Menopause and cardiovascular, metabolic and bone parameters

Thesis submitted to Victoria University for the

Degree of Doctor of Philosophy

By

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BSc, Grad Dip Sc

JANUARY 2002

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Declaration

I declare that the work described in this thesis has been of my own undertaking and are a record of original research. Below is a record of collaboration with other students detailing two chapters that were initiated prior to my involvement.

Chapter 4. Commenced by a Masters student, David Pagliaro, with my direct collaboration, upon which I took over the majority of the work and concluded the study.

Chapter 5. Commenced by an honours student, Ben Pang, upon which I concluded the study.

Chapters 3, 5, and 6 are wholly the work of my own and have not been submitted for any other degree or at any other institution.

This thesis is less than 100,000 words in length.

Suzy Honisett

Acknowledgments

I wish to sincerely thank Associate Professor Lilian Stojanovska who has provided support, guidance and encouragement for the period of this thesis. I am especially grateful to Lily for her continuing enthusiasm, friendship and understanding through these years. I would also like to express my gratitude to Associate Professor Paul Komesaroff, for his patience and support over the later period of my thesis, and for the opportunity to undertake studies within his laboratory. I am greatly indebted to both of you.

I am extremely grateful to Dr. Kathy Tangelakis and Dr. Bronwyn Kingwell for their patience and assistance over the duration of this thesis. My thanks also go to Professor John Wark and Associate Professor Krishnankutty Sudhir for their expert advice. It has been a privilege to work with all of you.

I have great pleasure in thanking my colleagues and friends at the Baker Medical Research Institute and Victoria University, Ms Tye Dawood, Mr Maro Williams and Mr David Pagliaro for their enduring support and friendship.

I cannot begin to express the thanks required for my husband Jeremy, and my mother Mary for their lasting enthusiasm, support and sacrifice. Without them I would not have been able to realise the completion of this thesis.

Publications

Parts of the work presented in this thesis have been reported in the following publications.Honisett, S., Stojanovska, L., Sudhir, K., Kingwell, B.A., Komesaroff, P.A. Rosiglitazone improves endothelial function in postmenopausal women with Type 2 diabetes mellitus. (Submitted to Diabetes).

Honisett, S., Tangalakis, K., Wark, J., Craven, R., Stojanovska, L. The effects of hormonal therapy and walking on bone turnover markers in postmenopausal sedentary women. (Submitted to Maturitus).

Honisett, S., Tangalakis, K., Wark, J., Craven, R., Stojanovska, L. The effect of hormonal therapy on bone turnover markers in 'Masters' trained postmenopausal women. (Submitted to Bone).

Honisett, S., Pang, B., Sudhir, Stojanovska, L., K., Komesaroff, P.A. The effect of progesterone on cardiovascular risk factors in postmenopausal women. (Submitted to Journal of Hypertension).

Parts of this work have been presented at:

- Annual Meeting of the Australasian Menopause Society, Perth 1997, Auckland 1998, Adelaide 2000, Melbourne 2001.

- Annual Meeting of the European Menopause Society, Florence 1998, Copenhagen 2000.Satellite Meeting for International Physiological Society, Diabetic Complications, Melbourne 2001.

- North American Menopause Society, New Orleans 2001.

Abbreviations

AGE	Advanced glycation end-products
ATP	Adenosine triphosphatase
BAP	Bone alkaline phosphatase
BMD	Bone mineral density
BMI	Body mass index
CEE	Conjugated equine oestrogen
CHD	Coronary heart disease
CSF	Colony stimulating factor
CV	Coefficient of variation
CVD	Cardiovascular disease
CVR	Cutaneous vascular reactivity
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DPD	Deoxypridinoline
EDHF	Endothelial derived hyperpolarizing factor
EDRF	Endothelial derived relaxing factor
FMD	Flow mediated dilation
FSH	Follicle stimulating hormone
GLUT	Glucose transporter
HDL	High density lipoprotein
HT	Hormonal therapy

IL-1	Interlukin-1
IL-6	Interlukin-6
LDL	low density lipoprotein
LH	Luteinising hormone
Lp(a)	Lipoprotein (a)
MAP	Mean arterial pressure
MAP-k	Mitogen activated protein kinase
MPA	Medroxyprogesterone acetate
mRNA	Messenger ribonucleic acid
NO	Nitric oxide
NOS	Nitric oxide synthase
OC	Osteocalcin
PBM	Peripheral blood monocytes
PPAR	Peroxisome proliferator activator receptor
PYR	Pyridinoline
SAC	Systemic arterial compliance
SBP	Systolic blood pressure
T2D	Type 2 diabetes mellitus
TNF	Tumor necrosis factor
VLDL	Very low density lipoprotein
VSMC	Vascular smooth muscle cells
VO_2 peak	Volume of expired oxygen -Measure of aerobic capacity

Synopsis

After menopause women experience an increased risk of developing cardiovascular disease, diabetes and osteoporosis. The work described in this thesis investigates the effects of exercise, hormonal therapy (HT) and rosiglitazone on cardiovascular risk variables, glucose and insulin levels and bone turnover markers. It was hypothesised that these interventions would independently improve parameters measured, whilst combined treatment would provide further benefit.

The effects of HT and exercise on the above mentioned parameters were investigated in healthy postmenopausal women by a randomised, double blind, placebo controlled trial. Initially sedentary women were administered HT for 8 weeks. The results of this trial demonstrated a reduction in bone resorption markers, whilst HT had no significant effects on fasting levels of glucose, insulin and cardiovascular variables, such as serum lipid concentrations, blood pressure and systemic arterial compliance (SAC). A moderate 12 week walking program was subsequently undertaken by these women, with no significant effects on any variables measured. These results suggest that HT alone, but not walking, is an effective treatment to reduce osteoporotic risk factors in healthy sedentary women, whilst combined treatments do not provide a significantly greater effect than HT alone.

A second trial investigated the effects of HT and exercise, by administrating HT to athletically trained postmenopausal women to determine whether HT would provide further benefit than high intensity exercise alone. A randomised, double blind, placebo controlled trial found 20 weeks of HT was effective in reducing bone resorption markers, whilst there were no significant effects on glucose and insulin levels or cardiovascular variables, mentioned above.

A cross sectional analysis involving healthy sedentary and athletically trained postmenopausal women was undertaken to identify differences in cardiovascular variables, glucose and insulin levels and bone turnover markers with high and low intensity exercise. The results from this study identified that athletically trained women had lower blood pressure and higher bone formation markers, whilst other cardiovascular variables, bone resorption markers, glucose and insulin levels were not significantly different between groups.

It was suggested from the above studies that neither walking, high intensity exercise nor HT significantly alters serum lipids, SAC or glucose and insulin levels in healthy sedentary and athletic postmenopausal women. To elicit changes in blood pressure and bone turnover markers exercise at high intensities, as opposed to walking, is required; however, HT provides further benefit to exercise in reducing risk factors for osteoporosis in athletically trained women.

There is limited information about the cardiovascular actions of progesterone alone. This was therefore investigated in healthy postmenopausal women not taking oestrogens. In a

randomised, double blind, cross over study, healthy postmenopausal women were tested before and after six weeks of treatment with micronised progesterone and matching placebo. Serum progesterone levels increased following progesterone administration, but there were no changes in oestradiol or any of the cardiovascular variables, such as serum lipid concentrations, blood pressure, SAC, cutaneous vascular reactivity (CVR) and flow mediated dilation of brachial artery (FMD). It appeared that in healthy women unprimed by oestrogen, micronised progesterone alone has no adverse or beneficial cardiovascular effects.

Atherosclerotic vascular disease is the primary cause of mortality in the Type 2 diabetic population. The effectiveness of antidiabetic thiazolidinediones, specifically rosiglitazone, in improving cardiovascular variables in postmenopausal women with Type 2 diabetes mellitus was investigated in this thesis. In a randomised, double blind study, women were administered 12 weeks of rosiglitazone or matching placebo. Rosiglitazone reduced fasting blood glucose, insulin, glycated haemoglobin, triglycerides, systolic blood pressure and mean arterial pressure. In addition, rosiglitazone increased FMD and SAC. It appears that rosiglitazone improves glycaemic control and improves cardiovascular variables in postmenopausal women with Type 2 diabetes mellitus.

The combined effects of rosiglitazone and HT were investigated in postmenopausal women with Type 2 diabetes mellitus. In a randomised, double blind, cross over study, women who were currently using rosiglitazone were administered 12 weeks of HT and matching placebo. The combination of rosiglitazone and HT treatment did not affect any of the above mentioned

cardiovascular variables, except FMD, which was reduced to levels experienced by women prior to commencing rosiglitazone. Potentially, from these results, the combined treatment of rosiglitazone and HT may be detrimental to endothelial function and should be considered cautiously.

There appears to have been no previous studies that have investigated the effects of rosiglitazone on bone metabolism in humans. It was therefore sought to determine its effects in a double blind study, involving women with established Type 2 diabetes mellitus randomly allocated to receive either 12 weeks of rosiglitazone or placebo. There were no significant effects of either treatment on any bone markers measured, indicating rosiglitazone has no detrimental or beneficial effects on bone metabolism. The addition of HT to rosiglitazone was also investigated to determine whether HT would maintain a beneficial effect on bone metabolism. Women receiving rosiglitazone were allocated to receive either 12 weeks of HT or placebo, following a randomised cross over design. Circulating FSH and LH were significantly reduced with the administration of HT. There was a significant reduction in one bone resorption marker from baseline levels with HT. No significant alterations in other bone turnover markers occurred as a result of adding HT to rosiglitazone treatment. It was concluded that the addition of HT to rosiglitazone treatment significantly reduced bone resorption, likely to be a beneficial effect.

1.0 General Introduction

1.1 Outline of thesis

The first chapter of this thesis provides a broad introduction to the endocrine changes occurring during and after menopause and reviews literature that focuses on how these alterations can influence cardiovascular functioning, glucose and insulin metabolism and bone metabolism. Available information on preventative and intervention strategies to reduce risks for the development of cardiovascular disease, osteoporosis, insulin resistance and diabetes mellitus will also be reviewed identifying any gaps within current literature. Subsequent chapters describe each study developed to address the knowledge gaps highlighted within the introductory. These chapters are forwarded by an introduction describing relevant literature to each particular study.

1.2 INTRODUCTION

Life expectancy and women's health issues in developed countries have changed significantly over the course of the twentieth century. As little as a hundred years ago the average life expectancy was approximately fifty years of age, hence, a women's life was mostly spent in her fertile years. The average age of menopause has not appreciably altered since this time. Life expectancy, however, has been greatly extended with an averaging over eighty years. Many women now live twenty to thirty years beyond menopause. As a result of extended longevity, there is a rise in chronic illnesses, exacerbated by oestrogen deficiency after menopause, including cardiovascular disease (CVD), osteoporosis, and metabolic disturbances such as insulin resistance and the development of Type 2 diabetes mellitus (T2D). Recent research has focused on both pharmacological and non-pharmacological treatment interventions for these chronic diseases, including exercise, hormonal therapy (HT) and more recently thiazolidinediones - exogenous ligands for PPAR gammas. This chapter will review the literature regarding these treatments- applied independently and in combination with each other.

1.3 MENOPAUSE

Women often welcome the cessation of menstruation at menopause as an end to the monthly 'curse'. This event signals the conclusion of a woman's fertile period of life. When it naturally occurs, menopause is due to depletion of approximately 2 million ovarian oogonia present at birth. The number of oogonia is rapidly reduced in the first decade of life; by the time menarche is reached only about 300,000 remain. This remaining 6% of oogonia are further reduced in number during a women's reproductive life by follicles being released during ovulation - in preparation for possible fertilisation - and by a poorly understood selection process termed 'atresia' (Carr 1998). Before the cessation of menstruation ovulation becomes increasingly irregular. This reduction in oogonia number and irregularity of ovulation are two factors that contribute to the attenuation with age of the rate at which ova are fertilised (Schwartz 1982).

Throughout the menstrual cycle, a variety of sex hormones are synthesised and secreted. Prior to, and during, menopause female sex hormone– oestrogens – fluctuate erratically, until physiologically low levels are recorded after menopause. During reproductive years the ovaries are the main sites of oestrogen biosynthesis. Steroid hormones such as oestrogens and progestins are synthesised in the ovaries from conversion of cholesterol, a process stimulated by secretion of pituitary gonadatrophins such as follicle stimulating hormone (FSH) and luteinising hormone (LH). This regulatory feedback loop involving steroid and gonadotrophic hormones is disturbed around the time of menopause. Significant reductions in ovarian oestrogen production occur due to the depletion of follicle numbers, whilst serum FSH and LH levels are chronically increased

after menopause. In addition to the feedback effects of oestrogen, another hormone, inhibin, is produced by the ovaries and negatively feeds back to the pituitary to control FSH secretion (Guyton 1991). Inhibin is thought to be important in causing the increase in FSH and LH with menopause (Robertson 2002). Biosynthesis of oestrogens also occurs via the aromatisation of adrenal androgens, providing a significant contribution to circulating oestrogen levels after menopause. The primary circulating oestrogens pre-menopause are oestradiol, whilst the main oestrogens after menopause are oestrone. Oestrone is primarily synthesised via the aromatisation of androstenedione in peripheral muscles and adipose tissue (Khaw 1992), and its conversion is increased with age and obesity (Carr 1998).

The reduction in circulating oestrogen levels around the time of menopause are associated with acute and chronic vasomotor and somatic disturbances. The symptoms of acute vasomotor disturbances are quite common among menopausal women and include sudden hot 'flushes' or 'flashes', increased or excessive perspiration and a transient elevation in heart rate. Approximately 39% of Australian postmenopausal women experience hot flushes (Dennerstein 1993), which are correlated with reduced oestrogen levels. These acute symptoms tend to stabilise when circulating oestrogen levels reach physiologically low concentrations. Chronic symptoms of menopause include decreased skin elasticity- most likely due to reduced collagen and connective tissue content (Barlow 1991), vaginal dryness, and atrophic vaginitis (Barlow 1991, Khaw 1992). Other chronic alterations include impaired insulin sensitivity and glucose tolerance, an increased risk of developing cardiovascular disease and osteopenia, which will be discussed in greater detail in the following chapter.

The average age of natural menopause in Australia is about 50 years (healthlisite 2001). Some women, however, undergo oopherectomy in conjunction with a hysterectomy at a younger age, surgically inducing acute and dramatic reductions in ovarian sex hormone synthesis and secretion. Many factors may affect the age at which natural menopause occurs. Women who are nulliparous tend to have an earlier menopause than women who have at least one live birth (Kato 1998). Women with a shorter menstrual cycle length (less than 26 days) have a mean menopause 2.2 years earlier than women with a menstrual cycle length equal to or longer than 33 days (Whelan 1990).

Currently, female life expectancy in Australia is approximately 81 years of age (Australian Bureau of Statistics 2001), hence, most women will live more than a third of their lives past menopause. The clinical implications of chronic symptoms associated with endocrine changes after menopause can be substantial, affecting quality of lifestyle and well being. Currently 2.3 million Australian women have experienced menopause, with this number increasing by 80,000 annually (Davis 1994). These figures will have a considerable impact on health services if chronic symptoms associated with menopause are not managed effectively. This chapter will review the literature related to cardiovascular disease risks, altered bone metabolism, and impaired glucose and insulin metabolism and Type 2 diabetes mellitus (T2D) in women after menopause, assessing information available on the efficiency of independent and combined treatments to prevent, ameliorate or manage these risks.

1.4 METABOLIC IMPLICATIONS OF MENOPAUSE

1.4.1 Endocrine influences of menopause on glucose and insulin metabolism

Increasing body weight, age and menopause are the main catalysts for alterations in glucose and insulin metabolism, which, if left untreated, may lead to the development of insulin resistance or T2D. Ovarian senescence is associated with increased circulating glucose concentrations, reduced plasma insulin response to glucose (Bailey 1980), and hyperinsulinaemia (Proudler 1992). The direct actions of sex hormones on glucose and insulin metabolism involve alterations in insulin receptor concentrations (De Pirro 1978), whereby varying sex hormone levels throughout the menstrual cycle influence receptor concentrations and pancreatic and islet insulin content and beta-cell numbers (Bailey 1980). These changes may be partially responsible for the fact that the risk of impaired glucose tolerance that rises 6% each year for women who reach menopause after 49 years of age, and for 44 % of non-obese postmenopausal women who experience insulin resistance (Lindheim 1993). Reduced participation in physical activity, increased adiposity and altered fat distribution often occur around the time of menopause; these too may be important factors that compound the effects of reduced oestrogen levels on altered glucose and insulin metabolism.

1.4.2 Adiposity and glucose and insulin metabolism.

Increased adiposity is common among women after menopause. As mentioned in Chapter 1.4.1, this may be influenced by a variety of factors including reduced participation in physical activity with increasing age and reduced oestrogen levels after menopause. Postmenopausal women have 36% greater trunk fat, 49% greater intra-abdominal fat and 22% more subcutaneous abdominal fat area in comparison to their pre-menopausal counterparts (Toth 2000). Increased adiposity has been positively associated with insulin resistance (Johnson 1992, Zavaroni 1994, Brochu 2000). A number of investigators have demonstrated a 35-40% increase in weight beyond a woman's ideal body weight. This has been associated with a 30-40% reduction in tissue sensitivity to insulin (DeFronzo 1978, Kolterman 1980, Bogardus 1984, Golay 1988, Bonadonna 1990).

The molecular mechanisms underlying the link between adiposity/obesity and insulin resistance/diabetes is not fully understood; however, a variety of factors may be involved. One contributing factor to this link is triglyceride rich adipose cells release free fatty acids providing an alternate substrate to glucose; this reduces the requirement for glucose as a fuel source resulting in decreased insulin stimulated glucose clearance and ultimately insulin resistance (Schwartz 1999). Increased storage of triglycerides in adipose tissue promotes the secretion of a number of peptides, such as tumour necrosis factor alpha (TNF aplha), resistin (Steppan 2001) and leptin (Rosenbaum 1996, Gower 2000), which lead to a variety of complications including insulin resistance. The identification of leptin receptors on pancreatic beta cells (Islam 2000) indicate; direct actions of leptin on insulin secretion; increased leptin levels inhibit glucose-stimulated

insulin secretion (Cases 2001). There is an inverse correlation between circulating oestrogen levels and leptin concentration (Rosenbaum 1996, Shimizu 1997, Gower 2000), which may reinforce the link between leptin and insulin resistance after menopause. An important association between adiposity and insulin resistance is clearly apparent, however, the specific mechanisms underlying this relationship are yet to be fully elucidated.

1.4.3 Exercise and glucose and insulin metabolism

The level of physical activity in which women participate is often reduced after menopause. Fitness or aerobic capacity is an independent predictor of insulin sensitivity; demonstrated by athletic postmenopausal women having 43% greater insulin sensitivity compared to their sedentary counterparts (Brown 2000). Exercise directly increases the expression of glucose transporters, which stimulate muscle glycogen synthesis and improve insulin sensitivity (Perseghin 1996). Indirectly, intense exercise increases energy expenditure and, therefore, is an independent determinant of total and regional body fatness (Hagberg 2000); as previously mentioned adiposity is one risk factor for impaired glucose and insulin metabolism.

Ovarian senescence, increased adiposity and a reduction in the level of habitual physical activity all have deleterious effects on glucose and insulin metabolism; a combination of these factors may further increase the risk of disturbed glucose and insulin metabolism.

1.4.4 Clinical implications of altered glucose and insulin metabolism

Altered glucose and insulin metabolism and insulin resistance, if left untreated, contribute to the pathogenesis of serious chronic diseases including the development of T2D and CVD.

T2D is an increasing health priority within Australia. It is proposed that 1.15 million Australians will be affected by diabetes by the year 2010 (Diabetes-Australia 1999). T2D is associated with the development of macrovascular complications including CVD (Phillips 1998, Sowers 1998). 65 – 80% of people with diabetes will have vascular complications and die from coronary heart disease (Diabetes-Australia 2002), whilst age and menopause significantly add to this vascular risk profile; these issues will be discussed in greater detail in chapter 1.5.

1.4.5 Treatment interventions for altered glucose and insulin metabolism

There are a variety of pharmacological and non-pharmacological interventions that are commonly used to treat disruptions to glucose and insulin metabolism and T2D, including weight loss, dietary alterations, exercise and HT. Ranges of oral hypoglycaemic agents are also commonly used to manage T2D; these include metformin, sulphonylureas and thiazolidinediones. HT, exercise and a member of the thiazolidinedione class of drug – rosiglitazone - will be reviewed as treatment options for postmenopausal women in the following sub-sections. Each treatment will

be looked at independently and as a combined regimen, and information regarding the efficacy of each treatment will be reviewed identifying any gaps in the literature.

1.4.5.1 Thiazolidinediones – rosiglitazone

Thiazolidinediones are a relatively new class of drugs that act as high affinity ligands for peroxisome proliferator-activator receptors (PPAR). There are three distinct PPARs – alpha, beta and gamma – each encoded by a separate gene and showing a distinct tissue distribution pattern. These receptors belong to the steroid-thyroid nuclear receptor super-family. Once activated they alter the transcription of numerous target genes by interacting with specific DNA response elements located upstream of responsive genes, as shown in Figure 1.1.

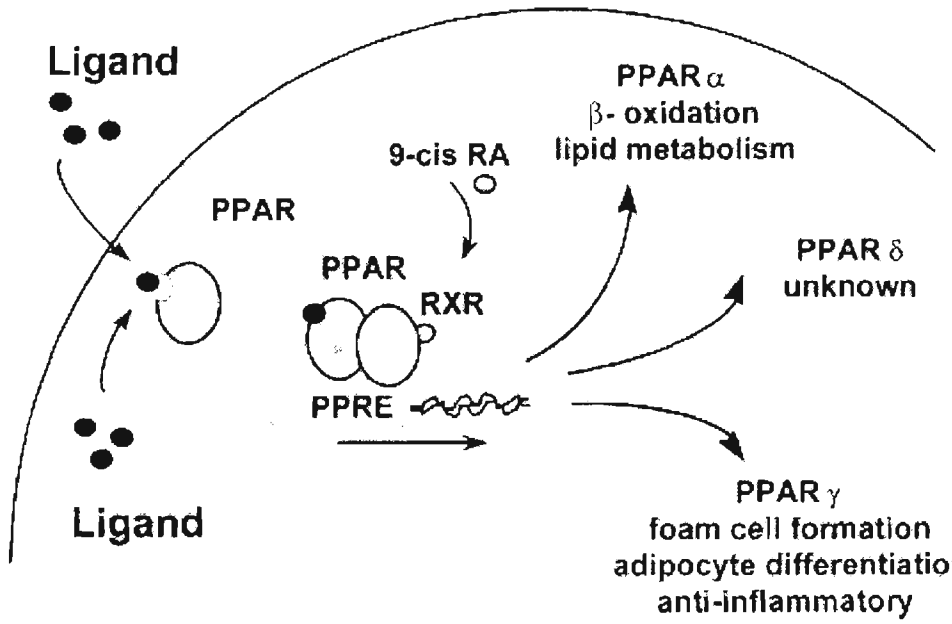


Figure 1.1. PPAR gamma on a cell nucleus activated by thiazolidinediones (Bishop-Bailey 2000).

Thiazolidinediones specifically activate PPAR gammas, predominantly expressed in adipocyte tissue, playing crucial roles in adipogenesis and insulin sensitisation. PPAR gamma regulated gene expression primarily occurs in adipose tissue, and is implicated in lipogenic pathways including acyl-CoA synthase, fatty acid transport protein, lipoprotein lipase and adipocyte fatty acid binding protein (Kersten 2000). In addition to target gene regulation, PPAR gamma activation increases adipocyte differentiation of immature adipocytes into mature fat storing cells. Adipocytes, as well as having an energy storage role, secrete a number of hormones – leptin and TNF alpha – and a signalling molecule – resistin (Steppan 2001) – that are regulated by PPAR gamma. The secretion of these messenger molecules by adipocytes have potent effects on insulin's action and may influence insulin sensitivity in peripheral tissue. The expression of each of leptin, TNF alpha and resistin (Steppan 2001) is markedly increased with obesity and diabetes. The activation of PPAR gammas provide a wide variety of metabolic effects such as, down-regulating ob gene expression and subsequently reducing leptin levels (De Vos 1996), down-regulating resistin gene expression and inhibiting the pathophysiological effects exerted by TNF alpha (Ohsumi 1994, Peraldi 1997). These actions further implicate the role of PPAR gammas in the link between adiposity and insulin resistance.

The beneficial actions of PPAR gamma activation on glucose and insulin metabolism have been demonstrated in diabetic model rat studies, which has shown that thiazolidinediones improve beta cell response to insulin secretagogues (Masuda 1995), increase insulin sensitivity by restoring the ability to suppress hepatic glucose production (O'Rourke 1997) and enhance peripheral glucose uptake (Lee 1994). These beneficial effects of thiazolidinediones on glucose

and insulin metabolism observed in experimental animals have been confirmed in a series of clinical double blind, placebo controlled studies in patients with T2D. Rosiglitazone reduces fasting plasma glucose levels (Fonseca 2000, Raskin 2000, Garber 2001, Lebovitz 2001), glycated haemoglobin (Fonseca 2000, Garber 2001, Lebovitz 2001) and circulating insulin levels (Raskin 2000, Garber 2001, Lebovitz 2001) and improves beta-cell function (Fonseca 2000, Lebovitz 2001). Furthermore, PPAR gamma activation may affect insulin signalling directly through the regulation of glucose homeostatic genes, such as the glucose transporter GLUT-4 (Wu 1998).

PPAR gammas are also expressed in all of the major cells in the vasculature, functioning to protect the vessels from injury (Hsueh 2001). The role of PPARs and the vasculature will be discussed in greater detail in Chapter 1.5.

The beneficial effects of PPAR gamma activation on glucose and insulin metabolism provides therapeutic potential for exogenous ligands of this receptor - such as thiazolidinediones - to reduce altered glucose and insulin metabolism and address the metabolic abnormalities of T2D.

