BLOOD PRESSURE AND THE EFFECT OF CALCIUM ENRICHED MILK IN HUMANS WITH NORMAL OR MILDLY ELEVATED BLOOD PRESSURE: METHODOLOGICAL CONSIDERATIONS

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ABSTRACT

The aim of the present study was to assess the influence of three different milk products on blood pressure in patients with mild hypertension who were not under medication or had ceased medication for the trial period under medical supervision. In addition, the effect of calcium on blood pressure was to be explored.

The initial objectives of the trial were not met because the trial was stopped due to an adverse coronary event in one volunteer. However, there was sufficient data to reinforce the validity of the methodology, especially the ambulatory blood pressure recording, and to ensure that all other aspects of the trial were achievable.

Subjects with elevated blood pressure could be recruited and undergo a series of tests to determine their physical parameters, have blood pressure taken by a variety of methods and on several occasions, and meaningful data obtained. In addition, a small, potentially beneficial, modification could be made to their diet, and the effect of this dietary change monitored by both blood pressure and blood profile changes. These changes were made with minimal disruption to their daily routine, and were generally well-received.

The present study confirmed that ambulatory blood pressure monitoring could be conducted with little intrusion into the lives of the subjects. The data obtained from a variety of methods was able to identify those subjects who presented with some of the cluster of factors which characterise Syndrome X.

These results, while only from a small sample group, strongly support the use this research methodology to provide an accurate representation of a population subgroup, such as those with elevated blood pressure. In addition, the effect of a dietary intervention on blood pressure and blood lipid profiles can be monitored in free-living subjects.

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CHAPTER ONE: INTRODUCTION

Elevated blood pressure is an increasing problem in industrialised nations, especially as the population ages (Levy, D., 1999) and becomes more obese (Whelton *et al.,* 1998: New Zealand Guidelines, 2000).

It has been demonstrated by numerous studies that adequate calcium lowers blood pressure (Buonopane *et al.*, 1992; Osbourne *et al.,* 1996; McCarron, 1998; Barr *et al.*, 2000). Sufficient calcium in the diet may have a greater effect on people who have elevated blood pressure. Known as hypertensives, these people usually submit to a drug regime to control their blood pressure. Simply drinking a daily glass of calcium-reinforced milk provides an attractive alternative. Calcium-enriched fat-free milk is currently available on the New Zealand market, as the benefits of sufficient calcium in the diet are well-recognised (Karanja *et al.,* 1994). These benefits are for both osteoporosis and blood pressure reduction (McCarron, 1998). The former relationship is well established, however the latter is somewhat controversial (Seely, 1991; Buonopane *et al.*, 1992; Osbourne et al., 1996; McCarron, 1998; Barr *et al.*, 2000).

Fat offers enhanced flavour to most pallets, therefore there is market resistance to fat-free products. This observation prompted the milk industry to explore the effect of a low-fat calcium-enriched milk that is currently unavailable on the market. The low fat content may offer improved flavour and therefore increased sales, but the health benefits of the calcium may be offset by the increased potential of raised blood cholesterol levels. This effect is especially important for those with elevated blood pressure, who may further compromise their health by consumption of such a higher fat product.

Control of blood lipids is a means of reducing the risk of cardiovascular disease (Karanja *et al.*, 1994). Dairy food consumption has been linked to the development of arteriosclerosis (McNamara, 1992), thus people with an increased risk of cardiovascular disease are usually encouraged to reduce their intake of these foods. However, the strong link between calcium and blood pressure begs the investigation whether the potential benefit of consuming calcium enriched milk outweighs any potential harm of dairy fat.

A nine-week trial was conducted to determine if drinking modified milk powder lowered blood pressure. The purpose of this study was to assess the influence of three different milk products on blood pressure in patients with mild hypertension who were not under medication or had ceased medication for the trial period under medical supervision. The three milk products were normal skim milk; normal skim milk with added calcium; and low fat milk (1.5% fat) with added calcium. These were formulated by the New Zealand Dairy Research Institute. In addition, the effect of the higher fat milk on blood lipids was also to be explored.

The 9-week trial was stopped prematurely after only three subjects had completed the measurements and, therefore, the number of subjects is limited. The purpose of the thesis was revised to assess in more detail the suitability of the various methodologies for this kind on intervention trial.

CHAPTER TWO: LITERATURE REVIEW

2.1. BLOOD PRESSURE

Blood pressure and the general mechanisms of control are well known. However, certain aspects are less well-defined and constituted the initial focus of the present study. Blood pressure is characterised by the complexity and, often, inconsistency of its response to external influences.

Blood pressure is conventionally described in mm mercury (Hg). Normal blood pressure is often regarded as a systolic blood pressure (SBP) of 120 mm Hg, and a diastolic blood pressure (DBP) of 80 mm Hg. However, a range is more descriptive; normal blood pressure being an SBP of less than 130 mm Hg and a DBP of less than 85 mm Hg. Upper limits for blood pressure are noted as being in the range of 129-135 mm Hg SBP and 80-85 mm Hg DBP for various populations (Ohkubo *et al.,* 1998, Schettini *et al.*, 1999). In New Zealand, mildly elevated blood pressure is regarded as an SBP range of 150-170 mm Hg and a DBP range of 90-100 mm Hg (New Zealand Guidelines, 2000).

2.1.1. Blood pressure regulation

The regulation of blood pressure is multifactorial. Most of the physical mechanisms are well understood and beyond the scope of this review. The focus of the present review is the impact of diet and, in particular, the role of minerals in blood pressure regulation.

2.1.2. Pulse pressure

Pulse pressure is the difference between SBP and DBP and is typically around 50 mm Hg. However, there is considerable physical activity-related variation. This variation in pulse pressure provides an indication of the health of the cardiovascular system. Increased pulse pressure generally occurs with age and is due to stiffening of the large artery walls. Verdeccia *et al.,* (1998), using ambulatory recording apparatus (see 3.2.2.), concluded that ambulatory pulse pressure was a potent marker for cardiovascular risk in hypertension. These authors note that morbidity was more closely predicted by the ambulatory method

than by office pulse pressure. The significance of this observation over the more usual SBP and DBP measures is unknown.

2.1.3. Diurnal variation

Blood pressure does not remain the same throughout the day and normally displays diurnal variation, where both SBP and DBP decrease at night. It is common to describe the people whose blood pressure falls > 10% at night as "dippers", while those whose blood pressure falls less as "non-dippers" (Kario, 1999). Blood pressure dipping at night is regarded as normal, whereas reduced diurnal blood pressure variation (< 10%), in addition to high blood pressure, is related to target organ damage and cardiovascular events (Kario *et al.*, 1999). Pickering *et al.* (1995) demonstrated that smokers displayed an exaggerated diurnal swing in blood pressure. Kario *et al.* (1999) noted that the determinants of diurnal variation are not well understood. Uzu *et al.* (1999) found that sodium restriction restored "dipping" in salt-sensitive hypertensive patients.

Differing levels of activity between night and day might provide the variation in blood pressure, however Kario *et al.* (1999) found that within-individual activity was more strongly related to HR than to blood pressure. These authors hypothesised an association of sleep activity to sleep blood pressure where dipping reflected differences in sleep quality. They found greater sleep activity amongst non-dippers compared to extreme dippers. Stergiou *et al.* (1997) found that ABP dipped during a mid-day siesta almost as much as during the night and also affected the amount of dipping observed at night. A lack of dipping may be also due to stress or to shift work (Adams *et al.*, 1998, Vrijkotte *et al.*, 2000). It is thought that less than adequate sleep quality may increase sympathetic activity to prevent dipping (Lusardi *et al.*, 1999). In addition, these authors noted increased blood pressure and HR after a night of disrupted sleep. Sympathetic cardiovascular control is not well-understood (Mancia *et al.*, 1997).

2.1.4. Morning surge

Associated with the diurnal variation is an increase in blood pressure known as the "morning surge". This increase from the low of the night begins prior to awakening and

increases more rapidly once awake. This phenomenon is thought to be the result of increased sympathetic activity before awakening (Elliot, 1999), or decreased parasympathetic activity (Pasic *et al.*, 1998) that may have a reduced moderating effect. Elliot (1999) suggested that the morning surge might be associated with cardiovascular events in elderly patients due to the relatively sudden elevation of blood pressure. Pasic *et al.* (1998) linked an exaggerated morning surge to feelings of hostility upon awakening.

2.2. HYPERTENSION

As previously mentioned (p.1), an increase in blood pressure, especially with age, is a common finding in industrialised societies (Pietinen & Aro, 1990). Known as hypertension, this condition has serious implications for public health in the future as the older population increases (Levy, D., 1999). However, there may be other reasons such as dietary factors. Hypertension increases, or accelerates, the risk of cardiovascular complications such as stroke and coronary heart disease (CHD) (Zafari & Wenger, 1998) and has also been linked to cognitive impairment in the elderly (Reavan *et al.*, 1990; Kilander *et al.*, 1998). Hypertension is one of the cluster of metabolic-related disorders that have been labelled "Syndrome X" (Reavan, 1988).

Table 2.1. Blood pressure and stages of hypertension

Blood pressure	Systolic (mm Hg)	Diastolic (mm Hg)
Normal	< 130	<85
High-normal	130 – 139	85 – 89
Hypertension	≥ 140	≥ 90
Stage 1: Mild	140 – 159	90 – 99
Stage 2: Moderate	160 – 179	100 – 109
Stage 3: Severe	180 – 209	110 – 119
Stage 4: Very Severe	≥ 210	≥ 120

Data from the Joint National Committee (1993), "The Fifth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure" Archives of Internal Medicine, 153: 161, cited in Heyward, 1997, p. 17.

Hypertensive patients may present with increased SBP giving a high pulse pressure, or

combined with an increased DBP. Hypertension is defined as a SBP of 140 mm Hg or greater, and a DBP of 90 mm Hg or greater. Stages of hypertension are shown in Table 2.1. The aetiology of most hypertension is unknown, but many factors are involved and contribute to high blood pressure. Genetics, race, age, obesity, smoking, stress, alcohol and certain dietary components are implicated as risk factors (Rusoff, 1987; Zafari & Wenger, 1998).

2.2.1. Types of Hypertension

There are two types of hypertension: Primary or essential hypertension is a persistently elevated blood pressure that has no readily identifiable cause. Approximately 90-95% of all hypertension is in this category: Secondary hypertension constitutes the remainder. A further, relatively common, acute elevation of blood pressure, known as white coat hypertension, is described in 2.2.2.5.

2.2.1.1. Primary hypertension

In the early stages of primary hypertension blood pressure elevations are intermittent and there is an exaggerated pressor response to moderate stimuli that would normally only produce limited blood pressure elevation. This response suggests an arteriolar spasm caused by a sensitised autonomic reaction. Treatment with drugs that block sympathetic activity slows progression of the disease, therefore early screening and diagnosis is emphasised. Blood pressure elevation later becomes sustained because the baroreceptors are reset. At this stage, even a normal autonomic stimulus is associated with an elevated pressure, so that primary hypertension is a treatable, but not curable, disease. Treatment can be by both pharmacological and non-pharmacological means (Ganong, 1989).

There is also a condition known as primary pulmonary hypertension that is a progressive disease. Right-heart function is diminished due to increased pulmonary vascular resistance (Gaine & Rubin, 1998). However, this disease and its implications are beyond the scope of this review.

2.2.1.2. Secondary hypertension

Secondary hypertension has an identifiable underlying cause such as aldosteronism and kidney disease. Aldosteronism is hypersecretion of aldosterone, which stimulates excess reabsorption of salt and water by the kidneys. In humans, deoxycorticosterone and aldosterone both elevate blood pressure, and hypertension is a prominent feature of primary hyperaldosteronism. Elevated aldosterone secretion is usually due to a tumour of the adrenal cortex. Kidney disease often causes obstruction of renal blood flow or damage to renal tissue, which may cause the kidneys to release excessive amounts of renin into the blood. Renin stimulates the secretion of angiotensin II, a powerful vasoconstrictor, and also aldosterone release. Constriction of one renal artery causes the development of sustained hypertension known as renal or Goldblatt hypertension. Expansion of the blood volume due to sodium retention also plays a role (Tortora & Grabowski, 1996).

2.2.1.3. Pregnancy-induced hypertension

About 10-15% of pregnancies in the USA result in pregnancy-induced hypertension. The major cause is pre-eclampsia in which the hypertension seems to result from impaired renal function. It typically appears near the end of the second trimester with associated high protein in the urine. When occurring with convulsions and coma, the condition is termed eclampsia. Hypertension is also a prominent symptom of pregnancy toxaemia and is thought to be caused by a, presently unknown, pressor polypeptide from the placenta (Ganong, 1989).

2.2.1.4. Malignant hypertension

Chronic hypertension can enter an accelerated phase in which necrotic arteriolar lesions develop and there is rapid degeneration with papilloedema, cerebral symptoms, and progressive renal failure. Triggered by hypertension due to any cause, it is fatal in less than two years without treatment. However, its progression can be stopped and reversed by appropriate antihypertensive therapy (Ganong, 1989).

2.2.1.5. White coat hypertension

Clinic blood pressure may not always provide an accurate portrayal of an individual's blood pressure, possibly resulting in inappropriate treatment for hypertension (Jula *et al.*, 1999). Kario *et al.* (1999) note that body position and physical activity are two major determinants of short term fluctuations of blood pressure. However, a further factor is the phenomenon of white coat hypertension, which is the acute elevation of blood pressure with an otherwise normal blood pressure profile (Owens *et al.*, 1999). The phenomenon is due to the presence of a doctor or medical professional.

White coat hypertension is common in hypertensive patients, in addition to the general population (Cesana & Zanchetti, 1995; Owens *et al.*, 1999; Schettini *et al.*, 1999). The incidence has been reported as ranging from 12 to 50%, however the figure of 25% is most often cited (Pickering, 1993; Mansoor & White, 1994; Owens *et al.*, 1999). A white coat effect is also noted with pulse pressure, while clinic blood pressure is being recorded (Verdecchia *et al.*, 1998). Mancia *et al.* (1985) demonstrated that white coat hypertension persists even after several visits.

White coat hypertension has been associated with morbidity, while others report no association at all (Owens *et al.*, 1999) or at least having an, at present, unknown pathological significance (Pickering, 1993). Owens *et al.* (1999) considered that there was a middle ground of substantially less risk than sustained hypertension, but a greater risk than that of a normotensive.

In addition to White Coat Hypertension, Pickering *et al.* (1988) and Schettini *et al.* (1999) report differences in blood pressure related to the order in which measurements by different staff were performed, noting a difference between whether a nurse or doctor was taking a blood pressure measurement. The latter, often recording a higher blood pressure. The patients were likely to be more at ease with the nurses, who they perceived to have a lesser social standing. These authors also note cultural differences between professional staff and subjects that may explain some white coat hypertension.

Elevated blood pressure should not be diagnosed from a single reading because of the aforementioned reasons. However, it is recommended that home blood pressure readings not be used for initial assessment of patients (New Zealand Guidelines, 2000).

2.2.3. Effects of hypertension

Hypertension is associated with a number of serious disorders. When blood pressure is high, the heart works harder against an increased after-load caused by the elevated blood pressure. To compensate, the cardiac muscle hypertrophies, thus left ventricular hypertrophy is a sign of chronic hypertension. The total oxygen consumption of the heart, already increased by the higher workload, is increased further because of the hypertrophy. Therefore, any decrease in coronary blood flow is likely to have more serious consequences in hypertensive patients than is found in normotensives. Coronary narrowing that does not produce symptoms or signs in a normal sized heart may produce *angina pectoris* or myocardial infarction when the heart is enlarged. Hypertension is also a factor in the development of artherosclerosis. In addition, continued high blood pressure may produce stroke or brain haemorrhage (Tortora & Grabowski, 1996).

2.2.4. Treatment of hypertension

A detailed description of the treatment of hypertension is beyond the scope of this review, however an overview of the recent history of treatment and general strategies is presented.

Prior to the introduction of effective hypotensive drugs almost 50 years ago, a patient's survival from hypertension left untreated related closely to blood pressure level (Littler, 1984). This author noted that the reduction of blood pressure seemed to be the basis of the therapeutic effect, rather than any intrinsic property of a therapeutic drug. Hansson (1999) found, in analysis of data obtained from the Hypertension Optimal Treatment (HOT) study, that optimal protection against major cardiovascular events was for an SBP in the range of 130-140 mm Hg and a DBP range of 80-85 mm Hg. Hansson (1999) concluded that the best the quality of life resulted from the lowest blood pressure. Comparing these values with those in Table 2.1., it is evident that these SBP values are in the high-normal range, while the DBP is in the normal range. Hansson (1999) is presumably providing these

figures as desirable targets for treatment of hypertensive patients rather than ideals as presented in Table 2.1. However, Vidt & Pohl (1999), analysing the same data, note that those patients who achieved a goal DBP of 80 mm Hg did not experience fewer cardiovascular events than those who had a goal DBP of 85 or 90 mm Hg. In addition, al-Roomi *et al.* (1990) and Minami *et al.* (1998) found that a low DBP, less than 80 mm Hg, might be associated with a higher risk of myocardial infarction and stroke, especially in elderly hypertensive patients and those with CHD.

Al-Roomi *et al.* (1990), Laragh (1992), Alderman *et al.* (1998) and Zafari & Wenger (1998) all found that blood pressure control *per se* did not prevent cardiovascular events, especially in hypertensive patients. These authors suggest additional preventative intervention or cause-specific therapy supporting the contention of Reavan (1991) with regard to Syndrome X (see 2.7). The consensus is to reduce blood pressure (Flack *et al.*, 1995) towards normal values, however the additional treatment required to further reduce high-normal blood pressure to normal may not be desirable.

2.2.4.1. Lifestyle changes

Medical practioners usually suggest lifestyle changes to reduce high blood pressure as first line of treatment against hypertension (Tortora & Grabowski, 1996). These strategies usually include one or more of the following; a weight loss regime, limiting alcohol intake, stress management and exercise. Doctors recommend stopping smoking as it is associated with increases in plasma triglycerides and decreased HDL concentration, in addition to the maintenance of higher blood pressures than non-smokers (Bolinder & de Faire, 1998), increasing the risk of CHD.

Doctors also advise patients to maintain recommended dietary intakes of potassium, calcium, and magnesium. As roughly half of hypertensive patients are salt-sensitive, the consensus is to reduce sodium intake (Whelton *et al.*, 1998). It is apparent that the elderly, and more severe hypertensives respond better to salt reduction (New Zealand Guidelines, 2000). However, McCarron (1997) has argued that the effectiveness of the sodium reduction is linked to the adequacy of the mineral content of the diet. If the RDAs of

calcium, potassium and magnesium are met or exceeded in the diet a concurrent high sodium intake is not associated with elevated blood pressure.

Weight-loss and exercise are highly recommended by medical professionals as blood pressure and obesity are directly related at all ages from childhood to adulthood (He *et al.* 2000). Those who gain weight with age seem to have the greatest risk of becoming hypertensive (Julius *et al.,* 2000). In addition, Julius *et al.* (2000) reported that those who have high blood pressure initially also tend to gain weight thereafter. Studying participants in the Trials of Hypertension Prevention (1987 - present), He *et al.* (2000) concluded that even relatively short-term weight loss and sodium intake may be effective in long term primary prevention of hypertension. After a follow-up of an average of 7 years, an 18-month weight-loss programme was associated with a 77% reduction in the incidence of hypertension compared to the control group. A corresponding sodium reduction programme resulted in a 35% reduction in hypertension. The mechanisms for the long-term beneficial effects were unknown. However, Reavan (1997) cautions against weight loss by low-fat, high-carbohydrate diets which can accentuate the metabolic disorders associated with Syndrome X, of which hypertension is one facet. Syndrome X is discussed further in 2.7.

A sedentary lifestyle is a major risk factor for cardiovascular disease (Burnham, 1998). In addition, exercise is a useful non-pharmacological tool in reducing the effects of hypertension (Motoyama *et al.,* 1998; Nho *et al.,* 1998), obesity and other disorders and diseases. However, Motoyama *et al.* (1998) also noted that the exercise benefit on hypertension is transitory , stopping when exercise ceases. Weight loss appears to be more effective than exercise for reduction of blood pressure.

2.2.4.2. Nutrition in the prevention of hypertension

Diet can influence the risk of developing hypertension and the resulting consequences. The Dietary Approaches to Stop Hypertension (DASH) study provided evidence that a very palatable low-fat diet rich in fruits, vegetables, and dairy products could lower blood pressure as much as any single drug treatment (Pietinen & Aro, 1990; Appel *et al.,* 1997; McCarron, 1998; Moore *et al.*, 1999). These diets also beneficially change the plasma cholesterol levels. The dietary approach is also supported by de Lorgeril *et al.* (1999) who

analysed data from the Lyon Diet Heart Study that focused on the efficacy of a Mediterranean Diet in reducing or preventing cardiovascular events. They also concluded that a high fibre, low fat diet provided a protective effect for patients following a myocardial infarction. Diets of this nature also have the added benefit of a possible weight-loss, which is also known to lower blood pressure. Other measures are salt reduction, and/or dietary supplementation or augmentation of various minerals. However, an overall healthy diet, as suggested previously, appears to have the most beneficial long term effect.

2.2.4.3. Pharmaceutical therapy

Although there is currently no cure for primary hypertension, certain forms of secondary hypertension can be treated by removing the underlying cause. These drug strategies are usually combined with lifestyle changes (described previously). Blood pressure reduction is carried out by the administration of a variety of specific drugs to reduce blood volume, reduce heart stroke rate and volume and to decrease peripheral resistance.

Diuretics increase elimination of water and sodium in the urine, thereby decreasing blood volume. Angiotensin converting enzyme (ACE) inhibitors are used to block formation of angiotensin II, promoting vasodilation and decreasing aldosterone secretion. ß-blockers inhibit renin secretion, decreasing HR and contractility. Vasodilators relax arterial smooth muscle causing vasodilation. Calcium channel blockers slow Ca^{2+} influx into vascular smooth muscle and myocardial fibres reducing the workload of the heart (Ganong, 1989; Tortora & Grabowski, 1996; New Zealand Guidelines, 2000).

2.3. MINERALS AND BLOOD PRESSURE

The role of minerals, especially calcium and magnesium, in the regulation of blood pressure is controversial. While McCarron (1983) viewed calcium as a key mineral in blood pressure control, subsequent research indicates the role of calcium to be inexorably linked with other minerals and the reactions highly integrated (McCarron, 1991). McCarron *et al.* (1998) later conclude that the focus should be on achieving the RDAs of a full array of minerals not just single nutrients. These other minerals are magnesium, potassium, sodium and chloride (Karppanen, 1991; Reusser & McCarron, 1994). Phosphorous also has

a role (McCarron *et al.,* 1984). Karppanen (1991) considered these essential minerals to have a central role in blood pressure regulation. In support, it is shown that low mineral intake characterises hypertensive patients (Karanja *et al.,* 1994).

2.3.1. Calcium

The adult human body contains about 1200 g of calcium, 99% of which is found in bones and teeth as calcium phosphate. The remainder is found in extracellular fluid, intracellular structures and cell membranes and is responsible for a number of regulatory functions, such as maintenance of normal heart beat, blood coagulation, hormone secretion, intracellular and membrane integrity, nerve conduction, muscle contraction, and enzyme activation (Tortora & Grabowski, 1996).

2.3.1.1. Plasma calcium

Plasma calcium concentration normally ranges between 9.0 to 10.0 mg/dL, with an average of 9.4 mg/dL. Plasma calcium is present in three forms with around 40% in combination with plasma proteins and is, thus non-diffusible. Approximately 10% is diffusible, but is combined with substances such as citrates and phosphates. The remainder is ionised and diffusible (Ganong, 1989).

2.3.1.2. Calcium regulation

Calcium is primarily regulated by three hormones (see Figure 2.1). These are 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$-D_3) formed from vitamin D, the primary action of which is to increase calcium absorption from the intestine. Parathyroid hormone (PTH), secreted by the parathyroid glands, mobilises calcium from bone and increases urinary phosphate excretion. Calcitonin, a serum calcium-reducing hormone secreted by the thyroid gland, has an opposite effect to PTH and inhibits bone resorption. Glucocorticoids, growth hormone, oestrogens and growth factors also affect calcium metabolism (Wood, 1999).

There is a readily exchangeable pool of plasma calcium and the, slowly mobilised, bone pool. However, this latter pool of calcium acts as a buffer against excess plasma calcium

and can remove an excess in approximately 70 minutes. A large amount of calcium is filtered in the kidneys, but 98-99% of the filtered calcium is reabsorbed. This emphasises the importance of the level of urinary calcium in determining the health of an individual.

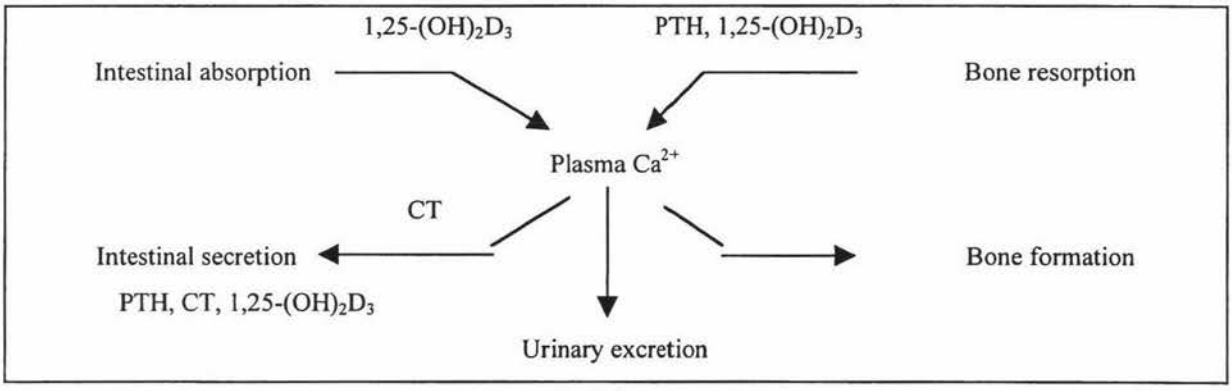

Figure 2.1. Hormonal control of plasma Ca^{2+} PTH; parathyroid hormone; 1,25-$(OH)_2D_3$; 1,25-dihydroxycholecalciferol; CT; calcitonin (from Ganong, 1989, p. 337)

2.3.1.3. Parathyroid hormone

Parathyroid hormone acts directly on bone to increase bone resorption and to mobilise. In addition, PTH also increases phosphate excretion in the urine, while reducing magnesium and hydrogen reabsorption. The PTH action on bones and the kidneys involves adenylate cyclase activation by membrane receptors and a G-protein increasing cAMP formation, which subsequently stimulates PTH secretion.

