *International Journal of Obesity,* **21**, 211-216.

Andres, R., Muller, D.C. and Sorkin, J.D. (1993) Long-term effects of change in body weight on all-cause mortality. A review. *Annals of Internal Medicine,* **119**, 737-743.

Apfelbaum, M., Bostsarron, J. and Lactis, D. (1971) Effect of calorie restriction and excessive calorie intake on energy expenditure. *American Journal of Clinical Nutrition,* **24**, 1205-1207.

Aristimuno, G.G., Foster, T.A., Voors, A.W., Sathanur, P.H., Srinivasan, S.S. and Berenson, G.S. (1984) Influence of persistent obesity in children on cardiovascular risk factors; the Bogalusa Heart Study. *Circulation,* **69**, 895-904.

Armstrong, B.K., Mann, J.I., Adelstein, A.M. and Eskin, F. (1975) Commodity consumption and ischaemic heart disease mortality, with special reference to dietary practices. *Journal of Chronic Diseases,* **28**, 455-469.

Ashley, F.W., Kannel, W.B. (1974) Relation of weight changes to changes in atherogenic traits: the Framingham Study. *Journal of Chronic Disease,* **27**, 103-114.

Ashwell, M.A. and Garrow, J.S. (1975) A survey of three slimming and weight control organisations in the U.K. *Nutrition,* **29**, 347-356.

Assman, G. and Schulte, H. (1992) Obesity and hyperlipidaemia: results from the Prospective Cardiovascular Munster Study, 502-511. In Obesity, Ed Bjorntorp, P. and Brodoff. Lippincott, New York.

Avellone, G., de Garbo, V., Cordova, R., Cucinella, G., de Simone, R., Raneli, G. and Bompiani, G. (1994) Evaluation of cardiovascular risk factors in overweight and obese subjects. *International Angiology,* **13**, 25-29.

Avenell, A., Richmond, P.R., Lean, M.E. and Reid, D.M. (1994) Bone loss associated with a high fibre weight reduction diet in postmenopausal women. *European Journal of Clinical Nutrition,* **48**, 561-566.

Aznar, J., Estelles, A., Tormo, G., Sapena, P., Tormo, V., Blanch, S., and Espana, F. (1988) Plasminogen activator inhibitor activity and other fibrinolytic variables in patients with coronary artery disease. *British Heart Journal,* **59**, 535-541,.

Bachman, F. (1994) Molecular aspects of plasminogen, plasminogen activators and plasmin. In: Bloom, A.L., Forbes, C.D., Thomas, D.P. and Tuddenham, E.G.D. (Eds.) *Haemostasis and Thrombosis*, 3rd edn. pp. 574-614. Edinburgh: Churchill Livingstone.

Bahadori, B., Neuer, E., Schumacher, M., Fruhwald, F., Eber, B., Klein, W., Toplak, H., Wascher, T.C. (1996) Prevalence of coronary-artery disease in obese versus lean men with angina-pectoris and positive exercise stress test. *American Journal of Cardiology*, **77**, 1000-1001.

Bajaj, S.P. and Mann, K.G. (1973) Simultaneous purification of bovine prothrombin and factor X. *Journal of Biological Chemistry,* **248**, 7729-7744.

Balleisen, L., Bailey, J., Epping, P.H., Schulte, H. and van de loo, J. (1985) Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population: 1. Baseline data on the relation to age, gender, body weight, smoking, alcohol, pill-using and menopause. *Thrombosis and Haemostasis,* **54**, 475-479.

Balleisen, L., Schulte, H., Assmann, G., Epping, P.H. and Vandeloo, J. (1987) Coagulation factors and the progress of coronary heart disease. *Lancet,* **2**, 461

Bannen, L.T., Beevers, D.G. and Wright, N. (1980) ABC of blood pressure reduction. The size of the problem. *British Medical Journal,* **281**, 921-924.

Barnes, A. (1988) The rheology of diabetes mellitus. In: Lowe, G.D.O. (Ed.) Clinical blood rheology, pp. 163-188. Florida: CRC Press.

Barnes, M.S. and Terry, R.D. (1991) Adherence to the cardiac diet: attitudes of patients after myocardial infarction. *Journal of the American Dietetic Association,* **91,** 1435-1437.

Baron, J.A., Mann, J.I. and Stukel, T. (1989a) Effect of weight loss on coagulation factors VII and X. *American Journal of Cardiology,* **64,** 519-522.

Baron, J.A., Schori, A., Crow, B., Carter, R. and Mann, J.I. (1989b) A randomised controlled trial of low carbohydrate and low fat/high fibre diets for weight loss. *American Journal of Public Health,* **76,** 1293-1296.

Barrett-Connor, E. (1985) Obesity, atherosclerosis and coronary artery disease. *Annals of Internal Medicine,* **103,** 1010-1019.

Barrett-Connor, E., Suarez, L., Khaw, K.T., Criqui, M.H. and Wingard, D.L. (1984) Ischaemic heart disease risk factors after age 50. *Journal of Chronic Diseases,* **37,** 903-908.

Barrett-Connor, E., Khaw, K.T. and Yen, S.S.C. (1986) A prospective study of dehydroepiandosterone sulphate, mortality, and cardiovascular disease. *New England Journal of Medicine,* **315,** 1519-1524.

Barrett-Connor, E. (1989) Epidemiology, obesity and NIDDM. *Epidemiology Reviews,* **11,** 172-181.

Barrett-Connor, E. and Goodman-Gruen, D. (1995) Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. *British Medical Journal,* **311,** 1193-1196.

Beales, P.L. and Kopelman, P.G. (1994) Options for the management of obesity. *Pharmoeconomics,* **5** (suppl 1), 18-32.

Beard, C.M., Orencia, A., Kottke, T. and Ballard, D.J. (1992) Body mass index and the initial manifestation of coronary heart disease in women aged 40-59 years. *International Journal of Epidemiology,* **21,** 656-664.

Becque, M.D., Katch, V.L. and Moffatt, R.J. (1986) Time course of skin fat plus fat compression in males and females. *Human Biology,* **58**, 33-42.

Belch, J.J.F., McArdle, B.M., Burns, P., Lowe, G.D.O. and Forbes, C.D. (1984) The effects of acute smoking on platelet behaviour, fibrinolysis and haemorheology in habitual smokers. *Thrombosis and Haemostasis,* **51**, 6-8.

Bennett, G.A. (1986) Behaviour therapy for obesity: a quantitative review of the effects of selected treatment characteristics to outcome. *Behaviour Therapy,* **17**, 554-562.

Bennett, N., Dodd, T., Flatley, J., Freeth, S. and Bolling, K. (1995) Health survey for England 1993. HMSO,London.

Berdanier, C.D., Parente, J.A., McIntosh, M.K. (1993) Is dehydroepiandosterone an anti-obesity agent? *FASEB Journal,* **7**, 414-419.

Bhathena, S.J., Berlin, E., Judd, J., Nair, P.P., Kennedy, B.W., Jones, J., Smith, P.M., Jones, Y., Taylor, P.R. and Campbell, W.S. (1989) Hormones regulating lipid and carbohydrate metabolism in premenopausal women: modulation by dietary lipids. *American Journal of Clinical Nutrition,* **49**, 752-757.

Bingham, S.A. and Cummings, J.H. (1985) Urine nitrogen as an independent validitory measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. *American Journal of Clinical Nutrition,* **42**, 1276-1289.

Bingham, S.A. (1987) The dietary assessment of individuals; methods, accuracy, new techniques and recommendations. *Nutrition Abstracts and Reviews,* **57**, 706-742.

Bingham, S.A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K.T., Sneyd, M.J., Key, T.J.A., Roe, L. and Day, N.E. (1994) Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24h recalls, food frequency questionaires and estimated-diet records. *British Journal of Nutrition,* **72**, 619-643.

Bitzen, P.O., Melander, A., Schersten, B. and Svensson, M. (1988) Efficacy of dietary regulation in primary health care patients with hyperglycaemia detected by screening. *Diabetic Medicine,* **5**, 634-639.

Bjorntorp, P. (1990) Obesity and adipose tissue distribution as risk factors for the development of disease- a review. *Infusiontherapie*, **17**, 24-27.

Bjorntorp, P. and Brodoff, B.N. (Eds.) (1992) *Obesity*, New York: Lippincott.

Black, A.E., Prentice, A.M. and Coward, W.A. (1986) Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Human Nutrition: Clinical Nutrition,* **40C**, 381-391.

Black, D.R., Gleser, L.J., Kooyers, K.J. (1990) A meta-analytic evaluation of couples weight loss programmes. *Health Psychology*, **9**, 3, 330-347.

Bladberg, E.M., Marckmann, P., Sandstrom, B. and Jespersen, J. (1994) Non-fasting factor VII coagulant activity (FVIIc) increased by a high fat diet. *Thrombosis and Haemostasis,* **71**, 755-758.

Blair, S.N. (1993) Evidence for success of exercise in weight loss and control. *Annals of Internal Medicine,* **119**, 702-706.

Bland, J.M. and Altman, D.G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* **1**, 307-310.

Blonk, M.C., Jacobs, M.A.J.M., Biesheuvel, E.H.E., Weda-Manak, W.L. and Heine, R.J. (1994) Influences of weight loss in type 2 diabetic patients: little long term benefit from group behaviour therapy and exercise training. *Diabetic Medicine,* **11**, 449-457.

Bloom, A.L., Forbes, C.D., Thomas, D.P. and Tuddenham, E.G.D. (1994) *Thrombosis and Haemostasis*, 3rd ed.

Blundell, J.E. (1984) Behaviour modification and exercise in the treatment of obesity. *Postgraduate Medical Journal,* suppl. 3, 37-49.

Bolton-Smith, C., Brown, C.A. and Tunstall-Pedoe, H. (1991a) Nutrient sources in non-manual and manual occupation groups. Results from the Scottish Heart Health Study. *Journal of Human Nutrition and Dietetic,s* **4**, 291-306.

Bolton-Smith, C., Casey, C.E., Gey, K.F., Smith, W.C.S. and Tunstall-Pedoe, H. (1991b) Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation with biochemical status in smokers and non-smokers. *British Journal of Nutrition,* **65**, 337-346.

Bolton-Smith, C., Woodward, M., Fenton, S., McCluskey, M. and Brown, C.A. (1995) Trans fatty acids in the Scottish diet. An assessment using a semi quantitative food frequency questionnaire. *British Journal of Nutrition,* **74**, 661-670.

Bonadonna, R.C. and DeFronzo, R.A. (1991) Glucose metabolism in obesity and type 2 diabetes. *Diabete and Metabolisme,* **17**, 112-135.

Bonifacj, C., Gerber, M., Scali, J. and Daures, J.P. (1997) Comparison of dietary assessment methods in a Southern French population: use of weighed records, estimated diet records and a food frequency questionnaire. *European Journal of Clinical Nutrition,* **51**, 217-231.

Bonora, E., Moghetti, P., Zancanaro, C., Cigolini, M., Querena, M., Cacciatori, V., Corgnati, A. and Muggeo, M. (1989) Estimates of *in vivo* insulin action in man: comparison of insulin tolerance tests with euglycaemic and hyperglycaemic glucose clamp studies. *Journal of Clinical Endocrinology and Metabolism,* **68**, 374-379.

Booth, N.A., Croll, A. and Bennett, B. (1990) The activity of plasminogen activator inhibitor-1 (PAI-1) of human platelets. *Fibrinolysis,* **4**, 138-140.

Brace, L.D., Gittler-Buffa, C., Miller, G.J., Cole, T.G., Schmeisser, D., Prewitt, T.E. and Bowen, P.E. (1994) Factor VII coagulant activity and cholesterol lowering in

premenopausal women consuming a long term cholesterol lowering diet. *Arterioslerosis and Thrombosis,* **14**, 1284-1289.

Bray, G.A. (1969) Effect of calorie restriction on energy expenditure in obese patients. *Lancet,* **ii**, 397-399.

Bray, G.A. (1990) Exercise and obesity. In: Bouchard, C., Shepherd, R.J., Stephens, T., Sutton, J.R. and McPherson, B.D. (Eds.) Exercise, fitness and health, pp. 497-510. Ilinois: Champaign.

Bray, G.A., Ryan, D.H., Gordon, D., Heidingsfelder, S., Macchiavelli, R., Wilson, K. (1995) Double blind randomised trial of sibutramine in overweight subjects. *International Journal of Obesity*, **19**, suppl. 2, 393.

Bray, G.A. (1996) Obesity an endocrine prespective. In DeGroot, L.J.(ed). Endocrinology, 2 Ed, W.B. Saunders, Philadelphia, .

Bremer, J.M., Scott, R.S., Lintott, C.J. (1994) Dexfenfluramine reduces cardiovascular risk factors. *International Journal of Obesity*, **18**, 199-205.

Bremer, J.M., Scott, R.S., Lintott, C.J. (1994) Dexfenfluramine reduces cardiovascular risk factors. *International Journal of Obesity*, **18**, 199-205.

Briley, M.B., Montgomery, D.H. and Blewett, J. (1992) Worksite nutrition education can lower total cholesterol levels and promote weight loss among police department employees. *Journal of the American Dietetic Association,* **92**, 1382-1385.

Broadhurst, P., Kelleher, C., Hughes, L., Imeson, J.D. and Raftery, E.B. (1990) Fibrinogen, factor VII clotting activity and coronary disease severity. *Atherosclerosis,* **85**, 169-173.

Brownell, K.D., Cohen, R., Stunkard, A., Felix, M. and Cooley, N. (1984) Weight loss competition at a worksite: impact on morale and effectiveness. *American Journal of Public Health,* **74**, 1283-1285.

Brownell, K. (1986) Public approaches to obesity and it's management. *Annual Review of Public Health,* **7**, 521-533.

Brownell, K.D. and Jeffery, R.W. (1987) Improving the long term weight loss. Pushing the lines of treatment. *Behaviour Therapy,* **18**, 353-374.

Brownell, K.D. and Rodin, J. (1994) Medical, metabolic and psychological effects of weight cycling. *Archives of Internal Medicine,* **154**, 1325-1330.

Burr, M.L., Gilbert, J.F., Holliday, J.F., Elwood, P.C., Fehily, A.M., Rogers, S., Sweetnam, P.M. and Deadman, N.M. (1989) Effects of changes in fat, fish and fibre intakes on death and myocardial infarction: Diet and Reinfarction Trial (DART). *Lancet,* **ii**, 757-761.

Burton, B.T., Foster, W.R., Van Itallie, T.B. (1985) Health implications of obesity: an NIH development conference. *International Journal of obesity*, **9**, 155-169.

Bush, A., Webster, J., Chalmers, G. (1988) The Harrow Slimming club: report on 1090 enrolments in 50 courses, 1977-1986. *Journal of Human Nutrition and Dietetics*, **1**, 429-436.

Cacciari, E., Balsamo, A., Palareti, G., Cassio, A., Argento, R., Poggi, M., Tassoni, P., Cicignani, A. and Tacconi, M. (1988) Haemorheologic and fibrinolytic evaluation in obese children and adolescents. *European Journal of Pediatrics,* **147**, 381-384.

Caimi, G., Lo-Presti, R., Serra, A., Catania, A., D'Asaro, S., Verga, S., Buscemi, S. and Sarno, A. (1991) Rheological determinants and red cell lipidaemic pattern in essential overweight, in overweight subjects with non-insulin dependent diabetes mellitus (NIDDM) and in obese subjects with impaired glucose tolerance (IGT). *Microcirculation and Endothelial Lymphatics,* **7**, 293-304.

Campbell, M.J., Elwood, P.C., Mackean, J. and Waters, W.E. (1985) Mortality, haemoglobin level and haematocrit in women. *Journal of Chronic Diseases,* **38**, 881-889.

Campbell, P.J. and Carlson, M.G. (1993) Impact of obesity on insulin action in NIDDM. *Diabetes,* **42**, 405-410.

Caprio Shovic, A., Adams, S., Dubitzky, J., Anacker, M. (1993) Effectiveness and dropout rate of a very low calorie diet program. *Journal of the American Dietetic Association*, **93**, 5, 583-584.

Carlson, L.A. and Bottiger, L.E. (1981) Serum triglycerides to be or not to be a risk factor for ischaemic heart disease. *Atherosclerosis,* **39**, 287-291.

Carter, C., McGee, D., Reed, D., Yano, K. and Stemmermann, G. (1983) Hematocrit and the risk of coronary heart disease: the Honolulu Heart Program. *American Heart Journal,* **105**, 674-679.

Castelli, W.P. (1996) Lipids, risk factors and ischaemic heart disease. *Atherosclerosis,* **124**, s1-s9.

Chakrabarti, R., Hocking, R.D., Fearnley, G.R., Mann, R.D., Attwell, T.N. and Jackson, D. (1968) Fibrinolytic activity and coronary artery disease. *Lancet,* **i**, 987-996.

Chan, J.M., Rimm, E.B., Colditz, G.A., Stampfer, M.J., Willett, W.C. (1994) Obesity, fat distribution and weight gain as factors for clinical diabetes in man. *Diabetes Care*, **17**, 9, 961-969.