1.4.5.2 Hormonal therapy

It may be expected that alterations in glucose and insulin metabolism, as a result of oestrogen deficiency after menopause, would be corrected with oestrogen supplementation such as with

HT. Previous investigations, however, have provided differing results (Cagnacci 1992, Crook 1997, Brown 2000, Ferrara 2001, Friday 2001). Transdermal administration of 17-beta oestradiol to healthy postmenopausal women reduced fasting insulin, improved islet response to a glucose challenge, elevated hepatic insulin clearance and ameliorated glucose tolerance (Cagnacci 1992). In contrast, studies of the effects of oral oestrogen supplementation alone on glucose and insulin metabolism in healthy postmenopausal women have produced varying results. One study demonstrated a reduction in fasting glucose levels with oral oestrogen, whilst no other alterations in metabolism were found (Cagnacci 1992). On the other hand, another clinical study showed a decrease in plasma insulin levels and a reduction in insulin sensitivity with oral oestrogen administration in postmenopausal women (Brown 2000). Comparisons of these studies are often difficult due to differences in the population group characteristics and the type and dosage of hormones administered. The route of administration is also an important variable which may impact substantially on the effectiveness of an HT regime.

Different doses of oestrogens can also have a considerable impact on glucose and insulin metabolism (Lindheim 1994, O'Sullivan 1995). High doses of oestrogens (conjugated equine oestrogens [CEE], 1.25 mg/day) in healthy postmenopausal women have caused deteriorations in insulin sensitivity (Lindheim 1994), whilst lower doses (CEE, 0.625 mg/day) either improved insulin sensitivity, or had no effect (The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial 1995).

Studies that have assessed the use of oestrogens in postmenopausal women with T2D have provided consistent glycaemic results, indicating significant reductions in glycated haemoglobin – an indication of glycaemic control (Andersson 1997, Brussaard 1997, Ferrara 2001, Friday 2001).

Progestins are generally prescribed in conjunction with oestrogen; the effects, however, of progestin incorporation on glucose and insulin metabolism appear to be dependent upon the type of progestins used. The combination of medroxyprogesterone acetate (MPA) or levonorgestrel with oestrogen increases insulin resistance (Godsland 1993, 1995), whilst norethisterone acetate combined with oestrogen (oral or transdermal) has no effect on glucose tolerance or insulin levels (Luotola 1986, Godsland 1993). To the best of my knowledge, no studies have investigated the effect of combined oestrogens and micronised progesterone on glucose and insulin metabolism in either healthy or T2D postmenopausal women.

The beneficial effects of combined transdermal oestrogens and selected progestins on glucose and insulin metabolism provide a therapeutic option to ameliorate altered glucose and insulin metabolism after menopause and improve glycaemic control with T2D. Careful selection, however, is required for the types of oestrogens and progestins, the dose and route of administration to ensure that altered metabolic situations are not exacerbated.

1.4.5.3 Exercise

There is a tendency to become more sedentary with increasing age, predisposing an individual to the development of impaired glucose tolerance (King 1984, Taylor 1984, Frisch 1986, Dowse 1991). A large population-based prospective study has showed that low cardiorespirtaory fitness was associate with an increased risk for impaired fasting glucose and T2D (Wei 1999); whilst lifestyle changes, including increased physical activity, can prevent the development of T2D in subjects with impaired glucose tolerance (Tuomilehto 2001). Exercise has beneficial direct (Houmard 1993, Hughes 1993, Houmard 1996, Cox 1999) and indirect (King 1988, Pollock 1997) influences on the metabolism of glucose and insulin and, therefore, may be used as a non-pharmacological intervention to prevent or reduce altered glucose and insulin metabolism.

The direct cellular effects of exercise are partially modulated by an increased GLUT-4 concentration (Houmard 1993, Hughes 1993, Houmard 1996) – an insulin sensitive glucose transporter - in skeletal muscle. The recruitment of GLUT-4 to the plasma membrane is independently stimulated by insulin and muscle contraction. The increase in GLUT-4 concentration in skeletal muscle with exercise facilitates the transport of glucose into muscle fibres (Abel 1996), constituting a molecular mechanism partially responsible for an increase in insulin-stimulated glucose transport-phosphorylation. This molecular mechanism contributes to a two-fold increase in insulin-stimulated glycogen synthesis in muscle (Perseghin 1996).

The indirect effects of exercise on glucose and insulin metabolism are associated with alterations in body composition. Participation in physical exercise increases energy expenditure, if all else is constant, influencing total and regional body fatness (Hagberg 2000). However, intense training, but not low- to moderate-intensity physical activity, is associated with markedly lower levels of total and regional body fat in postmenopausal women (Hagberg 2000). Body mass index (BMI), percent body fat and fat mass in postmenopausal women provides significant inverse correlations with insulin sensitivity (Brown 2000). BMI, abdominally distributed fat and physical activity are independent predictors of both impaired glucose tolerance and T2D in diverse ethnic groups (Dowse 1991). As discussed previously, adiposity is associated with impaired glucose and insulin metabolism and insulin resistance (Johnson 1992, Zavaroni 1994, Brochu 2000).

Insulin resistance is an important factor in the initiation and progression of impaired glucose tolerance and T2D. Interventions that improve the actions of insulin are beneficial in preventing or delaying the pathogenesis of altered metabolism. Physical training increases insulin sensitivity by more than 43% (Perseghin 1996), exceeding improvements reported from pharmacological agents such as metformin (Hother-Nielsen 1989, Widen 1992) or troglitazone (Nolan 1994), whilst exercise has added cardiovascular and bone benefits that will be discussed in Chapters 1.5 and 1.6.

1.4.5.4 Combined thiazolidinediones and hormonal therapy

A combined treatment of thiazolidinediones and HT, focusing on the effects on glucose and insulin metabolism, has not been reported in current literature. Provided both treatments work via nuclear receptors it would be of interest to determine whether a combined treatment would enhance, impair or have no effect in comparison to either treatment given alone. One point of interest is that thiazolidinediones inhibit the expression of aromatase and, therefore, oestrogen biosynthesis in adipose tissue, particularly adipose tissue present in the human breast (Rubin 2000). This has important implications for the potential therapeutic benefits of these ligands in the treatment and management of breast cancer. Nevertheless, whether thiazolidinediones affect the actions of HT is yet to be determined.

1.4.5.5 Combined exercise and hormonal therapy

The combined effects of HT and exercise on plasma glucose and insulin levels and insulin sensitivity in postmenopausal women remain unclear. One study that addressed this issue identified that physically active women using oral HT had lower fasting glucose, insulin and intravenous glucose tolerance tests in comparison to physically active women not using HT, whilst insulin sensitivity was greater in comparison to sedentary women taking HT (Brown 2000). These findings indicated that physical activity and HT may differentially and independently affect

glucose and insulin metabolism in healthy postmenopausal women. Further research, however, is required to establish the effects of different types and intensities of training and a variety of HT regimes, including transdermal HT on glucose and insulin metabolism.

1.4.6 Conclusions

Alterations in hormonal levels that occur with menopause predispose women to risks of impaired glucose tolerance and insulin resistance, a precursor to the development of T2D. Increasing adiposity and reduced levels of physical activity, often associated with menopause, are also risk factors for impaired glucose and insulin metabolism. Further, many women are challenged with many major life changes around the time of menopause - such as career changes, children leaving home, or a reversal in the care relationship with parents requiring support in older age. These factors may influence stress and lifestyle routines, which may compound other risk factors.

Transdermal HT and exercise are two treatments that affect glucose and insulin metabolism. Thiazolidinediones, such as rosiglitazone, is another treatment option for women with T2D. The individual effectiveness of these treatment regimens are well established, with each treatment providing favourable alterations to glucose and insulin metabolism in both healthy and T2D postmenopausal populations. It could be speculated that given each individual treatment is beneficial, a combination of treatments would provide added benefits. Few studies, however, have investigated the combinations of HT and varying levels of exercise in healthy postmenopausal women, and thiazolidinediones and HT in postmenopausal T2D populations. Therefore, further research is required concerning combined therapy, to ascertain any additive, synergistic or deleterious effects on glucose and insulin metabolism.

1.5 CARDIOVASCULAR IMPLICATIONS OF MENOPAUSE

1.5.1 Endocrine influences of menopause on the cardiovascular system

CVD is the leading cause of morbidity and mortality in western societies. It is a disease often associated with men, as males have 2.5 to 4.5 times greater risk of developing CVD than women of a similar age prior to menopause (Kalin 1990). After menopause, however, women experience a rapid increase in the incidence of cardiovascular events (Grodstein 1995). Reduced circulating oestrogens are the primary factor associated with the increased prevalence of CVD after menopause (Parrish 1967). An early natural menopause or oophorectomy increases the risk of developing CVD (Lobo 1990), whilst a delay in the onset of menopause reduces cardiovascular mortality risk by 2% for each year of delay (van der Schouw 1996). These proposed cardioprotective effects of oestrogens have not been consistently demonstrated in studies involving the exogenous administration of oestrogen to postmenopausal women. Therefore, considerable controversy remains regarding the use of HT as a cardioprotective agent. This will be discussed in greater detail in Chapter 1.5.5.1.

CVD is also the major cause of morbidity and mortality within the T2D population. T2D is characterised by insulin resistance, hyperinsulinaemia and an impaired beta cell response to

insulin with resulting glucose intolerance. Hyperinsulinaemia is associated with the development of high blood pressure, dislipidaemia, and CVD (DeFronzo 1991, Reaven 1991). T2D abrogates the cardioprotective effect of endogenous oestrogens in women (Hanes 1996). The risk of developing CVD after menopause is further increased with the addition of T2D (Manson 1991).

1.5.2 Menopause and lipoproteins

The aetiology and pathogenesis of atherosclerosis are diverse and complex. It is a progressive disease characterised by the accumulation of lipids and other fibrous elements in large arteries, as shown in Figure 1.3. Raised levels of atherosclerotic lipoproteins are a prerequisite for most forms of this disease (Lusis 2000). Menopause is associated with higher concentrations of total cholesterol, low density lipoprotein (LDL) cholesterol and lower concentrations of high density lipoprotein (HDL) cholesterol (Stevenson 1993). Greater circulating levels of LDL increase the likelihood that LDL will accumulate in the sub-endothelial matrix of arteries. Accumulated LDL is modified as a result of exposure to the oxidative waste of the vascular cells attributing to early lesion formation. In addition to LDL, other apoB-containing proteins, such as lipoprotein (a) [Lp (a)], can accumulate in the intima and promote atherosclerosis (Lusis 2000); high circulating levels are considered to be an independent risk factor for CVD (Newnham 1993). Circulating oestrogen levels influence Lp (a) (Newnham 1993). HDL provides protection against the pathogenesis of atherosclerosis by removing excess cholesterol from peripheral tissue and inhibiting lipoprotein oxidation (Lusis 2000). Hypertriglyceridaemia commonly occurs in patients with CHD (Cambien 1986, Austin 1989). Postmenopausal women have significantly higher levels of circulating

triglycerides (Stevenson 1993). Controversy continues, however, about whether an increase in plasma triglyceride levels is directly related to coronary heart disease (Austin 1989). The adverse changes in lipoprotein and cholesterol levels that occur after menopause appear to be independent of any effects of age and BMI (Stevenson 1993) and may partially explain the increased incidence of CVD after menopause.

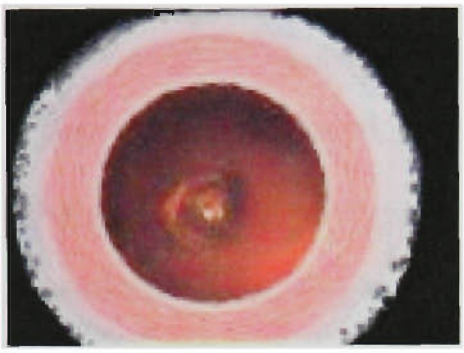


Figure 1.2.

A normal artery with no lipoprotein accumulation within the vessel wall.

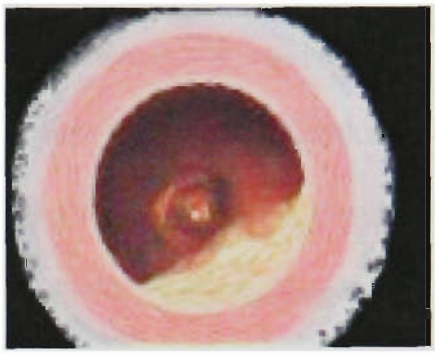


Figure 1.3.

An artery with plaque build up within the internal lumen of the artery (Nobel e-Museum 2000).

1.5.3 Menopause and vascular structure and function

The effects of oestrogens on cardiovascular parameters are complex and not fully elucidated (Farhat 1996). Observational data have shown that only 30 – 50% of the cardiovascular effects of oestrogens can be attributed to alterations in lipoprotein levels (Barrett-Connor 1991, Lobo 1991). Hence, oestrogens affect cardiovascular functioning by mechanisms beyond those involving lipoprotein metabolism. These other mechanisms include alterations to the structure and functioning of arteries, primarily regulated by endothelial function (Ross 1993); therefore, it is likely that endothelial dysfunction and modifications in arterial structure after menopause also contribute to the increased risk of CVD. The endothelium, as shown below in Figure 1.4, is comprised of a mono-layer of endothelial cells which act as a selectively permeable barrier between flowing blood and vascular tissue. The endothelium functions at a sensory and executive level producing effector molecules that assists in the maintenance of vascular homeostasis.

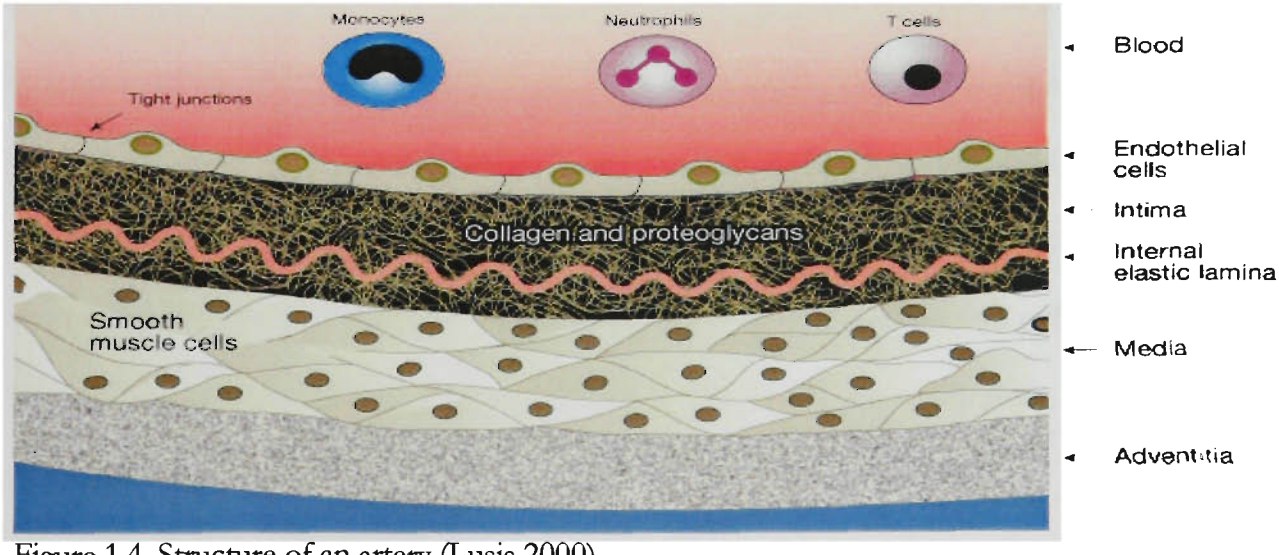


Figure 1.4. Structure of an artery (Lusis 2000).

Vascular regulation by the endothelium involves a complex and delicate balance between vasodilation and vasoconstriction, vascular cell growth promotion and inhibition and blood coagulation and fluidity. Vascular tone, reactivity and the release of vasodilatory mediators are partial aspects of the role of the endothelium; however, measuring endothelium-dependent vasodilation is an accessible and reproducible means of determining endothelial function. Menopause is associated with impaired endothelium dependent and independent-vasodilation, indicating that altered vascular function is not limited to disturbed endothelial function, but also involves vascular smooth muscle cells (VSMC) (Lim 1999). Nevertheless, not all investigators have demonstrated impaired endothelium independent vasodilation in postmenopausal women (Pinto 1997). Endothelial function begins to decline after 50 to 55 years of age in women compared with 40 years of age in men (Celermajer 1994). This suggests a benefit from circulating oestrogens before menopause, a hypothesis supported by studies demonstrating improvements in endothelium dependent dilation with oestrogen administration (Gilligan 1994). The predominant mechanism involved in oestrogen mediated improvements in endothelium dependent dilation appears to be due to an up-regulation of the transcription of nitric oxide synthase (NOs)- the enzyme responsible for the synthesis of nitric oxide (NO). The endothelium is involved in the synthesis of a variety of vasoactive substances that affect vascular tone; one of these substances, NO, plays a major role in maintaining vascular tone, inhibiting platelet aggregation and adhesion (Yao 1992), inhibiting atherosclerotic plaque development (Cohen 1995) and inhibiting the proliferation of VSMCs (Seki 1995). The advent of menopause is associated with reduced arterial NO activity, which can be restored to premenopausal levels with oestrogen supplementation (Majmudar 2000).

Previous studies have demonstrated a rapid decline in arterial elasticity, as measured by systemic arterial compliance (SAC), in perimenopausal women proportional to the waning of endogenous oestrogen levels (Laogun 1982, Karpanou 1996) and inversely correlated with circulating FSH levels (Waddell in press). The molecular mechanisms associated with these arterial mechanical changes in response to altered endogenous sex hormone levels are yet to be fully elucidated. It is known, however, that oestrogens exert positive effects via changes to the loading conditions on the elastin and collagen fibres and by structural changes in vessel walls (Van Buren 1992, Sudhir 1995, Volterrani 1995). Endothelium dependent vasodilation, mediated by NO may transfer the vascular wall stress from collagen fibres to more distensible elastin fibres, increasing the compliance of the vasculature. The influence of oestrogens on arterial compliance may also involve direct effects on the aorta or vasa vasorum (Stefanadis 1993). Chapter 1.5.5.1 will review in greater detail the molecular changes that occur with oestrogen supplementation.

1.5.4 Type 2 diabetes mellitus and cardiovascular disease

Epidemiological studies have identified a three to fourfold increase in the rates of coronary artery disease mortality in T2D patients compared to non-diabetics (Haffner 2000). A twofold increase is also apparent in the rate of coronary heart disease in patients with impaired glucose tolerance, demonstrating that increased insulin resistance and lesser degrees of hyperglycaemia augments the progression of atherosclerosis (Haffner 2000, Haffner 2000). Insulin resistance is associated with dyslipidaemia, hypertension and increased thrombotic potential (Reaven 1988, Laakso 1990, Juhan-Vague 1991, Reaven 1993), all of which are implicated in the pathogenesis of diabetic

vascular disease. The role, however, of hyperinsulinaemia and hyperglycaemia, characteristic of T2D, in the progression of diabetic vascular disease are not yet fully substantiated. Hyperinsulinaemia in response to insulin resistance is present well before the onset of hyperglycaemia in T2D. Although, the slow insidious development of T2D and the cluster of other cardiovascular risk factors often present with the diagnosis of T2D such as obesity, hypertension and dyslipidaemia make it difficult to determine the direct cause of diabetic vascular disease.

1.5.4.1 Hyperinsulinaemia

Insulin resistance, present with T2D and obesity, is often associated with hypertension (Jarrett 1978, Modan 1985, Turner 1985). Elevated blood pressure is both a CVD risk marker, indicating abnormal structure and function of the vasculature, and a risk contributor for CVD, accelerating age associated arterial degeneration (Wolinsky 1972, Avolio 1985). Interventions that improve the body's sensitivity to insulin, such as weight loss (Tuck 1981, Sowers 1982) and physical training (Horton 1981, Maiorano 1989), are effective in reducing blood pressure. This link between insulin resistance and hypertension is possibly due to elevated plasma insulin levels and the development of hyperinsulinaemia. Several potential mechanisms have been proposed to be responsible for this link between elevated plasma insulin levels and hypertension. These include hyperinsulinaemia: 1) inducing kidney sodium retention with the subsequent expansion of extracellular fluid volume causing hypertension; 2) stimulating the sympathetic nervous system, increasing blood pressure by augmenting cardiac output (by increased cardiac contractility and heart rate), increasing cardiopulmonary blood volume (via the constriction of large veins), directly

vasoconstricting resistant vessels and by increasing kidney sodium resorption (by the direct stimulation of renal tubular sodium resorption, stimulation of renin secretion and renal vasoconstriction); 3) enhancing sodium and calcium fluxes into vascular smooth muscle cells, subsequently, increasing vascular sensitivity to the vasoconstrictor effects of pressor amines; and 4) causing proliferation of the arteriolar smooth muscle cells which cause hypertrophy of the vascular wall and narrow the lumen of resistance vessels (reviewed in (DeFronzo 1991)).

These proposed mechanisms assume that insulin resistance is selective to specific tissues, whereas other tissue, such as that of the kidneys and sympathetic nervous system retain their insulin sensitivity (Hall 1994). Epidemiological studies have demonstrated that insulin is a primary risk factor in the development of CVD exerting its effects independently of dyslipidaemia and hypertension (Welborn 1979, Ducimetiere 1980, Fuller 1980). Insulin infusion promotes marked intimal and medial proliferation of canine femoral arteries and the accumulation of cholesterol and fatty acids (Cruz 1961). Insulin and insulin like growth factors have also been shown to increase collagen synthesis (an integral component of atherosclerotic lesions) (Weiss 1980). On the other hand, acute insulin administration acts as a vasodilator in most vascular beds (Anderson 1991, Sowers 1995). Therefore, the role of hyperinsulinaemia in the development of hypertension remains a contentious issue, requiring further research.

1.5.4.2 Hyperglycaemia

The specific nature of the associations between elevated glucose levels and diabetic vascular complications are not fully elucidated. A number of in vitro incubation studies have demonstrated that exposure of arteries to high glucose levels caused endothelial dysfunction similar to that observed in diabetic animal studies (Tesfamariam 1990, Tesfamariam 1991). Human studies have also demonstrated impaired endothelium-dependent vasodilation with diabetes (McVeigh 1992, Lim 1999). Endothelial dysfunction is likely to be potentiated by a wide variety of properties which may include; an increased production of vasoconstrictors (Mayhan 1991, Shimizu 1993); inactivation of endothelial derived relaxing factors by increased oxygen derived free radicals (Pieper 1992); decreased endothelium-dependent vasodilation via a reduced vasodilator substance, such as anginine (Pieper 1995, Rosen 1996) and a decreased responsiveness of vascular smooth muscle to endothelial derived relaxation factor (EDRF)s (Clarkson 1996, Lekakis 1997). These factors may occur concurrently or in isolation and effect vessels differently depending on the vessel size and location.

Chronic hyperglycaemia also results in the accelerated irreversible formation of advanced glycation end products (AGEs), which accumulate on collagen and are thought to play an important role in the progression of diabetic vascular disease. AGE accumulation increases collagen fibre rigidity, subsequently affecting arterial mechanical properties (Airaksinen 1993, Chappey 1997).

Dyslipidaemia associated with T2D is characterised by reduced serum HDL cholesterol and increased LDL cholesterol (Pyorala 1987, Jarret 1990). These alterations in HDL and LDL cholesterol levels are well established risk factors for coronary artery disease in diabetic and non-diabetic populations (The writing group for the Lipid Research Clinics Coronary Primary Prevention Trial Results II 1984, Anderson 1987, Frick 1987). Resistance to insulin stimulated glucose up-take and increased circulating insulin concentrations augment hepatic very-low density lipoprotein (VLDL) – triglyceride secretion, subsequently, causing hypertriglyceridaemia (Tobey 1981, Reaven 1991). An increase in plasma free fatty acid and glucose concentration, commonly associated with T2D (Golay 1988), provide more than an adequate supply of substrate to fuel VLDL synthesis also contributing to elevated triglyceride levels. The increased risk of CVD associated with T2D is due to a wide variety of abnormalities, with dyslipidaemia only partially accounting for this.

It is important to recognise that diabetes-induced atherosclerosis maintains a complex and diverse aetiology and pathogenesis. Therapies that target dyslipidemia and restore endothelial function in T2D populations are required to reduce the elevated risk of CVD experienced by this population group. A selection of treatment options will be reviewed in the following sub-sections.

1.5.5 Treatment options

1.5.5.1 Hormonal therapy

There is considerable controversy regarding the cardiovascular effects of exogenous oestrogen administration. Epidemiological evidence has demonstrated that HT containing oestrogens decrease the risk of CVD after menopause. The Nurses' Health Study followed 48,470 women over a 10 year period comparing current oestrogen use with having never used oestrogen (Stampfer 1991). It was found that women currently using oestrogen therapy had half the age adjusted risk of major coronary disease in comparison to women who had never used oestrogen (Stampfer 1991). There were, however, no associations with current oestrogen use and total stroke (Stampfer 1991). A decrease in the risk of first myocardial infarct in women using any non-contraceptive oestrogens was evident in one prospective cohort trial (Falkeborn 1992) whilst a 46% reduction in the risk of death - attributable to lower mortality from CVD - was associated with oestrogen use after menopause (Ettinger 1996). These above mentioned studies and a quantitative assessment of epidemiological evidence prior to 1991 (Stampfer 1991) suggest a protective effect of oestrogens on the cardiovascular system. In contrast, the HERS study – a large randomised placebo controlled trial – did not demonstrate any beneficial cardiovascular effects of combined therapy (Hulley 1998). However, there is the potential for selection bias in observational studies, as opposed to randomised clinical trials, which may confound results. This issue needs to be considered when comparing the outcomes of these trials. The HT administered in the HERS trial included MPA. One of the main questions raised as a result of this study was whether MPA attenuated the benefits of oestrogens. Nevertheless, the Amercian Heart

Association (Mosca 2001) and the International Menopause Society (Genazzani 2000) have recently developed guidelines indicating that HT does not have a role in cardioprotection.

The addition of a progestin to HT requires consideration as to the type of progestin given to ensure that the protective effects of oestrogens are not compromised. Progestin administration will be discussed in greater detail in Chapter 1.5.5.2.