The active transport of both Ca^{2+} and PO_4^{3-} from the intestine is increased by 1,25-$(OH)_2$-D_3. The formation of 1,25-$(OH)_2$-D3 in the kidneys is regulated by a feedback mechanism from plasma Ca^{2+} and PO_4^{3-}. Its formation is facilitated by PTH, thus when plasma Ca^{2+} is low, PTH secretion is increased. Conversely, when plasma Ca^{2+} is high, 1,25-$(OH)_2$-D_3 production is inhibited and, relatively inactive, 24,25-$(OH)_2$-D_3 is produced instead, thus reducing intestinal absorption of Ca^{2+}. Magnesium also appears to have a direct effect, with an acute decrease in plasma Mg^{2+} concentration stimulating PTH secretion (Wood, 1999).

2.3.1.4. Calcitonin

Calcitonin is a large polypeptide with a primary serum calcium-reducing effect, several times more rapidly than PTH increases serum Ca^{2+}. In addition, it has a secondary role in reducing circulating phosphate levels. Calcitonin receptors are found in bones and the kidneys. Calcitonin's calcium reducing-effect inhibits bone resorption. This action works, firstly by inhibition of the Ca^{2+} permeability of osteoclasts reducing their activity, and secondly, and more long term, by reducing the formation of new osteoclasts. Calcitonin also increases Ca^{2+} excretion in the urine, but this is considered a minor role.

Calcitonin and PTH are released in response to circulating Ca^{2+} passing a threshold of 9.5 mg/dL. After this point, the secretion of calcitonin into the circulation is directly proportional to the level of Ca^{2+}. Reduction of Ca^{2+} below 9.5 mg/dL causes a dramatic increase in plasma 1,25-$(OH)_2$-D_3. Other compounds, including oestrogen, also stimulate calcitonin secretion, thus contraceptives can have an effect on bone density (Ganong, 1989, Wood, 1999).

2.3.2. Calcium and blood pressure

It is well established that there is an inverse relationship between calcium and blood pressure in almost all populations that have been studied (McCarron, 1984; Hatton & McCarron, 1994). Investigation of this relationship has taken several forms.

Epidemiological data from large populations such as the National Health and Nutrition Examination Surveys (NHANES) -1, -2, -3, and -4 surveys have, and are, being examined for this relationship. Various weightings being applied for other factors, thus controversy surrounds the interpretation of this data.

Intervention studies have been applied to various groups and on both normo- or hypertensive subjects with varying interpretations of what levels of systolic and diastolic blood pressures constitute hypertension (see 2.2.1). In some studies the subjects have sufficient calcium in their diet and additional calcium is administered by adding to or substituting calcium-replete foods in the diet, or by giving calcium supplements.

Retrospective studies are where those who have insufficient calcium intake, either from their diets or by insufficient absorption or metabolism, are determined. The effect of the insufficient calcium intake, without attempts to redress the insufficiency, has been analysed as well as that of adding increased calcium by food or supplementation to these subjects.

2.3.2.1. Dietary calcium

A diet low in fat, yet replete in calcium, fruits and vegetables has been linked to reduced blood pressure (Karanja *et al.*, 1994; McCarron, 1998) reductions in osteoporosis and cancer (McCarron, 1998), and a decreased risk of stroke (Suter, 1999).

Research into the relationship of calcium to blood pressure is reviewed by Mikami *et al.* (1990, cited by Kynast-Gales & Massey, 1992) and Osbourne *et al.* (1996). The latter group conclude that dietary calcium played an integral role in normal blood pressure maintenance. In addition, they found that an adequate calcium intake might contribute to a reduction of high blood pressure. In contrast, Seely (1991) suggests that high consumption of calcium in Western society contributes to hypertension. Seely hypothesises that this effect is due to calcification of the aorta and the subsequent loss of elasticity. This has not been pursued by other authors who generally report either no effect or a reduction in blood pressure from appropriate calcium consumption. However, the observations of Seely (1991) may be related to the ionic hypothesis of Resnick (1999) or the impairment of calcium homeostasis reported by Levy, J. (1999) (see 2.3.3.6).

The relationship between dietary calcium intake and blood pressure has also been analysed in several studies, most of them conducted in the United States of America (USA) (Pietinen & Aro, 1990). The results of NHANES-1 (1971 - 1975) have been analysed by four independent groups using different statistical methods. McCarron *et al.* (1984) concluded that inadequate consumption of calcium and potassium were the primary nutritional markers of systolic hypertension. Harlan *et al.* (1984) also found an independent association between dietary calcium and blood pressure. However, in two later analyses (Gruchow *et al.* 1985 and Sempos *et al.* 1986, cited by Osbourne *et al.*, 1996) based on the same material, no association was found between calcium intake and blood pressure.

Hatton & McCarron (1994) report that diets with restricted calcium intake are associated with elevated blood pressure in epidemiological studies. Ackley *et al.* (1983), McCarron (1983) and Karanja *et al.* (1994) report that hypertensive patients consumed less calcium than normotensives. However, these authors also report that a diet deficient in calcium is almost always deficient in other minerals such as magnesium, and sodium. In contrast, Narayan *et al.* (1998), analysing the diet of a group of Native Americans, report higher blood pressure in those subjects who had higher levels of dietary calcium.

Outside the USA, population studies have also found that calcium intake has been inversely associated with blood pressure. This association is strongest in population groups where the mean calcium intake is relatively low. However, it is also evident in the Netherlands where the mean calcium intake is 1000-1200 mg/day, the recommended daily allowance (RDA) in the USA (Kromhout *et al.*, 1985; Kok *et al.*, 1986, cited by Pietinen & Aro, 1990). In addition, van Leer *et al.* (1995) found, in this population, that a diet rich in potassium and magnesium, as well as calcium, was associated with lower blood pressure.

Pietinen & Aro (1990) report that the most consistent finding in these studies has been an inverse association between consumption of dairy products and blood pressure. In addition to calcium, dairy products contain a variety of nutrients, and possibly other factors, which may affect blood pressure. Rusoff (1987) reports that low intake of calcium reflects avoidance of dairy products by older persons concerned with heart disease and that deficiency of calcium among North Americans is far more serious than the concern over the cholesterol content of dairy products. As noted earlier, additional deficiencies occur as a result of this avoidance as dairy products also are rich in potassium and provide some magnesium.

2.3.2.2. Calcium supplementation

Supplementation of calcium has a variable response in comparison to dairy food or calcium-enriched dairy food. Supplementation falls into two categories: The first is to add the mineral to the diet as an additive; the second is to make up either a shortfall of calcium in the diet or to enhance the calcium-rich elements of the existing diet to achieve RDA levels or exceed them. The physiological characteristics of the subjects who are

administered calcium supplementation may differ. These factors include dietary deficiency, sufficiency but with impaired absorption, or defective metabolism.

Bucher *et al.* (1996) conducted a systematic review of randomised controlled trials of the effect of calcium supplementation on blood pressure. Their analysis included strict criteria for trial inclusion. They found some evidence of an effect of calcium supplementation of between 1000-2000 mg/day might have an effect on systolic, but not diastolic blood pressure. However, this effect was smaller than that found for other blood pressure reduction strategies such as potassium supplementation and drugs. In contrast, a later meta-analysis by the same group (Griffith *et al.*, 1999), which included ten new trials, found small decreases in both systolic and diastolic blood pressure from calcium supplementation.

Sacks *et al.* (1998) examined the effect of supplementation of calcium, magnesium and magnesium on normotensives with low intakes of these minerals. That there exists a group of normotensives that are deficient in these minerals confounds the overall effect of minerals. Sacks *et al.* (1998) hypothesised that this group may be more sensitive to supplementation. However, they concluded that potassium, but not calcium or magnesium, supplementation had a small effect in lowering blood pressure in the sample group. These authors also concluded that supplementation with magnesium and calcium may interfere with the blood pressure lowering action of potassium. This hypothesis is also used to partially explain the differing results from the calcium supplementation trials. McCarron and Morris (1985, cited by Kynast-Gales & Massey, 1992) suggested that the degree of elevation of blood pressure may have an effect on the response to calcium supplementation, so that the higher the blood pressure, the greater the effect of the supplementation.

2.3.2.3. Calcium absorption

Calcium is actively transported out of the intestine by a system in the brush border of the epithelial cells that involves a calcium-dependant ATPase, regulated by 1,25-$(OH)_2$-D_3 and PTH (described in 2.1.1). Calcium absorption is high when calcium intake is low and decreased when the calcium intake is high. There is also some absorption by passive diffusion. A deficiency of vitamin D may lead to poor absorption of calcium from the

intestine, which may lead to hypocalcaemia and, further, to rickets where the bone structure becomes deficient.

Approximately one-third of the intake is absorbed in the intestines, and one-quarter is secreted in the gastrointestinal fluids, thus there is a positive calcium intake. McCarron (1997) points out that the amount of calcium in the diet may be sufficient, however, there may be factors that inhibit the rate or amount of absorption. Lactose enhances calcium absorption (Rusoff, 1987; Flynn, 1992). However, dietary compounds in other food groups decrease calcium absorption and excess calcium is known to decrease phosphate absorption. Calcium is poorly absorbed in the intestines because of the relative insolubility of many of the compounds that calcium forms. Calcium often binds with phosphates and oxalates or alkalis which form insoluble calcium soaps which are poorly absorbed. Some vegetables, which contain appreciable amounts of calcium, also contain oxalates (e.g. spinach, beet grains, rhubarb) or phytates (e.g. unmilled cereals) that bind the calcium as calcium-oxalate or calcium-phytate, that are poorly absorbed. This observation brings in to question the common practice of having milk and cereal together, if calcium intake is the primary goal of the milk intake. Rusoff (1987) recommended that calcium supplements be taken between meals to prevent the inhibition of calcium by these mechanisms and to minimise the inhibition of iron absorption by calcium.

Percent absorption or absorption fraction of calcium is higher with lower oral loads and decreasing significantly as the load increases (Heaney *et al.*, 1990, cited by Osbourne *et al.*, 1996). This is mainly due to the regulation in the amount of reabsorption in the kidneys by PTH. However, it has been suggested that there may a contribution from either the active and passive intestinal absorption mechanisms (Bronner & Pansu, 1999) or the dual transport mechanisms at the cellular level (Wood, 1999). The thresholds described earlier for these mechanisms may partially explain an apparent threshold of 800 mg/d calcium intake versus blood pressure noted by McCarron *et al.* (1984). This interdependence of minerals at the cellular level highlights the difficulty in isolating one mineral's effect from the effects of others (Flynn, 1992).

2.3.2.4. Bioavailability

There is no clear, widely accepted definition of bioavailability, especially when applied to dietary minerals and trace elements (Flynn, 1992; Whiting, 1996; Weink *et al.*, 1999). Weink *et al.* (1999) list five definitions for bioavailabilty from various sources:

- That portion of the total [substance] which is metabolisable.
- The measure of the proportion of the total in a food or diet that is digested, absorbed and metabolised by normal pathways.
- The proportion of the total [mineral] in a food, meal or diet that is utilised for normal body functions.
- The percentage of ingested [mineral] that becomes available for metabolic action.
- The measure of the ability of man and animals, or the effectivity, by which nutrients, in a given chemical form, are liberated from food in the presence of certain food components. It moreover includes intestinal absorption and transport of nutrients to organs and cells, where they finally fulfil their physiological function.

Mean calcium absorption from cows milk in healthy human adults has been variably reported as ranging from 21.4%, to 45.5% (Flynn, 1992) depending on a variety of other factors, such as age and concurrent diet.

2.3.2.5. Abnormal calcium metabolism

Abnormal calcium metabolism is suggested to be one of the contributory factors in primary hypertension (Grobee & Hoffman, 1986). Hatton & McCarron (1994) report that an apparent abnormality in calcium metabolism that occurs as a subset of hypertensive patients may contribute to hypertension. The deficit presents as increased circulating PTH, reduced serum phosphorous, low plasma renin activity and increased urinary calcium.

Lind *et al.* (1987) found evidence that the sympathetic nervous system was involved in abnormal calcium metabolism while studying ß-blockers as a hypertensive treatment,. This mechanism may be associated with the reduction in diurnal variation noted in some hypertensive patients. Julius *et al.* (2000) also reports increased sympathetic activity that may be the cause of obesity associated with hypertension and the possibility of a reverse relationship (see 2.6.2).

2.4. OTHER MINERALS

Kotchen & Kotchen (1997) report that analysis of NHANES-1 data found that only calcium and phosphorous intake and alcohol consumption were variables having a significant, consistent and independent relationship to blood pressure. In addition, differences were found between males and females.

Sacks *et al.* (1995) found that combinations of potassium, calcium and magnesium supplements did not have any apparent blood pressure lowering effect. Kotchen & Kotchen (1997), McCarron (1997) and McCarron *et al.* (1998) support the concept that maintaining an adequate dietary mineral intake, specifically of calcium, magnesium and potassium, protects against high blood pressure in humans.

2.4.1. Sodium

The consumption of salt alone was long regarded a leading cause of elevated blood pressure. However, after examining data from the INTERSALT study, McCarron (1991) viewed the effect of sodium in the diet in the development of hypertension as inconclusive and overemphasised. However, he later stated that sodium and calcium metabolism were closely linked (McCarron, 1997).

The salt sensitivity of blood pressure is related both to the anion ingested with sodium as well as to other components in the diet. In several experimental models of salt-sensitive hypertension and in humans, blood pressure is not increased by a high sodium intake provided with anions other than chloride. Epidemiological and clinical evidence suggests that sodium chloride-induced increases in blood pressure are augmented by diets deficient in potassium or calcium. The effect of dietary sodium on blood pressure is modulated by other components of the diet (Kotchen & Kotchen, 1997). Animal models show that the significant proportion of blood pressure variability in response to sodium chloride can be linked to adequacy of the mineral content of the diet (Kotchen & Kotchen, 1997; McCarron, 1997). Kotchen & Kotchen (1997) and McCarron (1997) report that when adults meet or exceeded the RDA's of calcium, potassium, and magnesium, the simultaneous ingestion of a diet high in sodium chloride is not associated with elevated

blood pressure. McCarron (1997) considered this association to imply some form of protective mechanism from a generally adequate mineral intake. When this situation is attained, a higher sodium chloride intake in these adults is most likely associated with the lowest blood pressure in the society (Kotchen & Kotchen, 1997).

2.4.2. Potassium

Low potassium intake is associated with high blood pressure (Whelton *et al.,* 1997). Both Krishna & Kapoor (1991) and Gallen *et al.* (1998) found that dietary potassium restriction increased hypertensive patients blood pressure. In addition, urinary excretion of calcium and phosphorous increased and serum PTH levels were raised. These authors considered that both sodium retention and calcium depletion might be contributory factors to the blood pressure increase while potassium is depleted.

2.4.3. Phosphorous

Foods high in protein, such as meat, milk, eggs and cereals, are also generally good sources of phosphorous (Wood, 1999). Phosphorous is readily absorbed in the intestines, for example, absorption of phosphorous from red meat is > 70%. However, phosphorous absorption is hindered by an excess of calcium. In this situation, calcium and phosphorous form almost insoluble calcium phosphate compounds in the intestine. However, calcium phosphate is a major structural component of bones and teeth.

Like calcium, phosphate is controlled by PTH around a plasma concentration threshold. Below a critical value of 1 mmol/L, no phosphate is excreted in the urine, however, above this concentration the amount of excretion is directly proportional to the plasma concentration.

2.5. DAIRY PRODUCTS

2.5.1. Introduction

Ackley *et al.* (1983) suggested that a component of dairy products had a protective effect against hypertension. These authors considered this to be calcium. However, as mentioned previously, Rusoff (1987) reported avoidance of dairy products by older persons and that additional deficiencies occur as dairy products are also rich in potassium and provide some magnesium. Some dairy products, such as cheese and butter, have sodium chloride added to varying degrees (Flynn, 1992) which may have deleterious effects if other minerals are deficient, as noted previously. However, cows milk and derived products, such as cheese and yoghurt, are excellent sources of calcium (McCarron, 1998). Significantly, attaining a daily dietary intake of calcium in excess of 300 mg/d is difficult to achieve without dairy products (Schaafsma, 1984, cited by Flynn, 1992; Wood, 1999).

Bioavailability of calcium was discussed in 2.3.3.5. Dairy products have a negative aspect in that they also contain significant amounts of dietary lipids that are reviewed in 2.6.

2.5.2. Milk

Milk is regarded as the best source of calcium for most populations. However, there appears to be an increasing group of people who are lactose intolerant (Wood, 1999) and, therefore, must seek calcium from other sources. The 20 essential minerals or trace elements for humans all occur in both human and cows milk (Flynn, 1992). These essential minerals include calcium (~1200 mg/L), magnesium (~120 mg/L), sodium (~500 mg/L), potassium (~1500 mg/L) and phosphorous (~950 mg/L). Van Beresteijn *et al.* (1990) found a small hypotensive effect with milk consumption that they attributed to the essential minerals found in milk. This effect was confirmed by Buonopane *et al.* (1992) and Barr *et al.* (2000) who also found reductions in blood pressure using skim milk. Buonopane *et al.* (1992) observed a reduction in serum cholesterol in a high cholesterol (≥ 190mg/dL) subgroup. However, Barr *et al.* (2000) found no change in blood cholesterol. The latter group were healthy and older.

2.5.3. Another Dairy Factor?

To further confound the mineral debate, in addition to the effect of calcium on blood pressure, there is an unknown factor in sour milk that was effective in reducing blood pressure in hypertensive Japanese (Hata *et al.*, 1996, Kawase *et al.*, 2000). Sour milk is produced by fermenting milk with bacteria such as *Lactobacillus helveticus*, *Lactobacillus casei, Streptoccus thermophilus or Saccharomyces cerevisiae.* Reduced SBP and DBP was observed in the sour milk group, but not in the placebo group (Hata *et al.*, 1996). Kawase *et al.* (2000) noted an increase in serum HDL and reduced TG. These effects have not yet been noted in non-Asians.

In the study of Hata *et al.* (1996), the calcium content was constant between both drinks and was lower than the effective dose for lowering blood pressure in previous reports. They concluded that the blood pressure lowering was due to another factor. This was considered to be one of two tripeptides found in the sour milk, both of which act as ACE-inhibitors. In addition, blood pressure remained decreased for 4 weeks after the study. They also found no significant differences in total cholesterol (TC), high density lipoproteins (HDL) or triglycerides (TG) between the sour milk and placebo groups. Hata *et al.* (1996) concluded that supplementation of the daily diet with sour milk was a practical and useful way to reduce hypertension.

2.5.4. Conclusion

Pietinen & Aro (1990) reported that the most consistent finding in the population studies was an inverse association between consumption of dairy products and blood pressure. In addition to calcium, dairy products contain a variety of nutrients and possibly other factors that may affect blood pressure.

Sacks *et al.* (1998) report that dietary intakes of potassium, calcium and magnesium have each been associated with reducing blood pressure. However, supplementation trials on normotensives have not demonstrated this effect convincingly. Supplementation would appear to be an ineffectual method for delivering calcium. It seems that the most effective

method is in the adequate or increased consumption of dairy products. However, the limiting effect of other foodstuffs should also be considered.

An inverse relationship has also been found, to varying degrees, when comparing blood pressure with magnesium, potassium and sodium. Therefore, it has been difficult to demonstrate the effectiveness of calcium alone. A parallel relationship exists between the intakes of calcium, potassium and other components of dairy products which hampers statistical interpretation of the data and, therefore, it is not possible to determine conclusively the independent effects of these nutrients from the results of cross-sectional epidemiological studies.

It is apparent that dairy products such as milk and cheese have a greater effect than supplementation with calcium alone. Dairy products also have magnesium and potassium, in addition to calcium, so that the effect is difficult to isolate from the effects of these other minerals or possibly other factors. These differences may contribute to the contradictory results from the effect of calcium on blood pressure. However, isolation of these factors may not be useful as there may be a synergistic effect between minerals as suggested by Green *et al.* (2000).

2.6. BLOOD LIPIDS

A significant risk factor for developing heart disease is a high blood cholesterol level. Fatty plaques build up on the walls of arteries and may lead to occlusion of the vessel. Lipoproteins are formed in the liver and intestines so that lipids, can be transported in the blood.

There are three classes of lipoproteins currently known. These are classified as low-density (LDL), high-density (HDL) and very low-density (VLDL). Each has varying composition of protein, triglycerides and cholesterol. The major cholesterol-carrying lipoprotein is LDL, therefore an elevated plasma LDL level is a major risk indicator for CHD. Reducing plasma LDL levels is the primary dietary intervention goal. The HDL group removes excess cholesterol from cells and transport it to the liver for elimination. This mechanism prevents

accumulation of cholesterol in the blood and so a high HDL level indicates a reduced risk of CHD. The VLDL group transports triglycerides from the liver to adipose tissue. However, deposition of the triglycerides transform these cells to the LDL form. Table 2.2 shows the levels of the three classes of lipoproteins that may be found in the blood, in addition to ratios that are indicative of risk factors for CHD.

Table 2.2. Blood lipid concentrations

	Desirable	Borderline	High	Very High
TG (mmol/L)	< 2.3	2.3 – 4.5	4.5 – 11.3	> 11.3
TC (mmol/L)	< 5.2	5.2 - 6.2	> 6.2	-
HDL (mmol/L)	> 1.0	0.9 – 1.0	< 0.9	-
VLDL (mmol/L)	< 0.5	0.5 – 0.9	0.9 – 2.3	> 2.3
LDL (mmol/L)	< 3.4	3.4 - 4.1	> 4.1	-
TC/HDL	< 4.5`	4.5 – 5.5	> 5.5	-
LDL/HDL	< 3.0	3 - 5	> 5.0	-

Data adapted from National Cholesterol Education Programme Committee (1993), "Summary of the Second report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation, and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)", *Journal of the American Medical Association*, 269: 3017, and New Zealand Guidelines, 2000.

Total cholesterol (TC) in blood plasma is also used as a risk indicator for CHD. The risk of myocardial infarction increases two-fold for every 1.3 mmol/L increase in TC once the level is over 5.2 mmol/L (Heyward, 1998). There is an inverse relationship between plasma HDL levels and the incidence of coronary heart disease. An HDL > 1.6 mmol/L is considered a negative risk factor. The New Zealand mean HDL is 1.5 ± 0.01 mmol/L. The ratio of TC to HDL, and LDL to HDL, are also important risk indicators of CHD. The abnormal lipid metabolism associated with the cluster of factors called Syndrome X (see 2.7) presents as low HDL and high TG.

Table 2.3 shows the relative risk associated with the inverse relationship between plasma HDL and the incidence of CHD. Average risk of CHD is 1.00. Risk values greater or lesser than 1.00 indicate varying degrees of risk (Cholestech L.D.X. manual: Cholestech Corporation, California USA).

Table 2.3. Relative Risk of Coronary Heart Disease for people 50-80 years of age

HDL	Relative Risk	
mmol/L	Women	Men
0.78	-	1.82
0.91	-	1.49
1.04	1.94	1.22
1.17	1.55	1.00
1.30	1.25	0.82
1.42	1.00	0.67
1.55	0.80	0.55
1.68	0.64	0.45
1.81	0.52	-

From Framingham Heart Study, cited in Cholestech manual, p.2 (Cholestech L.D.X., Cholestech Corporation, California USA).

It has been demonstrated that there is a direct relationship between the intake of a high saturated fat diet and blood cholesterol (Committee on Diet and Health Food and Nutrition Board, 1989, cited in the American College of Sports Medicine (ACSM) manual, 1998 p.6). These authors demonstrate that changes in saturated fat intake account for 60% of the change in blood cholesterol. Restriction of dietary fat, saturated fat and cholesterol are known to reduce blood cholesterol. A relationship has also been demonstrated between blood cholesterol levels and body weight (Denke *et al.,* 1993). Thus, reducing excess body weight has a two-fold benefit in that both blood cholesterol and blood pressure are reduced.

Fatty acids in the diet, lipoproteins, CHD and the various interactions associated with these factors are reviewed by McNamara (1992), who concludes that dietary fat saturation affected all aspects of lipoprotein metabolism and that the effects are complicated and contradictory due to the heterogeneous nature of the subjects. Saturated fatty acids, occurring with calcium in dairy foods, might be expected to raise the concentrations of total and LDL cholesterol in plasma. Karanja *et al.* (1994) suggested that the benefits of increased calcium in dairy products may be offset by the saturated fatty acids also found in diary products. However, Karanja *et al.* (1994) found that the study of hypertensive patients was confounded by the presence within this group of other risk factors associated with CHD and hypertension such as obesity and hyperlipidaemia. This group of factors is

otherwise known as Syndrome X (see 2.7). Karanja *et al.* (1994) found that increased saturated fatty acid intake from dairy sources did not significantly affect the plasma lipid or lipoprotein concentrations. Sacks *et al.* (1987) found no effect of replacing dietary fats with carbohydrate or linoleic acid in mild hypertensive patients. However, Karanja *et al.* (1994) report 'favorable changes' in plasma lipid and lipoprotein concentrations after ensuring adequate calcium intake. This may reinforce the desirability of consuming milk and dairy products and indicates a positive relationship between calcium consumption and less desirable nutrients as noted earlier. In New Zealand, Beaglehole & Jackson (1985) reported a reduction of death rates from CHD associated with the reduction in the consumption of dairy products. However this reduction is also associated with reduced smoking, increased activity, and improved control of hypertension in those patients with CHD. Therefore the contribution of dairy products to CHD is not clear.