Chenoweth, D.A. (1990) Health promotion programs examined through cost effectiveness analysis. *Occupational Health and Safety,* **59**, 40-41.

Chien, S. (1975) Biophysical behaviour of red cells in suspensions. In: Surgenor, D.M. (Ed.) *The red blood cell*, pp. 1031-1133. Academic Press,New York.

Chien, S. (1986) Blood rheology in myocardial infarction and hypertension. *Biorheology,* **23**, 633-653.

Chievitz, E. and Thiede, T. (1962) Complications and causes of death in polycythaemia vera. *Acta Medica Scandinavia,* **172**, 513-523.

Chmielewska, J. and Wiman, B. (1986) Determination of tissue plasminogen activator and its fast inhibitor in plasma. *Clinical Chemistry,* **32,** 482-485.

Ciuffetti, G., Mercuri, M., Mannarino, E., Robinson, M.K., Lennie, S.E. and Lowe, G.D.O. (1989) Peripheral vascular disease. Rheological variables during controlled ischaemia. *Circulation,* **80,** 348-352.

Clauss, A. (1957) Gerinnungsphysiologische schnellmethode zur bestimmung des fibrinogens. *Acta Haematologica,* **17,** 237-246.

Colditz, G.A., Willett, W.C., Rotnitzky, A. and Manson, J.E. (1995) Weight gain as a risk factor for clinical diabetes. *Annals of Internal Medicine,* **122,** 481-486.

Cole, T.G., Bowen, P.E., Schmeisser, D., Prewitt, T.E., Aye, P., Langenberg, P., Dolecek, T.A., Brace, L.D., Kamath, S. (1992) Differential reduction of plasma cholesterol by the American Heart Association Phase 3 diet in moderately hypercholesterolaemic, premenopausal women with different body mass indexes. *American Journal of Clinical Nutrition,* **55,** 385-394.

Coleman, W.P. (1991) Evaluation of the patient for liposculpture. *Journal of Dermatological Oncology,* **17,** 741-743.

Collen, D. and Juhan Vague, I. (1988) Fibrinolysis and atherosclerosis. *Seminars in Thrombosis and Hemostasis,* **14,** 180-183.

Committee on the medical aspects of food policy The use of very low calorie diets in the treatment of obesity. HMSO, London, 1987.

Committee on the medical aspects of food policy (1991) Dietary reference values for food energy and nutrients for the United Kingdom. Report 41, London, HMSO.

Compston, J.E., Laskery, M.A., Croucher, P.I., Coxon, A. and Krietzman, S. (1992) Effect of diet induced weight loss on total body bone mass. *Clinical Science,* **82,** 429-432.

Connelly, J.B., Cooper, J.A. and Meade, T.W. (1992) Strenuous exercise, plasma fibrinogen, and factor VII activity. *British Heart Journal,* **67**, 351-354.

Connelly, V.M., Gallagher, A., Kesson, L.M., (1995) A study of fluoxetine in obese elderly diabetic patients with type II diabetes. *Diabetic Medicine*, **12**, 416-418.

Cortellaro, M., Boschetti, C., Cofrancesco, E., Zanussi, C., Catalano, M., de Gaetano, G., Gabrielli, L., Lombardi, B., Specchia, G., Tavazzi, L., Tremoli, E., della Volpe, A., Polli, E. and Plat Study Group (1992) The PLAT study: hemostatic function in relation to atherothrombotic ischaemic events in vascular disease patients. *Arteriosclerosis and Thrombosis,* **12**, 1063-1070.

Cousins, J.H., Rubovits, D.S., Dunn, J.K., Reeves, R.S., Ramirez, A.G. and Foreyt, J.P. (1992) Family versus individually orientated intervention for weight loss in Mexican American women. *Public Health Reports,* **107**, 549-555.

Cowburn, G., Hillsdon, M., Hankey, C.R. (1997) Obesity management by life-style strategies in obesity. *British Medical Bulletin*, **53**, 2, 389-408.

Crave, J.C., Fimbel, S., Lejeune, H., Cugnardy, N., Dechaud, H. and Pugeat, E.A. (1995) Effects of diet and metformin administration on sex hormone binding globulin, androgens and insulin in hirsute and obese women. *Journal of Clinical Endocrinology and Metabolism,* **80**, 2057-2062.

Craveri, A., Tornaghi, G., Paganardi, L., Ranieri, R. and Giavardi, L. (1990) Weight loss does not affect the hemorheological and fibrinolytic changes in obesity. *Clinical Hemorheology,* **10**, 541-546.

Craveri, A., Tornaghi, G., Paganardi, L., Ranieri, R. and Cietto, S., Scaglioni, S., Bellu, R., Giovannini, M. (1992) Hemorheological changes in obese children and the effect of weight loss. *Clinical Hemorheology,* **12**, 573-578.

Cushman, M., Yanez, D., Psaty, B.M., Fried, L.P., Heiss, G., Lee, M., Polak, J.F., Savage, P.J. and Tracy, R.P. (1996) Association of fibrinogen and coagulation factors VII and VIII

with the cardiovascular risk factors in the elderly. *American Journal of Epidemiology* **143**, 665-676.

Darga, L.L., Carroll-Micheals, L., Borsford, S.J., Lucas, C.P. (1991) Fluoxetine's effect on weight loss in obese subjects. *American Journal of Clinical Nutrition*, **54**, 321-325.

Dattilo, A.M. and Kris-Etherton, P.M. (1992) Effects of weight reduction on blood lipids and lipoproteins: a meta analysis. *American Journal of Clinical Nutrition* **56**, 320-328.

Davis, B.R., Blaufox, M.D. and Oberman, A. (1993) Effect of weight reduction by dietary intervention in overweight persons with mild hypertension. *Archives of Internal Medicine,* **153**, 1773-1782.

De Pergola, G., Giagulli, V.A., Cospite, M.R., Giorgino, F., Cignarelli, M. and Giorgino, R. (1991) Low dehydroepiandosterone circulating levels in premenopausal obese women with very high body mass index. *Metabolism,* **40**, 187-190.

De Pergola, G., Triggiani, V., Cospite, M.R., Cignarelli, M., Guastamacchia, E., Giorgino, R. (1995) The free testosterone to dehydroepiandosterone molar ratio as a marker of visceral fat accumulation in premenopausal obese women. *International Journal of Obesity*, **18**, 659-664.

de Simone, G., Devereux, R.B., Chien, S., Alderman, M.H., Atlas, S.A. and Laragh, J.H. (1990) Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. *Circulation,* **81**, 107-117.

DeFronzo, R.A. and Ferrannini, E. (1991) Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia, and atherosclerotic cardiovascular disease. *Diabetes Care,* **14**, 173-194.

den Besten, C., Vansant, G., Weststrate, J.A. and Deurenberg, P. (1988) Resting metabolic rate and diet-induced thermogenesis in abdominal and gluteal-femoral obese women before and after weight reduction. *American Journal of Clinical Nutrition,* **47**, 840-847.

Denen, M.E., Hennessey, J.V., Markert, R.J. (1993) Outpatient evaluation of obesity in adults and children: a review of the performence of internal medicine/pediatrics residents. *Journal General Internal Medicine*, **8**, 5, 268-270.

Denke, M.A., Sempos, C.T. and Grundy, S.M. (1994) Excess body weight an under recognised contributor to dyslipidaemia in white American women. *Archives of Internal Medicine* **154**, 401-410.

Denke, M.A., Sempos, C.T., grundy, S.M. (1993) Excess body weight. An under recognised contributer to high blood cholesterol levels in white Amercian men. *Archives of Internal Medicine*, **153**, 9, 1093-1103.

Depres, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A., Bouchard, C. (1990) Regional distribution of body fat, plasma lipoproteins and cardiovascular disease. *Arteriosclerosis*, **87**, 203-210.

DePretti, E. and Forest, M.G. (1978) Patterns of plasma dehydroepiandosterone sulphate levels in humans from birth to adulthood. *Journal of Clinical Endocrinology and Metabolism,* **47**, 452-457.

Dietel, M. and Shahi, B. (1992) Morbid obesity: selection of patients for surgery. *Journal of the American College of Nutrition,* **11**, 457-462.

Dintenfass L. (1971) Blood microrheology: viscosity factors in blood flow. Butterworths, London.

Ditschuneit, H.H., Flechtner-Mors, M. and Adler, G. (1995) Fibrinogen in obesity before and after weight reduction. *Obesity Research,* **3**, 43-48.

Doar, J.W.H., Thompson, M.E., Wilde, C.E., Sewell, P.F.J. (1975) Influence of treatment of diet alone on oral glucose tolerance test and plasma sugar and insulin levels in patients with maturity onset diabetes. *Lancet* **i**, 1263-1266.

Doll, R. and Peto, R. (1976) Mortality in relation to smoking: 20 years observations on male British doctors. *British Medical Journal,* **2**, 1525-1536.

Drent, M.L. and Van der Veen, E.A. (1993) Lipase inhibition: a novel concept in the treatment of obesity. *International Journal of Obesity,* **17**, 241-244.

Drott, C. and Lundholm, K. (1992) Cardiac effects of calorie restriction-mechanisms and potential hazards. *International Journal of Obesity,* **16**, 481-486.

Durnin, J.V. and Womersley, J. (1974) Body fat assessed from total body density and its estimation from skin-fold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition,* **32**, 77-97.

Dustan, H.P., Weinser, R.L., James, L.D., Darnell, B.E., Hunter, G.R. (1990) Treatment of obesity hypertension (Abstract) Cardiovascular disease and epidemiology abstracts, 723.

Dwyer, J.T. and Lu, D. (1992) Popular diets for weight loss from nutritionally hazardous to healthful. In: Stunkard, A.J. and Wadden, T.A. (Eds.) Obesity: theory and therapy, 2nd ed. New York: Raven Press.

Dyer, R.G. (1994) Traditional treatment of obesity: does it work. *Balliere's Clinical Endocrinology and Metabolism,* **8**, 661-688.

ECAT Angina Pectoris Study Group (1993) ECAT angina pectoris study: baseline associations of haemostatic factors with extent of coronary arteriosclerosis and other coronary risk factors in 3000 patients with angina pectoris undergoing coronary angiography. *European Heart Journal,* **14**, 8-17.

Edington, J., Thorogood, M., Geekie, M., Ball, M. and Mann, J. (1988) Assessment of nutritional intake using dietary records with estimated weights. *Journal of Human Nutrition and Dietetics,* **2**, 407-414.

Ehrly, A.M. (1991) Haemorheology, microcirculatory flow and oxygen supply. Pathophysiological and therapeutic effects. *Revue Francaise de Gynecologie et d'Obstetrique, 86,* 2, 131-138.

Eliahou, H.E., Iaina, A, Gaon, T., Shochat, J., Modan, M. (1981) Body weight reductior

necessary to attain normotension in the overweight hypertensive patient. *International Journal of Obesity*, **5**, 157-163.

Eliasson, M., Asplund, K., Evrin, P.E., Lindahl, B. and Lundblad, D. (1994a) Hyperinsulinaemia predicts low tissue plasminogen activator activity in a healthy population: The Northern Sweden MONICA study. *Metabolism,* **43**, 1579-1586.

Eliasson, M., Evrin, P.E. and Lundblad, D. (1994b) Fibrinogen and fibrinolytic variables in relation to anthropometry, lipids and blood pressure. The Northern Sweden MONICA study. *Journal of Clinical Epidemiology,* **47**, 513-524.

Elliott, P. and Marmot, M. (1984) International studies of salt and blood pressure. *Annals of Clinical Research* **16**, 67-71.

Elwood, P.C., Yarnell, J.W.G., Pickering, J., Fehily, A.M. and O'Brian, J.R. (1993) Exercise, fibrinogen, and other risk factors for ischaemic heart disease. *British Heart Journal,* **69**, 183-187.

Erfurt, J.C., Foote, A. and Heirich, M.A. (1991) The cost effectiveness of work site wellness programmes for hypertension control, weight loss and smoking cessation. *Journal of Occupational Medicine,* **33**, 962-969.

Eritsland, J., Seljeflot, I., Abdelnoor, M. and Arnsen, H. (1994) Long-term influence of omega-3 fatty acids on fibrinolysis, fibrinogen, and serum lipids. *Fibrinolysis,* **8**, 120-125.

Ernst, E., Weihmayr, T., Schmid, M., Baumann, M. and Matrai, A. (1986) Cardiovascular risk factors and hemorheology, physical fitness, stress and obesity. *Atherosclerosis,* **59**, 263-269.

Ernst, E. and Matrai, A. (1987) Normalisation of hemorrheologic abnormalities during weight reduction in obese patients. *Nutrition,* **3**, 337-339.

Ernst, E., Weihmayr, T.H. and Resch, K.L. (1989) Changes in blood rheology of grossly obese individuals during a very low calorie diet. *International Journal of Obesity,* **13**, 167-168.

Ernst, E. (1990) Plasma fibrinogen - an independent cardiovascular risk factor. *Journal of Internal Medicine,* **227**, 365-372.

Ernst, E. (1991) Fibrinogen. *British Medical Journal,* **303**, 596-597.

Ernst, E. (1993) Commentary: Does weight loss reduce plasma fibrinogen? *British Heart Journal,* **70**, 116-118.

Ernst, E. and Resch, K.L. (1993) Fibrinogen as a cardiovascular risk factor: a meta analysis and review of the literature. *Annals of Internal Medicine,* **118**, 956-963.

Ernst, E. and Resch, K.L. (1995) Therapeutic interventions to lower plasma fibrinogen concentration. *European Heart Journal,* **16**, 47-53.

Everhart, J.E. (1993) Contributions of obesity and weight loss to gallstone disease. *Annals of Internal Medicine,* **119**, 1029-1035.

Expert Panel. (1988) Report of the National Cholesterol Education Programme Experyt Panel on detection, evaluation, and treatment of high blood cholesterol in adults. *Archives of Internal Medicine*, **148**, 36-69.

Fahraeus, R. (1929) The suspension stability of blood. *Physiological Reviews,* **9**, 241-274.

Fanari, P., Somazzi, R., Nasrawi, F., Ticozzelli, P., Grugni, G., Agosti, R. and Longhini, E. (1993) Haemorheological changes in obese adolescents after short term diet. *International Journal of Obesity,* **17**, 487-494.

Favaro, A. and Santonastoso, P. (1995) Effects of parents' psychological characteristics and eating behaviour on childhood obesity and dietary compliance. *Journal of Psychosomatic Research*, **39**, 2, 145-151.

Fehily, A.M., Milbank, J.E., Yarnell, J.W.G., Hayes, T.M., Kubiki, A.J. and Eastham, R.D. (1982) Dietary determinants of lipoproteins, total cholesterol, viscosity, fibrinogen, and blood pressure. *American Journal of Clinical Nutrition,* **36**, 890-896.

Ferlito, S., Palmero, A., Mazzone, D., Condorelli, M. and Papalia, D. (1990) Fibrinogeno e antitrombina III in soggetti obesi. *Minerva Endocrinologia,* **15**, 145-148.

Finer, N. (1992) Body weight evolution during dexfenfluramine treatment after initial weight control. *International Journal of Obesity*, **16**, s25-s29.

Fischer, G. (1991) Liposculpture II. Evaluation of the patient for liposculpture. *Journal of Dermatological Surgical Oncology,* **17**, 741-743.

Fletcher, A., Alkjaersig, N., Schonfeld, G. and Witztum, J. (1981) Fibrinogen catabolism in patients with type II and type IV hyperlipidaemia - effect of dietary and clofibrate treatment on laboratory findings. *Arteriosclerosis*, **1**, 202-209.

Flynn, T.J. and Walsh, M.F. (1993) Thirty month evaluation of a popular very low calorie diet. *Archives of Family Medicine,* **2**, 1042-1047.

Folsom, A.R., Wu, K.K., Davis, C.E., Conlan, M.G., Sorlie, P.D. and Szklo, M. (1991) Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. *Atherosclerosis*, **91**, 191-205.

Folsom, A., Conlan, M., Davis, C. and Wu, K. (1992) Relations between homeostatic variables and cardiovascular risk factors in middle-aged adults. *Annals of Epidemiology,* **2**, 481-494.

Folsom, A.R., Kaye, S.A., Sellers, T.A., Hong, C.P., Cerhan, J.R., Potter, J.D. and Prineas, R.J. (1993a) Body fat distribution and 5-year risk of death in older women. *Journal of the American Medical Association,* **269**, 483-487.

Folsom, A.R., Qamhieh, H.T., Wing, R., Jeffery, R.W., Stinson, V.L., Kuller, L.H. and Wu, K.K. (1993b) Impact of weight loss in plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. *Arteriosclerosis and Thrombosis,* **13**, 162-169.

Folsom, A.R. (1995) Epidemiology of fibrinogen. *European Heart Journal*, **16** (suppl A), 21-24.

Foreyt, J.B., Brummer, R.L., St Jeor, S.T., and Goodrick, G.K. Psychological correlates of weight fluctuation. *International Journal of Eating Disorders,* **17**, 263-275, 1995.

Foster, G.D., Wadden, T.A. and Peterson, F.J. (1992) A controlled comparison of three very low calorie diets: effects on weight, body composition and symptoms. *American Journal of Clinical Nutrition,* **55**, 811-817.