Lipoproteins

One of the proposed mechanisms for the potential protective effects of oestrogens is related to their influence on lipoprotein profiles. HT increases circulating HDL levels, decreases LDL (Lobo 1990, Newnham 1993, Subbiah 1993, 1995, Taskinen 1996) and Lp(a) levels (Kim 1994, Taskinen 1996). Acute oestradiol infusion in the brachial artery and 3 weeks of transdermal oestradiol also significantly inhibits the oxidation of LDL in postmenopausal women (Sack 1994), with the lag time of LDL peroxidation significantly prolonged after oestradiol infusion. Concern remains, however, as to whether HT elevates plasma triglyceride levels and the cardiovascular relevance of this (Lobo 1991, Nabulsi 1993).

Vascular effects

Oestrogens regulate target cells directly and indirectly. The direct effects of oestrogens involve gene and plasma membrane related transport mechanisms including the modulation of protein expression and ion transport mechanisms. Indirectly, oestrogens regulate cell homeostasis by affecting oxidation reactions. Oestrogens' actions are classically derived by binding and activating a nuclear receptor which trigger transcriptional regulation of protein synthesis within 10-60 minutes (Elderman 1975). Oestrogen receptors have been identified in human VSMCs (Losordo 1994) and on membranes of endothelial cells (Russell 2000). The presence of receptors indicates that oestrogens directly regulate vascular tone and structure. Oestrogens also exhibit acute non-genomic effects via activating membrane mechanisms that affect signal transduction, including interaction with calcium influx mechanisms, which inhibit calcium entry into cells (Collins 1993, Yamamoto 1995). Therefore, oestrogens have a diverse array of effects that may act via a number of potential mechanisms. These effects may be immediate, via the activation of signal transduction mechanisms, or delayed, by mechanisms involving protein synthesis.

Hormonal therapy and the vascular endothelium

The presence of oestrogen receptors in vascular endothelial tissue indicates that these cells are targets for these steroids. The endothelium produces a range of vasoactive substances such as EDRF – now known to be NO, prostacyclin and the as yet undefined endothelial derived

hyperpolarising factor (EDHF). These substances are involved in regulating vascular tone, cell proliferation and contribute to the thromboresistant properties of the vascular endothelium by inhibiting platelet aggregation and adhesion (Luscher 1994). Males, and women after menopause, have significantly lower levels of arterial NO activity in comparison to premenopausal women (Majmudar 2000). Oestrogen supplementation to postmenopausal women can improve vascular NO activity (Schray-Utz 1993, Caulin-Glaser 1994). Oestrogens may also potentiate cardioprotective effects by reducing LDL oxidation (Sack 1994), a process that inactivates NO (Simon 1990). Oestrogens also inhibit endothelial secretion of vasoconstrictor, endothelin –1, in rabbit coronary arteries (Jiang 1992). Therefore, the potential beneficial effects of oestrogens act by increasing NO synthesis and reversing its inhibition.

A number of studies have suggested that the potential cardioprotective effects of oestrogens are mediated by the stimulation of prostaglandin synthesis in the vessel wall, namely prostacyclin (Fogelberg 1990, Mikkola 1995). Prostacyclin, synthesised in endothelial cells, is a vasodilator that also inhibits platelet activation and is upregulated by oestrogens (Mendelsohn 1994). Oestrogens also enhance endothelial cell attachment, proliferation, migration and organisation into capillary like structures in vitro, while increasing angiogenesis in vivo (Schnaper 1996), although the clinical significance of these findings have not been established.

Hormonal therapy and vascular smooth muscles cells

The presence of oestrogen receptors on VSMCs indicates that this tissue is also a target for these steroids. In vitro, oestrogens induce significant coronary artery vasorelaxation after pre-contraction with a vasoconstrictor, thromboxane A2. Vasorelaxation occurs in arteries with and without endothelium and after nitric oxide synthase or cyclooxygenase inhibition (Chester 1995). Animal studies have also demonstrated an acute oestrogen induced dilation of canine and rabbit coronary arteries with either denudation of the endothelium or inhibition of EDRF, indicating that oestrogen mediates an endothelium-independent relaxation via non-genomic mechanisms (Jiang 1991, Sudhir 1995). The mechanisms of oestrogen mediated endothelium-independent vasorelaxation may include an inhibition of the response to vasoconstrictors, such as endothelin – 1 (Jiang 1992) or angiotensin II (Cheng 1992). Oestrogens also mediate vascular responses by mechanisms beyond gene transcription, which includes specific high affinity membrane receptors distinct from classical oestrogen receptor pathways. These non-genomic pathways include an inhibition of voltage-dependent calcium currents in VSMCs (Zhang 1994) and regulation of large conductance chloride channels (Hardy 1994). It is important to note, however, that most of the acute effects of oestrogens on smooth muscle cells occur with supraphysiological concentrations, which may not be appropriate for prolonged human administration. Oestrogens also attenuate VSMC proliferation and migration (Cheng 1991, Dubey 2000); this reduction is partly mediated by reducing mitogen activated protein kinase (MAP-k) activity and in part is oestrogen receptor mediated (Dubey 2000), although other mechanisms cannot be ruled out.

Other effects of oestrogens

Oestrogens have been shown to beneficially modulate sympathetic and parasympathetic nervous systems. Basal and stress induced plasma noradrenaline concentrations are elevated in postmenopausal women compared to premenopausal women (Lindheim 1992). These increased pressor and neuro-humoral responses in postmenopausal women are attenuated with oestrogen administration (Del Rio 1994).

Numerous studies have demonstrated increased vasodilation and vascular reactivity with oestrogen administration (Gilligan 1994, Reis 1994, Gilligan 1995, Volterrani 1995, Gerhard 1998) and HT (Lim 1999). Long term oestrogen (McGrath 1998) and HT (Rajkumar 1997) increased SAC, whilst women using HT (McGrath 1998) and oestrogen (Manolio 1993) had lower intimal medial thickening in comparison to non-users. In contrast, 48 weeks of treatment with 17β oestradiol had no effect on the progression of carotid intimal-medial thickness in postmenopausal women (Angerer 2001). The effects of oestrogens are diverse and complex, and to fully evaluate their roles and mechanisms is beyond the scope of this thesis. However, as previously mentioned, the effects of oestrogens on cardiovascular parameters are highly contoversial, with a great deal more research required prior to defining the role of HT in cardioprotection.

1.5.5.2 Hormonal therapy and Type 2 diabetes mellitus

Observational and prospective data have identified that HT has been associated with cardioprotection in healthy postmenopausal women (Stampfer 1991, Stampfer 1991, Falkeborn 1992, Ettinger 1996). Whether this potentially beneficial effect of HT can be extrapolated to a T2D population is controversial. A number of investigators have shown, in randomised clinical trials, HT reduces a number of risk factors for CVD in postmenopausal women with T2D, including an improved glycaemic control (Andersson 1997, Brussaard 1997, Friday 2001), improved lipoprotein profiles and reduced endothelial activation (Lim 1999). In contrast, in vitro studies have demonstrated that hyperglycaemia deceases the cardioprotective effects of oestrogens on NO production (Sowers 1997) and VSCM proliferation (Ling In press). Oestrogen administration has also produced neutral effects in vitro (Bolego 1999) and in postmenopausal women with T2D on endothelial function (Lim 1999, Manhem 2000, Saltevo 2000). Further research is required to substantiate the effects of HT in a diabetic population.

1.5.5.3 Progestins

Progestins are added to HT to reduce the risk of uterine hyperplasia and carcinoma associated with oestrogen administration (Grady 1992). Clinical trials to assess whether progestins augment, attenuate (Miller 1991, Williams 1994) or have no influence on the cardiovascular effects of oestrogens (Adams 1990, Gerhard 1998) have produced conflicting results. To further confound the issue there may be differences in the vascular effects between synthetic and natural progestins

(Miyagawa 1997), and progestins with differing androgenicity. A number of investigators have shown that progesterone itself does not have the harmful effects of androgenic progestins on measures of CVD risk, such as endothelial mediated dilation of coronary arteries (Williams 1990, Clarkson 1996), vascular reactivity (Miyagawa 1997) and lipoprotein profiles (Crook 1992, 1995) when combined with oestrogens.

The relationship between oestrogens and progestins and their combined effects on the cardiovascular system are complex. Oestrogens up-regulate the expression of both oestrogen and progestin receptors, whilst progestins down-regulate both progestin and oestrogen receptors and may also produce direct progestin receptor mediated effects that compromise the cardioprotective influences of oestrogens (Sarrel 1999). Progestin and oestrogen receptors are present in canine vascular tissue (Horowitz 1982), arterial endothelial cells and smooth muscle cells in baboon (Lin 1982) and in human myocardial fibres (Ingegno 1988). The presence of progestin receptors in vascular tissue suggests progestins may elicit direct effects on the vasculature.

Numerous observational studies have demonstrated oestrogen containing HT reduces the risks of cardiovascular disease (Bush 1987, Stampfer 1991, Stampfer 1991). In contrast, within therandomised clinical HERS study women with established coronary heart disease had a significantly higher rate of secondary coronary heart disease events - venous thrombo-embolic events - in the first year of HT compared to the placebo group after one year (Hulley 1998). This rate declined in subsequent years to produce an overall null effect after 4.1 years. The type of HT administered was oral oestrogen, CEE (0.625 mg) plus MPA (2.5 mg) daily. The actions of

progestin administration on cardiovascular parameters has provided conflicting evidence, and it has been proposed that progestins attenuated any benefit of oestrogens in this study.

The cardiovascular effects of progestin administration alone have been demonstrated in numerous animal studies and in vitro. Supraphysiological doses of synthetic progestins – norethisterone and MPA- in ovariectomised hypercholesterolaemic rabbits reduced intimal plaque size after twelve weeks (Spagnoli 1990, Hanke 1996). There was no effect, however, on aortic intimal plaque size after 12 weeks of progesterone treatment. Progesterone administration in vitro has been shown to elicit cardioprotective effects (Jiang 1992, Lee 1997, Morey 1997, Cheng 1999, Karas 2001, Otsuki 2001) and detrimental effects in vascular tissue (Miller 1991, Vazquez 1999). The cardiovascular effect of progestin administration is an important consideration to HT. Progesterone administration to oestrogen-unprimed women, however, has not been addressed, as a result further research is required.

1.5.5.4 Exercise

Exercise provides a favourable effect on lipid metabolism resulting in reduced concentrations of small dense LDL particles - a cholesterol abundant in individuals with coronary heart disease (CHD) (Houmard 1994) - and increased HDL cholesterol following three months to two years of exercise training (King 1995, Motoyama 1995). Serum triglycerides are reduced by 36% and VLDL by 31% with acute exercise training (Baumstark 1993).

Vascular tone is regulated during exercise and at rest to ensure sufficient blood supply equal to the metabolic demand by interplay between neural mechanisms, circulating hormones and local vasoactive substances. The relative roles of these mechanisms in large arteries and resistance vessels are altered with regular exercise as a result of training induced adaptations. These training adaptations contribute to an increased functional capacity and a reduction in morbidity and mortality associated with involvement in regular exercise (Blair 1995). The mechanisms involved in training induced vascular tone alterations include a reduction in sympathetic modulation of the vasculature (Meredith 1991, Oltman 1992, Grassi 1994, Sinoway 1996), a possible reduction in plasma renin activity (Vanhees 1984, Hespel 1988), alterations in the concentrations of vasoactive substances (Maeda 1994, Kingwell 1997), angiogenesis and remodelling of the exercising muscle and heart (Guyton 1985, Koller 1990).

A reduction in sympathetic modulation occurs with exercise via an attenuation of sympathetic nerve activity to central and chemoreflex mechanisms at rest and during exercise (Grassi 1994, Sinoway 1996), and by a reduction in the release (Meredith 1991) and reactivity to sympathetic neurotransmitters (Oltman 1992).

Studies of the effects of exercise on the renin-angiotensin system have produced differing results in various patient populations and training intensities. A negative correlation was demonstrated between physical work capacity and plasma renin activity with four months of training (Hespel 1988) and three months of training in individuals with ischaemic heart disease

(Vanhees 1984). In contrast, no associations were apparent with moderate training in normal individuals (Hagberg 1989) and those with moderate hypertension (Nelson 1986). Further research is required to substantiate this relationship and that of angiotensin on vascular reactivity.

Vasoactive substances, such as endothelin–1 and NO, appear to be altered in response to exercise. A redistribution of blood flow to working muscles occurs via a reduction in vasoconstrictor- endothelin-1- concentration in local muscle blood flow, whilst endothelin-1 concentrations are increased in local blood flow of non-working muscles (Maeda 1994). Increasing support for the role of altered NO synthesis, release and activity in response to training adaptations is becoming apparent. It appears, however, that the type and duration of exercise are two important training parameters that alter NO efficiency. Whole body dynamic exercise, such as cycling, may represent a greater stimulus for adaptations (Kingwell 1997) as opposed to the effect of training isolated muscle groups (Green 1994). It is possible that NO adaptations may play an important role in the initial stages of training with exercise training adaptations evolving from vasodilation with long term exercise, to represent a small or less significant adaptation (Kingwell 1998).

Exercise training influences vascular structural adaptations. Although the exact mechanisms responsible for these structural alterations remain to be fully elucidated, endothelium-dependent mechanisms appear to be responsible for changes in vascular structure in response to changes in blood flow (Guyton 1985, Koller 1990).

Observational data have indicated that vascular stiffening increases two to three folds with ageing (Vaitkevicius 1993); however, exercise can mitigate this age-associated stiffening. Older athletes potentially have significantly lower arterial stiffness in comparison to sedentary age matched controls (Vaitkevicius 1993).

These training adaptations on the vascular tone and arterial compliance minimise cardiac work and provide adequate coronary perfusion (Kass 1996). Together with training induced adaptations in lipid profiles and insulin sensitivity - to name a few of these adaptations - exercise training provides a non-pharmacological intervention to reduce cardiovascular risk factors. The most appropriate type, duration and intensity of exercise to employ in a postmenopausal population in order to gain cardioprotection have not been comprehensively addressed in the current literature. Further research is therefore required to clarify the effect of varying intensities, durations and types of exercises in postmenopausal women on surrogate markers of cardiovascular risk such as vascular stiffness.

1.5.5.5 Combined exercise and hormonal therapy

To the best of my knowledge, only one study has focused on the combined effects of exercise and HT on cardiovascular adaptations. Exercise and HT were demonstrated to be independently beneficial in relation to lipid metabolism, with oestrogen therapy alone having the greatest positive effect (Lindheim 1994). When exercise and oestrogen therapy were combined, no additive effect was evident. With only one study currently addressing this issue, it is difficult to determine

conclusively the cardiovascular effect of HT and exercise combined on cardiovascular risk factors in postmenopausal women. Therefore, further research is required that assesses the combined effects of these interventions on a wide variety of cardiovascular parameters.

1.5.5.6 Thiazolidinediones

Thiazolidinediones, initially developed as lipid lowing agents, also improve glycaemic control by attenuating circulating glucose, insulin and glycated haemoglobin levels. The subsequent amelioration of hyperinsulinaemia and hyperglycaemia may reduce cardiovascular risk by preventing the initiation and progression of endothelial dysfunction and the accumulation of AGEs associated with poor glycaemic control. This improvement in glycaemic control is one mechanism whereby thiazolidinediones may reduce cardiovascular risk. Thiazolidinediones also have a number of anti-atherogenic effects, independent of their influences on glucose and insulin metabolism. This section will overview the current literature available regarding the anti-atherogenic actions of thiazolidinediones.

Thiazolidinediones significantly reduce triglycerides and non-esterified fatty acids (NEFA) in insulin resistant animals (Fujita 1983, Fujiwara 1988, Kemnitz 1994, Lee 1994, Kaumi 1996, Sreenan 1996) by inhibiting triglycerides or VLDL synthesis in the liver (Fujiwara 1988) and increasing clearance in the periphery (Kaumi 1996). The hypolipidaemic effects of thiazolidinediones, however, are not limited to insulin resistant states, and have also been demonstrated in streptozotocin diabetic rats (Shimabukuro 1996). This indicates that the effects

of thiazolidinediones on lipids are not wholly reliant on insulin-sensitisation, suggesting that their actions are the result of numerous mechanisms. Thiazolidinediones also decrease plasma LDL and increase HDL in insulin resistant KKA mice (Castle 1993). Thiazolidinedione administration to patients with T2D increases HDL levels (Garber 2001, Pinaire 2001), however, an increase (Kreider 2001) and no change (Pinaire 2001) in plasma LDL levels has been demonstrated after eight and ten weeks of rosiglitazone respectively. Pioglitazone and rosigliatzone (two members of the thiazolidinediones class of drug) have both resulted in an increase in LDL particle size, from small dense atherogenic to large buoyant LDL particles, after eight to ten weeks of treatment. Therefore, evidence supports an effect of thiazolidinediones ameliorating many lipid metabolism abnormalities associated with insulin resistance.

PPAR gammas are present in all of the major cell types in the vasculature, including endothelial cells, VSMCs and monocytes/macrophages (Marx 1998, Marx 1998, Ricote 1998, Law 2000). Therefore, it would be expected that activation of these receptors by exogenous ligands, such as thiazolidinediones, would result in a direct effect on the vasculature.

Thiazolidinediones reduce blood pressure in various models of hypertension and insulin resistance. These hypotensive effects were reported in Zucker obese rats (Pershadsingh 1993), fructose fed rats (Buchanan 1995, Chen 1996) and obese monkeys (Kemnitz 1994). In these instances the reduction in blood pressure was associated with an improvement in insulin sensitivity. However, thiazolidinediones have also lowered blood pressure in insulin resistant hypertensive and normo-insulinaemic hypertensive rats (Zhang 1994), indicating that the blood

pressure lowering effect of thiazolidinediones may not always be associated with insulin-sensitisation. Impaired endothelial function plays a primary role in the development of hypertension (Walker 1997). Thiazolidinediones have been shown to improve endothelial function; this has been demonstrated by increased forearm blood flow in non-diabetic humans (Fujishima 1998), improved arterial endothelial function, measured by brachial artery vasoactivity in patients with occult diabetes, and the restoration of tonic vasorelaxant action of insulin in resistance arteries of Zucker fatty rats (Walker 1999). In contrast, endothelial function, measured by forearm plethysmography, was unchanged after eight weeks of thiazolidinedione treatment in lean and obese insulin resistant subjects (Tack 1998). These apparently conflicting observations may be accounted for by differences in methods used and differences in the patient populations studied. Thiazolidinediones also inhibit vasoconstriction, endothelin-1, mRNA expression and secretion in bovine vascular endothelial cells (Satoh 1999), possibly contributing to the hypotensive effect of thiazolidinediones.

Thiazolidinediones have been shown to influence endothelium-dependent and endothelium-independent vascular tone. An inhibition of extracellular calcium uptake by VSMC demonstrates the ability of thiazolidinediones to act as a calcium channel blocker by inhibiting L-type calcium channels (Zhang 1994, Song 1997, Goud 1998, Kawasaki 1998). The resultant amelioration of intracellular calcium overload in VSMC is one mechanism whereby thiazolidinediones may reduce the development of hypertension in T2D (Komers 1998). On the other hand, thiazolidinediones block glibenclamide-sensitive potassium channels (Mishra 1999), which may inhibit vasodilation in pathological conditions such as hypoxia. Troglitazone has also been shown to up-regulate cytokine-stimulated NO synthesis in VSMCs (Hattori 1999), which

may inhibit the action of numerous growth factors on VSMCs. This inhibition of NO synthesis potentiated by thiazolidinediones may have important implications for the suppression of restenosis and atherosclerosis.

Other properties of thiazolidinediones that may contribute to their blood pressure lowering actions include inhibitory effects on vascular smooth muscle cell proliferation (Dubey 1993, Law 1996, Yasunari 1997). Thiazolidinediones also inhibit VSMC migration (Law 1996, Yasunari 1997, Goetze 1999) from the media to the intima, where VSMCs proliferate and form a neointima with increased extracellular matrix production, leading to the development of organised atherosclerotic plaque (Ross 1993). This inhibition of VSMC migration is proposed to be acting downstream of cytoplasmic activation of MAP-k, by affecting the nucleus (Goetze 1999). VSMC migration and proliferation are crucial processes in the development of vascular remodelling, atherosclerosis and diabetic organ complications. The relevance of these actions are demonstrated in vivo, whereby troglitazone inhibits neointimal formation following balloon vascular injury (Law 1996).

The actions of thiazolidinediones provide immense therapeutic potential for the amelioration of altered lipoprotein metabolism, endothelium dependent and independent dysfunctions commonly associated with T2D, and ultimately reducing the risk of CVD with T2D.

1.5.5.7 Combined thiazolidinediones and hormonal therapy

A combined treatment of thiazolidinediones and HT, focusing on cardiovascular effects, has not been reported in current literature. It would be of interest to determine whether this combined treatment enhances, impairs or has no effect on cardiovascular risk parameters in comparison to either treatment given in isolation.

1.5.6 Measurement of vascular tone and endothelial function as modifiable targets for treatment

The arterial system is composed of an elaborate network of elastic tubes, which act to deliver blood from the heart to peripheral organs and tissue. Arteries have two primary functions, that as a conduit- a means of blood supply to the body and a cushioning function, whereby ventricular ejection of blood flow into the arteries is dampened by the elastic properties of the arterial walls (O'Rourke 1990). This cushioning function allows a constant even supply of blood- similar to the function of a Windkessel. The Windkessel was a storage tank used for fire fighting that applied both cushioning and conduit properties to provide a continuous outflow of water when water inflow to the tank was of a pulsatile nature. The air filled dome of the Windkessel acted as a cushion and the fire hose acted as a conduit.

The elastic distensibility of the aorta and some proximal arteries allows temporary storage of approximately 50% of left ventricular stroke volume during systole (O'Rourke 1990). This volume of blood is then driven further along the vascular system during ventricular diastole due to the elastic potential of arteries. The constant distension and recoiling actions reduces the pulsatility of blood flow and allows a constant distribution of blood around the body (O'Rourke 1990).

Arterial mechanical properties are important cardiovascular parameters that affect coronary blood flow, systolic and diastolic blood pressure and coronary perfusion. The measurement of these parameters by specific, reproducible and non-invasive methods provides modifiable targets for therapy.

1.5.6.1 Systemic arterial compliance (SAC)

The mechanical properties of arteries are assessed by techniques based on three principles, which include the change in arterial wall displacement in response to pressure within that vessel- SAC, pulse wave velocity and analyses of vascular pressure and flow in the frequency domain- vascular impedance. Within the scope of this thesis one technique, SAC, will be reviewed.

The compliance of arteries refers to the elastic potential of arterial walls and their ability to distend and recoil as a function of blood flow. SAC is a non-invasive procedure aimed at modelling large arterial mechanical properties. These properties are influenced by the elasticity of

load bearing fibres of the arterial wall, endothelial dysfunction and resultant arterial stiffening and the progression of atherosclerosis.

SAC is defined as the change in volume of an artery as a result of a change in pressure within that vessel. Non-invasive calculations of SAC were previously based upon the assumption that vessels have a similar behaviour to that of a Windkessel, whereby, an exponential decay occurs in diastolic pressure and that the arterial pressure-volume relation is linear. These assumptions do not take into consideration properties of the real vasculature that are not accounted for in the Windkessel model such as, wave reflection and tapering of arteries. Therefore, an alternative expression for SAC measurement was derived based on a two-element Windkessel model, but not conforming to an assumed diastolic pressure decay waveform (Liu 1986). This expression was based on measurements of the area under an arbitrary portion of the diastolic waveform, corresponding to a specific cardiac cycle, and the accompanying volume-change determined by measurement of stroke volume.

1.5.6.2 Flow mediated dilation (FMD)

Endothelial dysfunction is a major initiating event in the pathogenesis of cardiovascular disease, appearing prior to clinical symptoms being present (Celermajer 1992). Flow mediated dilation (FMD) is a well-established non-invasive measurement of endothelial function (Liang 1998, Raitakari 2000). This measurement assesses the outcome of the following: increased blood flow through resistance vessels increases the amount of shear stress exerted on the endothelium which

activates adenosine triphosphatase (ATP) sensitive potassium channels present on the surface of endothelial cells, promoting the synthesis of NO (Cooke 1991, Ohno 1993) and subsequent vasodilation. The resultant vasorelaxation in response to flow is endothelium dependent (Pohl 1986, Rubanyi 1986, Drexler 1989) and is termed "flow mediated dilation". FMD measured at the brachial artery reflects systemic endothelial function and is significantly associated with the extent of coronary artery disease (Neunteufl 1997). Therefore, FMD is an effective non-invasive measure of endothelial dysfunction, which may be used as a surrogate marker to determine coronary circulation.

1.5.6.3 Cutaneous vascular reactivity (CVR)

Another means of assessing endothelial function involves the measurement of cutaneous vascular reactivity with direct current iontophoresis. This technique utilises iontophoresis in order to apply vasoactive substances, such as acetylcholine and sodium nitroprusside, to the skin and investigates the effect of these agents on cutaneous perfusion. The effect these substances produce on small vessels 1-2 mm under the surface of the skin indicates the reactivity capacity of these vessels. Iontophoresis is a non-invasive method of introducing substances across the skin surface by the application of a small electric current; when a direct electrical current is applied, the vasoactive substance is repelled from the electrode of the same polarity, and therefore penetrates the skin, whilst the ions of the opposite polarity are not transferred (Sloan 1986). The response of the vessels to the vasoactive substance is measured by laser Doppler velocimetry, which reflects a frequency shift of low laser light caused by moving red blood cells within the cutaneous arterioles,

used as an index of blood flow (Saumet 1988). Traditionally, when measuring endothelial function with this technique the vasoactive substances used are acetylcholine for endothelium-dependent vasodilation and sodium nitroprusside for endothelium-independent vasodilation (Komesaroff 1998, Komesaroff 1999). Acetylcholine mediates an endothelium-dependent reaction via interactions with nicotinic receptors present on endothelial cells resulting in NO release and subsequently vasodilation. Sodium nitroprusside is a nitric oxide donor causing vasodilation by directly acting on VSMC.

1.5.7 Conclusions

With the advent of menopause, and the associated changes in circulating sex hormone levels, there is an increase in the risk of developing CVD. Indeed, there is a significant increase in the rate of cardiovascular related mortality and morbidity after menopause. The development of T2D abrogates any cardioprotective effects of oestrogens prior to menopause, with postmenopausal women with T2D experiencing an accumulated risk of disease.