Bullen *et al.* (1998) studied New Zealand Caucasians aged between 65-84 years old and found that the distribution of major cardiovascular risk factors is similar to that of other Western populations. Less than half engaged in physical activity, over one-thrid were regarded as overweight and 10% were obese. The TC levels were higher in women, with almost half of the women having a TC level of ≥ 6.5 mmol/L. Only 17% of the men were in this category. Around half of the study group had elevated blood pressure or were on antihypertensive medication. These authors concluded that there is considerable potential in prevention of cardiovascular disease amongst older people (Bullen *et al.*, 1998).

2.7. SYNDROME X

Syndrome X, also known as Insulin Resistance Syndrome, or the Metabolic Syndrome, is the term to describe a cluster of metabolic disorders with which hypertension is associated. These factors are often found grouped within the same individual. Reavan & Hoffman (1987) proposed that metabolic abnormalities of glucose and insulin, such as insulin-resistance and hyperinsulinaemia, were linked to hypertension. This association was later seen to include abnormal lipid and mineral metabolism, abdominal obesity, non-insulin-dependant diabetes mellitus, and increased risk of cardiovascular events (Reavan, 1991; Timar *et al.*, 2000). Impaired calcium homeostasis is also reported in many of the tissues associated with the disorders of Syndrome X (Levy, J., 1999).

Resnick (1999) has proposed a unifying ionic hypothesis to link the elements that comprise Syndrome X. Characteristics of this hypothesis are elevated cytosolic free calcium and reduced intracellular free magnesium which may alter tissue function. Thus, in blood vessels vasoconstriction occurs, cardiac muscle hypertrophies, fat and skeletal muscle develops insulin resistance and increased sympathetic nervous activity occurs.

The cardiovascular risks associated with hypertension are enhanced by obesity that, in turn, may be a result of metabolic disorders. In addition, obesity increases the chance of developing insulin-resistance and is suggested to be linked to altered lipid metabolism (Toft *et al.,* 1998). Reavan (1991) considered that therapy that does not address the metabolic disorders, in addition to the treating the elevated blood pressure, will not be effective in decreasing the risk of CHD. This is emphasised by Lind *et al.* (1991) reporting that some drugs commonly used against hypertension, such as thiazides and β-blockers, induce impairment in glucose and lipid metabolism. The cure may contribute to a worsening of the general health of the patient.

CHAPTER 3: METHODOLOGY

In this chapter the means of attaining physical data are outlined. Blood pressure measurement methods, determination of body composition by various means and fitness testing are reviewed.

3.1. BLOOD PRESSURE MONITORING

Non-invasive monitoring of blood pressure by various means is an essential tool for health professionals in determining the health and well being of their clients. Blood pressure can be measured in different environments, such as clinic, home or workplace, by different personnel, doctor, nurse, or the patient, and with different kinds of monitors, such as auscultatory, automatic or ambulatory (Jula *et al.*, 1999; Schettini *et al.*, 1999).

Pickering (1993) regarded any non-invasive blood pressure recording as a surrogate estimate of the true blood pressure. However, non-invasive devices have been validated with intra-arterial methods (Majahalme *et al.*, 1998) and by concurrent blood pressure recording with other devices (Jula *et al.*, 1999), indicating that the invasive approach is neither required nor practical for routine usage. The indirect measurement of blood pressure routinely performed by auscultation is prone to several sources of error which inevitably lead to inaccuracy (Reid *et al.*, 1991). In addition, there are factors of observer bias, blood pressure variability, as well as the potential of the white coat effect (Pickering, 1993).

3.1.1. Ambulatory blood pressure monitoring

The method of taking ABP using a portable recording device over varying periods, up to 24 hours or longer (Abitol *et al.*, 1997; Okutani *et al.*, 1997), has several advantages over the taking of a limited number of blood pressure recordings in a clinical setting. The variability of blood pressure limits the accuracy of the limited number of clinic recordings (Pickering, 1993). However, Jula *et al.* (1999) found that self-measured home blood pressure's averaged over four readings were as reliable as ambulatory monitoring for evaluating untreated hypertension. This approach was not practical for the present study.

Ambulatory blood pressure recording is able to provide highly repeatable information on blood pressure changes throughout the day and night concurrent with normal activities (Reid *et al.*, 1991; Cesana & Zanchetti, 1995) despite the presence of some artefacts and false readings. Ambulatory blood pressure is thought to be a more accurate assessment of the subject's blood pressure status than clinic visits. This is due primarily to the phenomenon of white coat hypertension found in clinic visits. Cesana & Zanchetti (1995) confirm that home and ambulatory blood pressures are lower than clinic. Staessen *et al.* (1999) considered that ABP could not be recommended for screening for hypertension due to the cost of the equipment and training required. However, Reid *et al.* (1991), Mansoor & White (1994), Kawano *et al.* (1998) and Staessen *et al.*, 1999) considered that ABP was useful in selection of patients for hypertensive drug-treatment and monitoring of treatments which may have an effect on blood pressure. In addition, the prognostic value of ABP is thought to be superior to that of office blood pressure (Ohkubo *et al.*, 1998; Verdecchia *et al.*, 1998; Verdecchia, 2000).

Twenty-four hour ABP recording demonstrates the diurnal variation of blood pressure (described in 2.1.2) better than repeated office measurements (Parati *et al.*, 1986). However, Abitol *et al.* (1997) thought that longer periods would be more informative. In addition, 24-hour ABP recording eliminates or reduces limitations of office measurement due to the objectiveness of the measurements by being automatic, the greatly increased number of readings and, often, by being located away from the clinical setting (Pickering, 1993).

Because of the diurnal variation in blood pressure it is common to divide night and day when analysing data from ABP. These are normally day: 6.00-22.00h; night: 22.00-6.00h (Verdecchia *et al.*, 1991; Schillaci *et al.*, 1994; Wallace *et al.*, 1997, 1999). Other divisions of night and day can be found, for example Belsha *et al.* (1997) used divisions of day: 8.00-21.00h and night: 21.00-8.00h for analysis of children and adolescents. These time differences may cause difficulty when comparing data from different studies.

Arguments can be made for not having arbitrary divisions of night and day. Okutani *et al.* (1997) considered that the mid-sleep time should be the reference point or biological zero

hour for all analysis. May *et al.* (1998) used individually defined day and night times. Abitol *et al.* (1997) synchronised subjects by sleep time. Other divisions, such as work and leisure, have also been used to study the effect of work-related stress on ABP (James *et al.*, 1991; Pickering *et al.*, 1996; Kristal-Boneh *et al.*, 1998). However, some researchers use shorter periods for ambulatory monitoring. For example, Majahalme *et al.* (1998) found that five hour periods during the day and night closely correlated with 24-hour recording periods. In addition, there is the effect of sleep disruption (Staessen *et al.*, 1995; Lusardi *et al.*, 1999) which may impact on the results obtained from full 24-hour monitoring.

Researchers have found that the mean ABP is not predictable from office blood pressure (Porchet *et al.*, 1986, cited by Reid *et al.*, 1991). However, the difference diminishes as more readings are taken due to regression towards the mean (Mansoor & White, 1994) as would be expected. There has been considerable debate as to reference values for ABP (Ohkuba *et al.*, 1998; Jula *et al.*, 1999; Schettini *et al.*, 1999). While several studies of large groups of people have taken place, Ohkubo *et al.* (1998) considered values derived from these cross sectional studies as distributions, not definitive values, in the same manner as weight for height tables. This group made a distinction between those values for blood pressure obtained from office blood pressure recording and those obtained from ABP recording. Normal range upper limits recommended for 24-hour ABP recording are reported to be in the range of 129-135 mm Hg SBP and 80-85 mm Hg DBP for various populations (Ohkubo *et al.*, 1998; Schettini *et al.*, 1999). Comparing these values with those in Table 2.1, it is evident that the SBP values described by these authors fall into the lower range of the high-normal, while the diastolic values are less than the high normal. However, using the Dynapulse 5000A (Pulse Metric Inc. California, USA) ABP recording device Green *et al.* (2000) note over-reading of ambulatory SBP by a mean of 8.25 mm Hg which may partially explain some of these observations by those using the same device. This result is in excess of the ± 5.0 mm Hg accuracy claimed by the manufacturers.

3.1.1.1. Inconvenience

While Moore *et al.* (1999) reported excellent acceptance of the ABP devices, wearing them can modify the behaviour of the subjects. For example, for an accurate recording it is essential that the subject remains still (Green & Madigan, unpublished observation) which

means both a behaviour modification for some subjects and an additional stress factor for others. The subjects stop their activity or are caught by surprise at the start of the measuring cycle. In addition, many subjects suffer sleep disruption while wearing the devices during sleep periods. Initial measurements made with an ambulatory device are sometimes elevated due, in part, to the novelty of the device and the white coat phenomenon. In contrast, Parati *et al.* (1986) found no alerting reaction due to cuff inflation and no resultant elevated blood pressure. It has also been found that the removal of the device can also cause blood pressure elevation (Owens *et al.*, 1999), perhaps due to further contact of the patient with a health professional (Pickering *et al.,* 1988; Schettini *et al.,* 1999).

Staessen *et al.* (1995) note that ABP recording is widely used despite artefacts due to cuff size, movement, body position, short-term blood pressure variability and sleep interference. However, these authors question the performance of the monitors under truly ambulatory conditions confirming the observation of Green & Madigan, (unpublished observation).

3.2. BODY COMPOSITION

3.2.1. Anthropometry

Anthropometry is the measurement of the size and proportion of the human physique. It is used to assess total and regional body composition, often in the field of nutrition, but also in the health and fitness industry. Compared to skinfold measures, described in 3.2.5., anthropometric methods are relatively simple, inexpensive, and do not require a high degree of technical skill and training (Heyward, 1997), and so it is the method of choice in the clinical setting (Pollock & Jackson, 1984).

The fat content of a body has physiological and medical importance (Durnin & Womersly, 1974). It is considered to affect mortality and morbidity, and can have an effect on the efficacy of drugs and anaesthetics. In addition, bodyfat affects an individual's ability to withstand cold temperatures and starvation, and varies with age, gender, ethnicity, exercise and diet. Reliable and accurate body composition techniques are needed for monitoring bodyfat content and, thus, nutritional status.

Measurement of height and body mass are among the most reliable of all anthropometric dimensions (Frisancho, 1981). In addition, there is the added advantage that these measurements are obtained in a non-invasive, socially acceptable manner. The measures of weight and height, and some circumferences, fulfil these parameters while providing valuable information. However, Lukaski *et al.* (1985) considered that the use of any indirect method for assessing body composition must result in prediction errors.

Fat, muscle mass and skeletal size affect circumferences of the body, therefore these measures are related to fat mass and fat-free mass (FFM). The latter includes everything apart from the fat (Durnin & Womersly, 1974). Skeletal size is directly related to FFM (Jackson & Pollock, 1978). Seip & Weltman (1991, cited by Heyward, 1997) report that anthropometric equations using only circumferences as predictors estimate the body fatness of obese individuals more accurately than skinfold prediction equations. Compared to skinfolds, circumferences and skeletal diameters can be measured with less error (Bray & Gray 1988a, cited by Heyward, 1997).

Body mass index (BMI, described in 3.2.3.) and waist to hip ratio (W/H, described in 3.2.4.1.) are often used to identify individuals at risk for obesity-related diseases which are associated with the cluster of conditions in Syndrome X (see 2.7.). These are diabetes, hypertension, hyperlipidaemia and cardiovascular disease. Reavan (1991) suggested that there might be a common mechanism for all these conditions. However, the common factor appears to be abdominal obesity, determined by waist circumferences and W/H that may result from metabolic disorders, inappropriate nutrition and/or lack of physical activity. Although obesity is associated with increased morbidity, it is not the absolute excess of adipose tissue, but the regional distribution of bodyfat. Abdominal fatness, irrespective of body size, will predispose such conditions. Paradoxically, it has been found that leanness, rather than obesity, is an indicator of mortality in old people (Burr *et al.*, 1982a, cited by Burr & Phillips, 1984). Fat-free mass is not constant between older and younger adults due to the variation of the constituents of the FFM (Williams *et al.*, 1995). In a study on over 60-year olds, Deurenberg *et al.* (1990) found that the BMI criterion were of reducing value as the subject aged, being more noticeable around 70 years of age and beyond (Burr & Phillips, 1984). This was because of the reduced lean body mass and increased fat mass that generally occurs with ageing. Muscle mass declines with age and at a similar rate in both

men and women (Frisancho, 1981). Associated with the age-related increased fat overall is greater fat deposition internally relative to subcutaneous fat. In addition, there may also be a reduction in height as the subject ages. As described in 3.2.3., changes in abdominal circumferences may better describe obesity and, thus, obesity-related risk factors in the elderly.

Weight and body compositions vary not only with age, gender, and height, but they are also influenced by variations in frame size. Including a frame size determination, such as small, medium or large, can enhance the use of BMI tables. Elbow breadth is often used as an indicator of frame size as Frisancho (1981) showed that elbow breadth is less affected by adiposity than other anthropometric dimensions. However, he questions the validity of commonly used tables that have frame size categories, such as the Metropolitan Life Tables of 1959 and 1983. He also points out that the arbitrary divisions between frame sizes were based on the 1959 measurement of 25-year old male soldiers limiting the applicability of these tables when applied to a heterogeneous population.

Population-specific anthropometric equations are valid for, and can only be applied to, individuals whose physical characteristics (age, gender, level of body fatness, differing ethnic groups and socio-economic circumstances) are similar to those in a specific population subgroup (Frisancho, 1981). For example, Bishop *et al.* (1981) and Frisancho (1981) compared data from NHANES-I with an earlier trial (Ten State Nutrition Survey 1968-70 from the Centre for Human Growth and Development and the Department of Anthropology, University of Michigan, USA) in which subjects were predominantly from regions of lower socio-economic status. Therefore, the triceps skinfolds and arm circumferences were generally less, indicating a lower nutritional status and so were not representative of the 'norms' established from the NHANES-1 data. Frisancho (1981) also points out that the Ten State Nutrition Survey did not include data for subjects older than 44 years and therefore was not applicable to older subjects.

Fukagawa *et al.*, (1990), comparing isotope dilution techniques, BIA and anthropometry, warned that the results from differing body composition assessment methods should not be used interchangeably. They considered that anthropometry was not adequate to assess

between-group differences in body fatness, but that skinfold measures could be used for within-group comparisons. The differences this conclusion is based on are slight and may be regarded as over critical, however the warning is valid.

Deurenberg *et al.* (1990) and Williams *et al.* (1995) report that BIA (described in 3.2.6.) was a better measure of FFM than the anthropometry. Lukaski *et al.* (1986) emphasised that, when comparing anthropometry to BIA, it is important to also consider that anthropometry used localised fat distribution to predict whole-body fat percentages, whereas BIA and densiometry actually used whole-body measures. Reported errors estimating body composition from anthropometry range from 3 to 9% bodyfat (Jackson & Pollock, 1977; Kushner *et al.*, 1984, cited by Segal, 1985; Lukaski *et al.*, 1986). In contrast, the prediction error of BMI for bodyfat percentage is ≥ 5.0% BF (Heyward, 1997).

3.2.1.1. Weight for height

Weight for height indices have been used for many years as an attempt to determine the 'ideal weight' for an individual. These are quick, non-invasive measures, but are deficient in that they cannot distinguish between fat tissue, muscularity or oedema. The best known of these indices are the weight-height index (W/H) and the BMI or Quetelet's Index (W/H^2) which was chosen for the present study. However, several other indices have been employed. For example, the Khosla-Lowe Index (W/H^3), the Benn Index (W/H^b, where b is an allometric exponent), Ponderal Index ($H/\sqrt[3]{W}$) and inverse Ponderal index ($H/W^{1/3}$).

To estimate total bodyfat from weight to height indexes, the index should be highly related to bodyfat, but independent of height. Based on data from NHANES-I and -II, Micozzi *et al.* (1986, cited by Heyward, 1997) reported that BMI is not significantly related to height, but is directly related to skinfold thickness, and the estimated fat area of the arm in adults.

Bloomfield *et al.* (1995) emphasised that these equations all provide measures of ponderosity, which should not be regarded as adiposity. For an individual of any given stature, body mass may vary according to the amount and density of lean body tissue as well as fat. However, a proportionally greater lean body mass and/or skeletal frame size can contribute to apparent excess body weight. Thus, many athletes would be considered

'overweight', yet skinfold tests show a sub-normal amount of adipose tissue. For example, Benkhe (1942, cited by Bloomfield *et al.,* 1995) reported that, in World War Two, 70% of a sample of American college football players were classified as overweight using weight for height indices. These players were rated as unsuitable for military service and classified as 'bad risks' by standard life insurance tables.

Obesity can be underestimated by the simple weight/height ratio. However, in contrast, obesity can be overestimated by the Ponderal index ($H/\sqrt[3]{W}$) because this equation is biased towards height and decreases with increasing obesity. BMI is regarded as the best indice for adults. However, it was not regarded as a valid index for children, people under 25, or over 65 years old, or pregnant or lactating women (Bloomfield *et al.,* 1995). As discussed earlier, differing fat deposition and muscularity amongst different age groups and genders contribute to these limitations. No subjects in the present study were outside of these parameters, thus they could be included in the standard BMI evaluations.

3.2.1.2. BMI Criteria

It is generally regarded that both men and women from 18 years onwards with a BMI between 20-25 have the least risk of morbidity and mortality. A BMI of greater than 40 indicates Grade III obesity (high risk), Grade II (moderate risk) is a BMI of 30-40, Grade I or low risk obesity) is a BMI of 25-29.9. Grade O (desirable range of body fatness) is a BMI of 20-24.9 (Andres *et al.*, 1985, cited by Brodie, 1996). These criteria will be discussed for each subject in Chapter 5.

3.2.1.3. Circumferences

3.2.1.3.1. Waist to hip ratio

The simplest anthropometric measure that reflects body type, is waist to hip ratio (W/H). W/H is calculated by dividing the waist or abdominal circumference by the hip circumference. The W/H can be used to distinguish between patterns of fat distribution in the upper and lower body (Brodie, 1996, Heyward, 1997). The W/H is strongly associated with visceral fat and appears to be an acceptable index of intra-abdominal fat (Seidell *et*

al., 1987, cited by Heyward, 1997). Abdominal obesity is strongly associated with hypertension (Reisin, 1990). A W/H greater than 0.9 for men and 0.8 for women indicates central or android fat distribution. If the W/H is accompanied by a high BMI (> 25), the risk for morbidity and mortality is compounded.

Umbilical circumference alone (ideal: < 100 cm men, < 90 cm women) is now also thought to be a powerful measure of abdominal obesity and health risk in Caucasians (Monash University, 1999). In ethnic groups where the build is slight, such as in many Asian countries, a lesser degree of abdominal fatness may still be associated with a risk of developing chronic diseases.

Brodie (1996) reports that the W/H is not closely related to total bodyfat ($r = 0.39$), but has a higher correlation with the amount of visceral adipose tissue in men ($r = 0.5$ to 0.8). He also reports high reliability in repeat measurements; as much as $r = 0.98$ for the hip circumference, $r = 0.90$ at the waist, and $r = 0.92$ for the W/H.

3.2.1.3.2. Mid-arm circumference

Age and sex specific percentile distributions for the upper arm circumferences, triceps skinfold (TSF) thickness, and mid-arm muscle circumferences of American adults were developed from cross-sectional data collected from NHANES-I (Bishop *et al.*, 1981). Age-related changes were also noted. These data were compared to data obtained from the Health Examination Survey (1960-62) which showed that, for women, all parameters were larger for the later group. However, for men, the figures were similar to the previous data. This observation indicates increasing adiposity amongst women, and, perhaps, decreased physical work amongst the men. Bishop *et al.* (1981) concluded that no single value for each parameter could be considered normal given the diverse nature of the subject group. Thus, they question the validity of currently used sex-specific norms for nutritional assessment of adults.

Frisancho (1981), also using data from NHANES-I for triceps skinfolds and upper arm circumference, recommended that assessments of nutritional status be made on the basis of

fat and muscle area rather than using direct skinfolds and arm circumference. Like Bishop *et al.* (1981), he also recommended replacing norms that were current at the time.

3.2.1.3.3. Mid-arm muscle circumference

The mid arm muscle circumference (MAMC) is a measure of muscle mass and, thus, nutritional status. MAMC is calculated by taking a measurement of the total mid-arm circumference (MAC) and deducting the layer of adipose tissue based on the TSF.

$$\text{MAMC (cm)} = \text{MAC (cm)} - \pi \times \text{TSF (cm)}$$

For adults, the standard normal values for MAMC are: 28 cm (men); 23 cm (women). Measurements of < 23 cm for men and < 18 cm for women are indicative of either borderline or depleted muscle mass.

3.2.1.3.4. Arm muscle area

Arm muscle area (AMA) is another indicator of nutritional status that can be derived from the MAC and TSF (Heymsfield *et al.*, 1982). Originally used to evaluate undernourished children in the field, several assumptions in the calculations are made. These are: that the mid-arm and mid-arm muscle are circular; that the TSF is double the average subcutaneous fat; that the bone atrophies in proportion to muscle under conditions of malnutrition. In addition, the nerve and blood vessels are unaccounted for, although the latter would not be a significant factor for longitudinal studies. Heymsfield *et al.* (1982) reports the errors from these assumptions can amount to an overestimation of 20-25% of AMA. When a correction factor is introduced to allow for the elliptical nature of the arm the overestimation is reduced to 10-15% (Heymsfield *et al.*, 1982). A further reduction of 5-10% is due to the cross-sectional area of bone. Thus, this group presented some new AMA equations for men and women respectively:

Male

$$\text{AMA (cm}^2\text{)} = [(\text{MAC (cm)} - \pi \times \text{TSF (cm)})^2 / 4\pi] - 10$$

Female

$$\text{AMA (cm}^2\text{)} = [(\text{MAC (cm)} - \pi \times \text{TSF (cm)})^2 / 4\pi] - 6.5$$

However, in this study Heymfield *et al.*, (1982) used the Metropolitan Life Tables of 1959 to determine the ideal body weights for these subjects and was perhaps unaware of the shortcomings of these tables as discussed in 3.6. Whether this has any significant bearing on the establishment of these equations is not known, but does indicate that due caution is exercised in interpretation of data obtained using these calculations.

3.2.1.4. Skinfolds

A skinfold thickness measurement is a relatively simple method for assessing total bodyfat. A skinfold indirectly measures the thickness of subcutaneous adipose tissue so that there is a relationship between skinfold thickness and body density. Thus, the sum of several skinfold thicknesses at multiple sites can be used to estimate total bodyfat (Jackson & Pollock, 1976, Quatrochi *et al.*, 1992, cited by Heyward, 1997). In 1951, Brözek & Keys were the first to use this relationship for assessing bodyfat content (Durnin & Rahaman, 1967).

Estimating bodyfat from skinfolds generally uses the convention of regarding the body as two compartments that are mutually exclusive so that one is the bodyfat while the other is the FFM. Calipers are normally used to measure the thickness of a compressed double fold of skin together with the entrapped subcutaneous adipose tissue. This form of assessment is based on a number of assumptions. These are: that the skinfold has a constant compressibility and proportionality in the fold; that fat constitutes a constant proportion of adipose tissue; that the ratio of subcutaneous to internal fat deposits remains fixed, and that there is consistency between individuals. (Bloomfield *et al.*, 1995).

The validity of these assumptions has been refuted by a number of authors (Frisancho, 1981; Lohman, 1981; Martin *et al.*, 1985). Investigating the compressibility of the skinfold Martin *et al.* (1985) by examined cadavers and demonstrated that this factor is dependent on the age and body size of the subject, as well as the state of hydration of the tissues. The assumption that the distribution of fat, both subcutaneously and internally, is similar for all individuals within each gender is questionable as older subjects of the same gender and body density have proportionally less subcutaneous fat than younger. Frisancho (1981) points out that after 40 years of age the compressibility of fat may result in overestimation

of muscle and an underestimation of fat area. Lean individuals have a higher proportion of internal fat and the proportion of internal fat decreases as overall body fatness increases (Lohman, 1981) and internal visceral adipose tissue may increase independently of subcutaneous fat (Enzi *et al.*, 1986). Thus, the correlation between skinfold thickness and body fatness decreases as bodyfat increases.

Within-individual variation of bodyfat distribution suggests the TSF alone may not adequately represent total bodyfat percentage for some subjects. However, the single measure of the triceps skinfold as an estimation of bodyfat is commonly used (Monash University, 1999). For Australian adults, who are similar to New Zealanders, the normal values are 2.5 mm for men, representing approximately 20 % body fat, and 18.0 mm for women or about 30% body fat (Monash University, 1999). Half or less of these measures indicates fat depletion. A TSF of over 20 mm for men and over 30 mm for women are considered obese. This measurement was combined with the data obtained from the mid-arm circumference.

Robson *et al.* (1971) and Zillikens & Conway (1990) explored ethnic differences in skinfold thickness. Their results demonstrated significant differences and indicated the importance of reference standards for each racial group.

Despite the limitations of the assumptions, the skinfold is regarded as a good measure of subcutaneous fat. It has been demonstrated that subcutaneous fat, assessed by skinfold measurements at 12 sites is similar to the value obtained from magnetic resonance imaging (Hayes *et al.*, 1988, cited by Heyward 1997). Skinfolds also have a high correlation ($r >$ 0.80) with results obtained from underwater weighing for body composition (Nieman, 1995). However, these studies would be undertaken with the strictest adherence to protocols and may not reflect conditions found in the field.