Fowkes, F.G.R., Housley, E., Cawood, E.H.H., Macintyre, C.C.A., Ruckley, C.V. and Prescott, R.J. (1991) Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *International Journal of Epidemiology,* **20**, 384-392.

Fowkes, F.G.R., Pell, J.P. and Donnan, P.T. (1994) Sex differences in susceptibility to aetiological factors for peripheral atherosclerosis: importance of blood viscosity and plasma fibrinogen. *Arterioscleriosis and Thrombosis,* **14**, 862-865.

Fowkes, F.G.R., Dunbar, J.T. and Lee, A.J. (1995) Risk factor profile of non smokers with peripheral artery disease. *Angiology* **46**, 657-662.

Francis, R.B., Kawanishi, D., Barnich, T., Mahrer, P., Rahimboola, S. and Feinstein, D.I. (1988) Impaired fibrinolysis in coronary artery disease. *American Heart Journal,* **115**, 756-780.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry,* **18**, 499-502.

Friedman, C.I. and Kimm, M.H. (1985) Obesity and its effect on reproductive function. *Clinical Obstetrics and Gynaecology,* **28**, 645-663.

Frost, G., Masters, K., King, C., Kelly, M., Hasan, U., Heavens, P., White, R. and Stanford, J. (1991) A new method of energy prescription to improve weight loss. *Journal of Human Nutrition and Dietetics,* **4**, 369-374.

Fuller, J.H., McCartney, P., Jarrett, R.J., Keen, H., Rose, G., Shipley, M.J., and Hamilton, P.J. (1979) Hyperglycaemia and coronary heart disease: the Whitehall study. *Journal of Chronic Diseases,* 32, 721-728.

Fulton, R.M. and Duckett, K. (1976) Plasma fibrinogen and thromboemboli after myocardial infarction. *Lancet,* **ii**, 1160-1164.

Gaffney, P. and Wong, M.Y. (1992) Collaborative study of proposed international standard for plasma fibrinogen measurements. *Thrombosis and Haemostasis,* **68**, 428-432.

Garb, J.R. and Stunkard, A.J. (1974) Effectiveness of a self help group in obesity control: a further assessment. *Archives of Internal Medicine,* **134**, 716-720.

Garfinkel, L. (1985) Overweight and cancer. *Annals of Internal Medicine,* **103**, 1034-1036.

Garnett, E.S., Barnard, D.L., Ford, J., Goodbody, R.A. and Woodhouse, M.A. (1969) Gross fragmentation of cardiac myofibrils after therapeutic starvation for obesity. *Lancet,* **i**, 914-916.

Garrison, R.J. and Castelli, W.P. (1985) Weight and 30 year mortality of men in the Framingham study. *Annals of Internal Medicine,* **103**, 1006-1009.

Garrow, J.S. (1981a) Treat obesity seriously: a clinical manual. Edinburgh: Churchill Livingstone.

Garrow, J.S. and Gardiner, G.T. (1981b) Maintenance of weight loss in obese patients after jaw wiring. *British Medical Journal,* **282**, 858-860.

Garrow, J.S. and Webster, J.D. (1986) Long-term results of severe obesity with jaw wiring and a waist cord. *Proceedings of the Nutrition Society,* **45**, 119A.

Garrow, J.S. (1988) Obesity and related diseases. Churchill Livingstone, London.

Garrow, J.S. and Webster, J.D. (1989) Effects of weight and metabolic rate of obese women on a 3.4 mJ (800 kcal) diet. *Lancet,* **1,** 1429-1431.

Garrow, J.S. (1991) The safety of dieting. *Proceedings of the Nutrition Society,* **50,** 493-499.

Garrow, J.S. and Summerbell, C. (1995) Meta-analysis: effect of exercise, with and without dieting on the body composition of overweight subjects. *European Journal of Clinical Nutrition,* **49,** 1-10.

Gelmini, G., Coiro, V., Manotti, C., Delsignore, R. and Passeri, M. (1989) Effect of diet and weight loss on whole blood filterability and plasma fibrinogen values in hypertensive obese postmenopausal women. *Acta Diabetologia Latina,* **26,** 329-335.

Glueck, C.J., Taylor, H.L., Jacobs, D., Morrison, J.A., Beaglehole, R., Williams, O.D. (1980) Plasma high density lipoprotein cholesterol: associations with measurements of body mass. *Circulation,* **IV,** 62-69.

Golay, A., Allaz, A.F., Morel, Y., de Tonnac, N., Tankova, S. and Reaven, G. (1996) Similar weight loss on high or low carbohydrate diets. *American Journal of Clinical Nutrition,* **63,** 174-178.

Goldstein, D.J. (1992) Beneficial health effects of modest weight loss. *International Journal of Obesity,* **16,** 397-415.

Goldstein, D.J., Rampey, A.H., Dornseif, B.E., levine, L.R., Potvin, J.H., Fludzinski, L.A. (1993) Fluoxetine: a randomised clinical trial in the maintenance of weight loss. *Obesity Research,* **1,** 92-98.

Graham, L.E., Taylor, C.B. and Hovel, M.R. (1983) A five year follow up of a behavioural weight loss program. *Journal of Consulting and Clinical Psychology,* **51,** 322-323.

Gram, J., Declerck, P.J., Sidelmann, J., Jespersen, J. and Kluft, C. (1993) Multicentre evaluation of commercial kit methods: plasminogen activator inhibitor activity. *Thrombosis and Haemostasis,* **70**, 852-857.

Gray, A., Felman, H.A., McKinlay, J.B. and Longscope, C. (1991) Age, disease, and changing sex hormone levels in middle-ages men: results of the Massachusetts male aging study. *Journal of Clinical Endocrinology and Metabolism,* **73**, 1016-1025.

Gregory, J., Foster, K., Tyler, H. and Wiseman, M. (1990) The dietary and nutritional survey of British adults, HMSO,London.

Grilo C.M. (1994) Physical activity and obesity. *Biomedcine and Pharmacotherapy,* **48**, 127-136.

Gris, J.C., Schved, J.F., Feugas, O., Aquilar-Martinez, P., Arnaud, A., Sanchez, N. and Sarlat, C. (1990) Impact of smoking, physical training and weight reduction on FVII, PAI-1 and haemostatic markers in sedentary men. *Thrombosis and Haemostasis,* **64**, 516-520.

Grodstein, F., Levine, R., Troy, L., Spencer, T., Colditz, G.A., Stampfer, M.J. (1996) Three year follow up of participants in a commercial weight loss program. *Archives of Internal Medicine,* **156**, 1302-1306.

Guy-Grand, B., Crepaldi, G., Vefebvre, P., Apfelbaum, M., Gries, A., Turner, P. (1989) International trial of long term dexfenfluramine in obesity. *Lancet*, **1**, 1142-1145.

Gwinup, G. (1975) The effect of exercise alone on the weight of obese women. *Archives of Internal Medicine,* **135**, 676-680.

Hadden, D.R., Montgomery, D.A.D., Skelly, R.J., Trimble, E.R., Weaver, J.A., Wilson, E.A. and Buchanan, K.D. (1975) Maturity onset diabetes mellitus - response to intensive dietary management. *British Medical Journal,* **ii**, 276-278.

Hadvary, P., Lengsfeld, H., Wolfer, H. (1988) Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipostatin. *Biochemical Journal*, **256**, 357-361.

Haffner, S.M., Applebaum, Bowden, D., Wahl, P.W. (1985) Epidemiological correlates of high density lipoprotein subfractions, apolipoproteins A-I, A-II, and D and lecithin acyltransferase. *Arteriosclerosis*, **5**, 169-177.

Haffner, S.M., Stern, M.P., Hazuda, H.P., Mitchell, B.D., Patterson, J.K. (1990) Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes. *Journal of the American Medical Association*, **263**, 2893-2898.

Haffner, S.M., Valdez, R.A., Mykkanen, L., Stern, M.P. and Katz, M.S. (1994) Decreased testosterone and dehydroepiandosterone sulphate concentrations are associated with increased insulin and glucose concentrations in non diabetic men. *Metabolism*, **43**, 599-603.

Hakala, P., Karvatti, R.L., Ronnemaa, T. (1993) Group versus individual weight reduction in the treatment of severe obesity - a five year follow - up study. *International Journal of Obesity*, **17**, 97-102.

Hallberg, L. and Svanborg, A. (1967) Cholesterol, phospholipids and triglycerides in plasma of 50 year old women. Influence of the menopause, body weight, skinfold thickness, weight-gain and diet in a random population sample. *Acta Medica Scandinavia*, **181**, 185-194.

Halverston, J.D. and Printen, K.J. (1985) Perspective: gastric resection for morbid obesity. *Surgery*, **100**, 126-128.

Hamm, P., Shekelle, R.B. and Stamler, J. (1989) Large fluctuations in body weight during young adulthood and the 25 year risk of coronary disease in men. *American Journal of Epidemiology*, **129**, 312-318.

Hamsten, A., Blomback, M., Wiman, B., Svensson, J., Szamosis, A., de Faire, U. and Mettinger, L. (1986) Haemostatic function in myocardial infarction. *British Heart Journal*, **55**, 58-66.

Hamsten, A., Iselius, L., de Faire, U. and Blomback, M. (1987) Genetic and cultural inheritance of plasma fibrinogen concentration. *Lancet,* **2**, 988-991.

Han, T.S., van Leer, E.M., Seidell, J.C., Lean, M.E.J. (1996) Waist circumference as a screening tool for cardiovascular risk factors: evaluation of receiver operating characteristics. *Obesity Research*, **4**, 533-547.

Han,T.S, van Leer, E.M., Seidell, J.C., Lean, M.E.J. (1995) Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. *British Medical Journal*, **311**, 1041-1045.

Han, T.S., Richmond, P.R., Avenell, A., Lean, M.E.J. (1997) Waist circumference reduction and cardiovascular benefits during weight loss in women. *International Journal of Obesity*, **21**, 127-134.

Hankey, C.R. and Lean, M.E.J. (1996) Estimation of energy requirements and reported dietary intakes in overweight subjects with ischaemic heart disease. *International Journal of Obesity,* **20**, 29 (Abstract).

Hankey, C.R., Rumley, A., Lowe, G.D.O., Woodward, M. and Lean, M.E.J. (1997a) Moderate weight loss improves red cell aggregation and factor VII activity in overweight subjects. *International Journal of Obesity,* **21**, 644-650.

Hankey, C.R., Wallace, A.M. and Lean, M.E.J. (1997b) Plasma lipids, dehydroepiandosterone sulphate and insulin concentrations in elderly overweight angina patients, and effect of weight loss. *International Journal of Obesity* **21**, 72-77.

Harding, P.E. (1980) Jaw wiring for obesity. *Lancet,* **1**, 534-535.

Harkness, J. (1981) Measurement of plasma viscosity. In: Lowe, G.D.O., Barbenel, J.C. and Forbes, C.D. (Eds.) Clinical aspects of blood viscosity and cell deformability, pp. 79-87, Berlin: Springer-Verlag.

Harkness, J. (1971) The viscosity of human blood plasma: its measurement in health and disease. *Biorheology,* **8**, 171-193.

Hartz, A.J., Barboriak, P.N., Wong, A. (1979) The association of obesity with infertility and related menstrual abnormalities in women. *International Journal of Obesity*, **3**, 57-61.

Hartz, A.J., Gottleib, M.S., Kuhn, E.M., and Rimm, A.A. (1993) The relationship between adjusted hospital mortality and the result of peer review. *Health Services Research*, **27**, 765-772.

Hayes, K.C., Pronczuk, A., Lindsay, S. and DiersenSchade, D. (1991) Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in non human primates. *American Journal of Clinical Nutrition*, **53**, 491-498.

Haynes, R.B., Harper, A.C., Costley, S.R., Johnston, S.R., Logan, A.G., Flanagan, P.T., Sackett, D.L. (1984) Failure of weight reduciton in to reduce mildly elevated blood pressure: a randomised trial. *Journal of Hypertension*, **2**, 535-539.

Health Education Board for Scotland (1996) Scotland's Health at Work. Edinburgh, HMSO.

Hegsted, D.M., McGandy, R.B. and Myers, M.L. (1965) Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Clinical Nutrition*, **17**, 281-295.

Heinrich, J., Wahrburg, U., Martin, H. and Assman, G. (1990) The effect of diets, rich in mono or polyunsaturated fatty acids, on lipid metabolism and haemostasis. *Fibrinolysis*, **4**, 76-78.

Heinrich, J., Balleisen, L., Schulte, H., Assman, G. and van de loo, J. (1994) Fibrinogen and factor VII activity in the prediction of coronary risk. *Arterioslerosis and Thrombosis*, **14**, 54-59.

Heitmann, B.L. and Lissner, L. (1995) Dietary underreporting by obese individuals - is it specific or non-specific? *British Medical Journal*, **311**, 986-989.

Heitzmann, C.A., Kaplan, R.M., Wilson, D.K., Sandler, J. (1987) Sex differences in weight loss among adults with type II diabetes mellitus. *Journal of Behavioural Medicine*, **10**, 197-211.

Heller, S.R., Clarke, P., Daly, H., Davis, H., McCulloch, D.K., Allison, S.P. and Tattersall, R.B. (1988) Group education for obese patients with type 2 diabetes: greater success at less cost. *Diabetic Medicine,* **5**, 552-556.

Hellsten, G., Boman, K., Saarem, K., Hallmans, G. and Nilsson, T.K. (1993) Effects on fibrinolytic activity of corn oil and a fish oil preparation enriched with omega-3-polyunsaturated fatty acids in a long term study. *Current Medical Research Opinion,* **13**, 133-139.

Hendrikx, A., Heyns, W. and De Moor, P. (1968) Influence of a low calorie diet and fasting on the metabolism of dehydroepiandosterone sulfate in adult obese subjects. *Journal of Clinical Endocrinology,* **28**, 1525-1533.

Hennrikus, J., Jeffery, R.W. (1996) Worksite intervention for weight control: a review of the literature. *American Journal of Health Promotion*, **10**, 6, 471-498.

Henry, R.R. and Gumbinier, B. (1991) Benefits and limitations of very low calorie diet therapy in obese NIDDM. *Diabetes Care,* **14**, 802-823.

Henry, R.R., Wallace, P. and Olefsky, J.M. (1985) Effects of weight loss on mechanisms of hyperglycaemia in obese non insulin dependent diabetes mellitus. *Diabetes,* **35**, 990-998.

Henry, R.R., Wallace, P. and Olefsky, J.M. (1986) Effects of weight loss on mechanisms of hyperglycaemia in obese non insulin dependent diabetes mellitus. *Diabetes,* **35**, 990-998.

Herbert, J. (1995) The age of dehydroepiandosterone. *Lancet,* **345**, 1193-1194.

Herranz, L., Mega, A., Gonzalez-Gancedo, P. and Pallardo, F. (1995) Dehydroepiandosterone sulphate, body fat distribution and insulin in obese men. *International Journal of Obesity,* **19,** 57-60.

Higgins, M., Kannel, W., Garrison, R., Pinsky, J. and Stokes, J., (1988) Hazards of obesity: the Framingham experience. *Acta Medica Scandinvia,* Suppl., **723,** 23-36.

Higgins, M., D'Agostino, R., Kannel, W. and Cobb, J. (1993) Benefits and adverse effects of weight loss: observations from the Framigham study. *Archives of Internal Medicine,* **119,** 758-763.

Hill, P.B. and Wynder, E.L. (1979) Effect of a vegetarian diet and dexamethosone on plasma prolactin, testosterone and dehydroepiandosterone in men and women. *Cancer Letters,* **7,** 273-282.

Hill, J.O., Drougas, H.J., Peters, J.C. (1994) Physical activity, fitness and moderate obesity. In: Bouchard C, Shephard RJ, Stephens T, eds. Physical activity, fitness and health: international proceedings and consensus statement 1992. Champaign: Human Kinetics Publishers, 684-691.

Hirvonen, T., Mannisto, S., Roos, E. and Pietinen, P. (1997) Increasing prevalence of dietary underreporting does not necessarily distort dietary surveys. *European Journal of Clinical Nutrition,* **51,** 297-301.

Hjermann, I., Velve, Byre, K., Holme, J., Leren, P. (1981) Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo Study Group of a randomised trial in healthy men. *Lancet,* **2,** 1303-1310.

Hoakson, J.E. and Austin, M.A. (1995) Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population based studies. *Journal of Cardiovascular Risk,* **3,** 213-219.

Holbrook, T.L., Barrett-Connor, E. and Wingard, D.L. (1989) The association of lifetime weight and weight control patterns with diabetes among men and women in the adult community. *International Journal of Obesity,* **13**, 723-729.

Holden, J.H., Darga, L.L., Olson, S.M., Stettner, D.C., Ardito, E.A., Lucas, C.P. (1992) Long term follow up of patients attending a combination very low calorie diet and behaviour therapy weight loss programme. *International Journal of Obesity,* **16**: 605-613.

Hoppe, R. and Ogden, J. (1997) Practice nurses' beliefs about obesity and weight related interventions in primary care. *International Journal of Obesity,* **21**, 141-146.