CVD has a diverse and complex aetiology and pathogenesis. The measurement of surrogate risk markers for cardiovascular disease, such as circulating lipid levels, endothelial function, vascular reactivity and SAC, can provide important information about the current risk of an individual and the efficiency of treatment interventions employed. Treatment interventions used to ameliorate altered cardiovascular parameters as a result of menopause and, or T2D include HT, exercise, thiazolidinediones and combined treatments of HT and exercise, and HT and thiazolidinediones. The cardiovascular effects of HT and exercise in isolation are well established and provide cardiovascular protection. The effects, however, of combined treatments of HT and exercise are not fully elucidated. The cardiovascular effects of thiazolidinediones are not well established in vivo, similarly, little is known about the combined treatments of HT and thiazolidinediones in vitro or in vivo. Therefore, further research is required concerning the affects thiazolidinediones in vivo and combination therapy, to ascertain any additive, synergistic or deleterious influences on cardiovascular risk factors within a population at high risk of developing cardiovascular disease.

1.6.1 Endocrine influences of menopause on bone metabolism

Currently, one of the most challenging public health issues is the maintenance of skeletal integrity with increasing age. With the worldwide trend for increasing life expectancy there is an escalating need for management and prevention techniques for the development of osteoporosis, to reduce the burden on individuals and health care costs related to fractures.

Osteoporosis, characterised by low bone mass and the deterioration of bone tissue micro-architecture, is mainly caused by alterations to the normal process of bone remodelling. This is a continuous process involving the removal of bone by bone resorbing osteoclasts, and the subsequent formation and mineralisation of bone by bone forming osteoblasts. The control of bone remodelling is complex and finely regulated by a number of factors, including mechanical and hormonal stimuli.

Peak bone mass is attained around the age of twenty-five to thirty years (Mosekilde 1997), and is twenty-five to thirty percent greater in men than in women. The level of bone mass acquired is determined by a range of factors, including genetic predisposition and the level of physical activity undertaken throughout childhood and adulthood (Gilsanz 1988). There is a

continuous loss of bone mass from the age of twenty-five to thirty years, which, if it becomes pronounced causes bone fragility and an increase in the risk of fractures. Compounding this normal age related bone loss are alterations in bone remodelling that become prominent around the time of menopause. One to three years after final menses is the period for greatest bone loss, whilst late peri-menopausal women, who have higher circulating sex hormone levels, experience comparatively lower lumbar spine bone loss (Guthrie 1998).

Oestrogen deficiency after natural menopause, or oophorectomy, lead to acceleration in the rate of bone loss. This is associated with an increase in bone resorption and bone formation, with the former exceeding the latter. Trabecular bone is the principal site of bone loss after menopause (Manolagas 1995). Reduced circulating oestrogen levels increases bone resorption by a number of mechanisms, including the stimulating of peripheral blood monocytes (PBM) that up-regulate the synthesis of cytokines - interlukin-1 (IL-1) and tumour necrosis factor alpha (TNF alpha). Both of these cytokines stimulate stromal osteoblastic cells to secrete interlukin-6 (IL-6) and colony stimulating factors (CSF), which enhance osteoclast differentiation and activation, increasing bone resorption. Oestrogen deficiency also directly stimulates stromal osteoblastic cells to increase the secretion of IL-6, causing osteoclast differentiation (Horowitz 1993). Low oestrogen levels not only increase the development of osteoclast progenitors, but also the development of osteoblast progenitors in bone marrow (Jilka 1994), providing a possible explanation for the increase in osteoblastic activity that follows menopause. The net increase in bone turnover is a major cause of bone loss after menopause.

Intestinal calcium absorption is reduced (Bullamore 1970) and urinary calcium excretion is increased (Young 1967) after menopause, also accelerating bone loss. Although in perimenopausal and early postmenopausal women calcium supplementation alone is not able to prevent progressive bone loss (Riis 1987, Dawson-Hughes 1990), it has an additive effect with HT on bone in early postmenopausal years (Ettinger 1987). Calcium supplementation can also reduce further fracture rates in elderly women with previous fractures by up to 45 % (Recker 1994).

Regular exercise can increase bone mass, density and strength (Gutin 1992, Kannus 1995). Therefore, after menopause if the level of physical activity in which women participate is reduced, the risk of developing osteopaenia will be increased. Epidemiological studies have demonstrated a negative association between physical activity and osteoporotic fracture risk (Cooper 1988, Paganini-Hill 1991, Zhang 1992, Jaglal 1993).

Oestrogen deficiency and less frequent and intense mechanical strain elicited by physical activity are two factors that affect bone metabolism and increase the risk of impaired bone strength and density and ultimately fractures.

1.6.2 Bone turnover markers as a measure of bone metabolism

Assessment of bone mineral density (BMD) changes, as a result of altered remodelling, is important for determining the risk of fracture in an individual or the effectiveness of a treatment employed. Dual energy X-ray absorptiometry (DXA) and quantitative computer tomography (QCT) remain accepted standard techniques for measurement of BMD. The measurement of biochemical measures of bone turnover have a complementary role to measures of BMD. These biochemical markers can be used to measure the short term responses of bone to interventions. Bone formation markers such as osteocalcin (OC) (Delmas 1992) and bone alkaline phosphatase (BAP) (Parfitt 1987) correlate well with rates of bone formation. Urinary excretion of free cross-links or peptide forms such as pyridinoline (PYR) and deoxypyridinoline (DPD) also correlate well with other measurements of bone resorption (Beardsworth 1990, Uebelhart 1990). Changes in bone turnover markers can predict alterations in bone density and rates of bone loss in postmenopausal populations (Slemenda 1987, Kelly 1989, Schlemmer 1994). The usefulness of these markers in predicting fracture are yet to be established. Therefore, bone turnover markers are a viable alternative for measuring bone mineral changes in postmenopausal populations.

1.6.3 Treatment interventions

1.6.3.1 Hormonal therapy

Clarification of the mechanisms of bone loss after menopause is important for the understanding of the physiological actions of oestrogens on bone metabolism. In vitro studies have identified receptors for oestrogen, or genes for oestrogen receptors in oestoblastic cell lines (Etienne 1990, Masuyama 1992), osteoclastic and preosteoclastic cells (Oursler 1991, Fiorelli 1995), osteocytes (Braidman 1995) and bone endothelial lines (Brandi 1993). The widespread presence of oestrogen receptors in bone cells indicates that oestrogens directly affect bone in a multifaceted fashion. The mechanisms of the responses of oestrogens have not been not fully elucidated, although, it is proposed that oestrogens elicit their responses by affecting the synthesis of local regulatory factors such as prostaglandins, growth factors and cytokines and alter the sensitivity to parathyroid hormone.

IL-6 plays an important role in the replication and differentiation of osteoclast progenitor cell precursors (Dorshkind 1990); therefore, the heightened production or action of this cytokine may be one mechanism involved in the pathological conditions of altered bone metabolism after menopause. Numerous studies have demonstrated that oestrogens suppress constitutive IL-6 secretion (Cheleuitte 1998), reduce the expression of IL-6 receptors (Abrahamsen 2000), suppress IL-6 production by bone marrow stromal cells and osteoblasts (Girasole 1992) and inhibit IL-6 gene through oestrogen receptor mediated indirect effects (Pottratz 1994). These

studies provide a molecular basis for the protective effects of oestrogens on bone metabolism after menopause.

Oestrogen administration has been shown to slow bone loss in postmenopausal women (Lindsay 1976, Horsman 1977, Recker 1977). Retardation of bone loss is apparent in measures of biochemical markers of bone formation and resorption, showing that oestrogen reduces bone turnover markers (Prestwood 1994, Prior 1997). Long term administration of oestrogen after menopause has shown a protective role against hip fracture (Kiel 1987) and the development of osteoporosis (Weiss 1980).

1.6.3.2 Exercise

It is well established that physical activity can increase bone mass, strength and density (Gutin 1992, Kannus 1995). A mechanical stimulus on bone, such as that elicited by physical activity, causes a rapid and transient rise in NO release (Pitsillides 1995), inhibiting osteoclast function (Brandi 1995). The inhibition of NO reduces the rate of mechanically induced bone formation by 66% (Turner 1996), whilst the administration of a NO donor can counteract bone loss associated with oestrogen deficiency (Wimalawansa 2000), indicating that NO plays an important role in the transduction of a mechanical stimulus into a biological response of bone (Ralston 1994, Turner 1996, Fox 1998).

The most appropriate type, intensity and duration of exercise to ameliorate oestrogen deficient bone loss after menopause, or increase bone density, have been controversial issues, with conflicting results from numerous studies. One year of brisk walking (Cavanaugh 1998) and lifetime volleyball involvement (Ito 2001) provided insufficient stimulus to alleviate menopause related spinal bone loss. Low impact aerobic exercise was unable to counteract forearm bone loss over two years (Prince 1991). In contrast, walking twice weekly for 50 minutes over 24 weeks provided no change in lumbar BMD (Humphries 2000). Walking plus weight training twice weekly over 24 weeks (Humphries 2000), high intensity strength training twice a week for one year (Nelson 1994), weighted vest jumping performed three times a week for 32 weeks a year, over five years (Snow 2000) and resistance training for sixteen weeks (Ryan 1998) all maintained hip and lumbar bone densities. It should be noted that in studies conducted over a period of less than 1 year it may be insufficient time to see, or rule out, a change in BMD, due to the error of the method. Exercise that provided higher BMD in postmenopausal women included one year of high impact aerobic exercise (Chow 1987, Welsh 1996), one year of aerobic exercise and strength training (Chow 1987) and eighteen months to two years of resistance training (Heinonen 1996, Kerr 2001). It must, however, be noted that variations in the effects of physical activity on bone in different studies are difficult to compare due to variations in the type, intensities and durations of exercise, participant characteristics and BMD assay techniques.

Of the above studies mentioned, only three measured bone turnover markers as an indication of alterations in bone remodelling. One study (Humphries 2000) demonstrated a significant increase in OC levels as a result of walking over twenty-four weeks, indicating an increase in bone formation. Another study (Welsh 1996) showed that following six months of

high impact aerobic exercise, urinary cross-links (PYR and DPD) were significantly reduced, indicating a reduction in bone resorption. However, measuring either formation or resorption markers alone does not provide sufficient information to determine net changes in whole body bone turnover. Only one study (Ryan 1998) measured both bone formation and resorption markers, identifying no change after sixteen weeks of resistance training, indicating bone was in a state of equilibrium maintaining bone density, which was supported by measures of BMD. The limited data available for the effects of exercise on bone turnover markers in postmenopausal women compound the conflicting data available for alteration in BMD with differing exercise regimens. Further research is required to determine the effect of differing exercise regimens on bone turnover markers to assess the most appropriate exercises to enhance or maintain bone integrity after menopause. Studies detailed in Chapters 3 and 4 will address these issues.

1.6.3.3 Combined exercise and hormonal therapy

Animal studies have shown that the effects of mechanical loading on bones are additionally influenced by sex hormones (Bagi 1993, Westerlind 1997, Zaman 1999, Zaman 2000). Mechanical strain and oestrogens share common elements in signal transduction pathways (Westerlind 1997). The combined effects of these treatments are the focus of three separate clinical studies involving postmenopausal women, providing differing results. One study identified that weight bearing exercise and HT independently increased BMD at sites of lumbar spine and proximal femur, whilst a combined treatment provided an additive effect on BMD at lumbar spine and Wards triangle, and were synergistic for total body BMD (Kohrt 1995). Resistance training and HT increased BMD at sites of radial mid-shaft, spine and total body, whilst HT alone

was associated with no change, or bone maintenance after one year of treatment (Notelovitz 1991). The effect of exercise alone was not measured in this study making it difficult to determine whether BMD accretion was due to the combination of treatments or due to the effect of exercise alone. In contrast, moderate exercise, including weight lifting, was not enough to stimulate increases in lumbar and proximal femur BMD over one year. HT significantly increased BMD over one year, whilst the combination of exercise and HT provided no greater benefit (Heikkinen 1991). Differences in subject characteristics, the type, intensity and duration of exercises and types and doses of HT make comparison between these three studies difficult.

One primary issue to consider is compliance to an exercise program. It is important to develop an exercise program that will elicit beneficial changes to bone metabolism without compromising a woman's involvement. Exercises employed in the above studies involved supervision and direction in structured exercise regimens, which was often associated with substantial drop out rates (Heikkinen 1991, Notelovitz 1991). Walking is an accessible and cost efficient exercise that can be administered at home; however, the effect of a walking regime in combination with HT has not been addressed.

1.6.3.4 Thiazolidinediones

Bone marrow possesses multi-potential progenitor stem cells, capable of differentiating into fibroblasts, adipocytes and osteogenic cells (Owen 1988). Osteoporosis and osteopenia are associated with an increase in marrow adipose tissue and a decrease in bone volume (Meunier 1971). A disproportionate differentiation of progenitors into adipocyte cell lineages at the expense of osteoblasts could be one mechanism accounting for a reduction in bone volume and bone strength with osteoporosis. This hypothesis is supported by one study that identified, by histomorphometric observations, a replacement of adipose in the functional bone cell population in osteoporosis (Meunier 1971).

Very few data are currently available assessing the effects of thiazolidinediones on bone. To the best of my knowledge, only two independent studies have addressed this issue. One in vitro study has shown that thiazolidinediones increase adipocyte differentiation in bone marrow stromal cell lines (Gimble 1996). The activation of bone marrow differentiation into adipocytes may compromise the integrity of bone strength and predispose an individual to an increased risk of developing osteoporosis. On the other hand, thiazolidinediones reduce osteoclast-like cell formation and inhibit bone resorption in a dose dependent manner in mice (Okazaki 1999). In summary, further in vitro research is required to substantiate the effects of thiazolidinediones on adipocyte differentiation in human bone marrow stromal cells. Human in vivo studies that demonstrate alterations to bone turnover or BMD as a result of thiazolidinediones administration are also required.

1.6.4 Conclusions

Altered bone remodelling after menopause increases the age associated risk of developing osteoporosis. Treatment interventions such as exercise and HT have provided varying degrees of protection against increased bone turnover and osteoporosis. Controversy remains, however, as to the most effective type, intensity and duration of exercise to provide the greatest protection without compromising compliance to an exercise regime. Few data are available in regards to combined HT and exercise training. Further research is required in this area to substantiate whether both treatments combined provide greater protection than either treatment in isolation. Data are lacking in regard to the effects of thiazolidinediones on bone, with research required in a clinical setting to determine the effects of these drugs on bone turnover or BMD.

1.7 OBJECTIVES OF THIS THESIS

The aims of this thesis are to determine the effects of high and low intensity exercise alone and in combination with HT on glucose and insulin metabolism, cardiovascular risk factors and bone turnover in postmenopausal women. These are described in detail in Chapters 3 and 4. Walking will be used as a low intensity exercise for previously sedentary individuals, due to the safety and ease of administration of this exercise. Alternatively, high intensity exercise will involve endurance running, jogging and cycling performed at a 'Masters' level.

The cardiovascular actions of progesterone will be addressed in postmenopausal women (Chapter 5) to ascertain the effects this steroid may have on the functioning of the endothelium, and on large and small vessels in the cardiovascular system.

The role of thiazolidinediones on glucose and insulin metabolism, cardiovascular risk factors and bone turnover will be assessed to determine the effects of this drug in a T2D population at high risk of cardiovascular disease and osteoporosis. Thiazolidinediones will also be combined with HT to determine any additive, synergistic or deleterious effects (Chapters 6, 7 and 8).

2.0 Description of methods

2.1 Outline of chapter

This chapter describes the techniques and equipment used for the purposes of the following intervention studies. All of the methods described within this section detail the exact calculations, calibrations and venues for each testing procedure unless otherwise stated within the chapter of each study.

2.2 ETHICS AND INFORMED CONSENT

Ethics approval was provided for each individual study by the Victoria University Ethics Committee, ethics was also approved for the two studies undertaken at the Baker Medical Research Institute from the Alfred Hospital Ethics Committee. Any changes made to the parameters of the studies were amended via an application for amendment to each of the ethics committees. Prior to involvement within studies, subjects signed an informed consent form. The appropriate informed consent form for each study is included in the Appendix 1.

2.3 ANTHROPOMETRIC MEASUREMENTS

Height was measured for individuals using a standard tape measure and expressed in centimetres, weight of subjects was measured using a Mercury scale (211FP, Thebarton South Australia) and was expresses in kilograms. Body mass index was calculated by using the following formula;

$$BMI = \text{weight (kilograms)} / (\text{Height(meters)})^2$$

2.4 HAEMODYNAMIC MEASUREMENTS

2.4.1 Brachial blood pressure and heart rate

Brachial systolic, diastolic and mean blood pressures and heart rate were measured using a Dinamap vital signs monitor (1846SX, Critikon).

2.4.2 Carotid blood pressure

Carotid blood pressure was measured by applanation tonometry of the right proximal carotid artery using a calibrated non-invasive Millar Mikro-Tip pressure transducer (model SPT 301, Millar Instruments, Houston, Texas). A pressure wave was obtained by placing the pressure transducer on the carotid artery. The pressures obtained by this method were calibrated against brachial mean artery pressure measurements made simultaneously using a Dinamap vital signs monitor (1846SX, Critikon, Florida, USA). Calibration was achieved by assuming that both the mean arterial blood pressure (MAP) and end-diastolic blood pressure remain constant throughout the vasculature, and therefore the central systolic blood pressure can be calculated. This method of using brachial calibration of carotid pressures has been validated against invasively obtained aortic root pressure signals (Cameron 1994). These measures were undertaken at the Alfred

Baker Medical Unit for studies detailed in chapter 3 and 4, and at the Baker Medical Research Institute, Menopause Clinic for studies detailed in chapter 5, 6 and 7.

2.5 MEASUREMENT OF ARTERIAL MECHANICAL PROPERTIES

2.5.1 Systemic arterial compliance

SAC was determined using calculations based on the area method (Liu 1986). SAC non-invasively estimates the change in volume of an artery as a result of a change in pressure within that vessel, via the simultaneous measurement of right aortic root driving pressure using a Miller Mikro Tip Pressure Transducer (Model SPT 301, Millar Instruments, Houston Texas), and volume of flow in the ascending aorta, using a Doppler Flow Velocimeter (Multidoplex MD1, Huntleigh Technology, Cardiff, U.K., 4 mHz) placed on the suprasternal notch at the base of the neck. The subject was laid supine and rested for 5-10 minutes in a dark room. Resting brachial blood pressure was measured every 3 minutes. Aortic root driving pressure, via applanation of the right carotid artery, was measured, whilst simultaneously measuring aortic flow within the region of the suprasternal notch for 2 minutes. During calculations, 10 waveforms were selected that best represented overall waveforms, and SAC was calculated using purpose written software (Pascal Turbo, Borland International, Inc). This method produces good repeatability (coefficient of variation- 9.2%) and is significantly correlated with intima-medial thickness (Liang 1998).

2.5.1.1 Calculated systemic arterial compliance

$$SAC = A_d / [R (P_s - P_d)]$$

A_d = Area under the diastolic portion of the blood pressure waveform (from end-systole to end-diastole).

R = the total peripheral resistance.

P_s = the end-diastolic blood pressure.

P_d = the end-diastolic blood pressure.

2.6 MEASUREMENT OF ENDOTHELIAL FUNCTION

2.6.1 Flow mediated dilation

The subject was laid supine, and rested for 5-10 minutes in a dark room. The subject was fasted and they had abstained from caffeine for > 8 hours prior to testing. One trained researcher used a high resolution (7.5 MHz) ultrasound (Powervision 7000, Toshiba, Japan) to obtain a longitudinal B mode image of the right brachial artery within 5 centimetres of the ante-cubital region, whilst simultaneous 3-lead electrocardiogram was recorded onto super VHS videotape using an in-built video-recorder (Model 9500 MDP, Sony, Japan) for analysis purposes. Through out

measurements the subject was instructed to remain completely still to reduce the movement of the brachial artery image being recorded.

A sphygmomanometer cuff (Dinamap 1846Sx, Critikon, Florida, USA) was placed around the forearm of the patient's right arm, within 2 centimetres of the ante-cubital region. A baseline image of the brachial artery was obtained and recorded for approximately 1 minute; after which the forearm cuff was inflated to 250 mmHg for 4.5 minutes whilst the image of the brachial artery was maintained. After the cuff was deflated recording of the brachial artery continued for 2 minutes.

When the subject's heart rate returned to baseline levels (generally after approximately 5-10 minutes) a second baseline recording of the brachial artery was obtained. This image was maintained whilst the subject was provided with an oral dose of 300ug glyceryl trinitrate (Anginine, Glaxo SmithKline, St Louis) that was dissolved under the subject's tongue. Once the oral dose was dissolved, recording of the brachial artery was maintained for 5 minutes.

An in-built measurement program within the ultrasound, which was calibrated against a 1 centimetre scale each time a measurement was made, allowed determination of the diameter of the brachial artery. The measurement of diameter was consistently determined at peak systole, represented by the ECG trace on the screen. Three consecutive measures of brachial artery diameter were made to determine an average diameter at baselines, whilst measurements recorded

post occlusion and glyceryl trinitrate treatment, were made at 30 second intervals to determine the peak increase in diameter.

After peak dilation of the brachial artery was determined, baseline diameters were used to express the increase in dilation as a percentage of the baseline using the formula;

100/ Baseline diameter * peak diameter – 100 = Percentage increase in diameter (%).

These measurements were undertaken at the Alfred Hospital Vascular Laboratory.

2.6.2 Cutaneous vascular reactivity

The subject was seated in a temperature controlled environment, and was rested for approximately 10 minutes prior to commencement of this measurement. The subject's right forearm was exposed and cleaned, and four rubber wells with wire insertions were placed along the forearm. Care was taken to ensure that the wells were not placed over predominant forearm hair, alterations in skin pigmentation or skin abrasions. These wells were 12 millimetres in diameter, and contained 0.5 millilitres in volume. Two wells were filled with solution containing Methyl cellulose gel (M-6385, Sigma Chemicals, St Louis) and 1% acetylcholine (BDH Chemicals, U.K.), whilst the following two wells were filled with solution containing Methyl cellulose gel (M-6385, Sigma Chemicals, St Louis) 1% Sodium Nitroprusside (David Bull Laboratories, Australia). Each well was connected to a battery operated voltage box (World Precision Instruments,

A36ODC, Sarasota, Florida, USA), which supplied a constant amplitude 0.1 milliamp of current to the well for 30 seconds.

Positioned above the subject's forearm, at a distance of 38 centimetres, was the Laser Doppler flow meter (Dual Channel Moor DT4 Laser Doppler Flow Meter, Moor instruments, England). This laser emitted a light at 632.8 nanometres directly into the middle of one well, which then subsequently scanned the region within the well over a period of 10 seconds, and repeated this scan 25 times at 14 second intervals. Subsequent wells were measured following the same procedure.

For each well, the subject was asked to remain still whilst the laser scanned two baseline measurements, after which a current was supplied to the well for 30 seconds, allowing iontophoresis of the solution into the subcutaneous layer of skin. The effect of this solution on small vessel dilation was graphically displayed onto a laptop (Toshiba, Satellite 310 CDS, Japan), using a Laser Doppler Perfusion Measurement Package (V3.01). This image was later processed using Laser Doppler Perfusion Image Processing Package (V3.0) which measured blood flow within a selected region of each of the 25 time point measurements. These individual blood flow points were subsequently transferred to a Microsoft Excel (Microsoft 2000) spreadsheet, and converted into a logarithmic equation to determine area under the curve. Data are expressed as arbitrary perfusion units (APU).

The measurements were undertaken at the Baker Medical Research Institute, Menopause Clinic.

2.7 MEASUREMENT OF AEROBIC CAPACITY

2.7.1 VO_2 peak

Aerobic fitness was determined via a VO_2 peak test. This test measures the volume of oxygen consumption during exercise to peak levels. Measurement to maximum levels were deemed unsafe within this population group, hence, a VO_2peak test was used instead of a VO_2max protocol. The laboratory where the trials were conducted was maintained at a constant temperature (20 $\pm$ 1°C) and humidity (44 $\pm$ 2%). A 12 lead ECG (Montara, X-scribe, Stress testing System, Milwaukee, U.S.A.) was used to monitor subjects heart rhythm and rate throughout the duration of the test. Subjects exercised on a stationary bicycle ergometer (Cybex Metabolic System, Met 100, Huntsville, USA) at a constant cadence of 70 rpm. Increments in cycling intensity occurred each minute, starting at 35 watts and increasing by 10 watts each minute, until exhaustion, or until the subject experienced discomfort or the practitioner observing the ECG monitor identified an abnormal rhythm or rate. Each subjects' oral gas expiration was measured via Vacumetric Vista Turbofit Software package (Version 3.2, Ametek, Pittsbergh, USA) to determine their respiratory exchange ratio and aerobic capacity, which was subsequently

charted through-out the duration of the exercise to ensure that their aerobic threshold was reached. Subjects were deemed to have reached their VO_2peak when at least two of the following criteria were achieved (a) a plateau of VO_2peak readings, (b) exercising heart rate to within 10 beats of subjects' maximal heart rate, (c) a respiratory exchange ratio (RER) of greater than 1.10.

These measurements were undertaken at Victoria University, Exercise Physiology Unit.

2.7.2 Anaerobic threshold

From baseline aerobic capacity measures, volume of expired carbon dioxide (mL/kg/min) was plotted against the volume of expired oxygen (mL/kg/min). A parallel graph, plotting ventilation (L/min) against oxygen consumption (mL/kg/min) was determined for the duration of the test. A line of best fit was determined for the initial linear rise of these graphs, and the point at which the graph strayed from this line was determined. It was at this point that anaerobic threshold was reached and the corresponding heart rate for this stage was noted. An example of the parallel graphs representing volume of expired carbon dioxide (mL/kg/min) plotted against the volume of expired oxygen (mL/kg/min) and ventilation (L/min) plotted against oxygen consumption (mL/kg/min), used to measure anaerobic capacity is included in Appendix 2.

2.8.1 Sex hormones

2.8.1.1 Oestradiol and progesterone

Venous blood samples were obtained from subjects for the determination of sex hormones. Commercial radioimmunoassay kits (Orion Diagnostica, Finland) were used to measure serum oestradiol (within run CV =9.4%, total CV =17.1%) for studies detailed in chapter 3 and 4 (outsourced to Western Hospital, Footscray, Australia) and chapters 5, 6, and 7 (the Alfred Pathology Service, Melbourne, Australia). Progesterone was measured using a sequential competitive immunoassay (Immulite 2000 Progesterone, U.S.A.) with coefficient of variation (CV) 17%.