3.2.1.4.1. Measurement

Measurements are improved by following standardised testing procedures with well-designed calipers (Edwards *et al.*, 1955; Lohman & Pollock, 1981). Major causes of inter-tester reliability are improper location and measurement of skinfold sites, difference in

anatomical site descriptions, locations and technique (Pollock & Jackson, 1984). The Anthropometric Standardisation Reference Manual (Lohman *et al.*, 1988, cited by Heyward, 1997) provides clear guidelines for making these measurements. However, Durnin *et al.* (1997) found that site location may vary by up to 20 mm and remain sufficiently accurate.

3.2.1.4.2. Derivations

There are over 100 population specific equations to predict body density from various combinations of skinfolds, circumferences and bony diameters (Nieman, 1995; Heyward, 1997). These equations were developed for relatively homogenous populations and are assumed to be valid only for individuals having similar characteristics such as age gender, ethnicity or level of physical activity. Population specific equations will tend to underestimate % BF in fatter individuals and overestimate in leaner individuals due to the normal distribution of a population.

3.2.2. Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) is a rapid, non-invasive, reliable, repeatable, and relatively inexpensive method for evaluating body composition (Lukaski *et al.*, 1985; Heyward, 1997). It is especially appropriate for large group studies, but is less so for individuals (Houtkooper *et al.*, 1996). Williams *et al.* (1995) found that BIA was a useful tool for measuring FFM in older adults, which compared well with anthropometry. However, Deurenberg *et al.* (1990) and Chumlea *et al.* (1993) question the accuracy of BIA when applied to the elderly due to the water content of the body decreasing with age, the intracellular water decreasing more rapidly than extracellular. In addition, they cite the changing fat distribution with age. These observations may have implications for the accuracy of BIA with older subjects such as those participating in the present study.

3.2.2.1. Total body water

The BIA method is a measure of total body resistance (Segal *et al.*, 1985). Estimations can be made on total body water (TBW) from the impedance measurement because of the

electrolytes in the body's water conduct current. A low-level electrical current is passed through the subject's body and the impedance is measured. A low frequency (~1 Hz) current passes mainly through extracellular fluids, whereas higher frequency (~50 Hz) current penetrates intra- and extracellular fluids. As TBW increases current flows more readily due to the decreased resistance. Thus, resistance will be greater in individuals with large amounts of bodyfat, since adipose tissue, with its relatively low water content, is a poor conductor of electrical current. Because the water content of the fat free body is relatively large (approximately 73%; see below), FFM can be predicted from TBW estimates.

3.2.2.2. Assumptions

Bioelectrical impedance analysis makes several assumptions that may contribute to errors. For the purpose of calculation it is assumed the body is shaped like a cylinder with a uniform length and cross-sectional area. However, as this is not so, resistance through different body segments will differ due to variations in composition (Deurenberg, 1996).

3.2.2.3. Validation

However, numerous validation studies comparing BIA with various methods of determining body composition have found BIA to be an accurate consistent method (Lukaski *et al.*, 1985, 1986; Williams *et al.*, 1985; Deurenberg *et al.*, 1990; Fukagawa *et al.*, 1990; Pierson *et al.*, 1991; Chumlea *et al.*, 1993). Segal *et al.* (1988) carried out a four-site cross-validation study to determine the consistency of the method at various stations. This study confirmed the validity of the procedure with respect to other methods of determining body composition. However, Segal *et al.* (1985) earlier found that the derivations provided unsatisfactory results, particularly in obese subjects, owing to the overestimation of FFM with increasing obesity. These workers also comment on the problem of the lack of an error-free benchmark method, so that none of the indirect body composition methods yielded a true value. This may be as a result of the application of factors, such as the hydration constant (see 3.13.1.4) derived from population statistics which, in this case, are from North America. This brings in to question the accuracy of data obtained using BIA techniques on other populations.

Baumgartner *et al.* (1989) showed that the impedance index for the arm alone could be almost as accurate as the whole body impedance, at least for young adults. Settle *et al.* (1980, cited by Chumlea *et al.*, 1993) found that approximately 85% of the whole body impedance could be accounted for by the sum of resistance for the arm and the leg despite these body parts being only 35% of the total body volume. This group suggested that the current in the trunk is not conducted in the same manner as in the arms and legs due to internal adipose deposits in the trunk. This observation has implications for when the fat distribution is focused on the trunk, as is likely with an older or obese subject, that may be additive for the increasing inaccuracy noted for such subjects.

3.2.2.4. Hydration constant

There is disagreement over the exact hydration constant, or proportion of water in the FFM, for use in calculation of FFM by the BIA apparatus. The values being; 72%, 73%, 73.2 % (Pace & Rathburn, 1945, Key & Brözek, 1953, Siri, 1956, 1961, all cited by Hewitt *et al.*, 1993). The hydration constant changes with age resulting in significant errors when body density is used to estimate fat percentage and FFM (Hewitt *et al.*, 1993). These variations also have implications for the calculations used by the BIA apparatus. Hewitt *et al.* (1993) concluded that the use of body density and the Siri two-compartment formula, which assumes 72%, may result in errors of up to 10% fat.

3.3. FITNESS TESTING

The principles behind fitness testing are generally well understood (Astrand, 1965; ACSM, 1998; Heyward, 1997). Several variables are used to evaluate cardiovascular fitness. These are oxygen consumption, HR and blood pressure. Submaximal activity at consecutive incremental levels correlates highly with increasing HR. Oxygen consumption at each workload is calculated using a validated predictive equation. Maximum rate of oxygen uptake is estimated using a linear regression of heart rate and oxygen consumption to an age-determined maximal HR (HR = 220 – age in years). A bicycle ergometer is a low stress, low risk method to administer such a test (ACSM, 1998; Heyward, 1997).

The maximum rate of oxygen uptake is then divided by weight to provide oxygen uptake per minute per kilogram of body weight (ml/kg/min) which is directly comparable amongst individuals. Ranges of maximum rate of oxygen uptake are presented in Table 3.1. Note that the values for men are consistently higher than that for women due to the, generally, greater muscle mass of men. In addition, the values decrease with age due, in part, to the loss of muscle mass.

Table 3.1. Maximal oxygen uptake (ml/kg/min)

	Age	Poor	Fair	Good	Excellent	Superior
Women	**20-29**	≤ 31	32 - 34	35 - 37	38 - 41	42 +
	30-39	≤ 29	30 - 32	33 - 35	36 - 39	40 +
	40-49	≤ 27	28 - 30	31 - 32	33 - 36	37 +
	50-59	≤ 24	25 - 27	28 - 29	30 - 32	33 +
	60+	≤ 23	24 - 25	26 - 27	28 - 31	32 +
Men	**20-29**	≤ 37	38 - 41	42 - 44	45 - 48	49 +
	30-39	≤ 35	36 - 39	40 - 42	43 - 47	48 +
	40-49	≤ 33	34 - 37	38 - 40	41 - 44	45 +
	50-59	≤ 30	31 - 34	35 - 37	38 - 41	42 +
	60+	≤ 26	27 - 30	31 - 34	35 - 38	39 +

From The Cooper Institute for Aerobics Research, Dallas Texas, USA, in Heyward (1999) p.48.

Confounding factors are the elevated HR often observed prior to the commencement of the exercise bout (Raglin *et al.*, 1993). This may flatten the oxygen uptake curve indicating the subject is fitter than they may be. Gadsboll *et al.* (1998) report that during exercise testing of hypertensive patients stroke volume was increased by recruiting pre-load reserve rather than by increased contractility as would normally be expected. This observation reinforces that heart function is compromised during hypertension.

3.3.1. Strength test

Handgrip strength correlates moderately with the total strength of 22 other muscles of the body ($r = 0.69$) (deVries, 1980, cited by Brodie, 1996). Therefore, it gives a reasonable prediction of an individual's total muscular strength ($r \geq 0.90$). Grip strength may vary

daily by between 2 and 12 % (Brodie, 1996). No allowance was made for occupational classes where grip strength may be enhanced by physical work. Like the exercise test, there may be a desire by some subjects to cease effort before they have exhausted themselves.

CHAPTER FOUR: PRELIMINARY TRIAL

4.1. INTRODUCTION

Ambulatory blood pressure (ABP) recording is a valid method for recording blood pressure and heart rate (HR) for varying lengths of time. Before the trial, described in Chapter 5, was undertaken it was necessary to become familiar with the ABP recording apparatus and to identify any shortcomings with its use. In addition, this pre-trial was used to determine whether to use the ambulatory monitors for 24 hours or a shorter period. Data obtained using this method are the ambulatory systolic and diastolic blood pressures (SBP, DBP), mean ambulatory blood pressure (MBP; 1/3 systolic + 2/3 diastolic) and heart rate (HR).

4.2. METHODS

Five subjects (age range: 18-41 years, mean age: 28.6 ± 9.2 years) were recruited from post-graduate students and staff at the Massey University Turitea campus. Each regarded themselves in normal health with normal blood pressure, however none were screened for this prior to beginning the trial. The subjects were asked to wear an ABP and HR recording device (Dynapulse 5000A, Pulse Metric Inc. California USA) for 24 hours on two occasions. In the pre-trial, the ABP was recorded for 24-hours. Day and night were divided using the day-night divisions described by Verdecchia *et al.* (1991), Schillaci *et al.* (1994), and Wallace *et al.* (1997, 1999). Readings were taken every 30 minutes from the time of fitting the device at 9.00 a.m. (9.00h) until 10.00 p.m. (22.00h), after which the readings were taken every two hours. One subject tested 20-minute sample times during the day and each hour during the night. The subjects were asked to continue with their normal activities, with the importance of keeping the arm still while the recording was taking place being emphasised. In addition, they were asked to record the time of their significant activities so that they could be correlated with the blood pressure and HR recordings.

The data was downloaded from the recording device to a personal computer and into an associated programme (Dynapulse 5000A, Pulse Metric Inc. California USA). This provided some analysis, but was not compatible with Apple McIntosh software (Apple

Computer Inc. California, USA). Therefore, the data was then exported to a spreadsheet programme (Excel, Microsoft Corporation, USA) for analysis.

4.2.1. Statistical Analysis

Each pair of 24-hour sessions were compared with a two-tailed, non-parametric Student's t-test using GraphPad Prism (GraphPad Prism, Version 2.01, GraphPad Software Inc., USA). This procedure was also carried out for day and night recordings separately. Each data point could not be compared to its corresponding point as no control for activity was made. Data from all 24-hour sessions were then pooled and standard deviation (SD) and standard error of the mean (SEM) was determined using Excel (Microsoft Corporation, USA).

4.3. RESULTS

4.3.1. 24-hour ABP and HR (individual)

There was only one complete 24-h record (Subject Five, day 2), and three almost complete records with one data point missing in each (Subject One, day 2; Subject Three, day 1 and 2; Subject Four, day 2). Only one data point is missing for the period 9.00-22.00h. However, most are from the after 6.00h day recording period near the end of the 24h recording period.

Table 4.1. Individual 24-hour ABP and HR means ± SEM

Subject	Day	1	2	3	4	5
SBP	1	131.0 ± 5.4	140.0 ± 5.2	120.0 ± 2.2	130.0 ± 3.2	120.0 ± 3.7
(mm Hg)	2	135.4 ± 2.4	140.0 ± 3.8	120.0 ± 1.6	120.0 ± 3.1	120.0 ± 2.8
DBP	1	73.0 ± 3.1	71.0 ± 3.3	62.0 ± 1.3	73.0 ± 2.0	69.0 ± 2.5
(mm Hg)	2	77.0 ± 2.2	75.0 ± 2.2	64.0 ± 1.9	69.0 ± 2.6	65.0 ± 2.5
MBP	1	91.2 ± 3.6	92.4 ± 3.7	81.1 ± 1.6	89.5 ± 2.1	85.3 ± 2.9
(mm Hg)	2	94.8 ± 2.1	94.6 ± 2.3	81.5 ± 1.7	84.9 ± 2.7	82.7 ± 3.8
HR	1	61 ± 3	64 ± 2	74 ± 2	61 ± 2	78 ± 4
(bpm)	2	60 ± 1	68 ± 2	78 ± 2	61 ± 1	79 ± 3

Table 4.1. presents the 24-h mean ABP and HR for each recording day. Note the similarity for most parameters. However, HR is most consistent between recording days for most subjects. There was no significance between means ($p > 0.05$) for any of the recording sessions. Two apparently aberrant data sets, due to physical activity and improper recording were removed. However, this made no significant difference of the mean for these recording sessions.

4.3.2. Day ABP and HR (individual)

Table 4.2. Individual day ABP and HR means ± SEM

Subject	Day	1	2	3	4	5
SBP	1	134.2 ± 6.2	140.7 ± 3.6	118.3 ± 2.6	134.2 ± 3.6	126.5 ± 3.4
(mm Hg)	2	137.8 ± 2.9	141.3 ± 4.1	121.0 ± 1.6	124.0 ± 3.3	125.3 ± 2.8
DBP	1	75.9 ± 3.1	75.8 ± 1.6	62.0 ± 1.5	74.2 ± 1.8	71.5 ± 1.9
(mm Hg)	2	80.6 ± 2.3	77.1 ± 1.7	66.3 ± 1.5	71.7 ± 2.7	69.1 ± 2.0
MBP	1	94.9 ± 3.6	95.2 ± 1.8	80.6 ± 2.0	91.8 ± 2.1	88.5 ± 2.1
(mm Hg)	2	98.9 ± 2.4	96.1 ± 2.2	84.2 ± 1.2	87.9 ± 2.9	87.0 ± 1.8
HR	1	63 ± 3	66 ± 3	74 ± 2	61 ± 2	81 ± 4
(bpm)	2	63 ± 2	70 ± 2	80 ± 3	60 ± 1	83 ± 3

Table 4.2 shows day (6.00-22.00h) ABP and HR. These are almost all higher than the 24-h ABP as would be expected. Individual SEM did not change significantly from the 24-h ABP. There were no significant differences between means ($p > 0.05$) for any comparisons between day data.

4.3.3. Night ABP and HR (individual)

Table 4.3. summarises individual night ABP and HR means ± SEM. Compared to 24-hour and day, HR is more comparable between sessions. There was no significant difference between means ($p > 0.05$) for each recording period. The SEM increases compared to 24-hour and day. However, during the night there were considerably reduced data points, that may contribute to the increased range of the SEM.

Table 4.3. Individual night ABP and HR means ± SEM

Subject	Day	1	2	3	4	5
SBP	1	116.0 ± 7.0	149.5±22.8	123.5 ± 3.7	118.2 ± 3.8	101.5 ± 2.5
(mm Hg)	2	130.9 ± 4.0	124.5 ± 4.5	112.0 ± 2.0	109.0 ± 3.6	108.0 ± 2.7
DBP	1	58.0 ± 1.7	55.2 ± 12.3	59.7 ± 2.7	68.2 ± 5.4	51.0 ± 3.0
(mm Hg)	2	69.1 ± 3.4	62.5 ± 10.5	53.5 ± 4.5	57.2 ± 2.9	48.5 ± 3.0
MBP	1	94.9 ± 3.6	81.7 ± 17.2	83.0 ± 2.3	83.4 ± 4.7	64.0 ± 3.0
(mm Hg)	2	98.9 ± 2.4	84.5 ± 6.5	71.5 ± 3.8	73.7 ± 2.7	65.5 ± 2.5
HR	1	51 ± 3	55 ± 4	73 ± 2	61 ± 5	57 ± 7
(bpm)	2	54 ± 2	53 ± 6	70 ± 4	62 ± 5	63 ± 3

4.3.4. Dipping (individual)

Summarising Table 4.4, percentage dipping ranged from -2.2 to 28.3% (mean 15.8 ± 8.8%). Dipping is regarded as > 10% reduction of MBP from the day mean MBP to the night mean MBP, so that 70% of the recording sessions demonstrated dipping. All, but one subject (3), demonstrated dipping to varying degrees. Subject Three showed an increase in the night mean MBP over the day mean MBP. Subject Five, a smoker, displays a considerably increased percentage of dipping than all others who are non-smokers.

Table 4.4. Percentage dipping calculated from night MBP change from the day MBP.

Subject	Day	1	2	3	4	5
SBP %	1	15.7	-5.3	-3.7	12.9	20.0
	2	6.5	13.6	7.5	12.8	13.9
DBP %	1	25.3	19.0	4.7	7.4	29.3
	2	16.4	4.7	19.8	17.1	30.4
MBP %	1	23.7	12.9	15.5	9.1	28.3
	2	13.6	-2.2	9.1	17.1	25.2

Except for isolated occasions, the recordings for each day were remarkably consistent, in spite of no allowance being made for activity. Blood pressure did not rise consistently throughout the day for all subjects. The DBP was relatively more consistent compared to

SBP. The SBP and HR were much more variable. There was no significance difference between means ($p > 0.05$).

4.3.5. Males and females

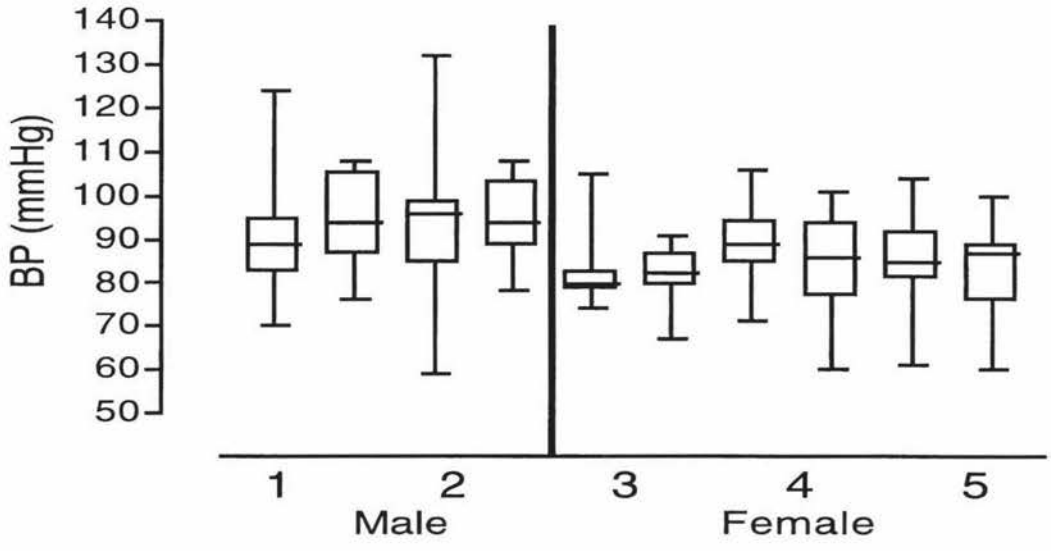

Figure 4.1. Individual recordings comparing males and females.

Figure 4.1. shows that the females demonstrated more consistent and lower MBP than the males, in addition to a reduced range of MBP.

4.3.6. 24-hour ABP (pooled)

Figure 4.2. illustrates that this group's pooled 24-h HR and SBP rose together during the day until a peak at 19.00h, there followed a precipitous decline until 21.00h. This time preceded the bedtime of 22.00h for all subjects. DBP remains relatively stable throughout the day until 18.00h. HR falls faster than DBP and at a similar rate as SBP after 19.00h. HR rises at a faster rate before the "awake" time of 6.00h than the rise of SBP or DBP ("morning surge"). Relaxation after 19.00h precipitated a fall in all parameters prior to sleep. However, after the sleep time of 22.00h all parameters were elevated for a short time and more variable than during the day.

The 24-h mean SBP was 127.2 ± 7.3 mm Hg (95% CI; 124.1-130.3), while the mean DBP was 68.3 ± 8.4 mm Hg (95% CI; 64.8-71.8) and the mean HR was 66.5 ± 7.1 beats per minute (bpm) (95% CI; 63.5-69.5).

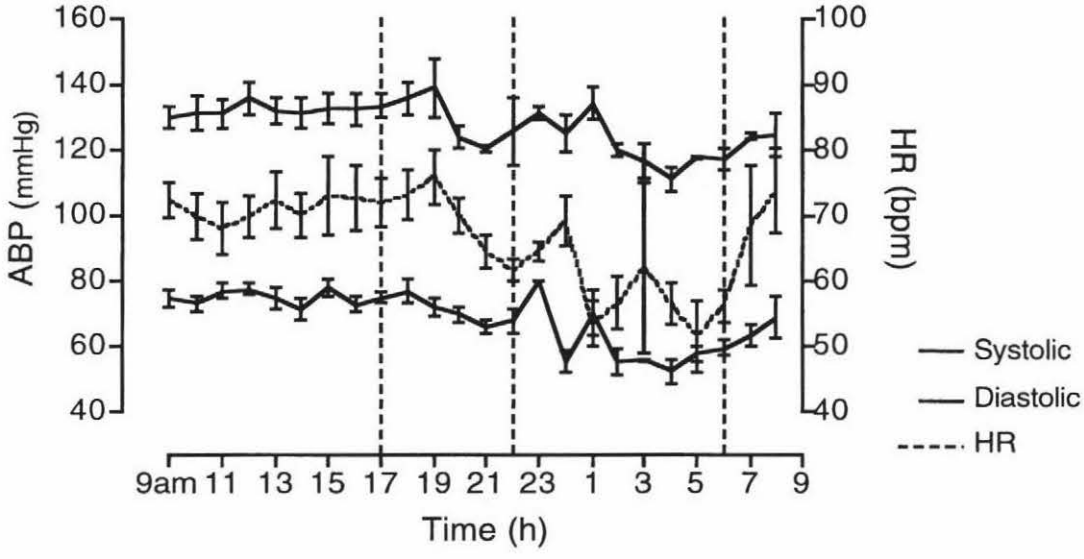

Figure 4.2. Pooled hourly mean ± SEM SBP/DBP/HR with time lines for ceasing work (17.00h), bedtime (22.00h) and awake (6.00h)

The pooled data in Figure 4.3. illustrates that most SBP was above 120 mm Hg, but below the hypertension "threshold" of 140 mm Hg. This level only falls below 120 mm Hg at 1.00h which may be regarded as the "middle of sleep" (Okutani *et al.*, 1997). However, SBP remains below 80 mm Hg almost throughout the day.

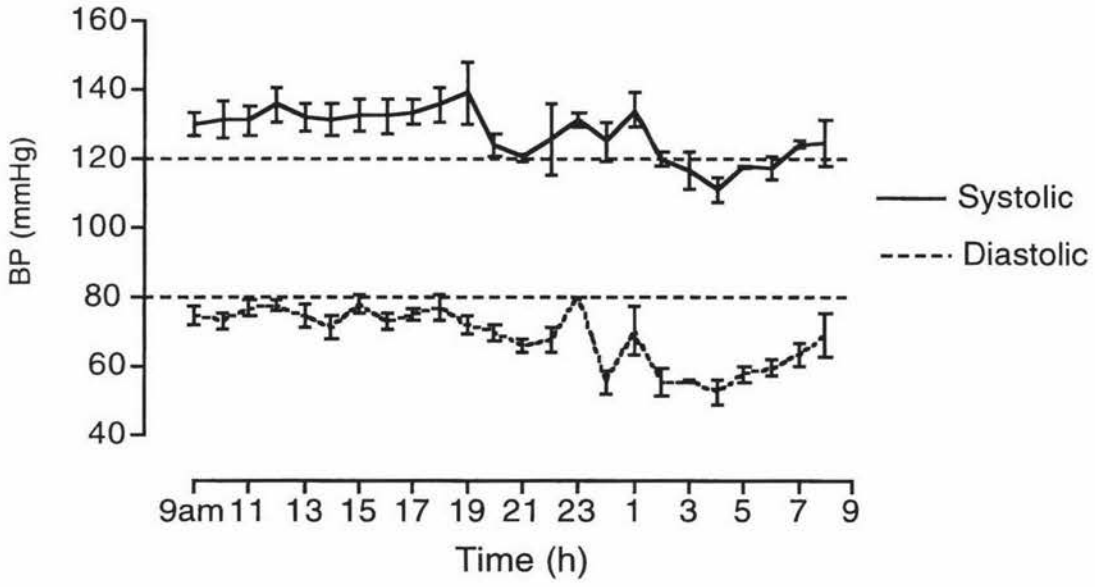

Figure 4.3. Pooled mean ± SEM SBP/DBP with BP 120/80 (mm Hg)

4.3.7. Day ABP and HR (pooled)

When the data were divided into day and night, comparison was made between recording days in the same manner as the 24-h recordings. Mean day SBP was 129.6 ± 6.0 mm Hg (95% CI; 126.4-132.8), mean day DBP was 71.6 ± 5.3 mm Hg (95% CI; 68.8-74.4) and mean day HR was 70.0 ± 4.6 bpm (95% CI; 67.6-72.5).

4.3.8. Night ABP and HR (pooled)

Mean night SBP was 122.5 ± 7.8 mm Hg (95% CI; 116.0-129.0), mean night DBP was 61.7 ± 9.8 mm Hg (95% CI; 53.5-69.8) and mean night HR was 59.4 ± 5.9 bpm (95% CI; 54.4-64.3).

4.3.9. Dipping (pooled)

Dipping is demonstrated in all pooled parameters as illustrated in Figure 4.3. Mean SBP dipped by 5.5%, mean DBP by over twice as much at 13.9% and mean MBP by 10.1%. HR

dipped by 15%. Dipping occurs significantly after 7.00 p.m. Compare these percentages with the range of individual percentages found in Table 4.4.

4.3.10. Smoking

Only one of the subjects was a cigarette smoker (Subject Five). The time and number of cigarettes smoked on each occasion were accurately recorded by Subject Five and are marked for each day in Figure 4.4. Raw data for MBP and HR are presented here, instead of the hourly means. Smoking has an effect on blood pressure and HR that is illustrated in Figure 4.4.