Horm, J. and Anderson, K. (1993) Who in America is trying to lose weight? *Annals of Internal Medicine*, **119**, 667-671.

Hostmark, A.T., Bjerkedal, T., Kieruff, P., Flaten, H. and Ulshagen, K. (1988) Fish oil and plasma fibrinogen. *British Medical Journal,* **297**, 180-181.

Houmard, J.A., McCulley, C., Roy, L.K., Bruner, R.K., McCammon, M.R. and Israel, R.G. (1994) Effects of exercise training on absolute and relative measurements of regional adiposity. *International Journal of Obesity,* **18**, 243-248.

Hoylaerts, M., Rijken, D.C., Lijnen, H.R. and Collen, D. (1982) Kinetics of the activation of plasminogen by human tissue plasminogen activator. The role of fibrin. *Journal of Biological Chemistry,* **257**, 2912-2919.

Hubert, H.B., Bloch, D.A., and Fries, J.F. (1993) Risk factors for physical disability in an aging cohort: The NHANES epidemiological followup study. *Journal of Rheumatology* **20**, 480-488.

Hughes, T.A., Gwynne, J.T., Switzer, B.R., Herbst, C. and White, G. (1984) Effect of caloric restriction and weight loss on glycaemic control, insulin release and resistance, and atherosclerotic risk in obese patients with type II diabetes mellitus. *American Journal of Medicine,* **77**, 7-17.

Humphries, S.E., Lane, A., Green, F., Cooper, J. and Miller, G.J. (1994) Factor VII coagulant activity and antigen levels in healthy men are determined by interaction between factor VII genotype and plasma triglyceride concentration. *Arteriosclerosis and Thrombosis,* **14**, 193-198.

Hymens, F.N., Sempos, E. and Saltsman (1993) Evidence for the success of calorie restriction in weight loss and control. *Annals of Internal Medicine,* **119**, 681-687.

International Committee for Standardisation in Haematology (1984) Recommendation for a selected method for the measurement of plasma viscosity. *Journal of Clinical Pathology,* **37**, 1147-1152.

International Committee for Standardisation in haematology and erythrocyte deformability (1986) Guidelines for measurement of blood viscosity and erythrocyte deformability. *Clinical Hemorheology,* **6**, 439-453.

Intersalt Cooperative Research Group (1988) Intersalt; an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *British Medical Journal,* **297**, 319-328.

Iyriboz, Y., Hearon, C.M., Edwards, K. (1994) Agreement between large and small cuffs in sphygmomanometry: a quantitative assessment. *Journal of Clinical Monitoring,* **10**, 127-133.

Jackson, I.M., Mowat, J.I. (1970) Hypothalamic-pituatory adrenal function in obesity and the cortisol secretion rate following prolonged starvation. *Acta Endocrinologia,* **63**, 415-422.

Jakubowicz, D.J., Beer, N.A., Beer, R.M. and Nestler, J.E. (1995) Disparate effects of weight reduction by diet on serum dehydroepiandosterone-sulfate levels in obese men. *Journal of Clinical Endocrinology and Metabolism,* **80**, 3373-3376.

James, W.P.T., Mascie Taylor, G.C., Norgan, N.G., Bistrian, B.R., Shetty, P.S. and Ferro Luzzi, A. (1994) The value of arm circumference measurements in assessing chronic

energy deficiency in Third World adults. *European Journal of Clinical Nutrition,* **48**, 883-894.

James, W.P.T., Ralph, A. and McNeill, G. (1990) Metabolism and nutritional adaptation to altered intakes of energy substrates. *American Journal of Clinical Nutrition,* **51**, 264-269.

Jansson, J.H., Nilsson, T.K. and Olofsson, B.O. (1991) Tissue plasminogen activator and other risk factors as predictors of cardiovascular events in patients with severe angina pectoris. *European Heart Journal,* **12**, 157-161.

Jansson, J.H., Olofsson, B.O., Torbjorn, K. and Nilsson, K. (1993) Predictive value of tissue plasminogen activator mass concentration on long term mortality in patients with coronary artery disease. *Circulation,* **88**, 2030-2034.

Jeffery, R.W., Gerber, W.M., Rosenthal, B.S. and Lindquist, R.A. (1983) Monetary contracts in weight control: effectiveness of group and individual contracts of varying size. *Journal of Consulting and Clinical Psychology,* **51**, 242-248.

JNC (1988) The report of the joint national committee on detection, evaluation and treatment of high blood pressure. *Archives of Internal Medicine,* **148**, 1023-1038.

Juhan-Vague, I., Moerman, B., De Cock, F., Aillaud, M.F. and Collen, D. (1984) Plasma levels of the specific inhibitor of tissue type plasminogen activator and urokinase in normal and pathological conditions. *Thrombosis Research,* **33**, 523-530.

Juhan Vague, I., Vague, P.H. and Alessi, M.C. (1987) Relationships between plasma insulin triglyceride, body mass index and plasminogen activator 1. *Diabete Metabolisme,* **13**, 331-336.

Juhan Vague, I., Alessi, M.C., Fossat, C., Valadier, J., Aillaud, M.F. and Serradimigni, A. (1988) Clinical relevance of high PAI-1 level in patients with ideopathic/recurrent deep vein thrombosis. *Fibrinolysis,* **2**, 85-87.

Juhan-Vague, I., Alessi, M.C., Joly, P., Thirion, X. and Vague, P. (1989a) Plasmiogen activator inhibitor-1 in angina pectoris. Influence of plasma insulin and acute phase response. *Arteriosclerosis* **9**, 362-367.

Juhan-Vague, I., Roul, C., Alessi, M.C., Ardissone, J.P., Heim, M. and Vague, P. (1989b) Increased plasminogen activator inhibitor in non insulin dependent diabetic patients - relationship with plasma insulin. *Thrombosis and Haemostasis* **61**, 370-373.

Juhan-Vague, J. and Vague, P. (1991) Hypofibrinolysis and insulin resistance. *Diabete Metabolisme,* **17**, 96-100.

Jung, R.T. and Chong, P. (1991) The management of obesity. *Clinical Endocrinology,* **35**, 11-20.

Kahn, H.S. and Williamson, D.F. (1991) Is race associated with weight change in the US after adjustment for income, education and marital factors? *American Journal of Clinical Nutrition,* **53**, 1566-1570.

Kalimi, M. and Regelson, W. (1990) The biologic role of dehydroepiandosterone (DHEA). Berlin, Walter de Gruyter and Co Limited.

Kanaley, J.A., Andresen Reid, M.L., Oenning, L., Kottke, B.A. and Jensen, M.D. (1993) Differential health benefits of weight loss in upper-body and lower-body obese women. *American Journal of Clinical Nutrition,* **57**, 20-26.

Kannel, W.B., Castelli, W.B. and Gordon, T. (1971) Serum cholesterol, lipoproteins and the risk of coronary heart disease. *Annals of Internal Medicine,* **74**, 1-12.

Kannel, W.B., Wolf, P.A., Castelli, W.P. and D'Agostino, R.B. (1987) Fibrinogen and risk of cardiovascular disease: the Framingham study. *Journal of the American Medical Association,* **258**, 1183-1186.

Kaplan, R.M., Wilson, D.K., Hartwell, S.L., Merino, K.L., Wallace, J.P. (1985) Prospective evaluation of HDL changes after diet and physical conditioning programs for patients with type II diabetes mellitus. *Diabetes Care*, **8**, 343-348.

Kaplan, R.M., Wilson, D.K., Hartwell, S.L., Merino, K.L., Wallace, J.P. (1985) Prospective evaluation of HDL changes after diet and physical conditioning programs for patients with type II diabetes mellitus. *Diabetes Care*, **8**, 343-348.

Kaufman, M.A. and Bhargava, A. (1990) Dietary weight reduction and seizures. *Neurology*, **40**, 1905-1906.

Kay, L.A. (1988) Essentials of haemostasis and thrombosis, Edinburgh: Churchill Livingstone.

Kelley, D.E., Wing, R., Buonocore, C., Sturis, J., Polonsky, K., Fitzsimmons, M.. (1993) Relative effects of calorie restriction and weight loss in non insulin dependant diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, **77**, 5, 1287-1293.

Kelly, F., Jones, S.P., Lee, I.K. (1995) Sibutramine: weight loss in depressed patients. *International Journal of Obesity*, **19**, 2, 397.

Kempen KPG, Saris WH, Westerterp KR. (1995) Energy balance during an 8-week energy-restricted diet with and without exercise in obese women. *American Journal of Clinical Nutrition*, **62**, 722-729.

Kent, A. and Bowyer, C. (1992) When weight gets out of control. *Doctor*, 48-49.

Keys, A., Anderson, J.T. and Grande, F. (1965) Serum cholesterol response to changes in the diet. *Metabolism*, **14**, 747-787.

Keys, A., Brozek, J., Henschel, A., Mickelson, O. and Taylor, H. (1950) The biology of human starvation. Minneapolis: Minnesota Press.

Kiddy, D.S., Hamilton-Fairley, D., Bush, A., Short, F., Anyaoku, V., Reed, M.J. and Franks, S. (1992) Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. *Clinical endocrinology*, **36**, 105-111.

King, A.C., Frey-Hewitt B., Dreon, D.M., Wood,P.D. (1989) Diet vs. exercise in weight maintenance: the effects of minimal intervention strategies on long term outcomes in men. *Archives of Internal Medicine*, **149**, 2741-2746.

King, A.C., Tribble, D.L. (1991) The role of exercise in weight regulation in nonathletes. *Sports Medicine,* **11**: 331-349.

Kluft, C., Cooper, P. and Jie, A.F.H. (1985) Evaluation of euglobulin methods for the study of blood fibrinolytic activity: results for patients with rheumatoid arthritis and in the post operative period. *Haemostasis,* **15**, 144-150.

Knottnerus, J.A., Swaen, G.M.H., Slagen, J.J.M., Volovics, A. and Durineck, J. (1988) Haematological parameters as risk factors for myocardial infarction. *Journal of Clinical Epidemiology,* **41**, 67-74.

Knowler, J.C., Bennet, P.H. and Peters, D.J. (1981) Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. *International Journal of Epidemiology,* **113**, 144-156.

Koenig, W., Ernst, E. and Matrai, A. (1987) Blood rheology associated with cardiovascular risk factors and chronic cardiovascular disease: results of an epidemiological cross-sectional study. *Proceedings of the American College of Angiology,* 986-995.

Koenig, W., Sund, M., Lowe, G.D.O., Lee, A.J., Resch, K.L., Tunstall-Pedoe, H., Keil, U. and Ernst, E. (1994) Geographical variations in plasma viscosity and relation to coronary event rates. *Lancet* **344**, 711-714.

Koivisto, V.A. and DeFronzo, R.A. (1984) Exercise in the treatment of type II diabetes. *Acta Endocrinologia,* **262**, 107-111.

Kopelman, P.G., Pilkington, T.R.E., White, N. and Jeffcoate, S.L. (1980) Abnormal sex steroid secretion and binding in massively obese women. *Clinical endocrinology,* **12**, 363-369.

Kopelman, P.G., White, N., Pilkington, T.R.E. and Jeffcoate, S.L. (1981) The effect of weight loss on sex steroid secretion and binding in massively obese women. *Clinical Endocrinology,* **14,** 113-116.

Kostis, J.B., Rosen, R.C., Brondolo, E., Taska, L., Smith, D.E. and Wilson, A.C. (1992) Superiority of non-pharmacological therapy compared to propranolol and placebo in men with mild hypertension: a randomised prospective trial. *American Heart Journal,* **123,** 466-474.

Kral, J.G. (1992) Overview of surgical techniques for treating obesity. *American Journal of Clinical Nutrition,* **55,** 552-555.

Kramer, F.M., Jeffery, R.W., Forster, J.L., Snell, M.K. (1989) Long term follow up of behavioural treatment for obesity. Patterns of weight regain among men and women. *International Journal of Obesity,* **13,** 123-136.

Krauss, R.M. (1991) Low density lipoprotein imbalances and risk of coronary artery disease. *Current Opinion in Lipidology,* **2,** 248-252.

Krebs-Smith, S.M., Heimendinger, J., Subar, A.F., Patterson, B.H. and Pivonka, E. (1995) Using food frequency questionnaires to estimate fruit and vegetable intake: association between the number of questions and total intakes. *Journal of Nutrition Education,* **27,** 80-85.

Kristal, A.R., Beresford, S.A.A. and Lazovich, D. (1994) Assessing change in diet intervention research. *American Journal of Clinical Nutrition,* **59,** 185s-189s.

Kromhout, D., Bosschieter, E.B. and Coulander, C.D. (1985) The inverse relation between fish consumption and 20 year mortality from coronary heart disease. *New England Journal of Medicine,* **312,** 1205-1209.

Kumanyika, S.K. and Charleston, J.B. (1992) Lose weight and win: a church based weight loss programme for blood pressure control among black women. *Patient Education and Counselling,* **19,** 19-32.

Kurtz, B.R., Givens, J.R., Komindr, S., Stevens, M.D., Karas, J.G., Bittle, J.B., Judge, D. and Kitabchi, A.E. (1987) Maintenance of normal circulating levels of 4-androstendione and dehydroepiandosterone in simple obesity despite increased metabolic clearance rates: evidence for a servo-control mechanism. *Journal of Clinical Endocrinology and Metabolism,* **64**, 1261-1267.

Kuyl, J.M., Slabber, M., Barnard, H.C., van Wyk, H.J. and Badenhorst, A.M. (1992) Observed changes in the plasma glucose, insulin, and C peptide response after an oral glucose load, and plasma lipid and fibrinogen levels in morbidly obese females during a prolonged calorie-restricted diet. *Clinical Biochemistry,* **25**, 336-337.

Kweider, M., Lowe, G.D.O., Murray, G.D., Kinane, D.F. and McGowan, D.A. (1993) Dental disease, fibrinogen, white cell count; links with myocardial infarction? *Scottish Medical Journal,* **38**, 73-74.

Landin, K., Stigendal, L., Eriksson, E., Krotiewski, M., Risberg, B., Tengborn, L. and Smith, U. (1990) Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism* **39**, 1044-1048.

Lane, M.A., Ingram, D.K., Ball, S.S., Roth, G.S. (1997) Dehydroepiandosterone sulfate: a biomarker of primate aging slowed by calorie restriction. *Journal of Clinical Endocrinology and Metabolism*, **82**, 7, 2093-2096.

Lasater, T.M., Sennett, L.L., Lefebvre, R.C., DeHart, K.L., Peterson, G., Carleton, R.A. (1991) Community based approaches to weight loss: The Pawtucket weight in. *Additive Behaviours*, **16**, 175-181.

Law, M.R., Frost, C.D. and Wald, N.J. (1991a) By how much does dietary salt reduction lower blood pressure? *British Medical Journal,* **302**, 811-818.

Law, M.R., Frost, C.D. and Wald, N.J. (1991b) Analysis of data from trials of salt restriction. *British Medical Journal,* **302**, 819-824.

Le Devehat, C., Khodabandehlou, T. and Dougny, M. (1992) Etudes des parametres hemorheologiques dans l'obesite isolee. *Diabetes Metabolism,* **18**, 43-47.

Lean, M.E. and James, W.P. (1986) Prescription of diabetic diets in the 1980s. *Lancet,* **1**, 723-725.

Lean, M.E., Powrie, J.K., Anderson, A.S. and Garthwaite, P.H. (1990) Obesity, weight loss and prognosis in type 2 diabetes. *Diabetic Medicine,* **7**, 228-233.

Lean, M.E.J., Han, T.S. and Morrison, C.E. (1995) Waist circumference as a measure for indicating need for weight management. *British Medical Journal,* **311**, 158-161.

Lean, M.E.J., Han, T.S., Deurenberg, P. (1996) Predicting body composition by densiometry from simple anthropometric measurements. *American Journal of Clinical Nutrition*, **63**, 4-14.

Lean, M.E.J., Han, T.S., Prvan, T., Richmond, P.R. and Avenell, A. (1997) Weight loss with high and low carbohydrate 1200 kcal diets in free living women. *European Journal of Clinical Nutrition,* **51**, 243-248.

Lean, M.E.J., Han, T.S., Seidell, J.C. (1998) Impairment of health and quality of life in people with large waist circumference. *Lancet*, **351**, 853-856.

Lee, A.J., Lowe, G.D.O., Smith, W.C.S. and Tunstall-Pedoe, H. (1993) Plasma fibrinogen in women: relationship with oral contraception, the menopause and hormone replacement therapy. *British Journal of Haematology,* **83**, 616-621.

Lee, A.J., Flanagan, P.A., Rumley, A., Fowkes, F.G.R. and Lowe, G.D.O. (1995) Relationship between alcohol intake and tissue plasminogen activator antigen and other haemostatic factors in the general population. *Fibrinolysis,* **8**, 49-54.

Lee, A.J., Smith, W.C.S., Lowe, G.D.O. and Tunstall-Pedoe, H. (1990) Plasma fibrinogen and coronary risk factors: the Scottish Heart Health study. *Journal of Clinical Epidemiology,* **43**, 913-919.