2.8.1.2 Follicle stimulating hormone and luteinizing hormone

FSH and LH were measured using a two-site chemiluminescent (sandwich) immunoassay (Ciba-Corning, Medfield, MA, USA) for studies detailed in chapter 3 and 4 (outsourced to Western Hospital, Footscray, Australia) (FSH within run CV =2.7%, total CV =3.6%, LH within run CV =4.3%, total CV =6.3%) and chapters 5, 6, and 7 (the Alfred Pathology Service, Melbourne, Australia).

2.8.2 Metabolic parameters

2.8.2.1 Insulin

Insulin assays for studies detailed in chapters 3 and 4 used a commercial radioimmunoassay kit (Pharmacia and Upjohn, Sweden) (CV 2.83%), whilst samples from studies 5, 6 and 7 were outsourced to the Alfred Pathology Services (IMx system, Japan, CV 6.0%).

2.8.2.2 Glycated haemoglobin

Glycated Haemoglobin was measured using a Borate affinity chromatography (in house method at Alfred Pathology, within run CV 0.82 – 0.46%, run to run CV 2.91 – 1.09%)

2.8.3 Lipids

Total cholesterol (CV 2.4%), HDL (CV 4.8%), LDL (CV 3.8%), triglycerides (CV 3.6%) and glucose (CV 4.5%) were measured using Cholestec L.D.X Lipid Profile plus Glucose (Cholestec, California).

2.8.4 Bone turnover markers

2.8.4.1 Bone resorption markers

Free PYR and DPD cross links were measured on morning void urine samples by an enzyme linked immunosorbent assay (ELISA) using rabbit antipyridinoline (Pyrilinks, Metra Biosystems, Palo Alto, CA, USA) and expressed as pyridinoline/creatinine ratio (PYR nmol/mmol Cr). Coefficients of variation (CV) for these crosslinks were; intra-assay PYR 4.2%, DPD 8.0%; inter-assay PYR 4.1%, DPD3.7%.

2.8.4.2 Bone formation markers

BAP was measured using the standard autoanalyser Elisa technique (Alkaphase B96, Metra Biosystems). Serum OC was measured by a two site Immunoradioimetric assay using the Active Human Osteocalcin IRMA kit (DSL-7600, CSL, Texas) (CV intra-assay 3.4%, inter-assay 3.4%).

All bone formation and resorption markers were outsourced to the Royal Melbourne Hospital, Endocrinology Department.

3.0 The effects of hormonal therapy and walking on glucose and insulin metabolism, cardiovascular risk factors and bone turnover in postmenopausal women.

3.1 Introduction

HT and exercise are two interventions that are commonly prescribed independently to reduce risk factors for the development of osteoporosis, cardiovascular disease and abnormal glucose and insulin metabolism. The effects of HT containing oestrogens have been well established in clinical trials, as discussed in Chapter 1. Few studies, however, have investigated the clinical effects of walking independently of, or in combination with, HT on these risk factors after menopause.

Exercise is often reduced with increasing age and after menopause. Walking, however, is a moderate exercise that can be employed safely by individuals within their own time constraints without costly outlays, providing a suitable exercise for previously sedentary individuals. The effects of a walking based exercise program after menopause on glucose and insulin metabolism and arterial mechanical properties are yet to be determined. Limited data are also available presenting the effects of walking on bone metabolism. Two studies have provided conflicting evidence, one study found that walking was unable to counteract age and menopause related reductions in BMD (Cavanaugh 1998). In contrast, another study concluded that walking

maintained BMD (Humphries 2000). No studies, to the best of my knowledge, have investigated the effects of walking alone on bone resorption and formation markers after menopause.

The potential interactive effects of HT and walking on bone metabolism, arterial mechanical properties and glucose and insulin metabolism have also been insufficiently characterised. There are no clinical data, to the best of my knowledge, that identify the combined effects of walking and HT on bone formation and resorption markers or arterial mechanical properties after menopause. Only one study has investigated the combination of exercise and HT on glucose and insulin metabolism. This study, which used a cross sectional design, involved women exercising and using a variety of HT regimes at the outset of the study, as a result of which the effects of HT and exercise were impossible to determine.

The purpose of this study was to evaluate the effects of walking and HT independently, and combined, on bone turnover markers, cardiovascular risk factors such as lipoprotein levels, blood pressure and arterial mechanical properties, and glucose and insulin metabolism in postmenopausal sedentary women.

3.2 METHODS

3.2.1 Subjects

Twenty eight postmenopausal women were recruited from the general public. Six women withdrew from the study: 1 woman moved away during the course of the study; 2 women suffered skin irritation caused from the transdermal oestrogen; 2 women withdrew due to lack of time available to continue involvement and woman was excluded prior to commencement due to excessive alcohol intake. Twenty two women completed the study. The criteria for inclusion were: between 1 and 5 years after menopause; FSH $\geq$ 30 pmol/L; age 45 to 60 years. The criteria for exclusion were: using any form of HT; previous hysterectomy or oophorectomy; family history of malignancies; established cardiovascular disease; involvement in any form of structured exercise; taking hypolipidemic or anti-hypertensive medication; smoking; taking any drug therapy that would affect gain or loss of bone mineral. All women were cleared for participation following a full physical examination by a clinician. All gave informed consent to participate in the study, which was approved by the Victoria University Human Research Ethics Committee (Appendix 1A).

The study followed a randomised double blind design. Women were randomly assigned into two groups: active treatment and placebo. Ten women assigned to the 'HT group' used transdermal oestradiol patches (Estraderm 50ug) changed twice weekly and oral MPA (5mg /day) (Pharmacia Pharmaceuticals, Rydalmere, NSW, Australia). The route of administration is an

important variable which may impact substantially on the effectiveness of an HT regime. As discussed in Chapter 1.4.5.2, transdermal oestrogen has provided positive effects on glucose and insulin metabolism and, therefore, was chosen as the route of choice for this study. Twelve women assigned to the 'placebo' group used placebo transdermal patches and oral placebo tablets.

3.2.2 Protocol

Subjects were tested three times over the duration of the 20-week study. Measurements were taken at baseline (T1), prior to commencement of exercise training at eight weeks (T2) and on completion of the study at twenty weeks (T3). Height and weight were determined for all subjects. At each time point aerobic capacity, SAC and blood pressures were recorded. Measurements also included oestradiol, LH and FSH, fasting glucose and insulin, total cholesterol, HLD, LDL and the bone formation markers BAP and OC. Urine samples were taken two hours post first morning void at the above intervals for measurement of the bone resorption markers PYR and DPD.

Subjects continued their specified treatment regimen throughout the twenty week period. After eight weeks subjects began an exercise training program. This program was individually based on each participant's baseline fitness level. From baseline aerobic capacity measures, volume of expired carbon dioxide (mL/kg/min) was plotted against the volume of expired oxygen (mL/kg/min). A parallel graph, plotting ventilation (L/min) against oxygen consumption (mL/kg/min) was constructed for the duration of the test. A line of best fit was determined for the initial linear rise of these graphs, and the point at which the corresponding data strayed from

this line was determined (example in Appendix 2). It was at this point that anaerobic threshold was reached and the corresponding heart rate for this stage was noted. Individuals underwent a 12 week walking program based on percentage of this anaerobic threshold, measured by participants by monitoring their heart rate (Appendix 3). This exercise-training program commenced at 70% of baseline anaerobic threshold and increased by 5% every second week to 95% of anaerobic threshold over the following twelve week period. The participants achieved the increments in heart rate by increasing their speed of walking, or walking on an incline. The duration of exercise at the specified heart rate commenced at 10 minutes and increased by 5 minutes every second week. A 5 minute warm up and cool down period preceded and followed each walking session. The frequency of exercise sessions performed commenced at 3 per week, and increased by 1 session per week after each 4 week period. All subjects participating in this study were asked to complete a two day diet recall (Appendix 4), and not change their general diet throughout their involvement it.

3.2.3 Assays

Biochemical assay techniques for oestradiol, FSH, LH, DPD, PYR, OC, BAP, insulin, glucose and total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides are described in Chapter 2.

3.2.4 Aerobic capacity and systemic arterial compliance

Techniques used to measure aerobic capacity, blood pressures and SAC are described in Chapter 2.7.1, 2.4.1 and 2.5.1 respectively.

3.2.5 Statistical analysis

Data were analysed using SPSS (10.0). Data are reported as mean $\pm$ standard error of the mean. Statistical significance was assesses using General Linear Model, repeated measures, using a repeated contrast to determine significance. Significance between groups was determined using a Student's unpaired t-test. Significance was reported at $p < 0.05$. Power calculations were based on an 80% power with an alpha level of 0.05. The size of change to be detected and the expected standard deviation of change were determined from previous studies within this laboratory (Nestel 1999, Williams 2001).

3.3 RESULTS

There were no significant differences in age (51.1 ± 2.1 vs. 51.6 ± 1.7 years) and time postmenopause (2.3 ± 1.1 vs. 1.6± 0.2 years) between groups taking HT and those taking placebo. Similarly, there were no significant differences in weight or body mass index (BMI) between or within groups for the duration of the study (Table 3.1).

The exercise program designed for these women was mild and graduated to ensure that previously sedentary women would not encounter injury throughout the training program; all women remained injury-free for the duration of their involvement. Walking was chosen as the mode of exercise due to its weight-bearing nature and ease of administration. It was also perceived that previously sedentary women would be more compliant with this mode of exercise, which was verified by subjects' diarising involvement in walking sessions. The moderate nature of the exercise program did not increase the aerobic capacity of the subjects, nor alter weight or BMI.

There were no significant changes to aerobic capacity as a result of exercise training in either treatment groups (Table 3.1).

Circulating oestradiol levels were significantly higher in the HT group in comparison with the placebo group at T2 (166.3 ± 36.8 vs 89.2 ± 17.1, pmol/L, p=0.014) and T3 (164.9 ± 29.9 vs 73.4 ± 18.3, pmol/L, p=0.001). The range of baseline oestrogen levels were large as indicated by

high SEM values. This large range in circulating oestrogen levels may have been due to the greater adiposity of these subjects, as indicated by weight and BMI values. Levels of FSH and LH were significantly reduced at T2 (77.1 ± 14.8 to 39.8 ± 15.8, pmol/L, p=0.001, 44.7 ± 8.9 to 18.4 ± 6.8, pmol/L, p=0.002 respectively) and T3 (77.1 ± 14.8 to 37.1 ± 14.6, pmol/L, p=0.001, 44.7 ± 8.9 to 19.6 ± 8.1, pmol/L, p=0.007 respectively) compared with baseline levels in the HT group (Table 3.1). The increased oestradiol and simultaneous significant reduction in plasma FSH and LH indicated that subjects were compliant with HT.

There were no significant differences in cardiovascular risk factors such as SAC, blood pressures, total cholesterol, HDL or LDL at baseline, or as a result of treatments or exercise (Table 3.2). Fasting glucose and insulin were also unchanged as a result of treatment or exercise in both groups (Table 3.2).

Bone resorption markers (DPD and PYR) were both significantly reduced from baseline levels with HT administration (13.7 ± 3.2 to 9.9 ± 2.4, nmol/mmol Cr, p=0.009, 75.5 ± 15.6 to 58.6 ±12.0, nmol/mmol Cr, p=0.04 respectively) (Table 3.3, Figures 3.1 and 3.2). Bone resorption markers were unchanged as a result of exercise. Although slightly reduced, neither BAP nor OC showed significant reductions as a result of HT, exercise or both combined, however, BAP was significantly lower in the HT group after 12 weeks of walking in comparison to the placebo group after walking (9.5 ± 4.1 vs 16.8 ± 3.1U/L, p=0.03). All subjects completed the two intervention phases of the study.

Table 3.1. Group characteristics of women as a function of treatment and walking

	Placebo	n=12		Hormone	Therapy	n=12
	Baseline	Placebo	Placebo and walking	Baseline	HT	HT and walking
	T1	T2	T3	T1	T2	T3
Weight, kg	69.4±4.7	69.7±4.9	69.3±4.8	71.8±7.8	72.1±7.6	72.5±7.5
BMI, kg/m^2	26.8±2.2	26.9±2.3	26.7±2.2	27.5±3.3	27.6±3.2	27.8±3.2
Oestradiol, pmol/L	77.6±12.0	89.2±17.1	73.4±18.3	100.0±21.5	166.3±36.8$^+$	164.9±29.9$^+$
FSH, m/u/mL	57.9±9.7	51.1±9.4	56.1±13.5	77.1±14.8	39.8±15.8*	37.1±14.6*
LH, m/u/mL	45.8±11.8	33.7±7.3	36.2±7.2	44.7±8.9	18.4±6.8*	19.6±8.1*
AC, mL/kg/min	20.6±1.9	21.2±1.7	21.6±1.7	21.4±2.6	22.3±2.7	22.9±1.9

BMI indicates body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; AC, aerobic capacity.* ($p < 0.05$) significantly different from T1 in HT group.$^+$($p<0.05$) significantly different from placebo group at similar time point.

Table 3.2. Cardiovascular and glucose and insulin metabolism parameters of women as a function of treatment and walking

	Placebo	n=12		Hormone	Therapy	n=12
	Baseline	Placebo	Placebo and walking	Baseline	HT	HT and walking
	T1	T2	T3	T1	T2	T3
TC, mmol/L	5.35±0.64	5.78±0.48	6.05±0.61	4.81±0.51	5.01±0.88	5.41±0.72
LDL, mmol/L	3.73±0.58	3.84±0.49	3.91±0.52	3.07±0.46	3.59±0.73	3.48±0.56
HDL, mmol/L	1.27±0.25	1.51±0.26	1.54±0.24	1.21±0.14	1.43±0.21	1.32±0.22
SBP, mmHg	112±6	109±7	106±4	121±7	118±5	115+6
DBP,mmHg	68±4	64±5	68±4	69±4	68±5	69±7
SAC, ACU	0.50±0.08	0.57±0.10	0.52±0.11	0.44±0.09	0.38±0.09	0.44±0.10
Glucose, mmol/L	4.86±0.13	4.8±0.57	4.92±0.14	4.71±0.14	4.49±0.15	4.64±0.14
Insulin, mU/L	6.89±0.84	10.10±1.12	9.59±1.3	7.77±1.69	8.16±0.95	7.76±1.76

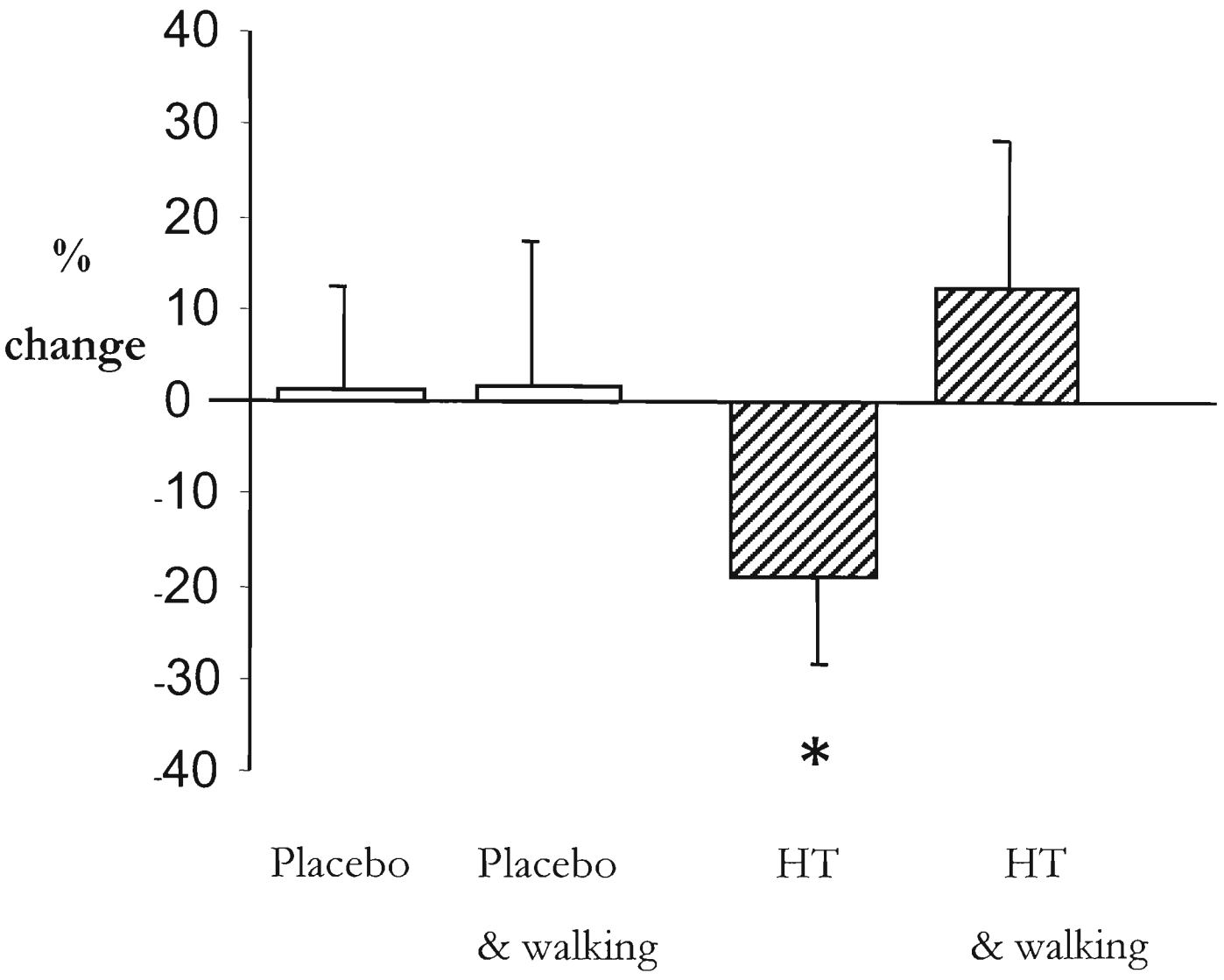
TC indicates total cholesterol; HDL, high densitylipoproteins; LDL, low density lipoproteins; SBP, systolic blood pressure; DBP, diastolic blood pressure; SAC, sytemic arterial compliance.

Table 3.3. Bone parameters of women as a function of treatment and walking

	Placebo	**n=12**		**Hormone**	**Therapy**	**n=12**
	Baseline	Placebo	Placebo and walking	Baseline	HT	HT and walking
	T1	**T2**	**T3**	**T1**	**T2**	**T3**
OC, ug/l	17.3±2.6	17.9±2.7	17.8±2.4	15.7±3.0	15.2±2.9	14.3±2.8
BAP, U/L	13.6±2.8	16.8±2.9	16.8±3.1	11.1±3.8	10.6±4.0	9.5±4.1^{+}
PYR, nmmol/mmol	81.0±16.9	79.6±15.9	78.1±13.6	75.5±15.6	58.6±12.0*	64.9±14.0
DPD, nmmol/mmol	15.6±3.1	16.0±3.3	14.1±3.0	13.7±3.2	9.9±2.4*	9.5±2.7

OC indicates osteocalcin; BAP, bone alkaline phosphatase; PYR, pyridinoline; DPD, deoxypyridinoline.* ($p < 0.05$) significantly different from T1 in HT group.$^{+}$($p<0.05$) significantly different from placebo group at similar time point.

Figure 3.1.


40
30
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*
Placebo
Placebo
& walking
HT
HT
& walking

Figure 3.1.

Urine Pyridinoline concentration- percent change- in placebo (n=12) and HT (n=10) groups with hormone or placebo treatment alone and with horomone or placebo treatment and walking. (* significantly different from baseline levels ($p<0.05$)

Figure 3.2.

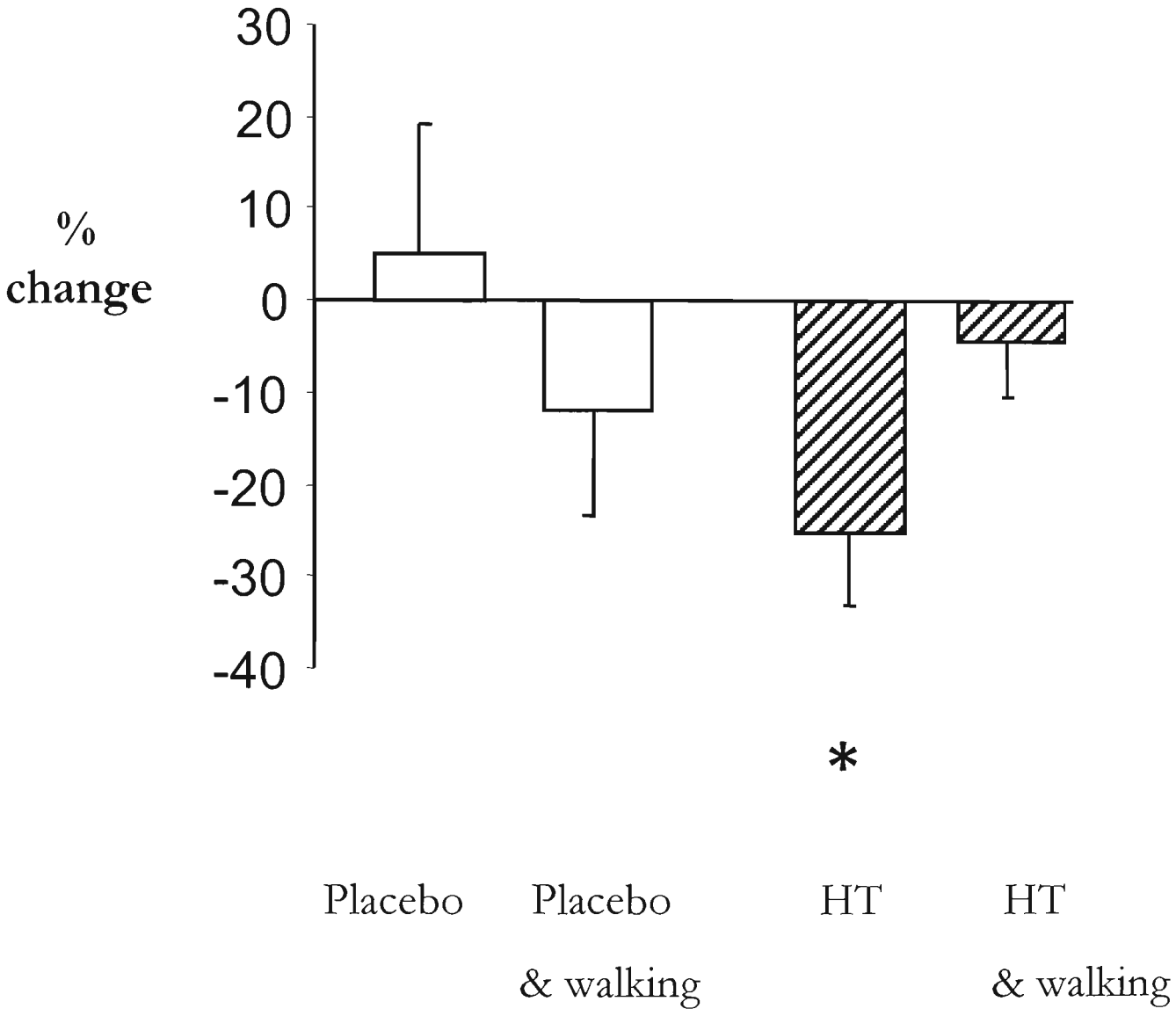

30
20
10
0
-10
-20
-30
-40
% change
*
Placebo
Placebo & walking
HT
HT & walking

Figure 3.2.

Urine Deoxypyridinoline concentration percentage change in placebo (n=12) and HT (n=10) groups with treatment alone and with treatment and walking. (* significantly different from baseline levels ($p<0.05$)

3.4 DISCUSSION

The main findings of this study were that HT significantly reduced bone resorption, whilst the addition of a walking program provided no further benefit to bone. HT and walking, independently and in combination, had no significant effects on cardiovascular risk factors or glucose and insulin metabolism in sedentary postmenopausal women.

Effects of HT on cardiovascular risk factors

The lack of change in total cholesterol, HDL and LDL levels as a result of HT in the current study is inconsistent with previous cross-sectional and randomised studies that have demonstrated significant alterations (Crook 1992, Nabulsi 1993, Lindheim 1994, 1995, Heikkinen 1997). The types of oestrogens used by women in these studies varied considerably, and included conjugated equine oestrogens, 17 beta oestradiol and oestradiol valerate, in both opposed and unopposed regimens and oral and transdermal regimes. In contrast, our study administered a homogenous regimen of HT, consisting of transdermal oestradiol and MPA. The magnitudes of changes in these previous studies were variable and may be dependent on the type and route of hormones used. The small sample size of this study may also be a factor to consider when assessing why changes in lipid levels were not seen.

There are a number of cross-sectional studies that show HT use is associated with higher SAC (Rajkumar 1997, Waddell 1999). The results of the current study, however, showed no change in these parameters with HT use. Age (Hirai 1989, Dart 1991, Gatzka 1998) and menopause (Waddell in press) are associated with increased arterial stiffening; therefore, it is likely that older women would have greater arterial stiffening than younger women who are fewer years past menopause. Previous studies involved women 5 to 8 years older, with lower circulating oestradiol and higher FSH levels and lower SAC measures in comparison to subjects within our study. It may be speculated that HT administration to older women with lower SAC may influence a greater vascular response in contrast to younger women with higher SAC and circulating oestradiol and FSH levels to begin with. In the previously mentioned studies, women were using a heterogenous range of HT at the initiation of the trial, whilst this study commenced women on a homogenous regime of opposed HT. This may be another factor contributing to the differences in the results between studies.

On the other hand, the lack of effect of HT on the cardiovascular risk markers measured may also be representative of a statistical Type II error; whereby, an alpha level of greater than 0.05 may have identified a significant association of treatment and cardiovascular risk parameters.