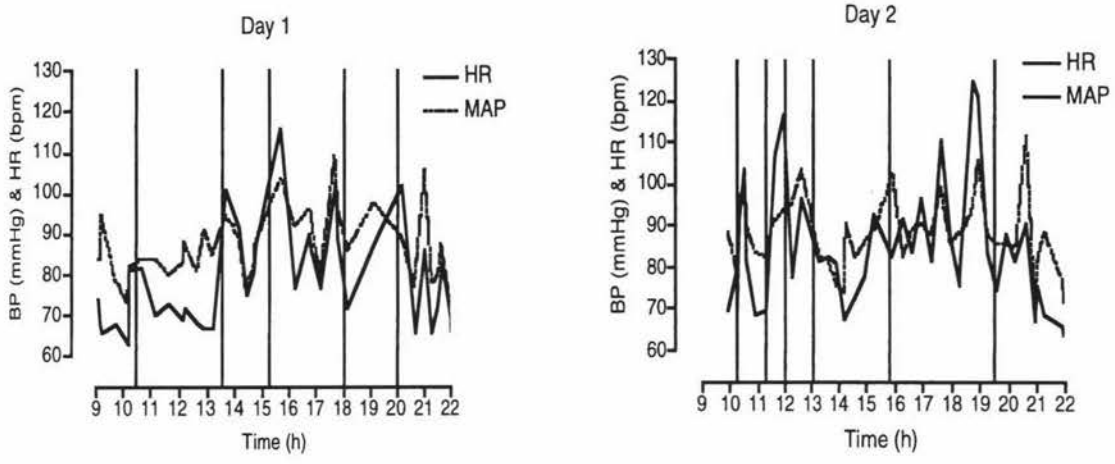

Figure 4.4. Subject Five MBP and HR compared to times of smoking for each recording day. Raw data shown.

There was an unexplained difference of response to the cigarette smoking between recording days. No allowance was made for concurrent activity, which may have contributed to the variation in response between days. On the first recording day, 40% of smoking sessions increased MBP and HR. However, one session of two cigarettes reduced HR. In contrast to the first day, 70% of the smoking decreased MBP, but only 50% decreased HR.

4.4. DISCUSSION

In this section, discussion of the points particular to the preliminary trial are presented. This trial was used to become familiar with the methods and ambulatory equipment. In addition, the results reinforced the decision to use a shorter ABP period as suggested by Staessen *et al.* (1995) and Lusardi *et al.* (1999).

4.4.1. Subjects

The subjects in the preliminary trial were all younger than those in the main trial, and all were, at least, moderately active. The males demonstrated slightly elevated mean blood pressure, however this may have been due to the greater amount of activity for these subjects. These subjects undertook the two 24-hour recording sessions with almost 100% completion. The main objection was sleep disruption.

4.4.2. Sleep disruption

In the preliminary trial, which ran for 24-hours, it was found that sleep was disrupted for all subjects, even at the two-hourly night reading protocol. One subject used the protocol of 20-minute day reading and each hour during the night. This subject experienced an almost complete lack of sleep and subsequent tiredness. Sleep disruption caused one subject to remove the ABP recording device early in the morning due to frustration at the lack of sleep. Another subject showed an increase in the night mean MBP over the day mean MBP, also indicative of disruption to sleep.

Despite sleep disruption, all subjects, but one, still demonstrated a reduction in blood pressure and HR in the night. The shorter recording period used by one subject was very disruptive to sleep and not recommended as a routine protocol. All parameters were more consistent during the day, when it would be expected that the opposite would be the case due to variations in activities between subjects. This indicates that sleep disruption is an important factor when using ambulatory recording.

The timing of readings is also important. There is, apparently, a lower limit of reading time

at night that exists when sleep becomes continuously disrupted. This factor would also need to be addressed when establishing a protocol involving night-time readings. The effect of sleep disruption noted by Staessen *et al.* (1999) and Lusardi *et al.* (1999) may preclude full 24-hour monitoring in some subjects. In addition, the elevated blood pressure and HR, following a night of poor sleep, described by Lusardi *et al.* (1999) may be contributory to further ill-health in these subjects. These authors also argue that a shorter recording period will provide sufficient information. Therefore, the decision was made to use a shorter time period for the main trial.

4.4.3. Night

There was no significant difference between measurements when only comparing night ABP. This result is surprising when viewing the ABP it would seem that the range of blood pressure increases and would demonstrate significant difference. However, this could have been as a result of the limited number of measurements.

A greater number of morning recordings would have demonstrated the "morning surge" more precisely, however it was apparent on some recordings. This begins prior to awakening and is thought to be the result of increased sympathetic activity (Elliot, 1999), or decreased parasympathetic activity (Pasic *et al.*, 1998).

4.4.4. Dipping

The pooled data shows a reduction in blood pressure and HR, which occurs from the cessation of work and immediate post-work activity, such as travelling home and preparing dinner, at around 19.00h. This occurred for both students and staff and is in agreement with the studies of the effect of extrinsic factors, such as stress and work, on blood pressure and HR (James *et al.*, 1991; Peiper *et al.* 1993; Pickering & James, 1993; Pickering *et al.*, 1996; Kristal-Boneh *et al.*, 1998). The only smoker demonstrated the greatest dipping and confirms the observation of Pickering *et al.* (1996).

All parameters dipped by varying amounts in the night in these healthy subjects. The sleep disruption may have contributed to this variation. The mechanism of this variation is not clear from this study.

A reduction in blood pressure before lying down precedes the later expected reduction, when the subjects lie down to sleep. Often, there is no distinction between lying down for sleep and sleeping. This tends to disregard the distinction between sleep and relaxation, and suggests a marked physical effect from relaxation, which is more pronounced than lying down to sleep.

4.4.5. Males and females

All, but one, of the female subjects results demonstrated no significant difference between sessions. The males both had greater variation in the range of their recordings, in addition to higher blood pressure. This could be a more active lifestyle for the males. However, due to the incomplete activity records it is not possible to confirm this.

4.4.6. Over-reading?

The daytime SBP was generally over 120 mm Hg and is noted in Figure 4.3. This result may have been due to the over-reading noted by Green *et al.* (2000) using the same ABP apparatus. Subtraction of their determined over-reading mean of 8.25 mm Hg from the pooled SBP essentially corrects this apparent blood pressure elevation. Caution is emphasised in taking SBP readings at face value.

4.5. CONCLUSION.

While ambulatory recording has some limitations, agreement between most sessions validates the repeated use of the device for blood pressure and HR recording. This result confirms observations by numerous authors (Reid *et al.*, 1991; Mansoor *et al.*, 1994; Ohkubo *et al.*, 1998; Jula *et al.*, 1999; Kario *et al.*, 1999). Ambulatory blood pressure recording can provide a sensitive profile of a subject's, or populations, blood pressure and HR. However, as emphasised by James & Pickering (1993), any interpretation of ABP

recording should be made according to the behavioural setting. In addition, weighting should be given for activity.

Improved validation of the data obtained would have been by measuring blood pressure by the auscultatory method. However, discrete blood pressure measurements made in this manner do not provide a complete blood pressure profile (Pickering, 1993) and may suffer from measurement errors (Reid *et al.,* 1991).

CHAPTER FIVE: MAIN TRIAL

5.1. INTRODUCTION

Control of blood lipids is a means of reducing the risk of cardiovascular disease. Dairy food consumption has been linked to the development of arteriosclerosis and, therefore, people with an increased risk of cardiovascular disease are usually encouraged to reduce their intake of these foods. However, the strong link between calcium and blood pressure leads to the investigation of whether the potential benefit of consuming calcium enriched milk outweighs any potential harm of dairy fat. Reduced-fat milk is generally regarded as more palatable than non-fat milk. Therefore, this study compared the impact on blood lipids of drinking reduced-fat milk with non-fat milks.

A nine-week trial for each subject was conducted to determine if drinking modified milk powder lowered blood pressure. The purpose of this study was to assess the influence of three different milk products on blood pressure in free-living people with mild hypertension, who were not under medication or had ceased medication for the trial period under medical supervision. The three milk products were normal skim milk; normal skim milk with added calcium; and low fat milk (1.5% fat) with added calcium. These were specially formulated by the New Zealand Dairy Research Institute (Appendix 1). This chapter presents the data obtained from this trial followed by a general discussion and conclusion.

5.2. METHODS

5.2.1. Subject recruitment

All subjects were patients of a local general practitioner (GP). Each of these patients had been diagnosed with mild hypertension. Potential subjects were given an information sheet outlining the objectives of the trial (Appendix 2). Each applicant underwent a physical examination by the GP to clear them as physically able to be involved in this trial. The upper limit for exclusion from the trial was a clinic blood pressure greater than 160/100 mm Hg. Exceptions were made at the discretion of the GP, who had full access to the

subject's medical history. Not all subjects were taking medication for their hypertension. Those subjects in the trial who were taking medication were asked to abstain from this medication once their involvement in the trial had started. In addition, they were to have their blood pressure monitored on several occasions as part of the trial. The subjects could not be allergic to milk and were not taking mineral supplements at the time of the study.

The subjects presented came to the Milk and Health Research Centre (MHRC) facilities at the Massey University Turitea campus, Palmerston North. Here they were first asked to sign an Informed Consent form to the procedures which were approved by both the Massey University Human Ethics Committee and the Manawatu-Whanganui Ethics Committee.

Information was obtained from the subjects including height and weight, girths, grip strength, and a fitness test. Combined with the 24-hour recall nutritional questionnaire, this information provided a general health profile of the subjects. At a later date, further blood pressures and blood samples were taken.

5.2.2. Physical data

5.2.2.1. Weight

Each subject was weighed using conventional scales to the nearest 0.2 kg. (Detecto, USA) The subjects were normally clothed, but without shoes, jackets or jerseys.

5.2.2.2. Height

Standing height was measured using a conventional stadiometer to the nearest 0.01 cm. The subject was asked to stand in bare or stocking feet with heels, buttocks, shoulders, and the back of the head in contact with the stadiometer. They were asked to stand straight to ensure the head remained level and with the head in the standardised position known as the Frankfort plane. The sliding bar was bought into contact with the subject's head and the height recorded. Combined with weight, the BMI was calculated.

5.2.2.3. Triceps skinfolds

The tricep skinfold thickness was measured using skinfold calipers (Harpenden Skinfold Caliper, British Industries Ltd. UK) to the nearest 0.5 mm. Bodyfat percentage was derived using the equations of Durnin and Womersley (1974).

5.2.2.4. Circumferences

The waist and hip circumferences were measured to derive the W/H using a conventional non-stretch tape measure . Dividing the W/H by the ideals provides a percentage above or below the ideal as an additional measure.

Mid-arm circumference was measured by conventional tape measure. The mid-arm point was found by determining the halfway point between the acromial process and the olecranon process. This point was marked as it was also used for the TSF measurement. From these measurements the upper arm muscle area could be determined. These provide an indication of the nutritional status of the subjects. A normative AMA value was calculated from New Zealand data (Russell *et al.*, 1999) using the formulae of Heymsfield *et al.* (1982).

5.2.2.5. Body composition

After a 3-hour fast, body composition was measured using total body bioelectrical impedance analysis (Biodynamics Model 310 Body Composition Analyser, Biodynamics Corporation, Seattle, Washington, USA).

5.2.2.6. Fitness test

The subjects undertook the exercise test on a cycle ergometer (Monark Ergomedic 818E, Bodyguard AB, Sweden) using the protocol of Astrand & Rohdahl (1965). This test involved cycling at low consecutive workloads (typically increments of 0.5, 1.0 and 1.5 Watts) for three minutes each workload, corresponding to very easy, easy and moderate in terms of the subject's perception of exertion.

Heart rate was monitored (Polar Vantage Heart Rate Monitor, Polar Electro Oy, Finland) continuously during the test, and the test was terminated if the HR exceeded 140 bpm. That was approximately 80% of the predicted maximum HR for this group of subjects. At the end of each work bout the HR at that time was recorded.

The subjects were monitored closely for breathlessness by engaging them in light conversation. The statement; "you may stop any time if you wish" was included several times in this conversation to confirm that people need not carry on beyond a level they felt comfortable with. If the predetermined maximum HR was reached, the subject became breathless or asked to quit then the workload was reduced, but the subject was to continue pedalling with no load for a while. The subject was to remain seated until the HR was returned to pre-exercise levels. This procedure was also used at the completion of the test.

5.2.2.7. Strength test

Forearm strength or handgrip strength was assessed by using a hand dynamometer (Lafayette Hand Dynamometer Model 78010 Lafayette Instrument Co. USA) on both hands using a standardised procedure. The method of Montoye & Lamphaier (1977, cited by Brodie, 1996) was used where the combined total of the left and right hand grip strength (kg) was divided by the body mass (kg). These were compared to age-related normative data.

5.2.3. Blood lipids

Appointments were made so that subjects could have a baseline blood sample by venepuncture (Becton-Dickinson Vacutainer Systems Europe, France) taken by the GP. These were whole blood (for serum) and another tube with EDTA (for plasma) to inhibit clotting. A later sample was taken by the same method at the end of the trial. These samples were sent to MHRC for analysis for total cholesterol (TC) and triglycerides (TG) by a commercial device (Cholestech L.D.X., Cholestech Corporation, California USA) which was first validated by an optics check. The samples were then centrifuged (3000 rpm for 10 minutes) to separate the plasma and serum. The EDTA sample was spun first at room temperature, while the whole blood was spun at 4^0 C. These samples were subsequently

frozen and stored for assay for of serum calcium, potassium, magnesium and sodium. However, these assays were not undertaken due to the cessation of the trial, thus this data is not currently available.

Fingerprick blood samples were taken after the baseline and end of the milk treatment periods for the measurement of blood lipids. These were analysed on site by the Cholestech device. The fingerprick procedure followed standard hygienic practice. Not all subjects had this form of analysis as this was to be taken at the second visit which did not occur for some subjects due to the cessation of the trial.

5.2.4. 24-hour food recall

The subjects were asked to recall their food intake for the last 24 hours to determine the composition of the food they had consumed during this period. The subjects were asked to compare volumes eaten with familiarly sized and shaped food containers that may, in part, reduce this problem. These data were analysed using the Australian and New Zealand Nutrient Database for calcium accessed via nutrient analysis software (FoodWorks v.2, Xyrex Software, Highgate Hill, Queensland, Australia). The subjects were then asked how this compared to their normal food intake. In addition, they were asked about their usual consumption of dairy products. Dietary histories were to be independently validated using measurements of urinary nitrogen and assessments of total energy expenditure. However, these assessments were not undertaken due to the cessation of the trial.

Accuracy of recall is questionable as subjects tend to under-report food intake in both quantity and content (Hirvonen, *et al.*, 1997). The accuracy of recall was tested by comparison of the reported energy intake as analysed by the FoodWorks programme and the cutoff criteria for reported energy intake presented by Goldberg *et al.* (1991). The 95% confidence limit was used for n =1 and 1-day reporting and assumes BMR is calculated from Schofield's equations (1985). Cutoff was 0.90 of reported energy intake, less than this indicates under-reporting.

5.2.5. Calcium intake

A short structured questionnaire based on common food items that contain significant quantities of calcium was used to assess calcium intake. Subjects were asked to recall their consumption of a selection of 15 foods over the last 24-hours. This questionaire was to be given to the subjects three times, at three weekly intervals, throughout the study. However, few were subsequently completed and therefore were not assessed.

5.2.6. Energy expenditure

Energy expenditure was to be calculated as the sum of basal metabolic rate (BMR), diet induced thermogenesis and energy cost of activity: The BMR was calculated from measurements of body weight (wt) and height (ht) using the equations of Schofield (1985).

30 – 60 years

Male BMR = 0.048 wt – 0.011 ht + 3.670

Female BMR = 0.034 wt + 0.006 ht + 3.530

Over 60 years

Male BMR = 0.038 wt + 4.068 ht – 3.491

Female BMR = 0.033 wt + 1.917 ht + 0.074

Diet-induced thermogenesis was assumed to be 20% of BMR. Activity energy expenditure was to be assessed from the habitual physical activity questionnaire of Baeke *et al.* (1982).

Total energy expenditure could not be calculated due to the missing or incomplete questionnaires. This omission occurred because these were meant to be handed out at the second visit at mid-treatment. However, due to the cessation of the trial, this did not occur in most cases. Instead, comparison is made of the BIA, the Schofield and FoodWorks-derived BMR scores. Activity reporting is also reliant on the memory of the subject. There was considerable likelihood of over-reporting by the subjects.

5.2.7. Urine collection

Subjects were asked to make three 24-hour urine collections during the baseline, intervention and washout periods. The urine was to be assayed for nitrogen as an independent marker for protein intake, calcium, potassium, magnesium, and sodium. The

latter minerals are indicators of the dietary intake of these minerals. In addition, the level of sodium excretion could help to explain any reduction in blood pressure due to calcium. However, these assays were not undertaken due to the cessation of the trial.

5.2.8. Estimation of cardiovascular risk

Using the data obtained from the blood lipid profile and ABP provided an opportunity to estimate the cardiovascular risk for each subject. The first method, based on data on 50 to 80 year old men and women from the Framingham Heart Study (1948-, cited in (Cholestech L.D.X. manual, p.2, Cholestech Corporation, California USA), used the HDL cholesterol level. These provided a Relative Risk score, with the average being 1.00. A higher score (to 1.94) meant a higher risk and *vice versa* (to 0.45). Scores were different for men and women, with the latter at consistently greater risk.

Further risk assessment was obtained using New Zealand Guidelines (2000). These tables were compiled from Recommendations for the Prevention of Coronary Heart Disease in Clinical Practice from the European Societies of Cardiology, Artherosclerosis and Hypertension. This was the 5-year risk of developing a cardiovascular disease (CVD). These tables distinguished between men and women, diabetics and non-diabetics, smokers and non-smokers, and compared blood pressure with TC in the case of non-smokers, or TC/HDL for smokers. Females, diabetics and smokers have increased risk of developing CVD in the next 5 years. The highest ABP and, TC or TC/HDL, was used for risk assessment in the present study. The ABP was chosen due to the greater number of readings and, therefore accuracy, as suggested by Abitol *et al.* (1997) and Okutania *et al.* (1997). Finally, the subjects body mass index (BMI) was compared for risk of developing CHD using the categories of Andres *et al.* (1985, cited by Brodie, 1996).

5.2.9. Blood pressure

5.2.9.1. Clinic and home blood pressure

Blood pressure was measured several times by two different methods. Firstly, auscultatory (Sphygmomanometer: CE 0124 Diplomat Presameter AG, Germany; Stethoscope:

Littman Cardiology III, 3M Corporation USA), and secondly, automated (A and D Digital Blood Pressure Meter UA-751, A and D Engineering Inc. USA). The former, for three repeat measures, and the latter twice in each position of seated and standing. The techniques followed standard practice. The auscultatory measurement involved taking the reading at the appearance of the first and the fourth Korotkov sound.

5.2.9.2. On-site blood pressure

An appointment was made at the initial collection of data to visit the subjects at a place of their choice. This was so further blood pressure measurements could be taken and the ABP recording device (Dynapulse 5000A, Pulse Metric Inc., California, USA) fitted. This was usually their place of residence, however some chose their workplace. Again auscultatory and automated blood pressures were taken both seated and standing. The ABP device was fitted in accordance with the manufacturers instructions. It was set for 20 measurements spread over 10 hours from approximately 9 a.m. The subjects were instructed to leave the device on, but that they could remove it any time if they so wished due to discomfort or other reason. It was stressed that they remain motionless when a measurement began.

5.2.10. Analysis by case study

After only three subjects had completed the trial, one had a myocardial infarction. This prompted the Principal Investigator to immediately suspend the trial for an indefinite time. Subsequently, the trial was stopped altogether and, therefore, the number of subjects is limited. It was proposed that it would be more suitable to analyse this material using the case study method. Each subject's data, although incomplete, is analysed separately. This approach is more appropriate than attempting to use statistical analysis, due to the lack of completed data. An initial power analysis indicates that over 50 subjects are required to obtain meaningful statistical data from this type of study.

In the following sections, the data for each subject is presented in case study format. The following areas are presented: Physical data obtained from the first visit; Basal metabolic rate comparisons; Activity is discussed where applicable; Mean blood pressures (SBP, DBP and MBP) and differences (where applicable); HR; Blood lipids; and % variations from

RDI (FoodWorks database). Following each section is a short note on key points, comparisons with norms and statistics, where applicable. Where possible, New Zealand normative data (NZ Food: NZ People, 1999) is included. A short summary concludes each case study with the subject's potential to develop CHD based on criteria developed from the Framingham Heart Study (1948-, cited in Cholestech L.D.X. manual, p.2, Cholestech Corporation, California USA). Two different people took the physical measurements, dietary recalls and blood pressures by the differing methods. This may account for some variations and omissions that were subsequently found.

5.2.11. Statistical analysis

Each subjects blood pressure measurements by all methods was entered into Prism (GraphPad Prism, Version 2.01, GraphPad Software Inc., USA). Blood pressure was divided into its components of systolic (SBP) and diastolic (DBP). Mean blood pressure (MBP) was calculated from these measures. The data were treated in several ways. All blood pressures were compared between methods by repeated-measures one-way ANOVA. The effect of the dietary intervention for the three subjects who completed the trial was considered by comparing the first and last measurements and tabulating the differences of the means. These means were compared by paired Student's t-test. In addition, repeated-measures one-way ANOVA with Tukey's *post hoc* test was carried out on the three blood pressure recording sessions.

5.3. SUBJECT DEMOGRAPHICS

There were 11 subjects aged from 38 to 60 years (mean age 53.1 ± 6.2 years): seven females (mean age 53.6 ± 7.3years) and four males (mean age 51.7 ± 1.5 years). Each subject completed at least one ABP recording over 24-hours. Five did two ABP recordings and only three did all three recordings, completing the study. Only five were on the milk intervention diet. Three different interventions were planned, so no comparison can be made between interventions. Any effect of an intervention would not be expected to be discernible.

5.4. SUBJECT ONE

5.4.1. Physical data

Subject One is a 60-year old female homemaker. As a Caucasian, she is one of approximately 25% of her peer population in New Zealand. She completed the trial.

Table 5.4.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.715	1.602 ± 0.26	**BMI:** 29.44	> 95th
Weight (kg)	86.6	69.0 ± 0.56	Overweight	75th
Waist (cm)	91.0	**W/H:** 0.81	**W/H:** 0.77	
Hip (cm)	118.0		**W/H%** 96.0	
MAC (cm)	34.0	30.8 ± 0.15		> 50th
TSF (mm)	22.32	25.6 ± 0.36	**% fat:** 39.0	50th
MAMC (cm)	26.99	23.00		> 50th
AMA (cm^2)	51.46	34.71		90th
$\dot{V}O_2$max(ml/kg/min)	19.89			Poor
Handgrip (kg)	**total:** 79.0		**Kg/wght:** 0.9	70th

For New Zealand data reflecting the subject's ethnic background, Subject One is taller and heavier than the average. Her W/H is nearing the recommended maximum of 0.8 for women. However, she has greater calculated MAMC and AMA than her peers. It may be that some of the additional weight is muscular, as indicated by the grip strength and grip/weight percentile that would be unusual for this age group. Her TSF is smaller than the mean yet her fat % is relatively high. Compared to the BIA-determined bodyfat of 36.2% these measures are similar. Her BMI suggests that she is overweight and emphasises the central distribution of most of her weight as indicated by her waist measurement. She is one of 38.9% of women regarded as overweight in her age and ethnic group (Table 5.4.1).

5.4.2. Blood pressure

The progress of the blood pressure over the course of the study follows an interesting pattern. The unexplained elevation at the second blood pressure recording session in all

parameters could possibly be explained by the subject's reaction to coming off her medication. Standing auscultatory blood pressure measures show an increase in blood pressure over the course of the study, whereas ABP means remain similar (Table 5.4.2).

Comparing all recording sessions by repeated-measures ANOVA found significant differences for SBP ($p = 0.0018$), DBP ($p = 0.0044$) and a highly significant difference for MBP ($p = 0.0001$). It is noted that HR remains independent from blood pressure. There was a highly significant difference between the mean HR obtained from the first and last sessions ($p = 0.0008$).

Table 5.4.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1); mid-intervention (2) and post-intervention (3)

Method	Day	SBP	DBP	MBP	HR
Clinic		140.7 ± 1.2	90.0 ± 2.0	105.8 ± 1.7	*
Auscultatory:	1	142.7 ± 2.3	88.7 ± 1.2	105.6 ± 1.3	*
sitting	2	168.0 ± 0.0	91.3 ± 5.8	115.7 ± 3.8	*
	3	137.0 ± 1.4	85.0 ± 1.4	101.3 ± 1.4	*
Digital:	1	137.5 ± 0.7	91.0 ± 9.9	105.4 ± 6.3	75 ± 1
sitting	2	164.5 ± 9.2	91.0 ± 4.2	114.3 ± 0.2	75 ± 16
	3	143.5 ± 7.8	83.5 ± 3.5	102.5 ± 0.2	*
Auscultatory:	1	140.7 ± 1.2	98.7 ± 1.2	111.5 ± 1.1	*
standing	2	161.3 ± 1.2	104.0 ± 2.0	121.9 ± 1.7	*
	3	151.0 ± 1.4	98.0 ± 0.0	114.5 ± 0.5	*
Digital:	1	138.5 ± 0.7	92.5 ± 0.7	106.8 ± 0.2	*
standing	2	161.0 ± 12.7	95.5 ± 3.5	116.2 ± 1.9	*
	3	137.5 ± 3.5	87.0 ± 1.4	102.8 ± 0.2	*
Ambulatory	1	155.1 ±15.5	86.1 ± 8.6	106.5 ± 10.0	72 ± 6
	2	156.9 ± 11.3	88.3 ± 10.9	110.7 ± 9.5	74 ± 9
	3	155.1 ± 15.5	86.1 ± 8.6	112.9 ± 8.5	68 ± 5

* data not obtained or not required.