Lee, I.M. and Paffenberger, R.S. (1992) Change in body weight and longevity. *Journal of the American Medical Association,* **268**, 2045-2049.

Leenen, R., van de Kooy, K., Seidell, J.C., Deurenberg, P. and Kopperschaer, H.P.F. (1994) Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy. *Journal of Clinical Endocrinology,* **78**, 551-555.

Leighton, R.F., Repka, F.J., Birk, T.J., Lynch, D.J., Bingle, J.F., Gohara, A.F., Saffran, J., Weaver, M.T., Brester, P.M., Walsh, M.E. (1990) The Toledo exercise and diet study: results at 26 weeks. *Archives of Internal Medicine*, **150**, 1016-1020.

Lew, E.A. and Garfinkel, L. (1979) Variations in mortality by weight among 750,000 men and women. *Journal of Chronic Diseases,* **32**, 563-569.

Licata, G., Scaglione, R., Avellone, G., Ganguzza, A., Corrao, S., Arnone, S. and Di Chiara, T. (1995) Hemostatic function in young subjects with central obesity: relationship with left ventricular function. *Metabolism,* **44**, 1417-1421.

Lipid Research Clinics (1980). Lipid research clinics population studies data book. Vol. 1. The prevelance study. Bethseda MD: National Institutes of Health. DHHS Publication, SO-1527.

Lissner, L, Odell, P.M., D'Agoatino, R.B., Stokes, J., Kreger, J., Belanger, A.J., Brownell, K.D. (1991) Variability of body weight and health outcomes in the Framingham population. *New England Journal of Medicine*, **324**, 1839-1844.

Lissner, L. and Lindroos, A.K. (1994) Is dietary underreporting macronutrient specific? *European Journal of Clinical Nutrition,* **48**, 453-454.

Lissner, L., Odell, P.M., Dagostino, R.B., Stokes, J., Kreger, B.E., Belanger, A.J. and Brownell, K.D. (1991) Variability of body weight and health outcomes in the Framingham population. *New England Journal of Medicine,* **324**, 1839-1844.

Litchenstein, A.H., Ausman, L.M., Carrasco, W., Jenner, J.L., Ordovas, J.M., Schaefer, E.J. (1994) Short-term consumption of a low fat diet beneficially affects plasma lipid concentrations only when accompanied by weight loss. *Arteriosclerosclerosis and Thrombosis*, **14**, 1751-1760.

Liu, G.C., Coulston, A.M., Lardinois, C.K., Hollenbeck, C.B., Moore, J.G., Reaven, G.M. (1985) Moderate weight loss and sulponylurea treatment of non insulin dependant diabetes. *Archives of Internal Medicine*, **145**, 665-669.

Livesey, G. and Elia, M. (1988) Estimation of energy expenditure, net carbohydrate utilisation, and net fat oxidation and synthesis by indirect calorimetery: evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition,* **47**, 608-629.

Long, S.D., O'Brian, K., MacDonald, K.G., Leggett-Frazier, N., Swanson, M.C., Pories, M.S., Caro, J.F. (1994). Weight loss in severely obese patients prevents the progression of impaired glucose tolerance to type II diabetes. *Diabetes Care*, **17**, 5, 372-75.

Longscope, C., Baker, R. and Johnstone, C.C. (1986) Androgen and oestrogen metabolism: relation to obesity. *Metabolism,* **35**, 235-237.

Loube, D.I., Loube, A.A. and Mitler, M.M. (1994) Weight loss for obstructive sleep apnoea: optimal therapy for obese patients. *Journal of the American Dietetic Association,* **94**, 1291-1295.

Lovejoy, J. and Digirolamo, M. (1992) Habitual dietary intake and insulin sensitivity in lean and obese adults. *American Journal of Clinical Nutrition,* **55**, 1174-1179.

Lowe, G.D.O. (1986) Blood rheology in arterial disease. *Clinical Science,* **71**, 137-146.

Lowe, G.D.O. (1987) Blood Rheology in vitro and in vivo. *Ballieres Clinical Haematology,* **1**, 597-636.

Lowe, G.D.O. (1988) Thrombotic disorders. In: Ludlum, C.A. (Ed.) Clinical Haematology, pp. 387-396. Churchill Livingstone.

Lowe, G.D.O. (1990) Pathophysiology of critical leg ischaemia. In: Dormandy, J.A. and Stock, G. (Eds.) *Critical leg ischaemia: Its pathophysiology and management.* pp. 17-38. Berlin: Springer Verlag.

Lowe, G.D.O. (1992) Blood viscosity and cardiovascular disease. *Thrombosis and Haemostasis,*. **67**, 494-498.

Lowe, G.D.O. (1996) Personnel communication.

Lowe, G.D.O., Drummond, M., Lorimer, A.R., Hutton, I., Forbes, C.D., Prentice, C.R.M. and Barbenel, J.C. (1980) Relation between the extent of coronary artery disease and blood viscosity. *British Medical Journal,* **i**, 673-674.

Lowe, G.D.O., Drummond, M.M., Forbes, C.D. and Barbenel, J.C. (1980) The effects of age and cigarette-smoking on blood and plasma viscosity in men. *Scottish Medical Journal,* **25**, 13-17.

Lowe, G.D.O., Drummond, M.M., Third, J.L.H.C., Bremner, W.F., Forbes, C.D., Prentice, C.R.M. and Lawrie, T.D.V. (1979) Increased plasma fibrinogen and platelet aggregates in type II hyperlipoproteinaemia. *Thrombosis and Haemostasis,* **42**, 1503-1507.

Lowe, G.D.O., McArdle, B.M., Stromberg, P., Lorimer, A.R., Forbes, C.D. and Prentice, C.R.M. (1982) Increased blood viscosity and fibrinolytic inhibitor in type II hyperlipoproteinaemia. *Lancet,* **i**, 54-55.

Lowe, G.D.O., Saniabadi, A., Turner, A., Lieberman, P., Pollock, J. and Drury, J. (1986) Studies on the haematocrit in peripheral arterial disease. In: Kiesewetter, H. and Jung, F. (Eds.) Blood fluidity and endothelial influences on microcirculation, 64th ed. pp. 969-974.

Lowe, G.D.O., Smith, W.C.S., Tunstall-Pedoe, H.D., Crombie, I.K., Lennie, S.E., Anderson, J. and Barbenel, J.C. (1988) Cardiovascular risk and haemorheology: results from the Scottish Heart Health Study and the MONICA project, Glasgow. *Clinical Hemorheology,* **8**, 518-524.

Lowe, G.D.O., Wood, D.A., Douglas, J.T., Riemersma, R.A., Macintyre, C.C.A., Takase, I., Tuddenham, E.G.D., Forbes, C.D., Elton, R.A. and Oliver, M.F. (1991) Relationships of plasma viscosity, coagulation and fibrinolysis to coronary risk factors and angina. *Thrombosis and Haemostasis,* **65**, 339-343.

Lowe, G.D.O., Lee, A.J., Rumley, A., Smith, W.C.S. and Tunstall-Pedoe, H. (1992) Epidemiology of haematocrit, white cell count, red cell aggregation and fibrinogen: the Glasgow Monica study. *Clinical Hemorheology,* **12**, 535-544.

Lowe, G.D.O., Fowkes, F.G.R., Dawes, J., Donnan, P.T., Lennie, S.E. and Housley, E. (1993) Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral artery disease and normal population in the Edinburgh Artery Study. *Circulation,* **87**, 1915-1920.

Lowe, G.D.O., Lee, A.J., Rumley, A., Price, J.F. and Fowkes, F.G.R. (1997) Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. *British Journal of Haematology* **96**, 168-173.

Luxenberg, M.N. and Mausolf, F.A. (1970) Retinal circulation in the hyperviscosity syndrome. *American Journal of Opthalmology,* **70**, 588-598.

MacMahon, S.W., Macdonald, G.J., Bernstein, L., Andrews, G., Blacket, R.B. (1985) Comparison of weight reduction with metoprolol in treatment of hypertension in young overweight patients. *Lancet* **1**, 1233-1236.

MacRury, S.M. (1990) Blood rheology in diabetes mellitus and its complications: assessment of new methods. M.D. Thesis, University of Glasgow, pp.1-123.

MacRury, S.M., Lennie, S.E., McColl, P., Balandra, R., MacCuish, A.C. and Lowe, G.D.O. (1993) Increased red cell aggregation in diabetes mellitus: association with cardiovascular risk factors. *Diabetic Medicine,* **10**, 21-26.

Mann, J.I., Doll, R., Thorogood, M., Vessey, M.P. and Waters, W.E. (1976) Risk factors for myocardial infarction in young women. *British Journal of preventitive Medicine,* **30**, 94-100.

Manning, R.M., Jung, R.T., Leese, G.P. and Newton, R.W. (1995) The comparison of four weight reducing strategies aimed at overweight diabetic persons. *Diabetic Medicine,* **12**, 409-415.

Manson, J.E., Colditz, G.A., Stampfer, M.J., Willett, W.C., Rosner, B., Monson, R.R., Speizer, F.E., Hennekens, C.H. (1990) A prospective study of obesity and risk of coronary heart disease in women. *New England Journal of Medicine*, **322**, 882-889.

Manson, J.E., Willett, W.C. and Stampfer, W.C. (1995) Body weight and mortality among women. *New England Journal of Medicine,* **333**, 677-685.

Marckmann, P., Jespersen, J., Leth, T. and Sandstrom, B. (1991) Effect of fish versus meat diet on blood lipids, coagulation and fibrinolysis in healthy young men. *Journal of Internal Medicine,* **229**, 317-323.

Marckmann, P., Sandstrom, B. and Jespersen, J. (1990) Effects of total fat content and fatty acid composition in diet on factor VII coagulant activity and blood lipids. *Atherosclerosis,* **80**, 227-233.

Marckmann, P., Sandstrom, B. and Jespersen, J. (1992) Fasting blood coagulation and fibrinolysis of young adults unchanged by reduction in dietary fat content. *Arteriosclerosis and Thrombosis,* **12**, 201-205.

Margetts, B.M., Cade, J.M. and Osmond, C. (1989) Comparison of a food frequency questionnaire with a diet record. *International Journal of Epidemiology,* **18**, 868-873.

Mathus-Vliegan, L.M.H., Res, A.M. (1993) Dexfenfluramine influences dietary compliance and eating behaviour, but dietary instruction may overrule its effect on food selection in obese subjects. *Journal of the American Dietetic Association*, **93**, 10, 1163-1164.

McFarland, R.J., Grundy, A., Gazet, J.C., Pilkington, T.R.E. (1987) The intragastric balloon: a novel idea proved ineffective. *British Journal of Surgery*, **74**, 137-139.

McLean, J.A. and Tobin, G. (1990) Animal and human calorimetry, 2nd ed. Cambridge: Cambridge University Press.

Meade, T.W., North, W.R.S., Chakrabarti, R., Haines, A.P. and Stirling, Y. (1977) Population-based distributions of haemostatic variables. *British Medical Bulletin,* **33**, 283-288.

Meade, T.W., Chakrabarti, R., Haines, A.P., North, W.R.S. and Stirling, Y. (1979) Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. *British Medical Journal,* **1**, 153-156.

Meade, T.W., Chakrabarti, R., Haines, A.P., North, W.R.S., Stirling, Y. and Thompson, S.G. (1980) Haemostatic function and cardiovascular death: early results of a prospective study. *Lancet,* **ii**, 1050-1054.

Meade, T.W. (1981) Epidemiology of atheroma and thrombosis. In: Bloom, A.L. and Thomas, D.P. (Eds.) *Haemostasis and Thrombosis*, Edinburgh: Churchill Livingstone.

Meade, T.M., Brozovic, M., Haines, A.P., Imeson, J.D., Mellows, S., Miller, G.J., North, W.R.S., Stirling, Y. and Thompson, S.G. (1986) Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart study. *Lancet,* **i**, 533-537.

Meade, T.W., Imeson, J. and Stirling, Y. (1987) Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. *Lancet,* 986-989.

Meade, T.W. (1987) Hypercoagulability and ischaemic heart disease. *Blood Reviews,* **1**, 2-8.

Meade, T.W. (1992) Thrombosis and cardiovascular disease. *Annals of Epidemiology,* **2,** 353-364.

Meade, T.W., Ruddock, V., Stirling, Y., Chakrabarti, R. and Miller, G.J. (1993) Fibrinolytic activity, clotting factors, and long term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet,* **342,** 1076-1079.

Mehrabian, M., Peter, J.B., Barnard, R.J. and Lusis, A.J. (1990) Dietary regulation of fibrinolytic factors. *Atherosclerosis* **84,** 25-32.

Merilainen, P.T. (1987) Metabolic monitor. *International Journal of Clinical Monitoring and Computing,* **4,** 167-177.

Mideon, C.J., Green, O.C., Eckert, J.P. (1963) Study of adrenocortical function in obesity. *Metabolism,* **12,** 718-722.

Miller, G.J., Martin, J.C., Webster, J., Wilkes, H., Miller, N.E., Wilkinson, W.H. and Meade, T.W. (1986) Association between dietary fat intake and plasma factor VII coagulant activity - a predictor of cardiovascular mortality. *Atherosclerosis,* **60,** 269-277.

Miller, G.J., Cruickshank, J.K., Ellis, L.J., Thompson, R.L., Wilkes, H.C., Stirling, Y., Mitropoulos, K.A., Allison, J.V., Fox, T.E. and Walker, A.O. (1989) Fat consumption and factor VII coagulant activity in middle-aged men. *Atherosclerosis,* **78,** 19-24.

Miller, G.J., Martin, J.C., Mitropoulos, K.A., Reeves, B.E.A., Thompson, R.L., Meade, T.W., Cooper, J.A. and Cruickshank, J.K. (1991) Plasma factor VII is activated by postprandial triglyceridaemia irrespective of dietary fat composition. *Atherosclerosis,* **86,** 163-171.

Miller, G.J., Stirling, Y., Esnouf, M.P., Heinrich, J., van de loo, J., Kienast, J., Wu, K.K., Morrissey, J.H., Meade, T.W., Martin, J.C., Imeson, J.D., Cooper, J.A. and Finch, A. (1994) Factor VII-deficient substrate plasmas depleted of protein C raise the sensitivity of the factor VII bioassays to activated factor VII: an international study. *Thrombosis and Haemostasis,* **71,** 38-48.

Ministry of Agriculture, F.A. (1993) Food portion sizes, 2nd edn. London, HMSO.

Mitchell, L.E., Sprecher, D.L., Borecki, L.B., Lasarzewski, P.M. and Rao, D.C. (1994) Evidence for an association between dehydroepiandosterone sulphate and nonfatal premature myocardial infarction in males. *Circulation,* **89**, 89-93.

Mitropoulos, K.A., Miller, G.A., Reeves, B.E.A., Wilkes, H.C. and Cruickshank, J.K. (1989) Factor VII coagulant activity is strongly associated with the plasma concentration of large lipoprotein particles. *Atherosclerosis,* **76**, 203-208.

Mitropoulos, K.A., Miller, G.J., Martin, J.C., Reeves, B.E.A. and Cooper, J. (1994) Dietary fat induces changes in factor VII coagulant activity through effects in plasma free stearic acid concentration. *Arteriosclerosis and Thrombosis,* **14**, 214-222.

Mitropolous, K.A., Armitage, J.M., Collins, R., Meade, T.W., Reeves, B.E.A., Wallendszuz, K.R., Wilson, S.S., Lawson, A. and Peto, R. (1997) Randomised placebo-controlled study of the effects of simvastatin on haemostatic variables, lipoproteins and free fatty acids. *European Heart Journal,* **18**, 235-241.

Morales, A.J., Nolan, J.J., Nelson, J.C. and Yen, S.S.C. (1994) Effects of replacement dose of dehydroepiandosterone in men and women of advancing age. *Journal of Clinical Endocrinology and Metabolism,* **78**, 1360-1367.

Morris, J.N., Clayton, D.G., Everitt, M.G., Semmence, A.M. and Burgess, E.H. (1990) Exercise in leisure time: coronary attack and death rates. *British Heart Journal,* **63**, 325-334.

Mortola, J.F. and Yen, S.S.C. (1990) The effects of oral dehydroepiandsterone on endocrine-metabolic parameters in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism,* **71**, 696-704.

Muller, A.D., Houwelingen, A.C., van Dam-Mieras, M.C.E., Bas, B.M. and Hornstra, G. (1989) Effect of a moderate fish intake on haemostatic parameters in healthy males. *Thrombosis and Haemostasis,* **58**, 468-475.

Munro, J.F., McCuish, A.C., Wilson, E.M., Duncan, L.J.P. (1968) Comparison of continuous and intermittent anorectic therapy in obesity. *British Medical Journal*, **1**, 352-354.

Murgatroyd, P.R., Shetty, P.S. and Prentice, A.M. (1993) Techniques for the measurement of human energy expenditure: a practical guide. *International Journal of Obesity*, **17**, 549-568.

Must, A., Jacques, P.F., Dallal, G.E., Bajema, C.J. and Dietz, W.H. (1992) Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935. *New England Journal of Medicine*, **327**, 1350-1355.