Effects of HT on glucose and insulin metabolism

A number of randomised clinical studies have demonstrated reduced plasma insulin levels and an improvement in insulin sensitivity with oestrogen administration (Bailey 1980, Lindheim 1994) while a prospective study that administered predominantly (66%) opposed heterogeneous HT and oral oestrogens (34%) has been associated with worsened insulin sensitivity (Brown 2000). Although there appears to be conflicting evidence available, the differences in study designs may contribute to the different outcomes of these studies. The results from the current study demonstrated no change in fasting glucose and insulin levels with opposed homogeneous HT; this may reflect altered glucose and insulin responses with varying dosages and types of hormones used.

Effects of HT on bone turnover markers

Alterations in levels of bone turnover markers precede changes in bone mass and density. The bone loss experienced in the initial six years after menopause is estimated to be approximately 15% (Mazzuoli 2000), imposing a considerable risk for the development of osteoporosis. Sex hormones play a regulatory role in bone metabolism (Jilka 1992, Manolagas 1995, Neer 1995). Our results demonstrated that bone resorption indices (PYR and DPD) were significantly reduced after eight weeks of HT (Figures 3.1 and 3.2). Bone formation markers (BAP and OC) remained unchanged as a result of HT or placebo after eight weeks. An effect of HT on bone

formation markers may have been masked by the addition of exercise after the eighth week, as urine analysed bone resorption markers respond earlier to treatment than serum analysed formation markers - 4 weeks vs. 12 weeks respectively (Kleerekoper 1998). These results support a previous study (Prior 1997) that demonstrated that oestrogens significantly suppressed bone resorption in ovariectomised women after one year of treatment, whilst bone formation remained unchanged.

Effects of walking on cardiovascular risk factors

Elevated blood pressure can indicate abnormal structure and function of the vasculature and contribute to the risk of CVD by accelerating age associated arterial degeneration (Wolinsky 1972, Avolio 1985). Many studies have demonstrated a reduction in blood pressure in a variety of population groups with walking (Kingwell 1993), and by employing a wide range of moderate intensity and duration exercise regimens (Hagberg 1983, Seals 1985, Jennings 1986, Nelson 1986, Meredith 1990). The walking regimen employed in this study had no significant effect on blood pressure. There were small decrements in SBP with walking in both HT and placebo groups, however, the SEM values for blood pressure within this study were comparatively large, indicating a greater number of subjects would be required for these results to reach significance. It is important to note that there were no significant changes in body weight with the exercise regime employed, which may be associate with the lack of change in measureds of blood pressure. It is possible that this intensity of exercise was insufficient to elicit blood pressure changes in postmenopausal women.

Previous studies have reported greater blood pressure reductions in response to exercise in hypertensive individuals as opposed to normotensive individuals (Hagberg 1983, Nelson 1986). The subjects within the current study were all normotensive, which may have influenced the response to exercise.

Training adaptations to vascular tone and arterial compliance are apparent with long term training (Kingwell 1995), after 4 weeks of moderate training (Cameron 1994) and after a single acute bout of moderate exercise (Kingwell 1997). Few data are available, however, regarding the effect of walking in previously sedentary individuals. The results of the current study indicate that walking may be insufficient in intensity to elicit vascular adaptations that are indicated by changes in SAC.

Effects of walking on glucose and insulin metabolism

The walking program in the current study had no effect on two predictors of insulin sensitivity, namely aerobic capacity and BMI; therefore, it is not surprising that fasting glucose and insulin levels were unchanged after twelve weeks of walking. There are many direct and indirect actions of exercise on glucose and insulin metabolism, as discussed in Chapter 1. However, an important note to make is that there may be a threshold for exercise, below which a metabolic effect does not occur. This hypothesis is supported by the results of a previous study that identified sedentary

and moderately physically active postmenopausal women had insulin sensitivity measures comparable to lean individuals with glucose intolerance, whilst athletically trained postmenopausal women had insulin sensitivity measures comparable to young lean healthy women (Brown 2000). Therefore a greater intensity or duration of exercise may be required to elicit changes to glucose and insulin metabolism.

Effects of walking on bone turnover markers

Weight-bearing exercise provides a stimulus of mechanical loading on bone cells, which induces bone formation (Evans 1996, Turner 1996). In our study moderate weight-bearing exercise alone in the form of walking (refer to Appendix 3) did not alter any bone formation or resorption indices. Although our preliminary power estimates indicated a sample size of 12 in each group would be sufficient, a retrospective analysis of the statistical power indicated that our study did not reach adequate power at its completion (BAP=0.05, OC=0.05, DPD=0.09, PYR=0.054). In order to reach an 80% power with the current exercise changes in turnover markers, a large increase in sample size (up to 2000 subjects) would have been required, indicating this level of exercise may be insufficient in these individuals to cause changes in bone turnover that would provide any significant benefit. In light of these preliminary findings further work is warranted to resolve the effects of exercise regimens on bone turnover markers.

Type and intensity of exercise

The intensity, frequency and duration of exercise employed are important parameters when considering an exercise training regimen. The most appropriate dimensions of exercise to employ in a postmenopausal population in order to gain cardioprotection have not been comprehensively addressed in the current literature available. Further research is therefore required that looks at surrogate markers of cardiovascular risks such as vascular stiffness.

Bone remodelling responses to exercise have been related in a dose dependent manner to the mechanical load placed on the bone (Chamay 1972). Exceptionally, exercises at intensities high enough to cause exercise-induced amenorrhoea in female athletes, are unable to counteract oestrogen deficient bone loss (Rencken 1996). Osteopenia develops despite intense exercise. Therefore, a strain threshold may exist for the stimulation of osteogenesis, as a result a moderate application of strain or load may be inadequate to reach the strain threshold, indicating that the magnitude of the strain may be more important than strain frequency. Brisk walking alone may provide an insufficient mechanical strain to overcome oestrogen deficiency after menopause. This may explain why in our study there were no changes in bone turnover markers in response to a walking based exercise training regimen with either HT or placebo treatment.

There have been numerous studies that have assessed bone mineral changes as a result of exercise in postmenopausal populations. The results of these studies, however, are conflicting.

Aerobic exercises, involving brisk walking and lifetime volleyball involvement, were unable to counteract an age and/or menopause related decline in BMD (Cavanaugh 1998, Humphries 2000, Ito 2001). Conversely, BMD was increased with exercise that involved vigorous walking, jogging, stair climbing, and high impact aerobics primarily at femoral neck (Kohrt 1995, Welsh 1996, Coupland 1999), with total body BMD also being augmented (Chow 1987, Coupland 1999) in a number of these studies. In contrast, long-term vertical jumping exercises using a weighted vest prevented hipbone loss over five years (Snow 2000). Resistance and strength training performed for at least one year increased regional BMD at sites of intertrochanter and hip (Kerr 2001), and total body (Chow 1987, Kerr 2001). Similar programs that were performed for one year or less observed no change in total bone mineral content (BMC) (Nelson 1994), BMD at lumbar spine (Pruitt 1995, Ryan 1998, Humphries 2000) or total hip BMD (Pruitt 1995). It should be noted, however, that differences in exercise type, intensity and frequency, and subject characteristics such as age, baseline BMD, and years since menopause often make it difficult to compare studies directly. It should also be noted that studies assessing BMD changes in under 12 months may be of insufficient duration to identify an effect of exercise training, given the error levels of the DEXA method.

The age range of the current study was small, with all women 51 to 52 years of age and 1.6 to 2.3 years after menopause. In previous exercise related studies, there have been large ranges for age and the number of years after menopause, with many involving women up to and over 10 years older than the women in the current study. With increasing age and years postmenopause there is an accretion in the amount of bone lost; therefore, if women are commencing exercise at lower bone densities there may be a greater effect of treatment. The earlier intervention of

exercise could partly explain the lack of exercise effect in the current study, as women were commencing exercise prior to having lost substantial BMD due to oestrogen deficiency.

In order for postmenopausal women to attenuate bone loss exercise involving a significant magnitude of mechanical strain may be required, with a greater effect occurring in women with lower BMD. Long term incorporation of this type of exercise may increase BMD after menopause.

The effects of combined HT and walking

Cardiovascular risk factors and glucose and insulin metabolism

The combined effect of HT and walking provided no changes to measures of body weight, cardiovascular risk, such as SAC, BP or lipoprotein levels, or glucose and insulin metabolism. One randomised clinical trial that investigated the effects of exercise on lipoprotein levels in postmenopausal women found no significant effect of exercise training regardless of whether women were using, or not using, HT (Klebanoff 1998). This study indicated that body weight was an important modulating factor, and suggested that heavier postmenopausal women did not respond as favourably to aerobic conditioning as postmenopausal women with lower body mass regardless of the presence of exogenous oestrogens. The oestrogen used within the above mentioned study, however, was an oral preparation, which may affect lipoprotein levels differently as opposed to a transdermal preparation.

Bone turnover markers

The addition of a brisk walking program to HT in this population of postmenopausal women provided no significant change to either resorption (figures 1a. and 1b.) or formation indices, indicating that HT was the primary stimulus for changes in bone turnover. Previous studies focusing on combined exercise and HT have shown conflicting results on measures of BMD. Prospective studies (Prince 1991, Heikkinen 1997) have demonstrated aerobic exercise in combination with HT, but not exercise alone, significantly increased BMD. Hence any benefit received by combined treatment appeared to be attributable to HT. On the other hand, HT and nine months of vigorous weight-bearing exercise were independently beneficial in increasing BMD, whilst combined treatment provided an additive benefit at sites of lumbar spine and Wards triangle (Kohrt 1995). HT and weight lifting exercises were also shown to significantly increase BMD of the total body in women who had undergone hysterectomy, but exercise was not assessed independently of HT (Notelovitz 1991). There have apparently been no studies that measure bone turnover markers as a result of these combined treatments.

Our study demonstrated no cardiovascular or glucose and insulin metabolism effect in the early years after menopause (1-5 years) with HT, or walking, independently. Similarly, both treatments combined do not alter measures of SAC, lipoproteins, fasting glucose or insulin. Our study also demonstrated that walking alone, at the intensities and duration prescribed, was an insufficient stimulus to reduce bone turnover in early postmenopausal women; whilst HT was an

effective treatment intervention to reduce bone turnover after menopause. The combination of HT and walking provided no added benefit in comparison to HT alone.

4.0 The effects of hormonal therapy and exercise on glucose and insulin metabolism, cardiovascular risk factors and bone turnover markers in Masters trained postmenopausal women

4.1 Introduction

Moderate to high levels of physical activity have been consistently associated with; improved glucose tolerance (if initially impaired) and insulin sensitivity; lower fasting and glucose stimulated plasma insulin levels (Seals 1984, Seals 1984, Kirwan 1993, Hersey 1994); improved lipoprotein profiles (Seals 1984, Seals 1984, Katzel 1995) and enhanced arterial mechanical properties (Ogawa 1992, Hagberg 1993, Vaitkevicius 1993, Cameron 1994). Cross sectional studies have demonstrated that a more active lifestyle is associated with higher BMD (Zhang 1992, Greendale 1995), whilst epidemiological studies have reported a significant negative correlation between physical activity and osteoporotic fracture risk (Cooper 1988, Paganini-Hill 1991). Therefore, moderate to high intensity physical activity provides a non-pharmacological intervention for protection against the increased risks of developing impaired glucose tolerance and CVD, whilst long term involvement in physical activity appears to reduce the risk of osteoporosis after menopause.

The cardioprotective effects of oestrogens on circulating lipoprotein levels, endothelial function and the structure and function of arteries are well established (Farhat 1996, Mendelsohn 1999). Oestrogens' beneficial effects on insulin and glucose metabolism (Silfverstolpe 1980, Notelovitz 1987, Cagnacci 1992, Lindheim 1994) and bone mass (Ettinger 1987, Lindsay 1990, Prince 1991) are also well founded.

Despite the positive independent associations of moderate to high intensity exercise and HT on cardiovascular risk factors, glucose and insulin metabolism and bone turnover, there are only a limited number of studies that have investigated the effects of combined treatments on these parameters in a postmenopausal population. It would be of interest to determine whether HT can provide further cardiovascular, metabolic and bone protection to athletic women after menopause, without negatively effecting aerobic capacity.

The aims of this study were to determine differences in cardiovascular risk factors, glucose and insulin metabolism and bone turnover after menopause in athletically trained women, compared to postmenopausal women with a sedentary lifestyle. It was also the aim of this study to determine the effect of HT administration in athletic postmenopausal women on the above mentioned parameters.

4.2 METHODS

4.2.1 Subjects

Twenty five sedentary postmenopausal women were recruited from the general public. The criteria for inclusion in the study were women 1-5 years after menopause, 45 to 60 years of age. The criteria for exclusion were: taking any form of hormonal therapy; previous hysterectomy or oophorectomy; family history of malignancies; established CVD; involved in any form of structured exercise; taking hypolipidaemic or anti-hypertensive medication; smoking; taking any drugs that would affect blood pressure or the gain, or loss, of bone mineral. One woman was excluded prior to commencement due to excessive alcohol intake. Twenty- four women completed baseline measurements.

Seventeen athletic postmenopausal women were recruited from athletic groups and 'Masters' athletic associations. Two women withdrew from the study: 1 woman moved away during the course of the study; 1 woman suffered skin irritation caused from the transdermal oestrogen. Sixteen women were measured at baseline and fifteen women completed the study. The criteria for inclusion were 1-5 years after menopause; 45 to 60 years of age; competing at 'Masters' level in athletics, or vigorously exercising at least 4 times a week with a VO_2 peak of greater than 30 $ml.min.kg^{-1}$. The criteria for exclusion were: taking any form of hormonal therapy; have had a hysterectomy; a family history of malignancies; established CVD; taking hypolipidaemic or anti-hypertensive medication; smoking; taking any drugs that would affect the

gain/loss of bone mineral. All women were cleared for participation following a full physical examination by a clinician. All women gave informed consent to participate in this study, which was approved by Victoria University Human Ethics Committee (refer to Appendix 1b for Informed Consent form).

Using a double blind randomised design; the athletic women were assigned into two groups. Eight women were assigned into the 'HT group', and commenced using transdermal oestradiol patches (Estraderm 50ug, Novartis, Basel, Switzerland) changed twice weekly, and oral MPA (5mg /day) (Pharmacia Pharmaceuticals, Rydalmere, NSW, Australia). Seven women were assigned to the 'placebo' group and commenced using placebo transdermal patches and oral placebo tablets.

4.2.2 Protocol

All women were measured at baseline (T1); the athletic women were also measured at the completion of the study after twenty weeks (T2). Height and weight were determined for all subjects. At both time points, aerobic capacity was measured, morning fasting blood samples were taken for analysis of oestradiol, LH and FSH, lipids, glucose and insulin and bone formation marker OC. BAP was not measured in this study due to lack of funding available for analysis. Urine samples were taken two hours post first morning void at both time points for analysis of bone resorption markers- PYR and DPD.

All athletic subjects participating in this study were asked to maintain their current training regimen. A two day diet recall (refer to Appendix 4) was documented by women prior to commencement within this study and women were asked to maintain their current diet throughout their involvement within the study.

4.2.3 Assays and measurements

Methods used for the analysis of oestradiol, FSH, LH, glucose and insulin, aerobic capacity, cholesterol and lipids, SAC, blood pressures, OC, DPD and PYR, BMI are discussed in Chapter 2. Methods.

4.2.4 Statistical analysis

Data were analysed using Sigma Stat.(Jandell). Data reported are mean $\pm$ standard error of the mean (SEM). An unpaired t-test was used to determine significance between groups at baseline. A Student's paired T-test was used to determine significance within the athletic groups. Significance was reported at $p < 0.05$. Power calculations were based on an 80% power with an alpha level of

0.05. The size of change to be detected and the expected standard deviation of change were determined from previous studies within this laboratory (Nestel 1999, Williams 2001).

Athletic vs. sedentary

At baseline, the athletic group had an aerobic capacity 72% higher (p=0.001) and BMI 18.8% lower (p=0.001) than their sedentary counterparts (Table 4.1). There were no differences between groups for age and years postmenopause. The athletic women had lower systolic blood pressure (12.3%, p=0.002), diastolic blood pressure (8.1%, p=0.04) and mean arterial pressure (8.1%, p=0.03) (Table 4.1). The athletic group tended to have higher circulating HDL levels (9%), however, this did not reach significance. There were no differences in SAC and circulating cholesterol or lipids between groups. Bone formation was significantly higher in the athletic group (54.2%, p=0.02), whilst there were no differences in resorption markers (Table 4.1).

The effect of HT in athletic women

There were no differences between the two athletic groups at baseline for age, BMI, serum sex hormones, fasting glucose or insulin, cardiovascular risk parameters or bone formation and DPD (Table 4.2). However, PYR was higher in the HT group in comparison to the placebo group at baseline. The athletic women taking HT increased circulating levels of oestradiol, whilst FSH levels were reduced indicating subjects were compliant with HT (Table 4.2).

Aerobic capacity

Measures of VO_2 peak were unchanged as a result of either HT or placebo treatment, indicating that subjects maintained their existing exercise training regimens throughout the duration of the trial (Table 4.2).

Glucose and insulin levels

Fasting glucose and insulin levels were unchanged as a result of HT and placebo (Table 4.2).

Cardiovascular risk parameters

Total cholesterol, LDL, HDL, triglycerides, SAC and blood pressures (SBP andDBP) were unchanged as a result of HT or placebo (Table 4.2).

Bone turnover markers

Resorption indices were significantly reduced; PYR decreased 29.4% ($p=0.006$), whilst DPD decreased 36.2% ($p=0.01$) after 20 weeks of HT (Figure 4.1). OC remained unchanged as a result of HT (Figure 4.2). There were no changes in bone turnover markers after 20 weeks of placebo.

Table 4.1. Characteristic of Sedentary and Athletic Groups

	Sedentary n=24	Athletic n=16	p– between groups
BMI, kg/m^2	28.15±3.06	22.86±1.20	0.001
Oestradiol, pmol/L	94.48±21.44	70.88±20.54	0.094
FSH, m/u/mL	68.43±12.94	71.62±13.69	0.395
AC, mL/kg/min	20.70±1.80	35.50±2.40	0.001
TC, mmol/L	5.61±0.57	5.64±0.51	0.730
LDL, mmol/L	3.53±0.53	3.49±0.45	0.601
HDL, mmol/L	1.56±0.24	1.7±0.20	0.346
SBP, mmHg	117±7	103±6	0.002
DBP, mmHg	69±4	63±4	0.044
MAP, mmHg	85±5	78±5	0.033
SAC, ACU	0.46±0.09	0.53±0.01	0.292
Glucose, mmol/L	5.04±0.35	5.46±0.41	0.081
Insulin, mU/L	6.34±1.09	5.47±0.41	0.170
OC, ug/L	18.08±4.75	27.88±7.38	0.016
PYR, nmol/mmol	75.13±15.13	75.30±10.30	0.764
DPD, nmol/mmol	13.76±2.88	16.32±	0.195

All values are mean±SEM. BMI indicates body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; AC, aerobic capacity; TC, total cholesterol; HDL, high density lipoproteins; LDL, low density lipoproteins; SBP, systolic blood pressure; DBP, diastolic blood pressure; SAC, sytemic arterial compliance; OC, osteocalcin; PYR, pyridinoline; DPD, deoxypyridinoline.

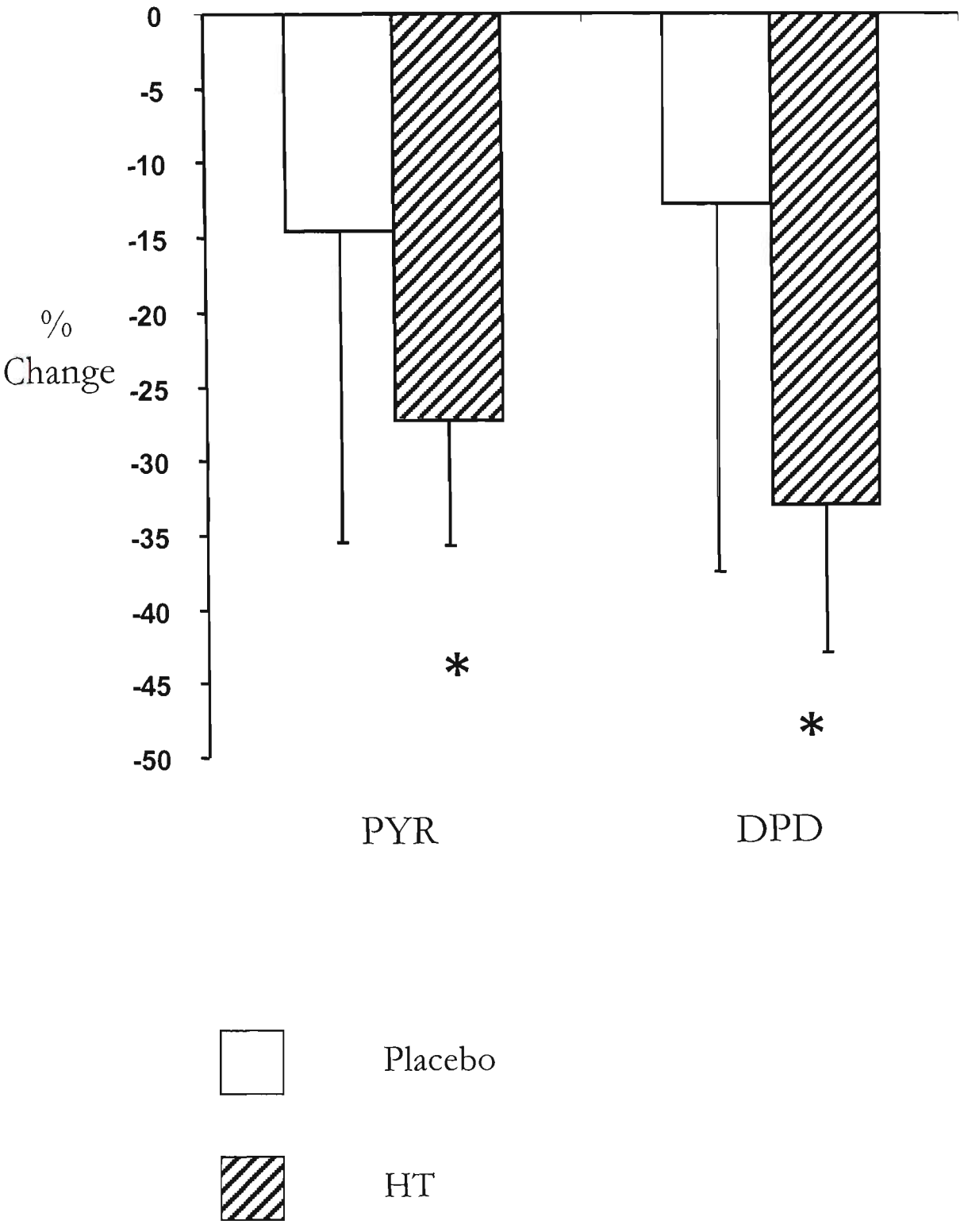
Table 4.2. Athletic womens's' characteristic at baseline and after treatment.

	Placebo		HT	
	Baseline	20 weeks	Baseline	20 weeks
BMI, kg/m^2	22.2±1.2		23.4±1.2	
Oestradiol, pmol/L	76.1±12.1	57.0±17.2	55.1±26.1	86.8±21.1
FSH, m/u/mL	70.8±13.5	72.6±13.9	73.3±13.7	48.6±10.5*
AC, mL/kg/min	35.6±3.0	34.6±3.6	35.2±2.0	37.0±2.0
TC, mmol/L	5.67±0.28	6.19±0.34	5.81±0.66	5.70±0.47
LDL, mmol/L	3.62±0.35	4.14±0.49	3.51±0.58	3.52±0.43
HDL, mmol/L	1.79±0.28	1.63±0.23	1.38±0.35	1.94±0.59
Trigs, mmol/L	0.87±0.16	0.86±0.08	0.96±0.14	0.73±0.07
SBP, mmHg	104±4	104±6	104±2	103±9
DBP, mmHg	64±2	63±3	63±5	60±5
MAP, mmHg	79±2	78±5	79±7	75±6
SAC, ACU	0.59±0.07	0.59±0.07	0.50±0.12	0.84±0.40
Glucose, mmol/L	5.7±0.4	6.0±0.2	5.3±0.4	5.6±0.5
Insulin, mU/L	5.9±1.3	6.9±1.9	8.4±3.3	8.9±3.3
PYR, nmol/mmol Cr	64.0±7.0	61.1±6.9	81.9±5.8	57.8±3.7*
DPD, nmol/mmol Cr	13.4±1.8	12.6±1.7	18.5±3.1	11.8±2.1*
OC, ug/L	32.5±9.9	24.7±5.5	31.2±10.2	32.3±11.8
AC, mL/kg/min	35.6±3.0	34.6±3.6	35.2±2.0	37.0±2.1

All values are mean±SEM. BMI indicates body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; AC, aerobic capacity; TC, total cholesterol; HDL, high densitylipoproteins; LDL, low density lipoproteins; SBP, systolic blood pressure; DBP, diastolic blood pressure; SAC, sytemic arterial compliance; OC, osteocalcin; BAP, bone

alkaline phosphatase; PYR, pyridinoline; DPD, deoxypyridinoline.* Significantly different ($p<0.05$) from baseline values.

Figure 4.1 Percent change in bone resorption indices from baseline.



4.4 DISCUSSION

The main findings of this research are that the administration of HT to athletic postmenopausal women reduces bone resorption markers without causing any adverse changes to cardiovascular risk factors or insulin and glucose metabolism. The results from this study also show that long-term involvement in high intensity physical activity is associated with significantly higher aerobic capacity, lower BMI, blood pressure, bone resorption indices and higher bone formation marker - OC, in comparison to sedentary postmenopausal women. There are no beneficial or deleterious associations with long-term high intensity exercise on lipid profiles, SAC or glucose and insulin metabolism.

Cardiovascular risk factors

The results of this study demonstrate no significant differences in lipid profiles between physically active and sedentary women. The results of cross sectional studies, however, may be influenced by a number of confounding variables, in this case these may include varying diets and genetic predispositions between the groups of women and the prospect of self selecting a more active lifestyle coinciding with favourable lipid profiles. The results of this study may also indicate that exercise has little effect on lipoprotein levels in postmenopausal women. Previous cross-sectional observational studies have demonstrated a positive dose response association between the volume

and intensity of physical activity and plasma HDL, and an inverse association with plasma triglycerides levels (Leon 1991, Durstine 1994, Williams 1996). There are also a number of studies that support the beneficial effects of exercise on lipoprotein levels (Baumstark 1993, Houmard 1994, King 1995, Motoyama 1995), however, these studies involved either men only (Baumstark 1993, Houmard 1994) or men and women (King 1995, Motoyama 1995).