5.4.2.1. Blood pressure differences

Table 5.4.3. Comparison of blood pressure (mm Hg) changes ± SD from baseline mean with post-intervention mean

Method	SBP	DBP	MBP
Auscultatory sitting	-5.7 ± 1.9	-3.7 ± 1.1	-4.5 ± 1.2
Digital: sitting	+6.0 ± 5.5	-7.5 ± 7.4	-3.2 ± 4.5
Auscultatory: standing	+10.3 ± 1.1	-11.7 ± 0.0	+2.9 ± 0.9
Digital: standing	-1.0 ± 2.6	-5.5 ± 1.1	-4.0 ± 0.3
Ambulatory	+6.0 ± 4.7	+4.0 ± 2.8	+6.4 ± 3.0

Differences between the baseline and post-intervention were tested by paired Student's t-test. All were non-significant ($p > 0.05$), except ABP MBP ($p < 0.05$) and standing auscultatory and digital that were highly significant ($p < 0.004$) (Table 5.4.3).

It would not be prudent to make an assumption from the consistent increases found with the ABP recording. As can be seen in Table 5.4.3. there was considerable difference between recording methods. There was expected to be some variation between standing and seated blood pressure measure, however the differences are not consistent.

5.4.3. Blood profile

Table 5.4.4. Blood lipid concentrations

	Start	3 weeks	6 weeks
TG (mmol/L)	1.40	1.50	1.32
TC (mmol/L)	5.01	5.81	6.04
HDL (mmol/L)	0.97	1.17	1.07
VLDL (mmol/L)	0.65	0.69	0.60
LDL (mmol/L)	3.40	3.95	4.37
TC/HDL	5.15	5.15	5.60
LDL/HDL	3.51	3.38	4.08

Subject One's TG level is low compared to the New Zealand mean TC for this age group of 6.5 ± 0.04 mmol/L. However, it can be seen that TC rises over the course of the

intervention. The increased TC has moved from a desirable range to high borderline. In turn this has moved her TC/HDL ratio into the high category. Her LDL concentration has also increased over the course of the trial. HDL has remained relatively constant, so that the ratio of LDL to HDL has worsened but has remained in the borderline category. The VLDL has remained consistently borderline as well (Table 5.4.4).

5.4.4. Estimation of cardiovascular risk

Due to the level of HDL Subjects One's Relative Risk score is a high 1.55. Taking the highest ABP mean and TC gives a 5-year CVD risk of 10-15% (NZ Guidelines). Her BMI places her at the high end of the low risk category (Grade I).

5.4.5. Reported food intake

Table 5.4.6. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	76
Thiamin	78
Riboflavin	66
Niacin Eq.	119
Vitamin C	142
Total Vitamin A Eq.	141
Potassium	104
Magnesium	76
Calcium	34
Phosphorous	73
Iron	112
Zinc	48

Reported food intake proportions were: protein - 10.96%; fat - 20.18%; carbohydrate – 66.86%. These proportions are low in protein with elevated carbohydrate and may be a deliberate dietary choice, however this was not known.

5.4.6. Basal metabolic rate

Table 5.4.7. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1492	1679	1417

These different analyses are similar, with the Schofield equation elevated by 12% (Table 5.4.7). Reported energy intake was 63% by FoodWorks and 84% of the Scofield score clearly showing under-reporting.

5.4.7. Activity

Subject one's activity was considerably less than average for her peers as she played no sport. While she kept busy in the home, it was mainly seated and there was not sufficient elevation of her HR to be regarded as healthy.

5.4.8. Summary

Subject One leads a very sedentary life, with poor fitness. Demonstrating several Syndrome X factors, her being over-weight, hypertension, poor blood TG profile and the estimation of CVD indicated that she is a very likely candidate for CHD.

5.5. SUBJECT TWO

5.5.1. Physical data

Subject Two is a 52-year-old male who works at a relatively physical job. As a Caucasian, he shares his hypertension with 43.7% of his peers. Subject Two completed the trial.

Subject Two is average for most parameters. He is apparently healthy despite his hypertension that is not obesity related. While not displaying large waist and hip measurements, the W/H % indicates he has some central adiposity. This may be born out by the fat percentage determined from the TSF that does not match his physical appearance

(from observation). The bodyfat from BIA of 19.9% may be closer to the actual percentage bodyfat. His active work may contribute to his good fitness and grip strength (Table 5.5.1).

Table 5.5.1. Summary of the physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.76	1.737 ± 0.37	**BMI:** 23.32	75th
Weight (kg)	72.24	80.9 ± 0.61	Healthy	25th
Waist (cm)	95.0	**W/H:** 0.94	**W/H:** 0.97	
Hip (cm)	98.0		**W/H %:** 108.0	
MAC (cm)	31.5	31.6 ± 0.13		50th
TSF (mm)	15.98	13.9 ± 0.28	**% fat:** 33.8	50th
MAMC (cm)	26.48	28.00		15th
AMA (cm^2)	45.80	49.01		5th
$\dot{V}O_2$max (ml/kg/min)	34.63			Good
Handgrip (kg)	**total:** 84.7		**Kg/weight:** 1.17	30th

5.5.2. Blood pressure

Subject Two recorded the highest blood pressures (Table 5.5.2.) despite enjoying an apparently robust health. His clinic blood pressure was low relative to the other methods, in contrast to usual observations. This result demonstrates how multiple blood pressure measures need to be taken to determine the extent of elevated blood pressure. However, he was also engaged in a great deal of work-related activity while the measurements were being taken. The ABP apparatus was especially sensitive to any disruption and multiple recordings were made on many occasions, which may have contributed to the high ABP results. There was a marked difference between the sitting and standing means. Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. Therefore, no *post hoc* tests were carried out ($p > 0.05$).

Table 5.5.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1); mid-intervention (2) and post-intervention (3)

Method	Day	SBP	DBP	MBP	HR
Clinic		144.0 ± 2.0	99.3 ± 3.1	113.1 ± 2.5	*
Auscultatory:	1	169.3 ± 1.2	102.7 ± 1.2	123.6 ± 1.0	*
sitting	2	183.3 ± 2.3	94.0 ± 2.0	122.5 ± 2.0	*
	3	172.7 ± 1.2	98.0 ± 0.0	121.7 ± 0.4	*
Digital:	1	178.51 ± 3.4	88.5 ± 0.7	117.3 ± 4.0	46 ± 0
sitting	2	160.0 ± 8.5	85.5 ± 3.5	109.2 ± 0.5	66 ± 1
	3	166.5 ± 6.4	101.0 ± 0.0	121.6 ± 2.1	76.± 1
Auscultatory:	1	188.7 ± 1.2	102.0 ± 3.5	129.6 ± 2.5	*
standing	2	183.3 ± 1.2	108.0 ± 2.0	131.8 ± 1.0	*
	3	180.7 ± 2.3	110.0 ± 0.0	132.2 ± 0.8	*
Digital:	1	191.0 ± 1.4	94.5 ± 0.7	125.4 ± 0.9	*
standing	2	168.0 ± 5.7	96.5 ± 4.9	119.1 ± 5.1	*
	3	183.0 ± 1.4	100.5 ± 4.9	126.7 ± 2.8	*
Ambulatory	1	178.9 ± 14.6	100.3 ± 10.1	125.9 ± 9.0	80 ± 7
	2	180.8 ± 11.3	98.3 ± 8.0	124.0 ± 8.1	84 ± 15
	3	185.8 ± 10.7	103.1 ± 8.0	129.3 ± 5.5	83 ± 5

* data not obtained or not required.

5.5.2.1. Blood pressure differences

Table 5.5.3. Comparison of blood pressure (mm Hg) changes ± SD from baseline mean with post-intervention mean

Method	SBP	DBP	MBP
Auscultatory: sitting	+3.3 ± 0.9	-4.7 ± 0.0	+2.0 ± 0.6
Digital: sitting	-12.01 ± 0.5	+12.5 ± 0.0	+4.3 ± 3.2
Auscultatory: standing	-8.0 ± 1.5	+8.0 ± 0.0	+2.7 ± 1.5
Digital: standing	-8.0 ± 1.4	+6.0 ± 3.5	+1.3 ± 2.1
Ambulatory	+6.9 ± 4.7	+2.9 ± 3.3	+3.5 ± 2.7

The results were inconsistent with a general reduction of SBP and an elevation of DBP resulting in a mean increase in MBP. Comparing the ABP baseline with post-intervention by paired Student's t-test showed no significance ($p > 0.05$) in all parameters.

5.5.3. Blood profile

Subject Two's HDL is satisfactory. However, the TG and TC are borderline, LDL and TC/HDL ratio are high. In addition, an LDL/HDL ratio that is also borderline. The VLDL increased from borderline to high (Table 5.5.4.).

Table 5.5.4. Blood lipid concentrations

	Start	3 weeks
TG (mmol/L)	1.81	2.33
TC (mmol/L)	5.38	6.89
HDL (mmol/L)	1.23	1.06
VLDL (mmol/L)	0.83	1.07
LDL (mmol/L)	3.31	4.76
TC/HDL	4.35	6.65
LDL/HDL	2.69	4.49

5.5.4. Estimation of cardiovascular risk

Relative Risk for Subject Two places him at average risk (1.00). His ABP and TC place him at 10-15% 5-year CVD risk (NZ Guidelines), however this is regarded as an under-estimation. His BMI is in Grade O for risk and, therefore, desirable.

5.5.5. Reported food intake

Proportions of reported food intake were: protein 14.93%; fat 25.67%; carbohydrate 58.6%. These were all in the desirable range. Significantly all nutrients except calcium were in ample proportion (Table 5.5.5.)

Table 5.5.5. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	159
Thiamin	259
Riboflavin	120
Niacin Eq.	211
Vitamin C	415
Total Vitamin A Eq.	68
Potassium	194
Magnesium	110
Calcium	74
Phosphorous	148
Iron	229
Zinc	114

5.5.6. Basal metabolic rate

Table 5.5.6. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1758	1709	1665

The BMR's, determined by three different methods, agree (Table 5.5.6). Reported energy intake is 93% (FoodWorks) and 97% (Schofield) indicating accurate food recall by the Goldberg *et al.* (1991) cutoff of 0.9.

5.5.7. Activity

This subject did not complete an activity questionnaire. He did not play sport, however he led the most active work and leisure lifestyle of all the subjects with much manual labour and walking.

5.5.8. Summary

This subject led an active lifestyle with a relatively physical job and appeared to be in outwardly good health. He is not overweight nor displaying any illnesses other than hypertension and a slightly poor blood TG profile. However, his high blood pressure and TC place him at risk for CHD. It may be that the blood pressure elevation, that appears to be work related, will also contribute to CHD.

5.6. SUBJECT THREE

5.6.1. Physical data

Subject Three is a female Caucasian 58 years of age. She has a busy professional career. This subject only completed two sessions for baseline and at three weeks.

Table 5.6.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.671	1.602 ± 0.26	**BMI:** 33.3	90th
Weight (kg)	93.0	69.0 ± 0.56	Obese	90th
Waist (cm)	84.0	**W/H:** 0.81	**W/H:** 0.69	
Hip (cm)	122.0		**W/H%:** 86.0	
MAC (cm)	37.0	30.8 ± 0.15		> 50th
TSF (cm)	28.48	25.6 ± 0.36	**% fat:** 42.4	75th
MAMC (cm)	28.05	23.00		> 50th
AMA (cm^2)	56.12	34.71		90th
$\dot{V}O_2$max (ml/kg/min)	23.55			Poor
Handgrip (kg)	**total:** 58.0		**Kg/weight:** 0.62	10th

Subject Three was quite overweight with a very high BMI. However, her weight was unevenly distributed as indicated by the waist and hip measurements. Her TSF, while only slightly above the average for her age group, indicated a high bodyfat %. This is reinforced by a similar BIA-determined bodyfat of 39.8%. Her fitness and relative strength are poor (Table 5.6.1).

5.6.2. Blood pressure

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$). Comparison between baseline and mid-intervention ABP by paired Student's t-test showed no significant difference ($p > 0.05$).

Clinic and ABP agree with the other methods being generally lower. This may be due to the unusual setting of both the clinic readings and the operation of the ABP device. The other recordings were done in the home setting and were less disruptive (Table 5.6.2).

Table 5.6.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1) and mid-intervention (2)

Method	Day	SBP	DBP	MBP	HR
Clinic		170.7 ± 1.2	102.7 ± 2.3	123.4 ± 15.2	*
Auscultatory:	1	169.3± 1.2	98.7± 5.8	121.0± 3.6	*
sitting	2	152.0± 0.0	82.0± 2.0	104.3± 1.3	*
Digital:	1	154.5± 2.1	90.0± 8.5	97.8± 0.7	59± 4
sitting	2	135.5± 3.5	80.5± 0.7	97.8± 0.7	72± 7
Auscultatory:	1	165.3± 1.2	82.0± 2.0	104.3± 1.3	*
standing	2	149.3± 2.3	94.7± 3.1	111.8± 2.0	*
Digital:	1	153.0± 7.1	102.5± 3.5	118.1± 0.0	*
standing	2	157.0± 9.9	99.0± 5.7	117.2± 7.0	*
Ambulatory	1	172.3 ± 15.7	101.5 ± 20.3	123.4 ± 15.2	73 ± 8
	2	171.1 ± 14.3	98.4 ± 6.4	120.8 ± 6.1	73 ± 7

* data not obtained or not required.

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. Therefore, no *post hoc* tests were carried out ($p > 0.05$). Comparison between baseline and mid-intervention ABP by paired Student's t-test showed no significant difference ($p > 0.05$).

Clinic and ABP agree with the other methods being generally lower. This may be due to the unusual setting of both the clinic readings and the operation of the ABP device. The other recordings were done in the home setting and were less disruptive (Table 5.6.4).

5.6.3. Blood profile

Table 5.6.3. Blood lipid concentrations

	Start	3 weeks
TG (mmol/L)	1.63	1.70
TC (mmol/L)	5.61	5.87
HDL (mmol/L)	1.46	1.49
VLDL (mmol/L)	0.75	0.78
LDL (mmol/L)	3.41	3.61
TC/HDL	3.80	4.00
LDL/HDL	2.34	2.42

Subject Three's blood lipids were good and belied her physical parameters. Her TG was in the desirable range. In comparison, her TC and LDL were in the low-borderline range. The HDL was good and the TC/HDL and LDL/HDL ratios were desirable. However, the VLDL was in the borderline range (Table 5.6.3.).

5.6.4. Estimation of cardiovascular risk

Her HDL level gave a relative risk score of approximately 1.00. Her ABP and TC give 10-15% 5-year risk of CVD (NZ Guidelines, 2000). Her BMI places her in the Grade II category of moderate risk.

5.6.5. Reported food intake

Proportions of reported food intake were: protein - 16.99%; fat - 31.49%; carbohydrate - 49.50%. These proportions of intake are good, except that fat is a little high. All, but two, nutrients are more than adequate. Her reported food intake is less than the estimated energy requirement.

Table 5.6.4. Reported food intake percentage of RDI.

Nutrient	Percent of RDI
Protein	184
Thiamin	101
Riboflavin	165
Niacin Eq.	136
Vitamin C	122
Total Vitamin A Eq.	93
Potassium	171
Magnesium	137
Calcium	176
Phosphorous	204
Iron	170
Zinc	93

5.6.6. Basal metabolic rate

Table 5.6.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1702	1608	1602

These basal metabolic rates agree well. Reported energy intake was 89% (FoodWorks) and almost 100% (Schofield) which indicates food recall was probably fairly accurate.

5.6.7. Activity

This subject, while active at work, played no sport. There was no data for her leisure time as she did not complete this part of the questionnaire.

5.6.8. Summary

This subject was obese, with poor fitness and no sport. Her blood lipid profile was acceptable and indicated only average risk of developing CHD. Her low reported food intake needs to be addressed before an accurate assessment can be made on her diet.

5.7. SUBJECT FOUR

5.7.1. Physical data

Subject Four was a Caucasian male 57 years of age. He was employed part-time but enjoys an active leisure time. With his hypertension, he joins 31.3% of his peers (Russell, *et al.*, 1999). Subject Four completed two sessions for baseline and 3-weeks.

Table 5.7.1. Summary of the physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.772	1.737 ± 0.37	**BMI:** 34.7	> 95th
Weight (kg)	109.0	80.9 ± 0.61	Obese	90th
Waist (cm)	121.0	**W/H:** 0.94	**W/H:** 1.07	
Hip (cm)	113.0		**W/H%:** 119.0	
MAC (cm)	40.5	31.6 ± 0.13		> 50th
TSF (mm)	28.58	13.9 ± 0.28	**% fat:** 41.5	75th
MAMC (cm)	37.64	28.00		> 50th
AMA (cm^2)	72.56	49.01		> 95th
$\dot{V}O_2$max (ml/kg/min)	29.17			Good
Handgrip (kg)	**total:** 119.0		**Kg/weight:** 1.09	> 90th

Subject Four was obese with a very high BMI. However, his fitness and relative strength were good. From his hip and waist measurements it is clear that much of this weight was centrally located. BIA-determined bodyfat is 24.5% and is considerably less than that derived from the TSF. The confounding factor is the low bodyfat % that may be due to the inaccuracies observed with BIA methods as the amount of bodyfat reaches high levels.

5.7.2. Blood pressure

Comparing the different methods by repeated measures ANOVA showed there was significant difference between methods for DBP ($p = 0.0247$) and MBP ($p = 0.0383$) with no significance for SBP ($p = 0.135$). No *post hoc* test was carried out ($p > 0.05$) Comparing ABP baseline with mid-intervention by paired Student's t-test found no significant difference ($p > 0.05$).

In each case the second home reading is less, while the second ABP is higher. The significance of this is not clear, however it may be due to the nature of the ABP recording process elevating blood pressure. Alternatively, the greater number of ABP readings ensure increased accuracy (Table 5.7.2).

Table 5.7.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1) and mid-intervention (2)

Method	Day	SBP	DBP	MBP	HR
Clinic		172.0 ± 2.0	115.7 ± 0.6	133.1 ± 1.0	*
Auscultatory:	1	170.7 ± 1.2	108.0 ± 0.0	127.6 ± 0.4	*
sitting	2	158.0 ± 2.8	105.0 ± 7.1	121.4 ± 5.6	*
Digital:	1	163.0 ± 7.1	108.5 ± 0.7	125.4 ± 2.8	71 ± 4
sitting	2	158.0 ±.2	103.0 ± 1.4	120.1 ± 2.3	74 ± 11
Auscultatory:	1	177.3 ± 1.2	116.0 ± 0.0	135.1 ± 0.4	*
standing	2	146.7 ± 5.8	110.0 ± 0.0	121.0 ± 1.9	*
Digital:	1	166.5 ± 13.4	111.5 ± 0.7	128.5 ± 4.0	*
standing	2	154.7 ± 9.5	103.7 ± 3.2	119.5 ± 5.2	*
Ambulatory	1	172.8 ± 14.2	103.5 ± 8.0	125.5 ± 8.6	72 ± 9
	2	177.7 ± 5.9	112.6 ± 13.4	133.8 ± 10.5	79 ± 9

* data not obtained or not required.

5.7.3. Blood profile

Table 5.7.3. Blood lipid concentrations

	Start	3 weeks
TG (mmol/L)	1.20	1.07
TC (mmol/L)	4.86	5.73
HDL (mmol/L)	1.14	1.01
VLDL (mmol/L)	0.55	0.49
LDL (mmol/L)	3.17	4.23
TC/HDL	4.30	5.70
LDL/HDL	2.78	4.19

Subject Four's TG levels were low, but TC, LDL, TC/HDL increased between readings from desirable to high. LDL/HDL increased from desirable to borderline. While it is difficult to infer from single readings it is obvious that the lipid profiles deteriorated considerably over the three weeks between readings. The contributory factors are unknown, but may be linked to the milk supplement or possibly poor diet.

5.7.4. Estimation of cardiovascular risk

The Relative Risk was > 1.94 from his highest HDL level. His ABP and TC gave a 5-year risk of CVD of 15-20% (NZ Guidelines, 2000). His BMI places him in the Grade II category of moderate risk.

5.7.5. Reported food intake

Table 5.7.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	110
Thiamin	104
Riboflavin	53
Niacin Eq.	118
Vitamin C	128
Total Vitamin A Eq.	211
Potassium	124
Magnesium	54
Calcium	85
Phosphorous	102
Iron	71
Zinc	60

Proportions of reported food intake were: protein - 26.92%; fat - 44.42%; carbohydrate - 27.16%. While these proportions are far from ideal, the subject reported a food intake of just 31% of calculated RDI that was less than half of BMR. The reason for this under-reporting is unknown, however it may explain the low values for some vitamins and minerals (Table 5.7.4.).

5.7.6. Basal metabolic rate

Table 5.7.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
2502	2083	2126

BIA is higher, but the subjects displayed considerable central adiposity which may explain this result compared to the other methods. Reported energy intake was just 31% (FoodWorks) and 102% (Schofield) which reveals some discrepancy with food reporting or an unbalanced diet.

5.7.7. Activity

This subject's work was not active, but he led an active life outside of work with a large amount of golf and dancing "as often as possible". However, this level of activity was not balanced by the reported food intake of just 31% of that required.

5.7.8. Summary

Subject Four presented in relatively poor physical shape, however his fitness and strength were good. He displayed considerable central adiposity with 34% greater W/H than is ideal. His blood profile deteriorated considerably from one sample to another. The cardiovascular risk was high. These factors indicate that he is highly susceptible to CHD. The low reported food intake needs to be addressed before an accurate conclusion can be made on his diet.

5.8. SUBJECT FIVE

5.8.1. Physical data

Subject Five is a female Caucasian 60 years of age. She was a busy professional with a relatively high workload. Her level of hypertension places her with 26.5% of her peers (Russell *et al.*, 1999). Subject Five completed the trial.

This subject was regarded as obese with a high BMI. However, she demonstrated good fitness. The TSF-determined bodyfat % was very high, and 23% higher than the BIA-determined bodyfat of 37.1%. (Table 5.8.1.)

Table 5.8.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.527	1.602 ± 0.26	**BMI:** 32.4	25th
Weight (kg)	61.8	69.0 ± 0.56	Obese	90th
Waist (cm)	79.5	**W/H:** 0.81	**W/H:** 0.85	
Hip (cm)	99.0		**W/H%:** 106.0	
MAC (cm)	32.0	30.8 ± 0.15		> 50th
TSF (mm)	35.67	25.6 ± 0.36	**% fat:** 45.7	25th
MAMC (cm)	21.42	23.00		15th
AMA (cm^2)	27.91	34.71		5th
$\dot{V}O_2$max (ml/kg/min)	26.72			Good
Handgrip (kg)	**total:** 57.5		**Kg/weight:**0.93	70th

5.8.2. Blood pressure

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$). However, the recordings were taken over a normal working day, where it is to be expected that blood pressure would be higher. As a professional, it would be expected that she was comfortable with situations where the unusual was taking place, such as clinic blood pressure (Table 5.8.2).

Table 5.8.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1); mid-intervention (2) and post-intervention (3)

Method	Day	SBP	DBP	MBP	HR
Clinic		140.7 ± 1.2	90.0 ± 6.0	105.8 ± 1.7	*
Auscultatory:	1	138.0 ± 2.0	89.3 ± 1.2	104.5 ± 2.0	*
sitting	2	140.0 ± 0.0	98.0 ± 2.8	110.9 ± 1.9	*
	3	150.0 ± 0.0	96.0 ± 0.0	112.9 ± 0.0	*
Digital:	1	146.7 ± 2.3	85.0 ± 5.6	104.5 ± 3.1	84 ± 2
sitting	2	147.0 ± 2.8	93.0 ± 1.4	109.9 ± 1.9	84 ± 7
	3	141.0 ± 1.4	88.5 ± 4.9	104.9 ± 3.7	87 ± 4
Auscultatory:	1	142.7 ± 3.1	95.3 ± 2.3	110.0 ± 1.0	*
standing	2	141.5 ± 4.9	99.0 ± 1.4	112.0 ± 0.7	*
	3	147.0 ± 4.2	106.0 ± 2.8	118.5 ± 3.3	*
Digital:	1	144.3 ± 3.8	98.3 ± 2.5	112.5 ± 0.9	*
standing	2	149.5 ± 9.2	96.5 ± 4.9	113.0 ± 6.3	*
	3	144.0 ± 0.0	101.0 ± 12.7	114.2 ± 8.4	*
Ambulatory	1	167.5 ± 20.1	97.3 ± 12.0	119.9 ± 12.1	81 ± 14
	2	153.2 ± 10.9	92.1 ± 9.4	112.5 ± 7.9	85 ± 7
	3	160.5 ± 27.0	88.7 ± 25.6	112.5 ± 22.5	80 ± 10

* data not obtained or not required.

5.8.2.1. Blood pressure differences

Table 5.8.3. Comparison of blood pressure changes (mm Hg) ± SD from baseline mean with post-intervention mean

Method	SBP	DBP	MBP
Auscultatory sitting	+12.0 ± 0.0	+6.7 ± 0.0	+8.4 ± 0.0
Digital: sitting	-5.7 ± 1.9	+3.5 ± 4.9	+0.5 ± 3.1
Auscultatory: standing	+4.3 ± 3.2	+10.7 ± 2.3	+8.5 ± 1.9
Digital: standing	-0.3 ± 0.0	+2.7 ± 7.0	+1.6 ± 4.5
Ambulatory	-7.0 ± 8.3	-8.5 ± 6.8	-7.4 ± 6.2

Table 5.8.3. shows that there was no consistent result. Testing for a difference with Student's t-test between the baseline and post-intervention found no significant difference

($p > 0.05$) except for auscultatory standing DBP and MBP ($p < 0.05$). No *post hoc* tests were carried out ($p > 0.05$).