National Institutes for Health (1992) Gastrointestinal surgery for severe obesity; National Institutes for Health consensus statement. *American Journal of Clinical Nutrition*, **55**, 615-619.

National Task Force on the Prevention and Treatment of Obesity (1993) Very low calorie diets. *Journal of the American Medical Association*, **270**, 967-974.

National Task Force on the Prevention and Treatment of Obesity (1994) Weight cycling. *Journal of the American Medical Association*, **272**, 1196-1202.

Neil, H.A.W., Roe, L., Godlee, R.J.P., Moore, J.W., Clark, G.M.G., Brown, J., Thorogood, M., Stratton, I.M., Lancaster, T., Mant, D., Fowler, G.H. (1995) Randomised trial of lipid lowering dietary advice in general practice: the effects on serum lipids, lipoproteins, and antioxidants. *British Medical Journal*, **310**, 569-573.

Nestler, J.E., Barlascini, C.O., Clore, J.N. and Blackard, W.G. (1988) Dehydroepiandsterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism*, **66**, 57-61.

Nestler, J.E., Usiskin, K.S., Banacini, C.O., Welty, D.F., Clore, J.N. and Blackard, W.G. (1989) Suppression of serum dehydroepiandosterone sulphate by insulin: an evaluation of possible mechanisms. *Journal of Clinical Endocrinology and Metabolism,* **69**, 1040-1046.

Nestler, J.E., Clore, J.N. and Blackard, W.G. (1991) Metabolism and actions of dehydroepiandosterone in humans. *Journal of Steroid Biochemistry,* **40**, 599-605.

Nestler, J.E., Clore, J.N. and Blackard, W.G. (1992) Dehydroepiandosterone: the missing link between hyperinsulinaemia and atherosclerosis. *FASEB Journal,* **6**, 3073-3075.

Nestler, J.E. (1993) Sex hormone-binding globulin: a marker for hyperinsulinaemia and/or insulin resistance? *Journal of Clinical Endocrinology and Metabolism,* **76**, 273-274.

Nilsson, I.M., Ljungner, H. and Tengborn, L. (1985) Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *British Medical Journal,* **290**, 1453-1456.

Nilsson, T.K., Sundell, I.B., Hellsten, G. and Hallmans, G. (1990) Reduced plasminogen activator inhibitor in high consumers of fruit and vegetables and root vegetables. *Journal of Internal Medicine,* **227**, 267-271.

Norell, S.E., Ahlbom, A., Feychting, M. and Pedersen, N.L. (1986) Fish consumption and mortality from coronary heart disease. *British Medical Journal* **293**, 496

Nutrition Sub-committee of the British Diabetic Association Professional Advisory Committee. (1992) Dietary recommendations for people with diabetes, an update for the 1990's. *Diabetic Medicine,* **9**, 189-202.

O'Kane, M., Wiles, P.G., Wales, J.K. (1993) Fluoxetine in the treatment of obese type ii diabetic patients. *Diabetic Medicine*, **11**, 105-110.

O'Dea, K., Tralanides, K. and Hopper, J.L. (1988) Impaired glucose tolerance: hyperinsulinaemia and hypertriglyceridaemia in Australian Aborigines from the dessert. *Diabetes Care*, **11**, 23-29.

Oberman, A., Wasserthheil-Smoller, S., and Langford, H.G. (1990) Pharmocologic and nutritional treatment of mild hypertension: changes in cardiovascular risk status. *Annals of Internal Medicine,* **112**, 879-883.

Ogston, C.M. and Ogston, D. (1966) Plasma fibrinogen and plasminogen levels in health and in ischaemic heart disease. *Journal of Clinical Pathology,* **19**, 352-356.

Ogston, D. (1985) Nutritional influences on the fibrinolytic system. *Proceedings of the Nutrition Society,* **44**, 379-384.

Olesfsky, J.M. and Kolterman, O.G. (1981) Mechanisms of insulin resistance in obesity and non insulin dependent diabetes. *American Journal of Medicine,* **70**, 151-168.

Orentreich, N., Brind, J.L., Rizer, R.L. and Vogelman, J.H. (1984) Age changes and sex differences in serum dehydroepiandosterone sulphate concentrations throughout adulthood. *Journal of Clinical Endocrinology,* **59**, 551-555.

Ornish, D., Brown, S.E., Scherwitz, B., Billings, J.H., Armstrong, W.T., Ports, T.A., McLanahan, S.M., Kirkeide, R.L., Brand, R.J. and Gould, K.L. (1990) Can lifestyle changes reverse coronary heart disease? *Lancet,* **336**, 129-133.

Osterman, J., Lin, T., Howard, R., Brown, K.A. and horning, C.A. (1992) Serum cholesterol levels during treatment of obese outpatients with a very low calorie diet. Effect of initial cholesterol levels. *International Journal of Obesity,* **16**, 49-58.

Ostermann, H. and van de loo, J. (1986) Factors of the haemostatic system of diabetic patients: a survey of controlled studies. *Haemostasis,* **16**, 386-416.

Oudenhoven, L.F.I.J., Brommer, E.P.J., Aronson, D.C., Raben, A.M.T.J., Frolich, M. and van Bockel, J.H. (1994) Impaired fibrinolysis in young adults with arterial occlusive disease: the relationship with hyperinsulinaemia and smoking. *Fibrinolysis,* **8**, 263-269.

Pacy, P.J., Webster, J.D., Pearson, M., garrow, J.S. (1987) A cross-sectional cost/benefit audit in hospital obesity clinic. *Human Nutrition - Applied Nutrition*, **41**, 1, 38-46.

Pahlavani, M.A., Haley-Zitlin, V., Richardson, A. (1994) Modulation of the aging processes by dietary restriction. Boca Raton, Florida, CRC Press, 143-156.

Palareti, G., Legnani, C., Poggi, M., Parenti, M., Babini, A.C., Biagi, R., Baraldi, L., Luchi, A., Capelli, M. and Coccheri, S. (1994) Prolonged very low calorie diet in obese subjects reduced factor VII and PAI but not fibrinogen levels. *Fibrinolysis*, **8**, 16-21.

Pamuk, E.R., Williamson, D.F., Madans, J., Serdula, M.K., Klienman, J.C. and Byers, T. (1992) Weight loss and mortality in a national cohort of adults. *American Journal of Epidemiology*, **136**, 686-697.

Parenti, M., Palareti, G., Babini, A.C., Poggi, M., Torricelli, P. and Melchionda, N. (1988) Effects of slimming on metabolic and haemorheologic patterns in a group of obese subjects. *International Journal of Obesity*, **12**, 179-184.

Pavlou K.N., Whatley, J.E., Jannace, P.W. (1989a) Physical activity as a supplement to a weight loss dietary regimen. *American Journal of Clinical Nutrition*, **49**, 1110-1114.

Pavlou, K.N., Krey S., Steffee, W.P. (1989b) Exercise as an adjunct to weight loss and maintenance in moderately obese subjects. *American Journal of Clinical Nutrition*, **49**, 1115-1123.

Pearson, G.C., De Looy, A.E. and Webster, J. (1991) Analysis of the dietetic treatment of obesity. *Journal of Human Nutrition and Dietetics*, **2**, 371-377.

Pearson, T.A., LaCava, J. and Weil, H.F.C. (1997) Epidemiology of thrombotic-hemostatic factors and their associations with cardiovascular disease. *American Journal of Clinical Nutrition*, **65**, 1674s-1682s.

Pedersen, O.D., Munkvad, S., Gram, J., Kluft, C. and Jespersen, J. (1993) Depression of factor XIII dependent fibrinolytic activity in survivors of acute myocardial infarction at risk of reinfarction. *European Heart Journal*, **14**, 785-789.

Perri, M.G., McAllister, D.A., Gange, J.J., Jordan, R.C., McAdoo, W.G. and Nezu, A.M. (1988) Effects of four different maintenance programs on the long term management of obesity. *Journal of Consulting and Clinical Psychology,* **56**, 529-534.

Perri, M.G., Nezu, A.M. and Viegner, B.J. (1992) Improving the long term management of obesity, New York: Willey Bioscience.

Perri, M.G., Sears, S.F. and Clark, J.E. (1993) Strategies for improving maintenance of weight loss. Towards a continuous care model for the treatment of obesity. *Diabetes Care,* **16**, 200-209.

Philips, J.M. and Philbin, C.A. (1992) Worksite weight loss, an effective and successful programme. *American Journal of Occupational Health Nursing,* **40**, 496-498.

Phinney, S.D., Tang, AB, Waggoner, CR, Tezanos-Pinto, RG, Davis, PA. (1991) The transient hypercholesterolaemia of major weight loss. *American Journal of Clinical Nutrition*, **53**, 1404-1410.

Pickart, L.R. and Thaler, M.M. (1976) Free fatty acids and albumin as mediators of thrombin stimulated fibrinogen synthesis. *American Journal of Physiology,* **230**, 996-1002.

Poggi, M., Palareti, G., Biagi, R., Legnani, C., Parenti, M., Babini, A.C. and Coccheri, S. (1994) Prolonged very low calorie diet in highly obese subjects reduces plasma viscosity and red cell aggregation but not fibrinogen. *International Journal of Obesity,* **18**, 490-496.

Poller, L. (1990) When to start oral anticoagulants and the optimal range. *Biologia y Clinica Hematologica,* **12**, 113-119.

Pontiroli, A.E., Calderara, A., Pacchioni, M., Cassisa, C. and Pozza, G. (1993) Weight loss reverses secondary failure of oral hypoglycaemic agents in obese non-insulin

dependent diabetic patients independently of the duration of the disease. *Diabetes Metabolisme,* **19**, 30-35.

Pooling Project Research Group (1978) Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events. *Journal of Chronic Diseases,* **31**, 201-306.

Pories, W.J., MacDonald, K.G., Flickinger, E.G., Dohm, G.L., Sinha, M.K., Barakat, H.A., May, H.J., Khazine, P., Swanson, M.S., Morgan, E., Legget-Frazier, N., Long, S.D., Brown, B.M., O'Brien, K. and Caro, J.F. (1992a) Is type II diabetes mellitus (NIDDM) a surgical disease. *Annals of Surgery,* **215**, 633-643.

Pories, W.J., MacDonald, K.G., Morgan, E., Sinha, M.K., Dohm, G.L., Swanson, M.S., Barakat, H.A., Khaznie, P.G., Legget-Frazier, N., Long, S.D., O'Brian, K.F. and Caro, J.F. (1992b) Surgical treatment of obesity and it's effect on diabetes: a 10 years follow up. *American Journal of Clinical Nutrition,* **55**, 582-585.

Potter van Loon, B.J., Radder, J.K., Frolich, M., Krans, H.M.J, Zwinderman, A.H., Meinders, A.E. (1992) Fluoxetine increases insulin action in obese non diabetic and in non insulin dependant diabetic individuals. *International Journal of Obesity*, **16**, 79-85.

Prasad, U.S., Walker W.S., Sang, C.T.M., Campanella, C., Cameron, E.W.J. (1991) Influence of obesity on the early and long term results of surgery for coronary artery disease. *European Journal of Cardiothoraic Surgery*, **5**, 67-73.

Pratt, C.A. (1989) Development of a screening questionnaire to study attrition in weight control programs. *Psychological Reports,* **71**, 1007-1016.

Prentice, A.M., Black, A.E., Coward, W.A., Davies, H.L., Goldberg, G.R. and Murgatroyd, P.R. (1986) High levels of energy expenditure in obese women. *British Journal of Nutrition,* **292**, 983-992.

Prentice, A.M., Jebb, S.A. and Goldberg, G.A. (1992) Effects of weight cycling on body composition. *American Journal of Clinical Nutrition,* **56**, 209s-216s.

Primrose, J.N., Davies, J.A., Prentice, C.R.M., Hughes, R. and Johnston, D. (1992) Reduction in factor VII, fibrinogen and plasminogen activator inhibitor-1 activity after surgical treatment of morbid obesity. *Thrombosis and Haemostasis,* **68**, 396-399.

Prowse, C.V. and McGregor, I.R. (1988) Regulation of the plasminogen activator level in blood. In: Kluft, C. (Ed.) Tissue-type plasminogen activator (tPA). pp. 49-60. CRC Press.

Pyorala, K., De Backer, G., Graham, I. and Task Force on Prevention of Coronary Disease in Clinical Practice (1994) Recommendations of the Task Force of the European Society of Cardiology, European Atherosclerosis Society and European Society of Hypertension. *European Heart Journal,* **15**, 1300-1331.

Racette SB, Schoeller DA, Kushner RF, Neil KM. (1995) Exercise enhances dietary compliance during moderate energy restriction in obese women. *American Journal of Clinical Nutrition,* **62**, 345-349.

Radack, K., Deck, C., Pharm, D. and Huster, G. (1989) Dietary supplementation with low dose fish oils lowers fibrinogen levels: a randomised, double blind controlled study. *Annals of Internal Medicine,*. **111**, 757-758.

Radcliffe, R. and Nemerson, Y. (1975) Activation and control of factor VII by activated factor X and thrombin and characterisation of a single chain form of factor VII. *Journal of Biological Chemistry,* **250**, 388-395.

Rainer, C., Kawanishi, D.T., Chandraratna, A.N., Bauersachs, R.M., Reid, C.L., Rahimtoola, S.H. and Meiselman, H.J. (1987) Changes in blood rheology in patients with stable angina pectoris as a result of coronary artery disease. *Circulation,* **76**, 15-20.

Rames, L.K., Clarke, W.R., Cannon, W.E. (1978) Normal blood pressures and the evaluation of sustained blood pressure elevation in childhood: the Muscatine study. *Pediatrics*, **61**, 245-252.

Ramhandany, E. and Baird, I.M. (1988) Gastric balloon versus sham procedure in obese subjects. *International Journal of Obesity,* **13**, 593-595.

Rampling, M.W., Feher, M.D., Sever, P.S. and Elkeles, R.S. (1989) Haemorheological disturbances in non insulin dependent diabetes and the effects of concomitant hypertension. *Clinical Hemorhelogy,* **9**, 101-107.

Ramsey, L.E., Ramsey, M.H., Hettiarachchi, J., Davies, D.L., Winchester, J. (1978) Weight reduction in a blood pressure clinic. *British Medical Journal, ii*, 244-245.

Rankinen, T., Rauramaa, R., Halonen, P. and Penttila, I.M. (1994a) Blood coagulation and fibrinolytic factors are unchanged by aerobic exercise or fat modified diet. *Fibrinolysis,* **8**, 48-53.

Rankinen, T., Rauramaa, R., Vaisanen, S., Penttila, I.M. and Uusitupa, M. (1994b) Relation of habitual diet and cardiorespiratory fitness to blood coagulation and fibrinolytic factors. *Thrombosis and Haemostasis,* **71**, 180-183.

Rao, L.V.M., Rapaport, S.I., Bajaj, S.P. (1986) Activation of human factor VII in the iniatiation tissue factor dependent coagulation. *Blood*, **68**, 685-691.

Reaven, G.M. (1985) Beneficial effects of moderate weight loss in older patients with non insulin dependant diabetes poorly controlled with insulin. *Journal of the American Geriatric Society*, **33**, 93-95.

Rebuffe-Scrive, M., Hendleer, R., Bracero, N., Cummings, N., McCarthy, S. and Rodin, J. (1994) Bio-behavioural effects of weight cycling. *International Journal of Obesity,* **18**, 651-658.

Reisin, E., Abel, R., Modan, M., Silverberg, D.S., Eliahou, H.E. and Modan, B. (1978) The effect of weight loss without salt restriction on the reduction in blood pressure in overweight hypertensive patients. *New England Journal of Medicine,* **298**, 1-6.

Rillaerts, E., van Sant, G., Van Gaal, L. and De Leeuw, I. (1989) Effect of weight reduction on blood viscosity parameters in obese women. *Clinical Hemorheology,* **9**, 983-987.

Rimm, E.B., Giovannucci, E.L, Stampfer, M.J., Colditz, G.A., Litin, L.B. and Willett, W.C. (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *American Journal of Epidemiology*, **135**, 10, 1114-1126.

Rissanen, A., Pietinen, P., Siljamaki-Ojansuu, U., Piirainen, H. and Reissel, P. (1985) Treatment of hypertension in obese patients: efficacy and feasibility of weight and salt reduction programmes. *Acta Medica Scandinavia,* **218**, 149-156.

Robertson, I., Philips, A., Mant, A., Thorogood, M., Fowler, G., Fuller, A., Yudkin, P. and Woods, M. (1992) Motivational effect of cholesterol measurement in general practice. *British Journal of General Practice,* **42**, 469-472.

Rocchini, A.P., Katch, V., Schork, A., Kelch, R.P. (1987) Insulin and blood pressure during weight loss in obese adolescents. *Hypertension*, **10**, 267-273.

Rocchini, A.P., Katch, V., Anderson, J., Hinderliter, J., Becque, D., Martin, M., Marks, C. (1988) Blood pressure on obese adolescents: effect of weight loss. *Pediatrics*, **82**, 16-

Rocha, E. and Paramo, J.A. (1994) The relationship between impaired fibrinolysis and coronary heart disease: a role for PAI. *Fibrinolysis,* **8**, 294-303.