The administration of HT to physically active women tended to increase circulating HDL levels (40%), however, this did not reach significance due to a small sample size. HT had no effect on total cholesterol, LDL or triglyceride levels when given to physically active women. Three prospective studies have examined the combined effects of exercise and oestrogens and HT providing conflicting results with respect to HDL, total cholesterol and triglyceride levels (Lindheim 1994, Binder 1996, Klebanoff 1998). These conflicting results may be influenced by differences in subject characteristics and the exercise parameters employed. These studies used a range of HT regimens including conjugated equine oestrogens and unopposed oestrogens that may also have influenced differences in lipoprotein changes. Other differences between previous studies and ours, that may be partially responsible for alternate lipoprotein results, are that other studies initiated exercise in previously sedentary women; whilst our study involved athletic women with a high training load. Further research is required to examine the effect of varying intensities, frequencies and types of exercises combined with HT and their influences on lipid profiles.

Modified arterial wall properties may influence determinants of circulatory function such as neural control mechanisms, coronary perfusion and cardiac work (Cox 1988). Increased

arterial stiffening with age (Hirai 1989, Dart 1991, Gatzka 1998) and menopause (Waddell in press) is caused by a combination of genetic, metabolic and hormonal alterations, reducing arterial compliance. Exercise can be seen to ameliorate age and menopause related arterial stiffening, with cross-sectional studies demonstrating endurance trained athletes have greater arterial compliance in comparison to age matched sedentary control subjects (Mohiaddin 1989, Cameron 1994, Kingwell 1995). Arterial stiffness has also been reported to be lower in older endurance trained individuals (Vaitkevicius 1993). Previous studies have primarily investigated the effect of initiating exercise on arterial mechanical properties in younger males (Cameron 1994, Kingwell 1995), or a male population of various ages (Vaitkevicius 1993). The results of this study, however, demonstrated no difference in SAC between athletic and sedentary women, which may be attributable to the limited power of the study, but may be indicate that after menopause exercise may be unable to mitigate the age and menopause associated arterial stiffening.

No study, to the best of my knowledge, has assessed the effects of combined treatment on blood pressure and SAC. Our results indicated athletic women given HT experienced a greater increase in SAC; however, this did not reach significance due to the small sample size. However, within these limitations of the current study HT appeared to have little effect. There were also trends for DBP and MAP to decrease with HT, however, samples sizes were small. Preliminary estimates indicated a sample size of 10 in each group would provide sufficient power to the study, however, a retrospective analysis indicated that our study did not reach statistical power at its completion (SAC = 0.176, SBP = 0.053, DBP = 0.108). Initial power calculations were based on an 30% change with 80% power, from previous studies within this laboratory. In order to reach an 80% power with the small current exercise and HT changes in arterial compliance, a large

increase in sample size (n=47 required for SAC, n=1768 required for SBP, n=99 for DBP) would be required. This power analysis indicates that the administration of HT to an athletic postmenopausal woman may have little individual benefit in improving SAC.

Fasting glucose and insulin levels

Aerobic capacity was shown to be an independent predictor of insulin sensitivity in a previous cross sectional study, which demonstrated athletic postmenopausal women had 43% greater insulin sensitivity compared to their sedentary counterparts (Brown 2000). Our results, however, showed no differences between sedentary and athletic postmenopausal women in fasting insulin and glucose levels. HT had no significant effects on plasma insulin and glucose levels in athletic women. One other study that has addressed the issue of combined HT and exercise in postmenopausal women identified insulin sensitivity and intravenous glucose tolerance were not different between athletic women using HT and those not using HT, whilst athletic women using HT had lower fasting plasma insulin levels (Brown 2000). The HT used by these women included opposed and unopposed oestrogens of various types. Differences in HT regimens may be a confounding factor responsible for the alternate outcomes of our study and that of the above mentioned study. These findings, and the results from our study indicate that the intense endurance-training regimen undertaken by athletic women may override the effects of HT on insulin sensitivity. On the other hand, an effect of HT may have been apparent in athletic women if their baseline glucose and insulin levels were high; however, the athletic women involved in our study had baseline fasting glucose and insulin levels within healthy ranges. This may be a possible

factor inhibiting further reductions. The results of this study and that of the previous study investigating HT and high intensity exercise are difficult to compare due to differences in study designs and HT regimens. Further randomised clinical trials are required to substantiate the combined effects of these treatments on glucose and insulin metabolism.

Insulin resistance is an important factor in the pathogenesis of impaired glucose tolerance and T2D. Therefore, interventions that improve the action of insulin would be beneficial in preventing or delaying the onset of these altered metabolic conditions.

Bone turnover

The results of our study demonstrate that athletic postmenopausal women have lower levels of bone resorption markers and higher bone formation marker -OC in comparison to sedentary postmenopausal women. Moreover, HT can further reduce bone resorption indices in athletic women. Markers of bone turnover can predict bone density and rates of bone loss in postmenopausal women (Kelly 1990, Mazess 1991, Seibel 1993, Henderson 1995).

There are a number of prospective studies that have yielded conflicting results when assessing the effect of exercise and HT combined on bone mineral density (BMD) in comparison to the independent effects of HT (Heikkinen 1991, Notelovitz 1991, Kohrt 1995) or exercise

(Heikkinen 1991, Prince 1991, Kohrt 1995). These studies are difficult to compare due to differences in subject characteristics – such as years postmenopause, age, and varied types, intensities and duration of exercises employed. One study demonstrated an additive effect of exercise and HT on BMD in older women, and proposed that exercise and oestrogen were acting by different mechanisms causing the additive effect of the combined treatments (Kohrt 1995). This interpretation, however, was speculative and requires further research to substantiate it.

In the initial 1-5 years after menopause, there are no differences in cardiovascular risk factors between sedentary women and women participating in high levels of physical activity. In the current study, the addition of HT to an active lifestyle did not appear to alter these risk factors. It is important to note, however, that with increasing age and an increase in years postmenopause, substantial differences in cardiovascular risk factors may become apparent.

There remains insufficient information regarding the effect of HT and various levels of exercise independently and combined on glucose and insulin metabolism. A dose response continuum would be beneficial, however, the effects of treatment may only be apparent if glucose and insulin metabolism are initially impaired.

This is the first study to our knowledge to assess bone turnover markers in response HT in an athletic postmenopausal population, indicating that HT can provide further benefit to bone turnover markers in comparison to exercise alone.

Ultimately the administration of HT to women participating in high levels of physical activity does not impair performance, measured by aerobic capacity, or detrimentally effect cardiovascular risk factors or glucose and insulin metabolism; however, an improvement in bone metabolism may be gained.

5.0 Progesterone effect on cardiovascular risk factors in postmenopausal women

5.1 Introduction

The cardioprotective effects of oestrogens in healthy postmenopausal women, on circulating lipoprotein levels, endothelial function and the structure and function of arteries, are well established (Farhat 1996, Mendelsohn 1999). However, much less is known about the cardiovascular influences of progestins. A number of human studies have examined the cardiovascular effects of progestins combined with oestrogens. These studies demonstrated progestins with androgenic properties abrogated the beneficial effect of oestrogens on lipoprotein metabolism (The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial 1995, Crook 1997) and vascular mechanical properties (Sorensen 1995), whilst progesterone and some 19-nor-progesterone derivatives did not adversely influence the cardioprotective effects of oestrogens (Conard 1995, 1995).

These data are supported by animal studies that have demonstrated progestins with androgenic properties, such as MPA, inhibit the beneficial effects of oestrogens on cardiovascular

functioning (Williams 1994, Miyagawa 1997). Progesterone, a non-androgenic progestin, did not reverse the cardioprotective effect of oestrogens (Williams 1994, Clarkson 1996, Miyagawa 1997).

Limited data are available that demonstrate the isolated effects of progestins. In vitro and animal studies have shown conflicting evidence that progesterone administered alone has beneficial (Jiang 1992, Lee 1997, Morey 1997, Cheng 1999, Karas 2001, Otsuki 2001) and detrimental effects on cardiovascular risk factors (Miller 1991, Vazquez 1999). The in vivo effects of progesterone administration alone are based on one study that demonstrated a reduction in forearm blood flow and an increase in local vascular resistance with short term progesterone administration in the form of vaginal cream in postmenopausal women (Mercuro 1999).

In spite of the lack of corroborative evidence there has been a rapid rise in the interest of natural therapies, such as progesterone supplementation as a remedy for menopausal symptoms. Numerous 'experts' have expounded the benefits of progesterone, as opposed to oestrogen, supplementation (Lee 2000) with few scientific or clinical data available to support this 'Progesterone Revolution'.

Therefore, the aim of this study was to determine the cardiovascular effects of orally administered non-androgenic micronised progesterone on oestrogen unprimed postmenopausal

women. Specifically we studied the effects of progesterone administration on surrogate measures of cardiovascular risk, such as lipoprotein profiles, vascular reactivity, flow mediated dilation and arterial compliance.

5.2.1 Subjects

Twenty postmenopausal women were recruited by local advertising. All women were postmenopause - confirmed by a serum FSH of $\geq$ 30 IU/L - and were between the ages of 49-69 years. Subjects were excluded if they: were smokers; were taking blood pressure medication; had established cardiovascular disease or a history of thromboembolism; had thyroid or hepatic abnormalities.

The study followed a randomised, double blind, cross over design. Subjects were randomly allocated to receive either progesterone (100mg /day, Iscovesco Besins) or placebo for a period of six weeks, after which, subjects crossed over to receive the alternate treatment for a period of six weeks. Subjects were requested to visit the Baker Medical Research Institute, Menopause Clinic at baseline, six weeks and after twelve weeks. At these time points a number of measurements were undertaken. These measurements included a fasting blood sample for analysis of cholesterol, triglycerides, oestradiol, FSH, LH and progesterone. Resting measures of blood pressure, FMD, CVR and SAC were recorded at these time points.

5.2.2 Assessment of endothelial function and large artery compliance

Endothelial function was measured non-invasively by FMD and CVR techniques. Large artery compliance was measured non-invasively by SAC technique. These techniques are described in Chapter 2.

5.2.3 Biochemical assays

Oestradiol, progesterone, FSH, LH, and lipoproteins assays are detailed in Chapter 2.

5.2.4 Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean. After testing for normality a Student's paired t-test was used to compare values at baseline and after each treatment, as reported in Table 5.1 and 5.2. We calculated that 20 subjects would provide 80% power for detecting a difference of absolute increase, >2.1% flow mediated dilation of the brachial artery between treatments, with $\alpha = 0.05$ on the basis of previous experiments (Sorensen 1995, Koh 1999).

5.3 RESULTS

Group characteristics are presented in Table 5.1 and demonstrate no differences in BMI or circulating oestradiol and LH levels as a result of treatment with either placebo or progesterone. Circulating progesterone levels increased from baseline levels, after 6 weeks of progesterone treatment, by approximately 10 fold; whilst FSH was significantly reduced with progesterone treatment. Lipoproteins and triglycerides remained unchanged after six weeks of placebo and progesterone.

Bood pressure

SBP, DBP and MAP did not differ significantly from baseline (Table 5.2) with either treatment.

Arterial mechanical properties

SAC, which provides a functional measure of compliance, was similar between baseline and after treatment with placebo and progesterone (Table 5.2).

Endothelial function

FMD and CVR (ACh), which non-invasively represent endothelial function in-vivo, were unchanged as a result of progesterone treatment (Table 5.2).

Endothelium independent function

CVR (SNP) non-invasively represents vascular reactivity by mechanism independent of the endothelium. This measure was unchanged as a result of six weeks of progesterone treatment (Table 5.2).

Table 5.1. Group characteristics

Variable	Baseline	Placebo	Progesterone	P values
Oestradiol, pmol/L	135.0 ±85.6	136.4 ±69.0	108.25 ±17.1	0.579
Progesterone, mmol/L	0.9 ± 0.2	1.2 ± 0.2	9.5 ± 2.3	0.001*
FSH, mmol/L	75.1 ± 11.4	71.9 ± 11.8	67.6 ± 10.0	0.001*
TC, mmol/L	5.56 ± 0.43	5.38 ± 0.36	5.35 ± 0.34	0.249
HDL, mmol/L	1.68 ± 0.24	1.64 ± 0.21	1.70 ± 0.23	0.817
LDL, mmol/L	3.24 ± 0.32	3.12 ± 0.28	3.06 ± 0.27	0.346
Trig, mmol/L	1.43 ± 0.34	1.38 ± 0.28	1.29 ± 0.29	0.452

Values are mean ± SEM. FSH indicates, follicle stimulating hormone; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; Trig, triglycerides. P values represent comparisons between baseline and after progesterone treatment.

Table 5.2. Cardiovascular parameters of groups

Variable	Baseline	Placebo	Progesterone	P value
SBP, mmHg	114 ± 9	112 ± 10	108 ± 8	0.31
DBP, mmHg	66 ± 3	65 ± 5	65 ±4	0.55
MAP, mmHg	83 ± 5	83 ± 8	81 ± 6	0.51
SAC, ACU	0.18 ± 0.04	0.21 ± 0.04	0.20 ± 0.04	0.36
FMD, %	10.1 ± 2.9	9.2 ± 3.1	9.5 ± 3.6	0.49
CVR, ACh	6132.2 ± 1819.3	8109.0 ± 3207.7	7067.8 ± 2272.2	0.48
CVR, SNP	7392.3 ± 2511.0	7958.7 ± 3378.0	7902.4 ± 2516.9	0.75

Values are mean ± SEM. SBP indicates, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SAC, systemic arterial compliance; ACU, arbitrary compliance units; FMD, flow mediated dilation; CVR, cutaneous vascular reactivity, ACh, acetylcholine; SNP, sodium nitro-prusside. P values represent comparisons between baseline and after progesterone treatment.

5.4 DISCUSSION

The main findings from this study were that progesterone, given to oestrogen unprimed postmenopausal women, did not affect cardiovascular risk factors such as brachial artery vasodilation, small vessel reactivity, arterial compliance, blood pressure, and cholesterol and lipid levels.

Progesterone receptors are expressed in vascular cells (Lin 1982, Ingegno 1988, Lee 1997), indicating progesterone may exert direct effects on the vasculature. In vitro studies have demonstrated conflicting results with progesterone administration. Progesterone inhibited the production of endothelin-1 in bovine aortic endothelial cells (Morey 1997), whilst supraphysiological doses inhibited endothelium independent relaxation by blocking calcium channels in vascular smooth muscle cells (Jiang 1992, Perusquia 1996). The effect of progesterone in vitro on vascular smooth muscle cell proliferation has provided inhibitory (Lee 1997, Morey 1997) and no significant effects (Suzuki 1996). It is important to note, however, that the differences in the type of cells used and the dosages of progesterone in the above studies may reflect the conflicting results, making comparisons between studies difficult.

A complex relationship exists between circulating oestrogens and progesterone levels, and their combined effect on the cardiovascular system. Increased circulating oestrogens up regulate progesterone receptor expression, while, increased circulating progesterone down regulates receptor expression. The relative number of receptor binding sites may affect the actions of each

hormone. Two recent studies have demonstrated in vitro, in progesterone receptor knock out mice, that progesterone administration had no significant effect on vascular smooth muscle cell proliferation in response to vascular injury (Karas 2001) and the rate of re-endothelialisation (Vazquez 1999). In contrast, progesterone administration to wild type mice induced increases in vascular injury (Karas 2001) and the rate of re-endothelialisation (Vazquez 1999). These results indicate that a down regulation or lack of progesterone receptors, which is likely to occur in an oestrogen deprived progesterone primed state, reduces or negates any negative or positive effects of progesterone on the vasculature.

It would be of interest to undertake studies of similar design comparing and contrasting the effects, if any, of synthetic androgenic progestagens.

Data from the current study have important implications for postmenopausal women who have physiologically low levels of circulating oestrogens and progesterone. The low levels of these hormones may down regulate the expression of progesterone receptors. The administration of exogenous progesterone further suppresses progesterone receptor expression. It is speculated that any adverse or beneficial cardiovascular effects of progesterone, as demonstrated in vitro, are unable to occur due to insufficient receptor binding, therefore, no significant physiological effect occurs as seen in the current study. The clinical significance of these results relates to the lack of effect physiological doses of progesterone supplementation have on cardiovascular risk factors in oestrogen unprimed women.

6.0 Rosiglitazone improves endothelial function and arterial compliance in postmenopausal women with Type 2 diabetes mellitus

6.1 Introduction

Diabetes, increasing age and ovarian senescence are associated with impaired endothelial function and altered arterial mechanical properties. Endothelial dysfunction plays an important role in the pathogenesis of vascular disease, whilst impaired arterial mechanical properties may adversely influence important circulatory functions such as cardiac afterload and coronary perfusion. Alterations in normal vascular structure and functioning, which commonly occur with T2D, are paramount in the development of micro– and macrovascular diseases that are the primary cause of mortality and morbidity in this population. Similarly, after menopause, women experience a rapid increase in the risk of cardiovascular disease.

A number of mechanisms underlying endothelial dysfunction have been demonstrated in various vascular beds within animal models of diabetes and in humans with Type 1 and 2 diabetes mellitus. These impaired mechanisms include: reduced substrate availability (Pieper 1995, Rosen

1996, Angulo 1998), impaired signal transduction pathways (Oyama 1986, Durante 1988, Cameron 1992, McNally 1994, Heygate 1995, Mayhan 1995, Fukao 1997, Mayhan 1997, Mayhan 1997, Gazis 1999), attenuated release, and increased destruction of EDRF (Pieper 1992), increased release of endothelial derived constricting factors (Tesfamariam 1989, Shimizu 1993) and a reduced sensitivity of vascular smooth muscle cells to EDRF (Calver 1992, McVeigh 1992, Zenere 1995, Clarkson 1996, Williams 1996, Lekakis 1997).

After menopause, cardioprotective hormones – oestrogens – are reduced to physiologically low levels. Oestrogens, as discussed in Chapter 1, confer their protection by mechanisms beyond lipoprotein metabolism. Some of these mechanisms include the inhibition of platelet aggregation, vasodilation, antioxidation (Sack 1994), reduced proliferation of VSMC and increased endothelial NO synthase expression and increased NO production (Skafar 1997). The endothelium plays a key role in the homeostasis of these processes. It is likely, therefore, that both with T2D and after menopause, the disturbance of the endothelial function may be a critical and initiating factor in the development of vascular disease.

Modified arterial wall properties, which develop with a variety of metabolic disturbances, may influence determinants of circulatory function such as coronary perfusion, neuronal control mechanisms and cardiac work (Cox 1988). Increased arterial stiffness, associated with diabetes (Salomaa 1995, Berry 1999, Kim 1999), is due to a variety of mechanisms. These mechanisms include the formation of AGEs, (Airaksinen 1993, Chappey 1997) and changes in gene and protein expression of various components of the extracellular matrix (Cooper 1997). Age (Hirai

1989, Dart 1991, Gatzka 1998) and menopause (Waddell in press) are also independent factors that are associated with increased arterial stiffness. Arterial stiffening, caused by a combination of genetic, metabolic and hormonal alterations, reduces arterial compliance and is associated with systolic hypertension, (O'Rourke 1990, Dart 1993) reduced coronary perfusion and coronary artery disease (Stefanadis 1987, Mohiaddin 1989, Waddell in press).

Endothelium-dependent vasodilation and SAC are widely used as accessible and reproducible parameters to measure endothelial function and arterial elasticity in differing pathological conditions. These two parameters may be modifiable targets for treatments, which aim to reduce cardiovascular disease risk factors associated with diabetes and menopause.

As discussed in Chapter 1., thiazolidinediones are well established in their ability to enhance peripheral glucose uptake (Lee 1994) and improve insulin sensitivity (Lee 1994, Masuda 1995, O'Rourke 1997) in animal models of diabetes. More recently they have also been shown to improve glycaemic control in clinical studies involving patients with Type 2 diabetes mellitus (Iwamoto 1996, Kumar 1996, Ghazzi 1997). Thiazolidinediones are also associated with a number of anti-atherogenic actions. These include improved lipoprotein profiles - via the reduction of oxidized LDL (Nagasaka 1995, Noguchi 1996) and circulating triglyceride levels (Fujiwara 1988, Castle 1993, Lee 1994, Kaumi 1996) and reduced vascular contractile responses - via a suppression of endothelin-1 levels and an inhibition of extracellular calcium uptake by VSMC by the inhibition of L-type calcium channels (Song 1997, Itoh 1999) and an inhibition of vascular smooth muscle cell proliferation and migration (Dubey 1993, Law 1996). Many of these

findings were obtained from in vitro and animal studies. The collective effects of thiazolidinediones in humans in a clinical setting, however, remain to be fully elucidated.

The aim of this study was to determine whether the proposed anti-atherogenic actions of thiazolidinediones, namely rosiglitazone, would improve endothelial function and increase arterial compliance in postmenopausal women with Type 2 diabetes mellitus.

6.2 METHODS

6.2.1 Subjects

Twenty three women with established Type 2 diabetes mellitus were recruited from Melbourne diabetes clinics, diabetes support groups and the general public. All women had a glycated haemoglobin level of $\geq$ 7%; were postmenopause - confirmed by a serum FSH of $\geq$ 30 IU/L and were between the ages of 49 and 69 years. Subjects were excluded if they: were smokers; were taking insulin; had established cardiovascular disease, or abnormalities of thyroid or hepatic function. Subject were currently controlling their diabetes by diet alone or with diabetic medication including Biguanides or Sulfonyureas.

The study followed a randomised, double blind, parallel design. A staff member who remained independent of the study undertook randomisation of subjects to the appropriate groups. Subjects were randomly allocated to receive either rosiglitazone (n=15) (4mg /day, GlaxoSmithKline, Victoria, Australia) or placebo (n=8) for a period of twelve weeks. Subjects were requested to visit the Baker Medical Research Institute, Menopause Clinic at baseline and after twelve weeks of treatment. At these time points they were assessed by a clinician to ensure that there were no contraindications to their involvement in the study, and to monitor their diabetic control and general health. Testing was undertaken at baseline and after 12 weeks. Measurements included a fasting blood glucose; insulin levels; glycated haemoglobin; total

cholesterol; HDL-C and LDL-C and triglycerides, resting measures of blood pressure, flow mediated dilation, cutaneous vascular reactivity and systemic arterial compliance.

6.2.2 Assessment of endothelial function and large arterial compliance

Endothelial function was measured non-invasively by FMD and CVR techniques. Large artery compliance was measured non-invasively by SAC technique. Blood pressure was also measured as a function of arterial mechanical properties. These techniques are described in Chapter 2.

6.2.3 Biochemical assays

Fasting glucose, insulin, glycated haemoglobin and lipoproteins assays are detailed in Chapter 2.

6.2.4 Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean. After testing for normality a Student's paired t-test was used to compare values at baseline and after each treatment, as reported in Table 1. A Spearman's correlation analysis was performed between measures of glycaemic control and cardiovascular risk factors to determine any significance correlations.

6.3 RESULTS

Baseline group characteristics are presented in Table 6.1, demonstrating no differences between the groups at baseline. BMI was unchanged as a result of treatment.

Glycaemic control

There were significant reductions in fasting glucose (8.69 ± 0.95 to 7.20 ± 0.86 mmol/L, p=0.004), insulin (11.77 ± 2.03 to 8.35 ± 1.45 mU/L, p=0.009) and glycated haemoglobin (7.73 ± 0.44 to 6.83 ± 0.36 %, p=0.001) with 12 weeks of rosiglitazone treatment. There were no changes in placebo group (Figure 6.1).

Lipoproteins

Total cholesterol, HDL and LDL levels remained unchanged after twelve weeks of placebo and rosiglitazone. Triglyceride levels were significantly reduced after twelve weeks of rosiglitazone treatment (2.30 ± 0.38 to 1.75 ± 0.23 mmol/L, p=0.005), whilst no significant changes were found as a result of placebo (Figure 6.1).

Blood pressure

There were significant reductions from baseline in SBP (131 ± 12 to 116 ± 10 mmHg, p=0.042) and MAP (95 ± 7 to 86 ±4 mmHg, p=0.002) (Figure 6.2.) with rosiglitazone treatment. SBP was significantly correlated with glucose levels (p=0.02), whilst DBP was significantly correlated with insulin levels (p=0.008) (Figure 6.4). There were no changes in blood pressure with placebo treatment.

Arterial mechanical properties

SAC was significantly increased (0.091 ± 0.021 to 0.121 ± 0.028 ACU, p=0.015) with rosiglitazone treatment (Figure 6.3). SAC was not significantly correlated with FMD (Figure 6.4). There were no changes in SAC with placebo treatment.

Endothelial function

FMD was increased (7.9 ± 2.3 to 15.3 ± 3.9 %, p=0.019) after twelve weeks of rosiglitazone (Figure 6.3). FMD was not significantly correlated with SAC (Figure 6.4). There were no changes in FMD with placebo treatment.

Table 6.1. Baseline group characteristics

Variable	Placebo N=8	Rosiglitazone n=15	p
Age, years	58.4 ± 2.8	56.3 ± 2.3	0.42.
BMI, $kg.m^{-2}$	30.63 ± 3.44	31.5 ± 3.12	0.77
Glucose, mmol/L	9.35 ± 1.73	9.15 ± 1.23	0.46
Insulin, U/L	11.53 ± 2.08	11.67 ± 1.95	0.71
HbA1c, %	7.8 ± 0.97	7.98 ± 0.62	0.81
Total cholesterol, mmol/L	5.05 ± 0.42	5.32 ± 0.49	0.58
HDL, mmol/L	1.27 ± 0.07	1.30 ± 0.13	0.71
LDL, mmol/L	2.84 ± 0.38	3.10 ± 0.41	0.85
Brachial SBP, mmHg	120 ± 8	130 ± 11	0.58
Brachial DBP, mmHg	69 ± 4	72 ± 5	0.42
MAP, mmHg	87 ± 5	94 ± 7	0.29
SAC, ACU	0.112 ± 0.024	0.091 ± 0.021	0.32
FMD - reactive hyperaemia, %	10.7 ± 2.1	7.9 ± 2.3	0.51

FMD – SNP, %	16.4 ± 3.3	17.4 ± 5.6	0.88

Values are mean ± SEM. B indicates baseline values; 12, values at 12 weeks; FSH, follicle stimulating hormone; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. P values represent comparisons between baseline.