5.8.3. Blood profile

Table 5.8.4. Blood lipid concentrations

	Start	3 weeks	6 weeks
TG (mmol/L)	1.10	0.76	1.57
TC (mmol/L)	5.01	5.94	4.40
HDL (mmol/L)	1.29	1.62	1.22
VLDL (mmol/L)	0.51	0.35	0.72
LDL (mmol/L)	3.22	3.98	2.46
TC/HDL	4.75	3.70	3.60
LDL/HDL	2.50	2.46	2.02

All parameters are generally good. The small elevations in the middle sample may be due to measurement variations or dietary factors. The blood profile generally improves from the first sample to the last with the critical LDL and ratios decreasing significantly. However, the VLDL decreases then increases to the borderline range.

5.8.4. Estimation of cardiovascular risk

Similar HDL levels between blood samples indicate that the Relative Risk of 1.55 remained constant. The ABP and TC give a 5-year risk of CVD of 10-15% (NZ Guidelines, 2000). Her BMI is also Grade II and indicates moderate risk.

5.8.5. Reported food intake

The 24-hour food recall indicated that reported food intake was less than BMR that may explain the low amounts of several nutrients. Significantly, calcium intake is very low. Proportions of food intake were: protein - 13.11%; fat - 26.62%; carbohydrate – 58.27%. These fall within the accepted ranges, despite the low reported intake.

Table 5.8.5. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	92
Thiamin	114
Riboflavin	77
Niacin Eq.	159
Vitamin C	118
Total Vitamin A Eq.	101
Potassium	91
Magnesium	60
Calcium	32
Phosphorous	64
Iron	139
Zinc	72

5.8.6. Basal metabolic rate

Table 5.8.6. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1181	1353	1221

Table 5.8.2. shows that the BMR determinations are similar. Reported energy intake was 70% (FoodWorks) and 90% (Schofield) indicating there was some under-reporting (Goldberg *et al.*, 1991).

5.8.7. Summary

This subject worked in a relatively highly stressed position. Other than her high blood pressure and obesity she apparently enjoyed good health. Displaying some of the elements of Syndrome X, she has quite a high chance of developing CHD or other obesity-related problem in the future.

5.9. SUBJECT SIX

5.9.1. Physical data

Subject Six is a professional 53-year-old Caucasian male leading a busy, life. His level of blood pressure puts him with 31.9% of his peers. Subject Six only completed two sessions.

Table 5.9.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.777	1.737 ± 0.37	**BMI:** 21.95	75th
Weight (kg)	69.3	80.9 ± 0.61	Healthy	10th
Waist (cm)	82.0	**W/H:** 0.94	**W/H:** 0.85	
Hip (cm)	97.0		**W/H%:** 94.0	
MAC (cm)	28.0	31.6 ± 0.13		15th
TSF (mm)	9.69	13.9 ± 0.28	**% fat:** 27.1	25th
MAMC (cm)	24.96	28.00		5th
AMA (cm^2)	39.56	49.01		< 5th
$\dot{V}O_2$max (ml/kg/min)	50.19			Superior

Subject Six was slightly built and healthy. A grip strength measure was not obtained for an unknown reason. The TSF-determined bodyfat % agrees moderately with BIA-determined bodyfat of 20.5%. The superior fitness category may have been a result of insufficient workload during the fitness test.

5.9.2. Blood pressure

Subject Six demonstrates high-normal blood pressure in all, but clinic and ABP recording. The ABP recording was undertaken during a normal working day, which may contribute to the apparent elevation. Each method is relatively consistent between days except for the standing auscultatory method, the second reading being taken during work time. However, this was not reflected by the other methods (Table 5.9.2).

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$). Each recording

day was compared with Student's t-test which demonstrated that there was no significance ($p > 0.05$) between recording days.

Table 5.9.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline (1) and mid-intervention (2)

Method	Day	SBP	DBP	MBP	HR
Clinic		142.0 ± 0.0	88.0 ± 0.0	104.9 ± 0.0	*
Auscultatory:	1	128.7 ± 1.2	84.7 ± 3.1	98.3 ± 1.7	*
sitting	2	131.0 ± 1.4	86.0 ± 2.8	100.0 ± 1.4	*
Digital:	1	131.0 ± 7.1	89.0 ± 1.4	102.0 ± 1.4	78 ± 18
sitting	2	130.5 ± 2.1	82.0 ± 2.8	97.2 ± 2.6	59 ± 1
Auscultatory:	1	118.0 ± 0.0	94.7 ± 4.6	101.4 ± 3.0	*
standing	2	131.5 ± 4.9	85.0 ± 7.1	99.5 ± 6.3	*
Digital:	1	118.0 ± 8.5	84.5 ± 6.4	94.7 ± 7.0	*
standing	2	118.0 ± 19.8	92.5 ± 2.1	100.0 ± 5.1	*
Ambulatory	1	157.1 ± 19.2	93.6 ± 4.9	112.2 ± 8.1	60 ± 9
	2	151.82 ± 3.2	86.31 ± 7.4	105.6 ± 18.5	57 ± 12

* data not obtained or not required.

5.9.3. Blood profile

Table 5.9.3. Blood lipid concentrations

	Start
TG (mmol/L)	1.30
TC (mmol/L)	5.82
HDL (mmol/L)	1.42
VLDL (mmol/L)	0.60
LDL (mmol/L)	3.81
TC/HDL	4.20
LDL/HDL	2.68

Subject six's TG, HDL, TC/HDL and LDL/HDL ratios are all in the desirable range. Total cholesterol is borderline. These data indicate a relatively healthy lipid profile (Table 5.9.3.).

5.9.4. Estimation of cardiovascular risk

Relative Risk is only 0.67, and the 5-year risk of CVD is 5-10% (NZ Guidelines). His BMI places him in the desirable range of risk.

5.9.5. Reported food intake

Reported food intake proportions were: protein - 13.13%; fat - 36.29%; carbohydrate - 41.40%. The protein content was acceptable, however the fat was too high with low carbohydrate. Estimated energy expenditure exceeded reported food intake by 45%. In fact, food intake was less than BMR, This result may indicate why most nutrients were less than the RDI (Table 5.9.4.).

Table 5.9.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	95
Thiamin	90
Riboflavin	90
Niacin Eq.	120
Vitamin C	41
Total Vitamin A Eq.	31
Potassium	94
Magnesium	80
Calcium	66
Phosphorous	118
Iron	151
Zinc	51

5.9.6. Basal metabolic rate

It is most unusual that the three BMR calculations arrive at almost the same figure. Reported energy intake was 69% (FoodWorks) and almost 100% (Schofield) indicating some discrepancy with food recall.

Table 5.9.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1675	1675	1670

5.9.7. Summary

This subject, while healthy and with only moderately elevated blood pressure, needs to address the insufficient dietary intake that may be a routine part of his busy schedule. This may cause health problems in the future.

5.10. SUBJECT SEVEN

5.10.1. Physical data

Subject Seven is a Caucasian female 54 years of age. She also led an active working life. Her hypertension puts her with 24.9% of her peers (Russell *et al.*, 1999). This subject did not complete the trial with only baseline data recorded.

Table 5.10.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.565	1.602 ± 0.26	**BMI:** 23.35	25th
Weight (kg)	57.2	69.0 ± 0.56	Healthy	25th
Waist (cm)	70.0	**W/H:** 0.81	**W/H:** 0.78	
Hip (cm)	90.0		**W/H%:** 97.5	
MAC (cm)	27.0	30.8 ± 0.15		15th
TSF (mm)	17.39	25.6 ± 0.36	**% fat:** 34.3	20th
MAMC (cm)	21.54	23.00		15th
AMA (cm^2)	30.41	34.71		10th
$\dot{V}O_2max$ (ml/kg/min)	33.76			Superior
Handgrip (kg)	**total:** 64.5		**Kg/weight:** 1.13	> 90th

This subject was slimmer than her New Zealand peers. Her BIA-determined bodyfat of 26.5% was less than that determined from the TSF. Her fitness was determined as superior,

however this is erroneous as can be seen from the very high HR that this subject exhibits during all recording sessions that skewed this result.

5.10.2. Blood pressure

This subject displayed both elevated blood pressure and HR. The clinic blood pressure was similar to the other non-ABP methods, but ABP was higher. Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$).

Table 5.10.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline

Method	Day	SBP	DBP	MBP	HR
Clinic		150.0 ± 0.0	110.0 ± 0.0	122.1 ± 0.0	*
Auscultatory: sitting	1	165.3 ± 1.2	112.0 ± 2.0	128.5 ± 1.4	*
Digital: sitting	1	149.5 ± 7.8	103.0 ± 1.4	117.3 ± 3.5	108 ± 6
Auscultatory: standing	1	158.7 ± 1.2	118.7 ± 7.0	130.7 ± 4.6	*
Digital: standing	1	158.5 ± 6.4	109.0 ± 12.7	124.2 ± 10.5	*
Ambulatory	1	171.7 ± 13.6	110.3 ± 9.0	129.1 ± 8.3	106 ± 16

* data not obtained or not required.

5.10.3. Blood profile

Subject Seven's TG, HDL, LDL and both ratios are well in the desirable range. The only elevated parameter is TC (Table 5.10.3.)

Table 5.10.3. Blood lipid concentrations

	Start
TG (mmol/L)	1.15
TC (mmol/L)	6.01
HDL (mmol/L)	2.15
VLDL (mmol/L)	0.52
LDL (mmol/L)	3.34
TC/HDL	2.80
LDL/HDL	1.55

5.10.4. Estimation of cardiovascular risk

The low HDL resulted in a Relative Risk less than 0.52. The 5-year CVD risk is 5-10%, however this may be regarded as an underestimation (NZ Guidelines, 2000). Her BMI places her in the desirable Grade O.

5.10.5. Reported food intake

Table 5.10.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	269
Thiamin	392
Riboflavin	282
Niacin Eq.	364
Vitamin C	477
Total Vitamin A Eq.	189
Potassium	291
Magnesium	164
Calcium	173
Phosphorous	224
Iron	149
Zinc	144

Reported food intake was: protein - 19.85%; fat - 32.44%; carbohydrate -45.75%. The intake of protein and fat is high, while carbohydrate is low. Intake of vitamins and minerals

are high. This subject consumed quite a large amount of fruit during the day, which may explain these results. Her reported food intake was higher than the estimated energy expenditure. If this trend were to continue extra weight may be a problem in the future.

5.10.6. Basal metabolic rate

Table 5.10.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1280	1317	1312

There is close agreement between the differing methods. The Schofield and FoodWorks BMR are almost identical. Reported energy intake was 135% (FoodWorks) and almost 100% (Schofield) indicating accurate recall, well above cutoff.

5.10.7. Summary

This subject demonstrated both elevated blood pressure and HR. However, she was in otherwise good health with a good blood lipid profile.

5.11. SUBJECT EIGHT

5.11.1. Physical data

Subject Eight is a Maori Male, 50 years of age who was moderately active. His blood pressure puts him with 26.2% of his peers (Russell *et al.*, 1999). Subject Eight did not complete the trial with only baseline data recorded.

This subject was the only Maori in this trial, therefore there is a lack of comparative data. This subject was obese, yet his TSF was less than his peers indicating a differing body composition compared with Caucasian New Zealanders. This result is confirmed by the BIA-determined bodyfat of 27.9%. In addition, his W/H is less than the mean for his peers. His BMI was just over the mean for his peers (31.6 ± 0.87, Russell *et al.*, 1999).

The fitness is shown as superior, however, in this case, it indicates that the workload during the fitness test was not sufficient to elevate the HR enough to provide an accurate determination of maximal oxygen uptake (Table 5.11.1.).

Table 5.11.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile*
Height (m)	1.78	1.723 ± 0.89	**BMI:** 32.4	75th
Weight (kg)	107.0	93.92.91	Obese	90th
Waist (cm)	104.0	**W/H:** 0.95	**W/H:** 0.85	
Hip (cm)	112.0		**W/H%:** 94.4	
MAC (cm)	40.0	34.9 ± 0.58		> 50th
TSF (mm)	13.53	17.6 ± 1.94	**% fat:** 31.6	75th
MAMC (cm)	35.75	28.0		> 50th
AMA (cm^2)	91.70	49.01		>95th
$\dot{V}O_2$max (ml/kg/min)	43.88			Superior
Handgrip (kg)	**total:** 99.0		**Kg/weight:**0.93	10th

* Non-Maori data used for percentiles

5.11.2. Blood pressure

While Table 5.11.2. suggests there to be quite a difference between means, comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$).

Only the clinic and ABP means classify this subject in the mild to moderate hypertensive range. All other means are normal to high-normal. The elevated clinic blood pressure may be the result of white coat phenomenon. The high ABP recording may be the result of there only being seven valid readings. The subject reported that the ABP cuff was too small and caused discomfort and that he removed the cuff and replaced it on several occasions causing missed readings. He appeared uncomfortable with the procedure.

It may be that the blood pressure recordings taken by the other methods in the subject's home were more accurate and that this subject's recruitment in this trial was erroneous.

Table 5.11.2. Baseline means for blood pressure (mm Hg) and HR (bpm) ± SD

Method	Day	SBP	DBP	MBP	HR
Clinic		150.0 ± 0.0	110.0 ± 0.0	122.1 ± 0.0	*
Auscultatory: sitting	1	137.0 ± 1.4	103.0 ± 4.2	113.2 ± 3.3	76 ± 1
Digital: sitting	1	130.5 ± 2.1	90.5 ± 3.5	102.8 ± 3.0	*
Auscultatory: standing	1	138.0 ± 2.8	100.0 ± 0.0	111.5 ± 0.9	*
Digital: standing	1	127.5 ± 0.7	95.0 ± 1.4	104.8 ± 0.7	*
Ambulatory	1	164.9 ± 30.1	103.6 ± 22.9	124.3 ± 20.8	81 ± 18

* data not obtained or not required.

5.11.3. Blood profile

Table 5.11.3. Blood lipid concentrations

	Start
TG (mmol/L)	0.59
TC (mmol/L)	4.14
HDL (mmol/L)	1.06
VLDL (mmol/L)	0.27
LDL (mmol/L)	2.81
TC/HDL	3.90
LDL/HDL	2.65

Subject Eight's TG is extremely low, the TC, LDL and VLDL are low. The HDL is good, thus both ratios are in the desirable range. New Zealand Maori male mean HDL is 1.1 ± 0.05 (SEM) (Russell *et al.*, 1999), so that Subject Eight is just below average. However, his TC is well below average for his peers (6.42 ± 0.23 (SEM), Russell *et al.*, 1999).

5.11.4. Estimation of cardiovascular risk

The Relative Risk is 1.22 and the 5-year risk of CVD is 5-10% (NZ Guidelines, 2000). His BMI places him in Grade II with moderate risk.

5.11.5. Reported food intake

Table 5.11.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	175
Thiamin	129
Riboflavin	53
Niacin Eq.	140
Vitamin C	62
Total Vitamin A Eq.	39
Potassium	98
Magnesium	72
Calcium	62
Phosphorous	116
Iron	243
Zinc	132

Reported food intake proportions were: protein - 16.52%; fat - 23.51%; carbohydrate - 57.90%. These fall well within healthy guidelines, especially the low fat. Significantly, calcium and magnesium are low with some vitamins also low. Overall food intake was higher than BMR, but less than the estimated energy expenditure. This may indicate some under-reporting of food intake (Table 5.11.5.).

5.11.6. Basal metabolic rate

All differing methods agree. Reported energy intake was 80% FoodWorks) and almost 100% (Schofield) indicating some inaccuracy in food recall (Table 5.11.5.).

Table 5.11.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
2345	2110	2103

5.11.7. Summary

This subject, while obese, has moderate risk of CHD. These data indicate that there are sufficient differences between Caucasians and Maori subjects to require more study of Maori in such a trial. There is also a suggestion that cultural and social differences may cause an erroneous elevation of blood pressure in the clinic setting.

5.12. SUBJECT NINE

5.12.1. Physical data

At 38-years old, Subject Nine was the youngest participant of the study. A female, she worked full-time and was the only current smoker in the study. Her hypertension puts her with just 2.5% of her peers (Russell *et al.*, 1999). Subject Nine did not complete the trial with only baseline data recorded.

Table 5.12.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.658	1.638 ± 0.30	**BMI:** 24.74	75th
Weight (kg)	68.0	66.6 ± 0.65	Healthy	50th
Waist (cm)	71.0	**W/H:** 0.89	**W/H:** 0.71	
Hip (cm)	100.0		**W/H%:** 88.8	
MAC (cm)	29.5	29.6 ± 0.19		> 50th
TSF (mm)	34.78	24.8 ± 0.46	**% fat:** 39.3	90th
MAMC (cm)	18.57	23.00		< 5th
AMA (cm^2)	20.95	31.33		< 5th
$\dot{V}O_2max$ (ml/kg/min)	25.34			Poor
Handgrip (kg)	**total:** 65.5		**Kg/weight:**0.96	70th

This subject was in good shape with average parameters relative to her New Zealand peers. However, she had poor fitness that her smoking may contribute to. Her TSF-determined bodyfat % was much higher than that of the BIA bodyfat of 23.1% (Table 5.12.1.).

5.12.2. Blood pressure

Table 5.12.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline

Method	Day	SBP	DBP	MBP	HR
Clinic		168.0 ± 0.0	112.0 ± 0.0	129.4 ± 0.0	*
Auscultatory: sitting	1	183.3 ± 3.1	123.3 ± 2.3	141.9 ± 0.7	*
Digital: sitting	1	177.5 ± 9.2	117.0 ± 4.2	135.8 ± 5.8	96 ± 7
Auscultatory: standing	1	188.7 ± 1.2	140.7 ± 1.2	155.1 ± 0.7	*
Digital: standing	1	186.0 ± 5.7	134.5 ± 3.5	150.2 ± 4.2	*
Ambulatory	1	173.8 ± 20.9	112.7 ± 14.2	133.3 ± 14.1	110 ± 9

* data not obtained or not required.

For Table 5.12.2. comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$).

This subject demonstrated quite high blood pressure and HR by all methods. These results may have been due to the white coat effect for all non-ABP readings. In addition, she wore the ABP device while working a fairly physical and demanding job. A significant contributory factor may also have been her smoking.

5.12.3. Blood profile

Subject Nine displayed low TG and borderline TC and LDL. The ratios are good and HDL is in the desirable range.

Table 5.12.3. Blood lipid concentrations

	Start
TG (mmol/L)	< 0.51
TC (mmol/L)	5.68
HDL (mmol/L)	1.44
VLDL (mmol/L)	N/A
LDL (mmol/L)	4.05
TC/HDL	4.00
LDL/HDL	2.81

5.12.4. Estimated cardiovascular risk

The HDL level gives a Relative Risk of 1.00. The 5-year risk of CVD is 5-10% (NZ Guidelines). Smoking puts this subject into a higher risk category. Her BMI places her at the high end of Grade O for risk.

5.12.5. Reported food intake

Table 5.12.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	169
Thiamin	172
Riboflavin	215
Niacin Eq.	312
Vitamin C	686
Total Vitamin A Eq.	138
Potassium	201
Magnesium	99
Calcium	86
Phosphorous	131
Iron	114
Zinc	107

This subject reported very high intake of vitamin C. A consumption that may have been

deliberate or unintentional. Calcium was slightly lower than RDI. Other nutrients are sufficient or in excess of requirements. Her proportion of food intake was: protein - 14.46%; fat - 42.11%; carbohydrate - 34.71%. The fat content is quite high, while the carbohydrates are very low. Her intake was slightly greater than that required for her weight and activity.

5.12.6. Basal metabolic rate

Table 5.12.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1591	1402	1400

Both the Schofield and FoodWorks BMR agree remarkably. However, the BIA-determined BMR is quite a bit higher. The reason for this is unknown. Reported energy intake was 109% (FoodWorks) and effectively 100% (Schofield) indicating fairly accurate food recall (Table 5.12.5.).

5.12.7. Summary

This subject's very high blood pressure, smoking and busy workplace may contribute to a risk of CHD in spite of her good blood profile and relative youth.

5.13. SUBJECT TEN

5.13.1. Physical data

Subject Ten is a Caucasian female 52 years of age. She also worked full-time. Her blood pressure places her with 26.5% of her peers (Russell *et al.*, 1999). This subject did not complete the trial with only baseline data recorded.

This subject was regarded as obese. Her TSF-determined bodyfat % agreed with the BIA bodyfat of 43.0%. She is one of 21.7% of her peers in this category (Russell *et al.*, 1999).

Table 5.13.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.564	1.602 ± 0.26	**BMI:** 32.4	25th
Weight (kg)	79.2	69.0 ± 0.56	Obese	90th
Waist (cm)	92.0	**W/H:** 0.81	**W/H:** 0.85	
Hip (cm)	108.0		**W/H%:** 106.3	
MAC (cm)	34.0	30.8 ± 0.15		> 50th
TSF (mm)	37.99	25.6 ± 0.36	**% fat:** > 46.0	90th
MAMC (cm)	22.06	23.00		50th
AMA (cm^2)	32.24	34.71		10th
$\dot{V}O_2max$ (ml/kg/min)	21.31			Poor
Handgrip (kg)	**total:** 53.5		**Kg/weight:**0.68	10th

5.13.2. Blood pressure

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$).

Table 5.13.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline

Method	Day	SBP	DBP	MBP	HR
Clinic		153.5 ± 3.5	97.0 ± 1.4	114.7 ± 0.2	84.0 ± 0.0
Auscultatory: sitting	1	143.3 ± 1.2	98.7 ± 1.2	112.4 ± 1.0	71.5 ± 7.8
Digital: sitting	1	138.0 ± 2.8	94.5 ± 4.9	107.9 ± 2.3	*
Auscultatory: standing	1	154.0 ± 0.0	108.7 ± 2.3	122.5 ± 1.5	*
Digital: standing	1	137.0 ± 1.4	104.5 ± 3.5	114.2 ± 1.9	*
Ambulatory	1	154.5 ± 15.2	85.7 ±14.7	109.7 ± 13.4	74.3 ± 10.1

* data not obtained or not required.

5.13.3. Blood profile

Almost all blood parameters are in the desirable range. However, the VLDL is barely in the borderline range (Table 5.13.3.).

Table 5.13.3. Blood lipid concentrations

	Start
TG (mmol/L)	1.18
TC (mmol/L)	5.10
HDL (mmol/L)	1.17
VLDL (mmol/L)	0.54
LDL (mmol/L)	3.39
TC/HDL	4.30
LDL/HDL	2.90

5.13.4. Estimation of cardiovascular risk

The HDL gives a Relative Risk of 1.55, and the 5-year risk of CHD is 2.5-5% (NZ Guidelines). Her BMI places her in Grade II obesity with moderate risk.

5.13.5. Reported food intake

This subject apparently ate less than half of the 24-hour RDI and estimated energy expenditure for food intake that may explain the low individual nutrient intakes shown in Table 5.13.4. Her reported food intake was lower than any determined BMR. This may have been due to the extreme under-reporting of food intake or another factor that was not clear. The proportion of food intake was: protein - 25.43%; fat - 20.58%; carbohydrate - 52.39%. Protein intake was high, but fat and carbohydrate were acceptable.

Table 5.13.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	141
Thiamin	82
Riboflavin	53
Niacin Eq.	219
Vitamin C	48
Total Vitamin A Eq.	22
Potassium	75
Magnesium	67
Calcium	33
Phosphorous	77
Iron	31
Zinc	40

5.13.6. Basal metabolic rate

These estimates of BMR agreed (Table 5.13.4.). However, the BIA-determined BMR is much less than that from the other methods. Reported energy intake was 49% (FoodWorks) and almost 100% (Schofield) indicating a food discrepancy or inappropriate diet.

Table 5.13.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1373	1497	1491

5.13.7. Summary

This subject demonstrates some risk of CHD or other health problems due to obesity and poor fitness. The HDL level indicates some risk of CHD despite the acceptable nature of the other parameters in the blood sample. Dietary analysis would not be accurate without further investigation into the very low food intake.

5.14. SUBJECT ELEVEN

5.14.1. Physical data

Subject Eleven is a 50-year old female Caucasian. Her hypertension is shared by 24.9% of her peers. She did not complete the trial with only baseline data recorded.

Table 5.14.1. Summary of physical data

Parameter	Data	Norm	Calculated	Percentile
Height (m)	1.633	1.602 ± 0.26	**BMI:** 22.12	75th
Weight (kg)	59.0	69.0 ± 0.56	Healthy	25th
Waist (cm)	75.0	**W/H:** 0.81	**W/H:** 0.82	
Hip (cm)	92.0		**W/H%:** 101.9	
MAC (cm)	26.0	30.8 ± 0.15		15th
TSF (mm)	22.65	25.6 ± 0.36	**% fat:** 38.9	50th
MAMC (cm)	18.88	23.00		< 5th
AMA (cm^2)	21.88	34.71		< 5th
$\dot{V}O_2max$ (ml/kg/min)	23.93			Poor
Handgrip (kg)	**Total:** 54.5		**Kg/weight:** 0.93	70th

This subject was generally healthy, in spite of her elevated blood pressure. Her weight and BMI are less than the New Zealand means. The TSF-determined bodyfat % is considerably higher than that of the BIA bodyfat of 26.5%. This may have been due to the relatively small size of her arms that may cause the BIA device to underestimate bodyfat. Her AMA was considerably less than the average.

5.14.2. Blood pressure

Comparison of all means by repeated-measures ANOVA found no significant difference ($p = 1.0000$) between methods. No *post hoc* tests were carried out ($p > 0.05$). However, ABP mean appears slightly elevated, while the standing digital is lower and does not agree with the auscultatory standing for an unknown reason (Table 5.14.2.).