Rogers, J., Mitchell, G.W. (1952) The relation of obesity to menstrual disturbances. *New England Journal of Medicine*, **247**, 53-56.

Rose, G.A. (1962) The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. *Bulletin of the World Health Organisation* **27**, 645-658.

Rosengren, A., Wilhelmsen, L., Welin, T., Tsipogianni, A., Teger-Nillson, A.C. and Wedel, H. (1990) Social influences and cardiovascular risk factors as determinants of plasma fibrinogen concentrations in a general population sample of middle aged men. *British Medical Journal*, **300**, 634-638.

Rosenthal, M.B., Barnard, R.J., Rose, D.P., Inkeles, S., Hall, J., Pritikin, N. (1985) Effects of a high complex carbohydrate low fat low cholesterol diet on levels of serum lipids and estradiol. *American Medical Journal*, **78**, 23-27.

Royal College of Physicians (1983) Obesity, a report of the Royal College of Physicians, London.

Royal Society of Chemistry and Ministry of Agriculture and Fisheries (1991) McCance and Widdowson's The composition of foods. 5th ed, Cambridge.

Ruddock, V. and Meade, T.W. (1994) Factor VII activity and ischaemic heart disease: fatal and non-fatal events. *Quarterly Journal of Medicine,* **87**, 403-406.

Saad, M.F., Knowler, W.C. and Petit, D.J. (1988) The natural history of impaired glucose tolerance in Pima Indians. *New England Journal of Medicine,* **319**, 1500-1506.

Safer, D.J. (1991) Diet, behaviour modification and exercise: a review of obesity treatment from a long term perspective. *Southern Medical Journal,* **84**, 1470-1473.

Salomma, V., Rasi, V., Pekkanen, J., Jauhiainen, M., Vantera, E., Pietinen, P., Korhonen, H., Kuulasmaa, K. and Ehnholm, C. (1993) The effects of saturated fatty acids and n-6 fatty polyunsaturated fat on postprandial lipaemia and hemostatic activity. *Atherosclerosis,* **103**, 1-11.

Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I.A., Packard, C.J. (1997) Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstetrics and Gynacology*, **89**, 3, 403-408.

Sayler, M.E., Goldstein, D.J., Roback, P.J., Atkinson, R.L. (1994) Evaluating success of weight loss programs, worth an application to fluoxetine weight reduction clinical trial data. *International Journal of Obesity*, **18**, 742-751.

Schaefer, E.J., Lamon-Fava, S., Ausman, L.M., Ordovas, J.M., Clevidence, B.A., Judd, J.T., Goldin, B.R., Woods, M., Gorbach, S., Litchenstein, A.H. (1997) Individual

variability in lipoprotein cholesterol response to National Cholesterol Education Programme Step 2 diets. *American Journal of Clinical Nutrition*, **65**, 823-830.

Schmid-Schonbein, H. (1981) Factors promoting and factors preventing fluidity of blood. In: Effros, R.M., Schmid-Schonbein, H. and Ditzel, J. (Eds.) Microcirculation, current physiologic, medical and surgical concepts, pp. 249-269. New York: Academic Press.

Schmid-Schonbein, H., Volger, E., Teitel, P., Kiesewetter, H., Dauer, U. and Heilmann, L. (1982) New hemorheological techniques for the routine laboratory. *Clinical Hemorheology*, **2**, 93-105.

Schobersberger, W., Wirleitner, B., Puschendorf, B., Koller, A., Villiger, B., Frey, W. and Mair, J. (1996) Influence of a ultra-marathon race at moderate altitude on coagulation and fibrinolysis. *Fibrinolysis*, **10**, 37-42.

Schofield, W.N., Schofield, C. and James, W.P.T. (1985) Basal metabolic rate - review and prediction, together with annotated bibliography of source material. *Human Nutrition : Applied Nutrition*, **39C**, 5-96.

Schonfeld, G., Patsch, W. and Rudell, L.L. (1982) Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *American Journal of Clinical Investigation* **69**, 1072-1080.

Schotte, D.E. and Stunkard, A.J. (1990) The effects of weight reduction on blood pressure in 301 obese patients. *Archives of Internal Medicine*, **150**, 1701-1704.

Scottish Intercollegiate Guidelines Network (1996) Obesity in Scotland. Integrating prevention with weight management. Edinburgh: Scottish Intercollegiate Guidelines Network.

Scottish Office (1996) Scotland's Health a challenge to us all. Eating for Health: a diet action plan for Scotland. Scottish Office Department of Health, Edinburgh, HMSO.

Seidell, J.C., Cigolini, M., Charzewska, J., Ellsinger, B-M, Deslypere, J.P., Cruz, A. (1988) Fat distribution in European men: a comparison of anthropometric measurements in relation to cardiovascular risk factors. *International Journal of Obesity*, **16**, 17-22.

Seidell, J.C., Cigolini, M., Charzewska, J. (1991) Fat distribution and gender differences in serum lipids in men and women from four European communities. *Atherosclerosis*, **87**, 203-210.

Seidell, J.C. (1996) Personal communication.

Sever, P., Beevers, G., Bulpitt, C., Lever, A., Ramsay, L., Reid, J., Swales, J. (1993) Management guidelines in essential hypertension: report of the second working party of the British Hypertension Society. *British Medical Journal*, **306**, 983-987.

Shackleton, C.H.L. (1985) Mass spectrometry: application to steroid and peptide research. *Endocrine Reviews*, **6**, 441-486.

Shahar, E., Folsom, A.R., Wu, K.K., Dennis, B.H., Shimakawa, T., Conlan, M., Davis, C.E. and Williams, O.D. (1993) Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypercoagulable profile. The atherosclerosis in communities (ARIC) study. *Arteriosclerosis and Thrombosis,*. **13**, 1205-1212.

Shekelle, R.B., Missell, L.V., Paul, O., Shryock, A.M. and Stamler, J. (1985) Fish consumption and mortality from coronary disease. *New England Journal of Medicine*, **313**, 820-824.

Shiffman, M.L., Sugarman, H.J., Kellum, J.M., Brewer, W.H. and Moore, E.W. (1990) Gallstone formation after rapid weight loss: a prospective study in patients undergoing gastric bypass surgery for treatment of morbid obesity. *American Journal of Gastroenterology*, **86**, 1000-1005.

Shimokata, S., Muller, D.C., Andres, R. (1980) Studies in the distribution of body fat. III Effects of cigarette smoking. *Journal of the American Medical Association*, **261**, 1169-1175.

Sikand, G., Kando, A. and Foreyt, J.P. (1988) Two year follow up with a very low calorie diet and exercise training. *Journal of the American Dietetic Association,* **88**, 487-488.

Silverstone, T., Goodall, E. (1992) Centrally acting anorectic drugs: a clinical perspective. *American Journal of Clinical Nutrition*, **55**, 211s-214s.

Simon, E.S., Razavian, S.M., Beyec, J., Atger, V., Paul, J.L., Simon, A., moatti, N. and Levensen, J. (1995) Influence of lipoprotein sub-fractions on dextran and fibrinogen induced erythrocyte aggregation. *Clinical Hemorheology,* **15**, 667-676.

Simpson, G.K., Farquhar, D.L., Carr, P., Galloway, S., Stewart, I.C., Donald, P., Steven, F. and Munro, J.F. (1986) Intermittent protein sparing fast with abdominal belting. *International Journal of obesity,* **10**, 247-254.

Simpson, H.C.R., Meade, T.W., Stirling, Y., Mann, J.I., Chakrabarti, R. and Woolf, L. (1983) Hypertriglyceridaemia and hypercoagulability. *Lancet,* **i**, 786-790.

Singh, R.B., Jackson, D.M.A., Wils, R., Davies, J., Wise, P.H. (1992). Delayed diagnosis in non insulin dependant diabetes mellitus. *British Medical Journal, 304, 1154-5.*

Siscovick, D.S., LaPorte, R.E. and Newman, J.M. (1985) The disease specific benefits and risks of physical activity and exercise. *Public Health Reports,* **100**, 180-188.

Sjostrom, L., Lissner, L., Backman, L., Bengtsson, C., Bouchard, C., Dahlgren, S., Jonsson, E., Lindstedt, S., Narbro, K., Naslund, I., Olbe, L., Sjostrom, D., Stenlopf, K., Sullivan, M. and Wedel, H. (1994) SOS- Swedish obesity study. An intervention study of obesity. *International Journal of obesity,* **18**, 14 (Abstract).

Skender, M.L., Goodrick, G.K., Del-Junco, D.J., Reeves, R.S., Darnell, L. and Gotto, A.M. (1996) Comparison of 2-year weight loss trends in behavioural treatments for obesity: diet, exercise and combination interventions. *Journal of the American Dietetic Association,* **4**, 342-346.

Slabber, M., Barnard, H.C., Kuyl, J.M. and Badenhorst, C.J. (1992) Effect of a short-term very low calorie diet on plasma lipids, fibrinogen, and factor VII in obese subjects. *Clinical Biochemistry,* **25**, 334-337.

Slowinska-Srzednicka, J., Zgliczynski, S., Sosynska, P., Srzednicki, M., Biernacka, M., Woroszylska, M., Ruzyllo, M. and Sadowski, Z. (1989) Decreased plasma dehydroepiandosterone sulphate and dehydroepiandosterone concentrations in young men after myocardial infarction. *Atherosclerosis,* **79**, 197-203.

Small M., Kluft C, MacCuish A., Lowe G.D.O. (1989) Tissue plasminogen activator inhibition in Diabetes Mellitus. *Diabetes Care*, **12**, 9, 655-658.

Smith, W.C.S., Crombie, I.K., Tavendale, R.T., Gulland, S.K. and Tunstall-Pedoe, H. (1988) Urinary electrolyte excretion, alcohol consumption and blood pressure in the Scottish Heart Health Study. *British Medical Journal,* **297**, 329-330.

Sonne-Holm, S., Sorensen, T.I.A., Jensen, G. and Schnohr, P. (1989) Independent effects of weight change and attained body weight on the prevalence of arterial hypertension in obese and non obese men. *British Medical Journal,* **299**, 767-770.

Sonnichsen, A.C., Richter, W.O. and Schwandt, P. (1992) Benefit from hypocaloric diet in obese men depends on the extent of weight-loss regarding cholesterol, and on a simultaneous change in body fat distribution regarding insulin sensitivity and glucose tolerance. *Metabolism,* **41**, 1035-1039.

Sopko, G., Leon, A.S., Jacobs, D.R., Poster, N., Moy, J., Kuba, K., Anderson, J.T., Casal, D., McNally, C., Frantz, I. (1985) The effects of exercise and weight loss on plasma lipids in young obese men. *Metabolism*, **34**, 227-236.

Sorlie, P.D., Garcia-Palmieri, M.R., Costas, R. and Havlik, R.J. (1981) Haematocrit and the risk of coronary heart disease: the Puerto Rico Heart Health Programme. *American Heart Journal,* **101**, 456-461.

Sowers, J.R., Nyby M., Stern, N., Beck, F., Baron, S, Catania, R and Vlachis N. (1982) Blood pressure and hormone changes associated with weight reduction in the obese. *Hypertension*, *4*, 686-691.

Sprengers, E.D. and Kluft, C. (1987) Plasminogen activator inhibitors. *Blood,* **69**, 381-387.

Stamler, R, Stamler J, Reidslinger WF, Algara G and Roberts RH. (1978) Weight and blood pressure: findings in hypertension screening of 1 million Americans. *Journal of the American Medical Association*, **240**, 1607-1610.

Stanik, S. and Marcus, R. (1990) Insulin secretion improves following dietary control of plasma glucose in severely obese patients. *Metabolism*, **29**, 346-350.

Stewart, A.J., Wahlqvist, M.L., Stewart, B.J., Oliphant, R.C., Ireland, P.D. (1990) Fatty acid pattern outcomes of a nutritional program for overweight and hyperlipidaemic Australian men. *Journal of the American College of Nutrition*, **9**, 2, 107-113

Stuart, J. and Nash, G.B. (1990) Red cell deformability and haematological disorders. *Blood Reviews,* **4**, 141-147.

Study Group of the European Athersclerosis Society. (1987) Strategies for the prevention of coronary heart disease. A policy statement of the European Atherosclerosis Society. *European Heart Journal,* **8**, 77-88.

Stunkard, A.J., Craighead, L.W., O'Brien, R. (1980) Controlled trial therapy, pharmacotherapy, and their combination in the treatment of obesity. *Lancet*, **1**, 394-386.

Stunkard, A.J. and Wadden, T.A. (1992) Psychological aspects of human obesity. In Obesity, Editors Bjorntorp, P. and Brodoff, B.N. Lippencott, New York.

Sullivan, M., Karlsson, J., Sjostrom, L., Backman, L., Bengtsson, C., Bouchard, C., Dahlgren, S., Jonsson, E., Larsson, B., Lindstedt, S. (1993). Swedish Obesity Study (SOS): an intervention study of obesity. Baseline evaluation of health and psychological

functioning in the first 1743 subjects examined. *International Journal of Obesity*, **17**, 9, 503-512.

Sundell, I.B., Dahlgren, S., Ranby, M., Lundin, E., Stenling, R. and Nilsson, T.K. (1989) Reduction of elevated plasminogen activator inhibitor levels during modest weight loss. *Fibrinolysis*, **3**, 51-53.

Sylvan, A., Rutegard, J.N., Janunger, K.G., Sjolund, B., Nilsson, T.K. (1992) Normal plasminogen activator inhibitor levels at log term follow-up after jejuno-ileal bypass surgery in morbidly obese individuals. *Metabolism*, **41**, 12, 1370-1372.

Tauber-Lassen, E., Damsbo, P., Henriksen, J.E., Pamvig, B. and Bech-Nielsen, H. (1990) Improvement of glycaemic control and weight loss in type 2 (non insulin dependent) diabetes mellitus after one year of dexfenfluramine treatment. *Diabetilogia*, **335**, A124 (Abstract).

Tchernof, A., Labrie, F., Belanger, A. and Depres, J.P. (1996) Obesity and metabolic complications: contribution of dehydroepiandosterone and other steroid hormones. *Journal of Endocrinology*, **150**, s155-s164.

Teufal, N.I. (1992) Diet and activity patterns of male and female co-workers: should worksite health promotion programmes assume homogeneity? *Women and Health*, **19**, 31-54.

Tholstrup, T., Marckmann, P., Jespersen, J. and Sandstrom, B. (1994a) Fat high in stearic acid favourably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *American Journal of Clinical Nutrition*, 59, 371-379.

Tholstrup, T., Marckmann, P., Jespersen, J., Vessby, B., Jart, A. and Sandstrom, B. (1994b) Effect on blood lipids, coagulation, and fibrinolysis of fat high in myristic acid and a fat high in palmitic acid. in palmitic acid or high in myristic and lauric acids. *American Journal of Clinical Nutrition*, **60**, 919-925.

Tholstrup, T., Andreasen, K., Sanstrom, B. (1996) Acute effect of high-fat meals either rich in stearic or mystric acidon hemostatic factors in healthy young men. *American Journal of Clinical Nutrition*, **64**, 2, 168-176.

Thompson, S.G., Kienast, J., Pyke, S.D., Haverkate, F. and van de Loo, J.C. (1995) Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group (ECAT). *New England Journal of Medicine,* **332**, 635-641.

Thomson, J.M. (1992) Blood collection and preparation: pre-analytical variation. In: Jespersen, J., Bertina, R.M. and Haverkate, F. (Eds.) ECAT assay procedures a manual of laboratory techniques. European concerted action on thrombosis and disabilities of the European communities. pp. 13-20, Dordrecht, Kluwer Academic.

Tikkanen, M.J. (1996) The menopause and hormone replacement therapy: lipids, lipoproteins, coagulation and fibrinolytic factors. *Journal of the Climacteric and Postmenopause,* **23**, 209-216.

Tozzi, E., Tozzi-Ciancarelli, M.G., Di Massimo, C., Mascoli, A., Gentile, T. and de Matteis, F. (1994) Hemorheological parameters and body weight in obese children. *Clinical Hemorheology,* **14**, 203-211.

Troiano, R.P., Frongillo, E.A., Sobal, J., Levitsky, D.A. (1996) The relationship between body weight and mortality: a quantitative analysis of combined information from existing studies. *International Journal of Obesity,* **20**, 1, 63-75.

Tuck, M.L., Swers, J., Dornfeld, L., Kledzik, G. and Maxwell, M. (1981) The effect of weight reduction on blood pressure, plasma renninactivity and plasma aldosterone levels in obese patients. *New England Journal of Medicine*, **304**, 930-933.

UK PDS (1990) Prospective diabetes study 7. Response of fasting plasma glucose to diet therapy in newly presenting type II diabetic patients. *Metabolism: Clinical and Experimental,* **39**, 905-912.

UK PDS (1995) Relative efficacy of randomly allocated diet, sulphonylurea, insulin or metformin in patients with newly diagnosed non-insulin dependent diabetes. *British Medical Journal,* **310**, 83-88.