Table 5.14.2. Summary of means for blood pressure (mm Hg) and HR (bpm) ± SD for baseline

Method		SBP	DBP	MBP	HR
Clinic		165.0 ± 0.0	102.0 ± 0.0	121.8 ± 0.0	*
Auscultatory: sitting	1	160.0 ± 14.1	104.0 ± 5.7	121.4 ± 0.9	*
Digital: sitting	1	165.5 ± 2.1	100.0 ± 7.1	120.6 ± 4.0	72 ± 7
Auscultatory: standing	1	166.0 ± 8.5	97.0 ± 1.4	118.8 ± 1.9	*
Digital: standing	1	152.5 ± 2.1	86.5 ± 2.1	107.4 ± 0.7	*
Ambulatory	1	173.5 ± 17.1	97.0 ± 8.5	121.2 ± 8.2	72 ± 7

* data not obtained or not required.

5.14.3. Blood profile

Subject Eleven's blood lipid profile was mixed with high VLDL and borderline TG and TC. However, LDL, HDL, LDL/HDL and TC/HDL were desirable (Table 5.14.3.).

Table 5.14.3. Blood lipid concentrations

	Start
TG (mmol/L)	2.78
TC (mmol/L)	5.44
HDL (mmol/L)	1.37
VLDL (mmol/L)	1.27
LDL (mmol/L)	2.79
TC/HDL	4.00
LDL/HDL	2.04

5.14.4. Estimation of cardiovascular risk

The Relative Risk was 1.00 and the 5-year risk of CVD was 5-10% (NZ Guidelines). HER BMI is in Grade O.

5.14.5. Reported food intake

Table 5.14.4. Reported food intake percentage of RDI

Nutrient	Percent of RDI
Protein	161
Thiamin	169
Riboflavin	151
Niacin Eq.	250
Vitamin C	999
Total Vitamin A Eq.	121
Potassium	199
Magnesium	122
Calcium	93
Phosphorous	151
Iron	74
Zinc	68

This subject consumed an astonishing amount of Vitamin C, it is not known whether this was deliberate or unintentional. Calcium, phosphorous and iron were slightly less than recommended, zinc more so (Table 5.14.4.). The 24-hour food recall determined that her diet was made up of the following proportions: protein - 16.03%; fat - 24.58%; carbohydrate - 56.98%. These proportions are in agreement with general recommendations. In addition, her reported food intake agreed well with the estimated energy expenditure.

5.14.6. Basal metabolic rate

Table 5.14.5. BMR by different methods (kcal/day)

BIA	Schofield	FoodWorks 24h recall
1320	1330	1326

There is remarkable agreement with the three methods of determining BMR. Reported energy intake was 99% (FoodWorks) and almost 100% (Schofield) indicating reasonable accuracy with food intake recall.

5.14.7. Summary

This subject was generally healthy in spite of her elevated blood pressure. From these data she did not fit the profile that characterises Syndrome X.

5.15. DISCUSSION

5.15.1. Subjects

The subjects were older, almost twice the mean age of the preliminary trial. All subjects were recruited specifically for their elevated blood pressure and this was observed by most methods of blood pressure recording, and which fulfilled this objective of the trial. Almost half the subjects were obese. Some were very unfit, yet others had apparently excellent cardiovascular fitness. Some subjects had poor blood profiles, while others did not. Almost all had moderate to high estimation of cardiovascular risk.

These subjects represented a cross-section of their age group. There was no single apparent cause for their hypertension. However, many demonstrated the cluster of factors that characterise Syndrome X. None of the subjects were exceptionally different from the standards of their peers, despite some normative data coming from North American studies.

5.15.2. Physical

Six of the 11 subjects were overweight, with five of these regarded as obese. Of these overweight subjects, five were female. In the healthy weight range, three were female and two were males. Otherwise these subjects were within the averages for New Zealanders as outlined in Russell, *et al.* (1999).

For some subjects, even if the BMI was acceptable, their W/H was not. Central adiposity is associated with hypertension (Reisen, 1990), and is a risk factor for CHD (Monash University, 1999).

5.15.3. Fitness

Generally, fitness was poor, especially amongst the women. The superior fitness of two subjects may have been due to an elevated HR. This may cause flattening of the oxygen uptake curve and indicate these subjects were fitter than in reality (Raglin *et al.*, 1993). Another subject had such poor fitness, that she had to terminate the fitness test and was unable to complete the task.

5.15.4. Basal metabolic rate

Most had similar derived BMR values, despite these being calculated by three different methods. This observation, in part, validates each of these methods.

5.15.5. Blood pressure

All, but two subjects, had elevated blood pressure. These two were in the high range of normal, and one had distinctive elevation with the clinic and ABP recording. The difference between clinic and other methods is demonstrated in almost all cases. However, most of these differences were not statistically significant.

5.15.6. Intervention

If there was an effect on blood pressure of any intervention it is not obvious due to the few subjects who completed the trial. For this reason, no attempt has been made to match treatments with subjects for those who did complete the trial.

5.15.7. Blood profile

The subjects had generally acceptable blood lipid profiles. However, for most of the subjects this assessment is based only on a single sample and cannot be accepted as an absolute, and would need to be repeated to make a valid assessment.

The blood profile of the smoker, paradoxically, had a low TG level and a desirable level of HDL. In contrast, it would be expected to have an elevated TG and low HDL (Bolinder & de Faire, 1998).

5.15.8. 24-hour food intake recall

The 24-hour food recall is deficient, in some respects, as some subjects appeared to grossly under-report their food intake or had reason to eat considerably less in the 24-hour period than their BMR might suggest. There was no apparent reason for these cases. However, this observation was confirmed by several means.

Many subjects also had higher proportions of fat in their diets than is recommended. One of the lifestyle treatments of elevated blood pressure is to ensure the diet is replete in fruits and vegetables (Pietinen & Aro, 1990; Appel *et al.*, 1997; McCarron, 1998; de Lorgeril *et al.*, 1999; Moore *et al.*, 1999). These diets also beneficially change the plasma cholesterol levels. A dietary change to increase proportions of fruits and vegetables is recommended for these subjects.

5.15.9. Mineral deficiencies

5.15.9.1. Calcium

Eight of the eleven subjects were deficient in calcium to varying degrees. Of these, five were women. Two subjects had extremely low reported 24-hour food intakes that may contribute to these low values. One subject had acceptable calcium intake with the remaining three having an excess intake.

5.15.9.2. Magnesium

Five subjects, two of who were male, had low magnesium intake to varying degrees. Three further females had sufficient magnesium. The remainder had an excess of magnesium in their reported diets.

5.15.9.3. Potassium

One female subject was low in potassium, with five (one male and four females) more being acceptable. The remainder had an excess of potassium intake.

5.15.9.4. Phosphorous

Two female subjects reported low intake of phosphorous, with a further four (one male and three females) having acceptable intake. The remainder reported an excess intake.

5.15.10. Cardiovascular risk

Cardiovascular risk was investigated by three methods that all generally agreed. Many subjects had a Relative Risk score greater than 1.00. The 5-year risk was greater than 2.5% in all cases, but commonly 10-15%. Five subjects (two male and three female) were in each of the BMI categories of Grade O (desirable) and Grade II obesity (moderate risk). One female was in Grade I (low risk). Therefore the latter grading was almost evenly split.

5.15.11. Conclusion

This group fulfilled the objectives sought for subjects in the trial. These subjects represented a cross-section of their age group for elevated blood pressure. None of the subjects were exceptionally different from the standards of their peers.

The elevated blood pressure combined with obesity and poor cardiovascular fitness indicated that some subjects have a high risk of poor health, and CHD in the future. This is supported by the mineral deficiencies, calcium in particular, recognised to be associated with elevated blood pressure. There was no single apparent cause for their hypertension, however these subjects generally fit an at-risk group with many displaying characteristics of the cluster of conditions associated with Syndrome X (Reavan, 1991; Timar *et al.*, 2000).

CHAPTER SIX: GENERAL DISCUSSION & CONCLUSION

While no inference can be made to the wider population, due the small numbers involved in this trial, there are many points of interest that may have relevance to the further conduct of similar trials.

6.1. BLOOD PRESSURE

Issues relating to the advantages and limitations of ambulatory apparatus and monitoring are discussed in the literature review. However, the results of this experiment raise several points.

6.1.1. Blood pressure recording methods.

In the main trial several methods of blood pressure recording were used. Each is commonly used in the clinical setting. In the present study these rarely gave significant differences from the other methods. However, for some subjects, a difference between clinic blood pressure versus and other methods was demonstrated. This is discussed presently.

The non-ABP blood pressures were taken in the same order to minimise the effect noted by Pickering (1988) and Schettini (1999). These authors observed variations in blood pressure dependant upon the order in which different methods were used.

6.1.2. Use of the ambulatory apparatus

All subjects in the preliminary trial found the apparatus easy to use and relatively comfortable. However, some of the subjects in the main trial had difficulty with it. The size of the cuff was an issue for two subjects who had larger arms than was appropriate for the cuff. It was necessary to tape the cuff in place otherwise it would slip down and loosen. A larger cuff was available, which would have eliminated these problems, but was not tried. Several found part of the cuff twisted and caught the underlying skin causing discomfort. All found it relatively easy to carry on daily tasks as normal, however the need to remain stationary while a recording was taking place made some behaviour modification

necessary as described by Green & Madigan (unpulished observation). Discomfort was also experienced by some subjects when a reading was missed, due to the cuff re-inflating one minute later at a higher pressure and yet again if the second reading failed. In the main trial one subject removed the cuff on several occasions and subsequently refitted it. However, they may not have returned the cuff to its original tension, which may have contributed to some measurement variation. In contrast to the observation of Parati *et al.* (1986), some were caught by surprise when the cuff inflated which may have temporarily elevated blood pressure. However, this would have occurred after the reading and may have subsided by the time of the next reading. Anticipating the cuff inflations may also have an effect on artificially elevating blood pressure.

In the preliminary trial, nearly all subjects recorded SBP of > 120 mm Hg and DBP < 80 mm Hg. These parameters are commonly classed as normal, so this consistency may be indicative of a shortcoming in the validation of the apparatus as suggested Green *et al.* (2000).

The associated software was "user friendly" and downloading of data was straight-forward. Information was readily exportable to other programmes. Battery life was limited, so that they needed routine replacement between subjects.

6.1.3. White coat hypertension

White coat hypertension may have been a factor for some of the subjects as demonstrated by differences between clinic and other blood pressure recording methods. Of particular note was the Maori subject, who may have had his blood pressure elevated in clinic situations. His home blood pressure readings were consistently lower than that for clinic or ABP, in contrast to the observation of Cesana & Zanchetti (1995), who found both home and ABP to be lower than clinic in white coat hypertension. This result was quite distinct and may have been for reasons of discomfort with the professional setting. This observation reinforces the social and cultural differences as described by Parati *et al.* (1986).

6.1.4. Activity records

Activity records returned in both trials were insufficiently complete to compare with all recording times. Some subjects in the preliminary trial were non-compliant and did not return a record at all. Some significant events were recorded, whereas others appear to have been missed. Activity that was recorded seemed to have less of an effect on DBP than SBP. Heart rate variability was more activity dependent than blood pressure.

Paired-analysis of each data point at the same time could not be carried out due to the varied nature of each day's activities and the lack of accurate activity records. However, this could be controlled for in future studies and the requirement for more comprehensive activity records emphasised. In the preliminary trial, the need for correction for individual activity diminished when the data is pooled, however, there was surprising agreement between the two recording sessions.

6.2. BODY COMPOSITION

6.2.1. Anthropometry

The anthropological methods chosen were simple with a minimum of equipment, but were informative and with high validity (Frisancho, 1981; Pollock & Jackson, 1984; Heyward, 1997.

The anthropometry provided useful measures that enabled comparison of the subjects with established normative data. While there are some issues with the methodology and interpretation of the data obtained, in general the methods obtained useful information. The comparative validity of the data increases with a greater number of subjects. The subjects did not consist of the ethnic mix that represents the population demographics of New Zealand. The subjects were predominantly Caucasian, with only one Maori. For this latter subject, some anthropometric data was limited and emphasises the caution of Frisancho (1981), who considered that anthropometric equations could only be applied to those of a similar ethnic group, in addition to age, gender, level of body fatness and socio-economic status.

6.2.2. Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) was chosen as a rapid, non-invasive, reliable, repeatable method for evaluating body composition (Lukaski *et al.*, 1985; Heyward, 1997). This approach is regarded as appropriate for older adults, such as were subjects in the present study, and which compares well with anthropometry (Williams *et al.*, 1995).

Deurenberg *et al.* (1990) and Williams *et al.* (1995) considered that BIA was a better measure of the fat percentage than anthropometry. In the present study, the BIA fat percentage was generally lower than that determined by TSF, especially for women. That difference may have been due to variation in regional fat distribution as suggested by Lukaski *et al.* (1986). Fukagawa *et al.*, (1990) warned that the results from differing body composition assessment methods should not be used interchangeably. With the BIA and TSF fat percentage estimates there was sufficient inconsistency to reinforce this warning.

Houtkooper *et al.* (1996) suggested that BIA is especially appropriate for large group studies, but is less so for individuals. As a large group was an objective of the present study, this approach was valid, however, as suggested, the validity is reduced for the individual analysis as became required.

This latter point of view may be emphasised by the BIA measurements obtained from the present study. In no case was the BIA-determined fat percentage greater than that determined from the TSF. However, as the BIA determination is essentially whole body it may be more accurate than the single TSF bodyfat determination. All male BMR determinations by BIA were higher than that by the other two methods. However, only three of seven females were higher. This observation may reflect the different fat distribution in the women having an effect on the fat percentage determination.

6.3. HYPERTENSION

The subjects in the main trial had elevated blood pressure, which was adequately demonstrated by most methods of blood pressure recording. Some of the subjects were manual workers so that during work effort there blood pressure would be elevated similarly

to weight-lifting exercise (Sale *et al.,* 1994). One of these subjects already had very high blood pressure. The additive effect on the risk of CHD is severe.

Many of those subjects with elevated blood pressure were also obese. Pietinen & Aro (1990) point out that weight loss will give around 5 mm Hg blood pressure reduction of both SBP and DBP.

6.4. INTERVENTION

If there was an effect on blood pressure by any intervention it is not obvious due to the few subjects who completed the trial. While it may be tempting to make a conclusion from these data, it is prudent to not do so. However, it has been demonstrated that sufficient calcium in the diet (McCarron, 1998), especially from dairy products (Ackley *et al.*, 1983; Pietinen & Aro, 1990), can lower elevated blood pressure and protect against the effects of excesses of other nutrients (McCarron, 1997). The age group, represented by the subjects in the main trial typically have reduced calcium intakes due to avoidance of dairy products (Rusoff, 1987).

The skim milk might have been expected to lower blood pressure in these subjects (van Beresteijn *et al.,* 1990; Buonopane *et al.*, 1992; McCarron, 1998, Barr *et al.*, 2000). However, it was not expected to raise blood cholesterol (Barr *et al.*, 2000) sufficiently to warrant caution in the new products use. In fact, Buonopane *et al.* (1992) observed a reduction in total cholesterol in a high total cholesterol subgroup fed skim milk.

6.5. MINERAL DEFICIENCIES

There were a variety of levels of reported mineral intakes. Almost all subjects with deficient mineral intake were females. However, these intakes were based on a single 24-hour dietary recall with inherent limitations. It is significant that the mineral found to be most consistently deficient was calcium. These results partially support the inverse relationship of blood pressure to calcium intake (McCarron, 1984; Hatton & McCarron, 1994).

6.6. CONCLUSION

The initial objectives of the trial were not met due to the early termination. However, there was sufficient data to reinforce the validity of the methodology, especially the ambulatory blood pressure recording, and to ensure that all other aspects of the trial were achievable.

Subjects with mild hypertension could be recruited into a trial and undergo a series of tests to determine their physical parameters, have blood pressure taken by a variety of methods and on several occasions, and meaningful data obtained. In addition, a small, potentially beneficial, modification could be made to their diet, and the effect of this dietary change monitored by both blood pressure and blood profile changes. These changes were made with minimal disruption to their daily routine, and were generally well received.

The ambulatory blood pressure recording apparatus could be used with little intrusion into the lives of the subjects for varying lengths of time. There was little reported difficulty in wearing the apparatus, other than cuff size and stability. However, most discomfort was caused from sleep disruption. In addition, the need to remain still for recordings caused some difficulty for subjects who were working and contributed to some missed readings. Generally, the equipment presented no difficulties in its use.

The blood pressure data, combined with the physical data, BIA and the blood profile were able to identify those subjects who presented with some of the cluster of factors which typify Syndrome X. These results, while only from a small sample group, strongly support the use this type of research methodology to provide an accurate representation of a population subgroup, such as those with elevated blood pressure. In addition, the effect of a dietary intervention on blood pressure and blood lipid profiles can be monitored in free-living subjects.

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APPENDIX ONE

Calcium-enriched milk for Blood Pressure trial

Prepared by the New Zealand Dairy Research Institute

Blend 1 – Standard SMP

Blend 2 – Ca-enriched SMP providing 2.4g Ca/100g powder

Blend 3 – Ca-enriched low-fat SMP providing 2.4g Ca/100g powder

Targets:

Calcium 1200 mg/100g

Low-fat blend 12% fat (provides 1.5% fat in milk reconstituted at 12.5% TS

	Total solids (w/w)	Calcium content (%)	Protein content (%)	Ash content (%)	Lactose content (%)	Fat content (%)
ALAMIN	94.00	28.00	7.00	77.00	4.00	0.00
SMP	96.00	1.22	38.70	7.30	48.50	0.60
WMP	97.50	0.96	30.20	4.30	34.80	28.00

MP = Milk powder

SMP = Skim milk powder

WMP = Whole milk powder

ALAMIN is a commercial calcium preparation made from fresh whey.

APPENDIX TWO

The influence of calcium-enriched milk on blood pressure and blood lipids

INFORMATION SHEET

Who are we?
The Milk and Health Research Centre is a joint venture between Massey University, the New Zealand Dairy Research Institute and the New Zealand Dairy Board. Its role is to carry out research into the links between drinking milk and human health. Dr is one of their Senior Research Scientists and is interested in the effect of cows' milk on blood pressure.

We are now collaborating with to extend our studies to people with mildly high blood pressure.

Why are we doing this trial?
Control of blood pressure is an important means of reducing the risk of stroke or heart disease. Lowering blood pressure reduces the risk of a stroke or heart attack. This has huge implications for public health, not only in developed countries such as New Zealand where heart disease is a leading cause of death, but also on a global scale.

High blood pressure has been linked to eating an inappropriate balance of certain minerals, namely, sodium, calcium, potassium and magnesium. Increasing dietary calcium seems to help to reduce the risk of hypertension in some people.

Milk is a natural source of calcium. Drinking milk may therefore be a means of reducing the risk of high blood pressure and the associated risk of a stroke and heart disease. These benefits of milk are only now becoming recognised. The purpose of this study is to assess the influence of 2 different calcium-enriched milk products on blood pressure:

- Normal skim milk
- Low fat milk (1.5% fat)

Each will be enriched with calcium to provide all the daily needs of calcium in 2 serves.

At the moment we are conducting a clinical trial of calcium-enriched milk on blood pressure in people with normal or low blood pressure. However, the scientific and medical literature suggests that the beneficial effect of calcium on blood pressure is greater in people with high blood pressure than in people with normal or low blood pressure. Therefore, we would now like to extend our ongoing trial to include observations in people with raised blood pressure.

Control of blood lipids is another very important means of reducing the risk of cardiovascular disease. Dairy food consumption has been linked to the development of atherosclerosis and so people with an increased risk of cardiovascular disease are

usually encouraged to reduce their intake of these foods. However, the strong link between calcium and blood pressure has prompted us to investigate whether the potential benefit of consuming calcium-enriched milk outweighs any potential harm of dairy fat. Reduced fat milk is generally more palatable than non-fat (skim) milk. Therefore, we would like to compare the impact on blood lipids of drinking reduced fat milk with non-fat milk.

Would you like to take part?
We would like to recruit people who are currently taking medication for mild hypertension. It is also necessary that participants:

- are not allergic to milk (i.e. not lactose intolerant)
- are not taking mineral supplements
- consult with Dr about coming off existing treatment of raised blood pressure for the duration of the trial

If you are interested in taking part please contact or Dr who will be happy to discuss the project and answer your questions.

Contact details:

Massey University
Private Bag 11222
Palmerston North

Telephone:)
Fax:
e-mail:

What is involved?
This is a 9-week clinical trial in which you will be asked to discontinue your current treatment for raised blood pressure for 8 weeks. During the last 6 weeks off medication you will be asked to replace your usual liquid milk with one of our test milks.

The test milk will either be non-fat (skim) milk or low fat (1.5% fat) milk. Both milks will be enriched with calcium to provide all your daily requirements for calcium. The New Zealand Dairy Research Institute who will make up the milk will label the milk with a colour. At the end of the study the New Zealand Dairy Research Institute will reveal the real identity of each milk. Consequently neither you, nor we, know who is consuming which milk until the trial is over. This will help us to avoid bias when we make our measurements.

The test milk will be provided as a powder. You will be asked to make 2 servings of milk up each day by adding 3 scoops (50g) of powder to 2 cups of water.

What are we going to measure?
Body composition/Nutritional status:
We would like to measure:

- *Body weight* will be measured using conventional weighing scales. You will be asked to remove your shoes and jumper or jacket.
- *Standing height* will be measured using a conventional height meter
- *Waist to hip ratio* will be derived from measurements of waist and hip circumference made using a conventional measuring tape.
- *Upper arm circumference* will be measured using a conventional measuring tape
- *Skinfold thickness* will be measured using a pair of calipers. These are designed to lightly pinch a fold of skin. We would like to measure your skinfold thickness upper arm.
- *Body composition* measurements will be made using a conventional meter for assessing body composition in human subjects. It involves sticking two small sensor pads on your right foot and two more on your right hand. These are the attached to the analyser find out how much fat is in your body. It takes about 5 minutes to make a measurement and is completely painless and entirely safe

The food you eat (dietary history)
We would like to know about the kinds of food you normally eat. To find out we would like to ask you some questions about the food you usually eat. This will take about half an hour.

Your calcium intake
We would like to know how much calcium you consume. To find out we would like you to complete a short questionnaire which asks you whether you consumed certain foods and in what amount during the previous 24 hours. This will only take about 5 minutes to complete.

How much energy you burn up each day
We would like to find out how much energy you expend in a normal day. This will involve completing a short questionnaire that asks about your daily physical activities. This will take about 10 minutes to complete.

Physical fitness
We would like to find out how fit you are. To do this we would like you to perform a short fitness test that involves cycling for 3 minutes at each of 3 low levels of exercise i.e. very easy, easy and moderate in terms of how you feel. Therefore, the exercise will not be strenuous. It will be sufficiently demanding so that you feel that your breathing is getting deeper, but we do not want you to become breathless during the test. Heart rate will be measured continuously during the test by attaching sensing pads to your chest.

Blood Pressure
Blood pressure will be measured when you visit the Research Centre. Blood pressure will also be measured at home using inflatable arm cuffs that are connected to a lightweight measuring device. This is attached to a belt that you wear round your waist. The machine will record your blood pressure automatically. It will be set to record blood pressure every hour during the day (7.00am – 11.00pm and every 2 hours during the night).

Urine

You will be asked to make three 24-hour collections so that we can measure how much nitrogen, calcium, potassium, magnesium, and sodium your urine contains. From these results we can find out about your consumption of protein, energy and minerals.

Blood

You will be asked to attend the Ruahine Medical Centre on four occasions to give a blood sample. This blood will be used to obtain a complete profile of the fat and some of the minerals in your blood.

Is there insurance to cover me if I suffer personal injury?

Yes, the New Zealand Dairy Board has taken out an insurance policy that covers you if you are unlucky enough to suffer medical misadventure while taking part in this trial. The ACC (Accident Rehabilitation and Compensation Insurance Corporation) is unable to cover you because this trial is funded by the New Zealand Dairy Board principally for its own benefit.

When will all these measurements made?

Milk, Blood Pressure and Blood Lipids
INFORMATION FOR PARTICIPANTS

Time	Treatment		Measurements
	Prescription	**Milk Intervention**	
Start (week 0)	Yes. Continue with your prescribed treatment	No. Drink your usual milk	1. Baseline measurements of health, food intake and fitness at Massey University 2. Blood sample taken at Ruahine Medical Centre 3. Other measurements (made at home or at Massey) • Office blood pressure • 24 hour blood pressure • Calcium intake questionnaire
Drug Washout (2 weeks) End of week 2	No. Stop taking your prescribed treatment	No. Continue drinking your usual milk	1. Blood sample taken at Ruahine Medical Centre 2. Other measurements (made at home or at Massey) • Office blood pressure • 24 hour blood pressure • Calcium intake questionnaire
Milk Intervention (6 weeks) End of weeks 3 **and** 6	No. Continue not to take your prescribed treatment	Yes. Replace your usual milk with the Test Milk	1. Blood sample taken at Ruahine Medical Centre 2. Other measurements (made at home or at Massey) • Office blood pressure • 24 hour blood pressure • Calcium intake questionnaire

Are any of the procedures harmful or painful?
This study involves routine clinical and laboratory testing procedures, which are widely used around the world. Blood sampling carries a small risk of bruising or damage to the vein and may be slightly painful. None of the laboratory procedures are harmful or painful. However, if you are not used to any physical exercise you may feel a little muscle stiffness on the day after the fitness test.

Who will see the information about me?
Personal information about individual participants will be confidential to the research team. When data from all the volunteers has been pooled it will be used in presentations to academic societies, scientific publications and reports to the New Zealand Dairy Board. We will give you a summary of these findings, if you would like one.

Personal data that can be traced to particular individuals will be disposed of at the end of the trial. Data filed on paper will be shredded and electronic data will be deleted from our entire computer records and databases.

Will I get any financial compensation?
We will reimburse all your travel costs.