Umachdran, V., Ranjadayalan, K., Ambepityia, G., Marchant, B. and Kopelman, P.G. (1991) Ageing, autonomic function, and the perception of angina. *British Heart Journal* **66**, 15-18.

Urano, T., Kojima, Y., Takahashi, M., Serizawa, K., Sakakibara, K., Takada, Y. and Takada, A. (1993) Impaired fibrinolysis in hypertension and obesity due to a high plasminogen activator inhibitor-1 level in plasma. *Japanese Journal of Physiology,* **43**, 221-228.

Usiskin, K.S., Butterworth, S., Clore, J.N., Arad, Y., Ginsberg, H.N., Blackard, W.G. and Nestler, J.E. (1990) Lack of effect of dehydroepiandosterone in obese men. *International Journal of Obesity,* **14**, 457-463.

Uusitupa, M.I.J., Laasko, M., Sarland, H., Majander, H., Takala, J. and Penttila, I. (1990) Effects of very low calorie diet on metabolic control and cardiovascular risk factors in the treatment of obese non-insulin dependent diabetes. *American Journal of Clinical Nutrition,* **51**, 768-773.

Vague, P., Juhan-Vague, I., Aillaud, M.F., Badier, C., Viard, R., Alessi, M.C. and Collen, D. (1986) Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level and relative body weight in normal and obese subjects. *Metabolism* **35**, 250-253.

Vague, P., Juhan-Vague, I., Charbert, V., Alessi, M.C. and Atlan, C. (1989) Fat distribution and plasminogen activator inhibitor activity in non diabetic obese women. *Metabolism,* **38**, 913-915.

van Gaal, L., Vansant, G., Moremaens, M. and DeLeeuw, I. (1995) Lipid changes after long term weight reduction, Influence of gender and body fat distribution. *Journal of the American College of Nutrition,* **14**, 382-386.

van Gaal, L., Wauters, M.A. and De Leew, I. (1997) The beneficial effects of modest weight loss on cardiovascular risk factors. *International Journal of Obesity,* **21**, s5-s9.

Van Itallie, T.B. (1985) Health implications of overweight and obesity in the United States. *Annals of Internal Medicine,* **103**, 983-988.

Velthuis-te Wierik, E.J.M., Meijer, P., Kluft, C. and van den Berg, H. (1995a) Beneficial effect of a moderately energy-restricted diet on fibrinolytic factors in non obese men. *Metabolism* **44**, 1548-1552.

Velthuis-te Wierik, E.J.M., Westerterp, K.R. and van den Berg, H. (1995b) Impact of a moderately energy-restricted diet on energy metabolism and body composition in non-obese men. *International Journal of Obesity* **19**, 318-324.

Vessby, B., Boberg, M., Karlstrom, B., Lithell, H. and Werner, I. (1984) Improved metabolic control in overweight diabetic patients. *Acta Medica Scandinavia,* **216**, 67-74.

Volkamar, F.R., Stunkard, A.J., Woolston, J., Bailey, J.F. (1981) High attrition rates in commercial weight reduction programmes. *Archives of Internal Medicine*, **141**, 426-428.

Wadden, T.A., Stunkard, A.J., Brownell, K.D. and Day, S.C. (1985) A comparison of two very low calorie diets: protein sparing modified fast versus protein formula liquid diet. *American Journal of Clinical Nutrition,* **41**, 533-539.

Wadden, T.A., Sternberg, J.A., Letizia, K.A., Stunkard, A.J. and Foster, G.D. (1989) Treatment of obesity by very low calorie diet, behaviour therapy and their combination: a five year perspective. *International Journal of obesity,* **13**, 39-46.

Wadden, T.A. (1993a) Treatment of obesity by moderate and severe caloric restriction: results of clinical trials. *Annals of Internal Medicine,* **119**, 688-693.

Wadden, T.A. (1993b) The treatment of obesity, an overview. In: Stunkard, A.J. and Wadden, T.A. (Eds.) *Obesity: theory and therapy*, 2nd edn. pp. 197-217. New York: Raven Press.

Wade, J., Milner, J., Krondl, M. (1981) Evidence for a physiological regulation of food selection and nutrient intake in twins. *American Journal of Clinical Nutrition*, **34**, 143-147.

Waki, M., Heshka, S. and Heymsfield, S.B. (1992) Long term lipid lowering behaviour modification and weight loss in obese women. *Nutrition,* **9**, 23-28.

Wannamethee, G., Shaper, A.G. and Whincup, P.H. (1994) Ischaemic heart disease: association with haematocrit in the British Regional Heart Study. *Journal of Epidemiology Community Health,* **48**, 112-118.

Ward, K.D., Sparrow, D., Vokonas, P.S., Willett, W.C., Landsberg, L. and Weiss, S.T. (1994) The relationship of abdominal obesity hyperinsulinaemia and saturated fat intake to serum lipid levels: the normative ageing study. *International Journal of Obesity,* **18**, 137-144.

Wassertheil-Smoller, S., Blaufox, M.D., Oberman, A.S., Langford, H.G., Davis, B.R. and Wylie-Rosett, J. (1992) The trial of antihypertensive interventions and management (TAIM) study. Adequate weight loss alone and combined with drug therapy in the treatment of mild hypertension. *Archives of Internal Medicine,* **152**, 131-136.

Watts, N.B., Spanheimer, R.G., Digirolamo, M., Gehart, S.S.P., Musey, V.C., Siddiq, Y.K. and Philips, L.S. (1990) Prediction of glucose response to weight loss in patients with non insulin dependent diabetes mellitus. *Archives of Internal Medicine,* **150**, 803-806.

Weaver, J.U., Holly, J.M., Noonan, K., Giadom, C.G., White, N., Virdee, S., and Wass, J.A. (1990) Decreased sex hormone binding globulin (SHBG) and insulin-like growth factor binding protein (IGFBP-1) in extreme obesity. *Clinical endocrinology,* **33**, 415-422.

Weinsier. R.L., James, R.D., Darnell, B.E., Wooldridge, N.H., Birch, R., Hunter, G.R., Bartolucci, A.A. (1992) Lipid and insulin concentration in obese postmenpausal women: separate effects of energy restrictionand weight loss. *American Journal of Clinical Nutrition*, **56**, 44-49.

Weintraub, M., Sundareson, P.R., Madan, M. (1992) Long term weight control study 1 (weeks 0-34): the enhancement of behaviour modification, calorie restriction and exercise by fenfluramine plus phentermine versus placebo. *Clinical Pharmacological Therapy*, **51**, 586-594.

Weintraub, M., Sundaresan, P.R., Schuster, B., Moscucci, M., Stein, E.C. (1992) Long term weight control study III (weeks 104-156). An open label study dose adjustment of fenfluramine and phenteramine. *Clinical Pharmacological Therapy*, **51**, 602-607.

WHO (1985) Technical Commitee on Diabetes World Health Organsisation criteria for diagnosis of diabetes. WHO, Geneva.

WHO (1987) Measuring obesity, classification and distribution of anthropometric data. 125, WHO, Copenhagen.

WHO (1989) WHO MONICA project: objectives and design. *Journal of Clinical Epidemiology,* **18**, 29-37.

WHO (1995) Physical status: the use and interpretation of anthropometry. Technical Report Series report **834**, 1-452.

Wilhelmsen, L., Svardsudd, K., Korstan-Bengtsen, K., Larsson, B., Welin, L. and Tibblin, G. (1984) Fibrinogen as a risk factor for stroke and myocardial infarction. *New England Journal of Medicine,* **311**, 501-505.

Wilkes, H.C., Meade, T.W., Barzegar, S., Foley, A.J., Hughes, L.O., Bauer, K.A., Rosenberg, R.D. and Miller, G.J. (1992) Gemfibrozil reduces plasma prothrombin fragment F1+2 concentration, a marker of coagulability in patients with coronary heart disease. *Thrombosis and Haemostasis,* **67**, 503-506.

Willett, W.C., Manson, J.E., Stampfer, M.J., Colditz, G.A., Rosner, B., Speizer, F.E. and Hennekens, C.H. (1995) Weight, weight change and coronary heart disease in women: risk within the normal weight range. *Journal of the American Medical Association,* **273**, 461-465.

Willey, K.A., Molyneaux, L.M., Overland, J.E., Yue, D.K. (1991) The effects of dexfenfluramine on blood glucose control in patients with type 2 diabetes. *Diabetic Medicine*, 9, 341-343.

Williams, P.D., Puddey, I.B., Beilin, L.J. and Vandongen, R. (1993) Genetic influences on plasma catecholamines in human twins. *Journal of Clinical Endocrinology and Metabolism,* **77**, 794-799.

Williamson, D.F. (1993) Descriptive epidemiology of body weight and weight change in US adults. *Annals of Internal Medicine,* **119**, 646-649.

Williamson, D.F., Serdula, M.K., Anda, R.F., Levy, A. and Byers, T. (1992) Weight loss attempts in adults, goals, duration and rate of weight loss. *American Journal of Public Health,* **82**, 1251-1257.

Williamson, D.F. and Pamuk, E.R. (1993) The association between weight loss and increased longevity. A review of the evidence. *Annals of Internal Medicine,* **119**, 731-736.

Williamson, D.F., Pamuk, E., Thun, M., Flanders, D., Byers, T. and Heath, C. (1995) Prospective study of intentional weight loss in never smoking US white women. *American Journal of Epidemiology,* **141**, 1128-1141.

Wilson, E.A., Hadden, D.R., Merrett, J.D., Montgmery, D.A.D., Weaver, J.A. (1980) Dietary management of maturity onset diabetes. *British Medical Journal*, 1367-1369.

Wing, R., Epstein, L.H., Kaplan, R.M., Nowalk. M.P., Koeske, R., Hagg S. (1985) Behaviour change, weight loss, and physiological improvements in type II diabetic patients. *Journal of Consulting and Clinical Psycology*, **53**, 111-122.

Wing, R., Koeske, R., Epstein, L.H., Nowalk, M.P., Gooding, W. and Becker, D. (1987) Long term effects of modest weight loss in type 2 diabetic patients. *Archives of Internal Medicine*, **147**, 1749-1753.

Wing, R.R., Shoemaker, M., Marcus, M.D.M., McDermott, M., Gooding, W. (1990) Variables associated with weight loss and improvements in glycaemic control in type II diabetic patients. *International Journal of Obesity*, **14**, 495-503.

Wing, R.R., Jeffery, R.W., Burton, L.R., Thorson, C., Kuller, L.H. and Folsom, A.R. (1992) Change in waist-hip ratio with weight loss and its association with change in cardiovascular risk factors. *American Journal of Clinical Nutrition,* **55**, 1086-1092.

Wing, R.R. (1992) Weight cycling in humans: a review of the literature. *Annals of Behavioural Medicine,* **14**, 113-119.

Wing, R.R. (1993) Behavioural treatment of obesity, it's application to type 2 diabetes. *Diabetes Care,* **16**, 193-199.

Wing, R.R. and Greeno, C.G. (1994) Behavioural and psychosocial aspects of obesity and it's treatment. *Ballieres Clinical Endocrinology,* **8**, 689-703.

Wing, R.R., Blair, E., Marcus, M., Epstein, E.H. and Harvey, J. (1994) Year long weight loss treatment for obese patients with type 2 diabetes: diets including an intermittent low calorie diet can improve outcome. *American Journal of Medicine,* **97**, 354-362.

Wing, R.R., Blair, E.H., Bonomi, P., Marcus, M.D., Watanabe, R. and Bergman, R.N. (1994) Calorie restriction *per se* is a significant factor in improvements in glycaemic control and insulin sensitivity during weight loss in NIDDM diabetes. *Diabetes Care,* **17**, 30-36.

Wing, R.R. and Jeffery, R.W. (1995) Effect of modest weight loss on changes in cardiovascular risk factors: are there differences between men and women or between weight loss and maintenance? *International Journal of Obesity*, **19**, 67-73.

Winocour, P.H., Kaluvya, S., Brown, L., Farrer, M., Millar, J.P., Neil, H.A.W. and Alberti, K.G.M.M. (1991) The association of different measures of insulinaemia with vascular risk factors in healthy normoglycaemic normotensive non obese men and women. *Quarterly Journal of Medicine* **79**, 539-560.

Wittenberg, C., Erman, A., Sulkes, J., Abramson, E., Boner, G. (1994) Which cuff size is preferable for blood pressure monitoring in most hypertensive patients? *Journal of Human Hypertension*, **8**, 819-822.

Wolf, R.N. and Grundy, S.M. (1983) Influence of weight reduction on plasma lipoproteins in obese patients. *Arteriosclerosis*, **3**, 160-169.

Wolf, R.N. and Grundy, S.M. (1983) Influence of weight reduction on plasma lipoproteins in obese patients. *Arteriosclerosis*, **3**, 160-169.

Wolfe, B.L. (1992) Long term maintenance following attainment of goal weight: a preliminary investigation. *Addictive Behaviour*, **17**, 469-477.

Wolffenbuttel, B.H.R., Weber, R.F.A, van Koetsveld, P.M., Verschoor, L. (1989) Limitations of dietary therapy in patients with non insulin dependant diabetes mellitus. *International Journal of Obesity*, **13**, 173-182.

Wood, P.D., Stefanick, M.L., Dreon, D.M. (1988) Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. *New England Journal of Medicine*, **319**, 1173-1179.

Wood, P.D., Stefanick, M.L., Williams, P.T., Haskell, W.L. (1991) The effects on plasma lipoproteins of a prudent weight reducing diet with or without exercise, in overweight men and women. *New England Journal of Medicine*, **7**, 461-466.

Woodhouse, P.R., Khaw, K.T., Plummer, M., Foley, A., and Meade, T.W. (1994) Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. *Lancet,* 343:435-439.

Woolf, B.C. (1954) Statistical aspects of dietary surveys. *Proceedings of the Nutrition Society,* **12,** 82-94.

Wosornu, D., Allardyce, W., Ballantyne, D. and Tansey, P. (1992) Influence of power and aerobic exercise on haemostatic factors after coronary artery surgery. *British Heart Journal,* **68,** 181-186.

Wright, J., Wood, B. and Hale, G. (1981) Evaluation of group versus individual nutrition education in overweight patients with myocardial infarction. *Australia and New Zealand Journal of Medicine,* **11,** 497-501.

Wu, K.K., Folsom, A.R., Heiss, G., Davis, C.E., Conlan, M. and Barnes, R. (1990) Hemostatic factors and carotid atherosclerosis the Atherosclerosis in communities study. *Circulation,* **82,** 445 (Abstract).

Wysocki, M., Krotkiewski, M., Braide, M. and Bagge, U. (1991) Hemorheological disturbances, metabolic parameters and blood pressure in different types of obesity. *Atherosclerosis,* **88,** 21-28.

Yang, H., Petersen, G.M., Roth, M.P., Schoenfield, L.J. and Marks, J.W. (1992) Risk factors for gallstone formation during rapid loss of weight. *Digestive Diseases Sciences,* **37,** 912-918.

Yao, S.T., Hobbs, J.T. and Irvine, W.T. (1969) Ankle systolic pressure measurements in arterial disease affecting the lower extremities. *British Journal of Surgery,* **56,** 676-679.

Yao, S.T. (1973) New techniques in objective arterial evaluation. *Archives of surgery* **106,** 600-604.

Yarnell, J.W.G., Fehily, A.M., Milbank, J.E., Sweetnam, P.M. and Walker, C.L. (1983) A short dietary questionnaire for use in an epidemiological survey: comparison with weighed dietary records. *Human Nutrition: Applied Nutrition,* **37A**, 103-112.

Yarnell, J.W.G., Baker, I.A., Sweetnam, P.M., Bainton, D., O'Brien, J.R., Whitehead, P.J., Elwood, P.C. (1991) Fibrinogen, viscosity and white blood cell count are major risk factors for ischaemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation*, **83**, 836-844.

Yen, S.S, Chaney, C., Judd, H.L. (1976) Functional aberrations of the hypothalamic-pituatory system in polycystic ovary syndrome. A consideration of the pathogenensis. In: James, V.H., Serio, M., Guisti, G. *The Endocrine function of the human ovary.* New York Academic Press, 373-381.

Young, E.A. (1992) National Dairy Council award for excellence in medical and dental education lecture. Perspectives on nutrition in medical education. *American Journal of Clinical Nutrition,* **56**, 745-751.

Yudkin, J.S. and Oswald, G.A. (1987) Hyperglycaemia, diabetes and myocardial infarction. *Diabetic Medicine,* 13-18.

Zandee, G.L. and Oermann, M.H. (1996) Effectiveness of contingency contracting. *American Association for Occupational Health Nursing Journal*, **44**, 4, 183-188.

Zimmet, P., Dowse, G. and Bennett, P. (1991) Hyperinsulinaemia is a predictor of non insulin dependent diabetes mellitus. *Diabete and Metabolisme,* **17**, 101-108.

Zitoun, D., Bara, L., Basdevant, A. and Samama, M.M. (1996) Levels of factor FVIIc associated with decreased tissue factor pathway inhibitor and increased plasminogen activator inhibitor-1 in dyslipidaemias. *Arteriosclerosis and Thrombosis,* **1**, 77-81.