Figure 6.1. Blood pressure

Blood pressure, percent changes with treatment. Open boxes represent placebo group, shaded boxes represent rosiglitazone group. *p<0.05.

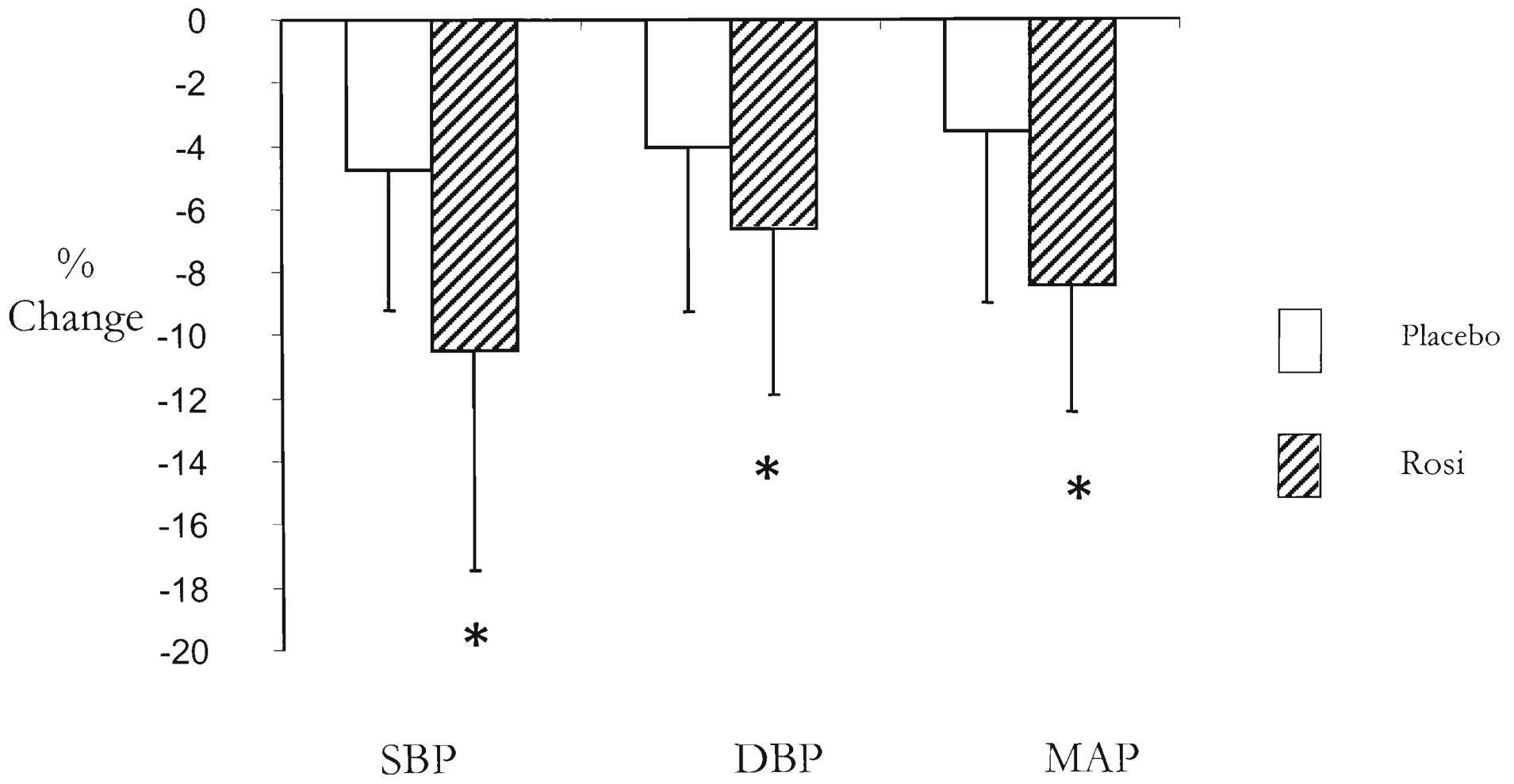


Figure 6.2. Triglyceride levels and glycaemic control.

Percentage change with treatment. Open boxes represent placebo group, shaded boxes represent rosiglitazone group. *p<0.05.

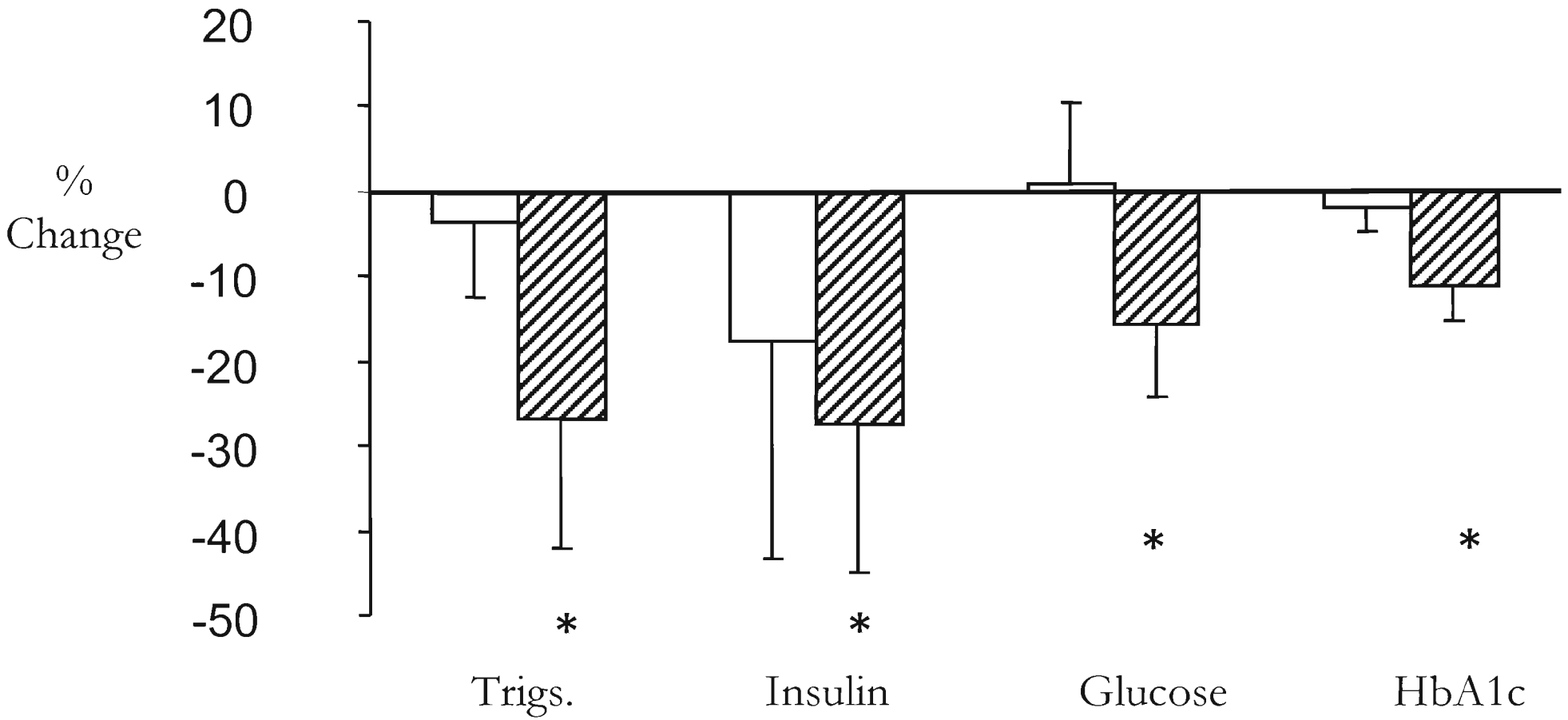


Figure 6.3. Endothelial function and large arterial compliance.

Percent change with treatment. Open boxes represent placebo group, shaded boxes represent rosiglitazone group. *p<0.05.

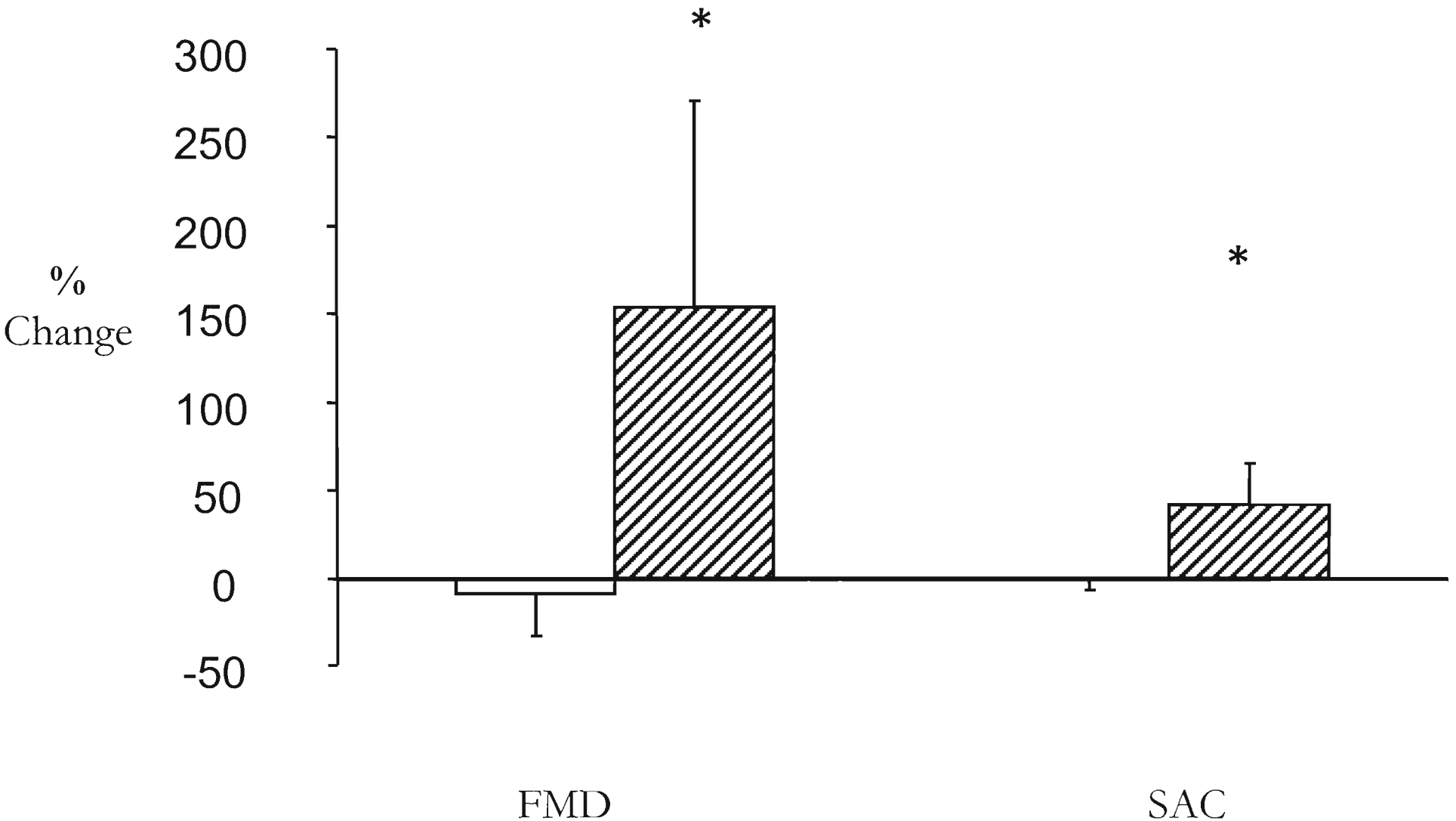


Figure 6.4 Spearman Rank Order Correlation

	HbA1c	insulin	SBP	DBP	MAP	FMD	SAC
glucose	0.265	0.0110	-0.579	-0.464	-0.300	0.411	-0.0643
	0.332	0.964	0.0231	0.0782	0.269	0.124	0.812
	15	14	15	15	15	15	15
HbA1c		-0.548	0.122	0.0894	0.0840	-0.352	-0.0375
		0.0410	0.657	0.743	0.753	0.189	0.883
		14	15	15	15	15	15
insulin			0.008	-0.670	0.0154	0.327	0.0901
			0.964	0.008	0.952	0.244	0.750
			14	14	14	14	14
SBP				0.424	0.534	-0.424	-0.0197
				0.110	0.038	0.110	0.934
				15	15	15	15
DBP					0.343	-0.589	-0.279
					0.204	0.0201	0.306
					15	15	15
MAP						-0.407	-0.0214
						0.127	0.934
						15	15
FMD							0.0821
							0.763
							15
SAC							

The pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

6.4 DISCUSSION

The main findings of this study were that for postmenopausal women with T2D, rosiglitazone improves glycaemic control, endothelial function and compliance of large proximal arteries. Correlation analysis suggests that improved endothelial function and large artery compliance were not directly associated with improved glycaemic control, and that rosiglitazone was acting independently on these parameters.

The role of thiazolidinediones in improving glycaemic control has been well established in animal models and more recently in human studies of T2D. The results from the present study reinforce this glycaemic effect of rosiglitazone. Prospective studies have demonstrated that high blood glucose levels are associated with future cardiovascular events (Gordon 1972, Barrett-Connor 1984), and that high plasma insulin levels are a risk factor for coronary heart disease (Welborn 1979, Ducimetiere 1980). Although strict glycaemic control delays the onset and moderates the progression of vascular complications (The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group 2000), it is possible that thiazolidinediones improve cardiovascular risk factors independent of improvements in glycaemic control. The anti-atherogenic effects of thiazolidinediones, indicated by improvements in blood pressure, endothelial function and arterial stiffness, beyond that which is due to improved glycaemic control, have not been demonstrated in human studies until now.

Hypertriglyceridemia, commonly associated with T2D, is an independent risk factor for coronary heart disease (Hokanson 1996), which can be markedly attenuated with thiazolidinedione treatment (Fujiwara 1988, Lee 1994, Kaumi 1996). Serum triglycerides levels are reduced by an inhibition of hepatic triglyceride and very-low density lipoprotein synthesis (Fujiwara 1988), and an increased clearance in the periphery (Kaumi 1996). Rosiglitazone had no effect on HDL, LDL or total cholesterol levels in the current study. In contrast, previous studies have demonstrated elevations in HDL and LDL levels (Fonseca 2000, Raskin 2000, Lebovitz 2001) with rosiglitazone and troglitazone in patients with T2D. These studies, however, did have larger sample sizes than the current study, which may have influenced the differences in outcomes.

Hyperinsulinaemia may stimulate sympathetic activity (Anderson 1991, Anderson 1992), enhance proliferation of VSMC (Stout 1975, Pfeifle 1981) and induce sodium retention (Skott 1991), which tends to increase blood pressure. T2D is commonly associated with hypertension, an association possibly mediated by shared causal factors including insulin resistance, obesity, inactivity and diet. Hypertension in Type 2 diabetic patients can significantly increase the risk of coronary heart disease, stroke and peripheral vascular disease (Arauz-Pacheco 1996). This study demonstrated a significant reduction in systolic, diastolic and mean arterial pressures after 12 weeks of rosiglitazone treatment. In addition, insulin levels may also affect arterial compliance; therefore, reductions in circulating insulin levels, demonstrated with rosiglitazone treatment, although not correlating significantly with improved SAC, may have provided a partial improvement in SAC.

Hyperglycaemia is associated with increased arterial stiffness with T2D (Salomaa 1995). The accumulations of advanced glycation end products on collagen fibres are the result of long term hyperglycaemia, affecting arterial mechanical properties (Chappey 1997) and arterial elasticity (Airaksinen 1993). Although a non linear relationship exists between SAC and mean arterial pressure, correlation analysis showed that the observed increase in SAC was not directly associated with either the improvement in glycaemic control, or the reduction in blood pressure, indicating that rosiglitazone was acting directly on arterial mechanical properties.

There are numerous mechanisms that affect arterial compliance, including VSMC tone, and modifications in arterial wall structure. Enhanced endothelial vasodilation was not significantly correlated with SAC improvements suggesting that the functioning of the endothelium and arterial compliance may not be directly related.

The functions of the endothelium encompass a myriad of activities that extend beyond vascular tone and reactivity, and the release of vasoactive substances. Nonetheless, endothelium dependent vasodilation can be used as an accessible and reproducible tool to determine endothelial function in vivo. The current study demonstrates an increased endothelium-derived vasodilation in response to shear stress, indicating an improved ability of the endothelium to respond to these stimuli, possibly conferring commensurate benefit in many aspects of endothelial function. The mechanisms for these novel in vivo improvements in endothelial function are unknown at present.

In summary, rosiglitazone improves endothelial function and arterial compliance in postmenopausal women with T2D. These improvements could not be fully accounted for by the differences in blood pressure, lipids, or simple indexes of glycaemic control. In addition, neither of these parameters were significantly associated with the other, indicating that endothelial function and arterial compliance are both improved independently of the other, improving the cardiovascular risk profiles of postmenopausal women with T2D.

7.0 The effects of rosiglitazone and hormonal therapy on cardiovascular risk factors in postmenopausal women with Type 2 diabetes mellitus.

7.1 Introduction

Rosiglitazone improves glycaemic control, indicated by reduced; glycated haemoglobin (Fonseca 2000, Raskin 2000, Lebovitz 2001); fasting glucose (Fonseca 2000, Raskin 2000, Garber 2001, Lebovitz 2001) and insulin levels (Fonseca 2000, Garber 2001, Lebovitz 2001) in patients with T2D, as discussed in Chapter 1 and demonstrated in Chapter 6. Good glycaemic control and the subsequent amelioration of hyperinsulinaemia and hyperglycaemia can delay the onset of vascular complications (The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group 2000). Rosiglitazone, however, also has a number of anti-atherogenic effects independent of its influences on glucose and insulin metabolism, as discussed in Chapter 6. Rosiglitazone, therefore, provides an effective treatment for populations at high risk of developing CVD, such as postmenopausal women with T2D.

HT has been associated with cardioprotection in healthy postmenopausal women (Stampfer 1991, Stampfer 1991, Falkeborn 1992, Ettinger 1996). Whether this potentially beneficial effect of HT can be extrapolated to a T2D population has created considerable controversy. A number of investigators have shown HT reduces a number of risk factors for

CVD in postmenopausal women with T2D, including an improved glycaemic control (Andersson 1997, Brussaard 1997, Ferrara 2001, Friday 2001), decreased circulating LDL, increased HDL levels and reduced soluble intercellular adhesion molecules (Lim 1999), thereby suggesting a reduction in endothelial activation. On the other hand, in vitro studies have demonstrated hyperglycaemia attenuates the ability of oestrogen to stimulate NO production (Sowers 1997) and inhibit VSCM proliferation (Ling In press). Oestrogen administration has also provided neutral effects on a range of endothelial functions in vitro (Bolego 1999) and in postmenopausal women with T2D (Lim 1999, Saltevo 2000). Reducing hyperglycaemia by the administration of rosiglitazone and combining this treatment with HT may provide some added benefits to cardiovascular risk factors in comparison to either treatment given in isolation. However, to the best of my knowledge, there are no studies that have addressed this issue.

The aim of this study was to determine whether HT administered in conjunction with rosiglitazone would provide further cardioprotection in postmenopausal women with T2D as opposed to rosiglitazone treatment alone.

7.2.1 Subjects

Twelve women with established T2D who were currently taking rosiglitazone (4mg/day) were recruited from a previous rosiglitazone trial. All women were postmenopause - confirmed by a serum FSH of $\geq$ 30 IU/L and were between the ages of 49 and 69 years. Subjects were excluded if they: were smokers; were taking insulin; had established cardiovascular disease; abnormalities of thyroid or hepatic function.

The study followed a randomised, double blind, cross over design. Subjects continued rosiglitazone treatment (4mg/day GlaxoSmithKline, Victoria, Australia) and were randomly allocated to receive either HT (transdermal oestradiol, 50ug, 3M Pharmaceuticals and oral micronised progesterone 100mg, Iscovesco Besnins) or placebo (patch and tablet) for a period of twelve weeks, after which subjects crossed over to the alternate treatment whilst continuing rosiglitazone treatment. Subjects were requested to visit the Baker Medical Research Institute, Menopause Clinic at baseline, after twelve weeks and twenty-four weeks of treatment. At these time points subjects were assessed by a clinician to ensure that there were no contraindications to their involvement in the study and to monitor their diabetic control and general health. A number of measurements were also undertaken at these time points. Measurements included oestradiol; FSH; LH; fasting blood glucose; insulin levels; glycated haemoglobin; total cholesterol; HDL-C and LDL-C; triglycerides; resting measures of blood pressure; FMD; CVR and SAC.

7.2.2 Assessment of endothelial function and large arterial compliance

Techniques used for the measurement and analysis of endothelial function and arterial mechanical properties are detailed in Chapter 2.4, 2.5 and 2.6.

7.2.3 Biochemical assays

Biochemical assays used for the analysis of oestradiol, FSH, LH, glucose, insulin glycated haemoglobin, total cholesterol, HDL-C and LDL-C and triglycerides are discussed in detail in Chapter 2.8.

7.2.4 Statistical analysis

Data were analysed using Sigma Stat 2.0 (Jandell). Data reported are mean $\pm$ standard error of the mean. A paired t-test was used to determine within group significance, whilst an unpaired t-test was used to determine differences between groups. Significance was reported at $p < 0.05$. Power calculations were based on an 80% power with an alpha level of 0.05. The size of change to be detected and the expected standard deviation of change were determined from previous studies within this laboratory (Nestel 1999, Williams 2001).

7.3 RESULTS

The age range of women involved was 49 – 69 years of age. BMI was unaffected by treatment (Table 7.1). There was a trend for serum oestradiol levels to increases with the administration of HT, whilst circulating FSH and LH were significantly reduced (Table 7.1).

There were no significant alterations to total cholesterol, HDL – C, LDL – C or triglycerides with either HT or placebo treatment (Table 7.1). Nor were there any changes in fasting glucose, insulin or glycated haemoglobin with treatment (Table 7.1).

Arterial mechanical properties, measured by SBP, DBP, MAP and SAC were unchanged as a result of either HT or placebo treatment (Table 7.1). Small vessel reactivity as measured by CVR in response to acetylcholine and sodium nitroprusside (SNP) were unchanged by treatment (Table 7.1). Brachial artery diameter, measured by FMD, was significantly reduced from baseline ($p<0.05$) in response to 12 weeks of HT treatment (Figure 7.1). There was no effect of order of treatment.

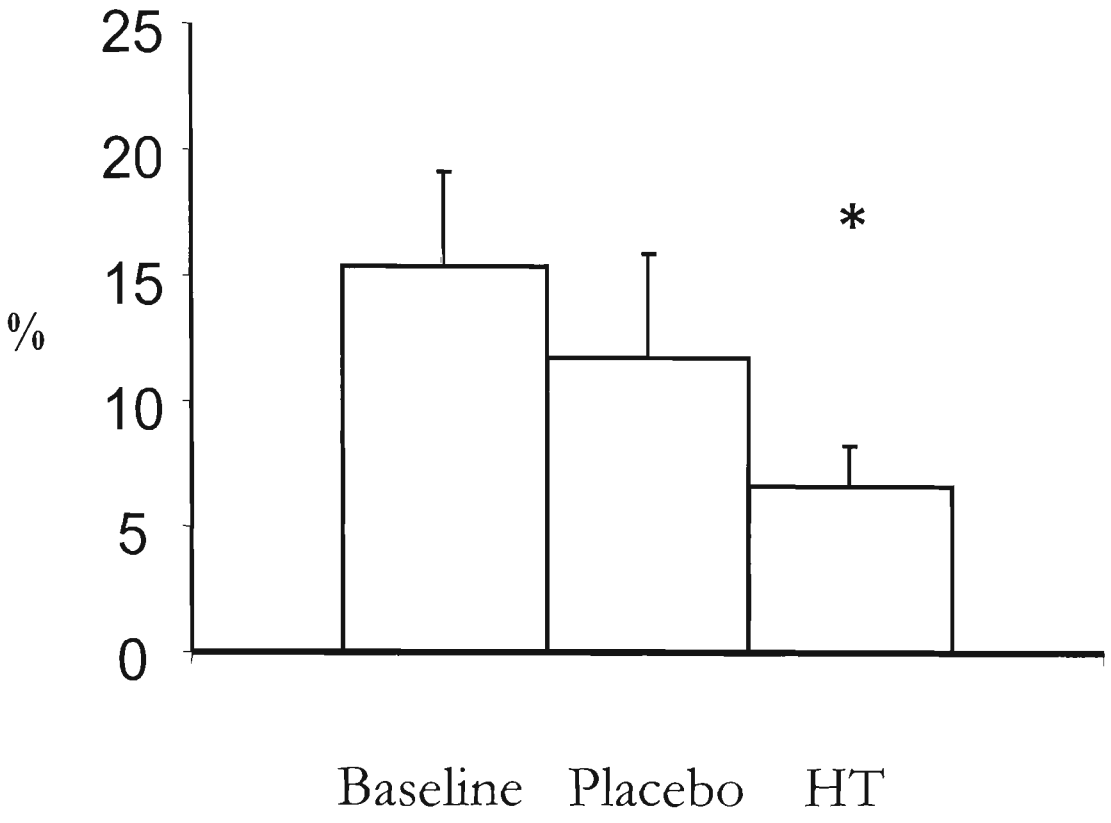
Table 7.1. Group Characteristics

Variable	Baseline	Placebo	Hormonal therapy
BMI, $kg.m^2$	31.67 ± 2.51	32.45 ± 2.90	32.70 ± 3.00
Oestradiol, pmol/L	78.45 ± 23.25	76.42 ± 16.57	125.36 ± 33.01
FSH, U/L	55.28 ± 9.31	63.54 ± 11.23	40.26 ± 7.47
LH, U/L	34.37 ± 7.24	35.97 ± 7.31	26.85 ± 8.45
Glucose, mmol/L	7.20 ± 0.87	7.88 ± 0.95	7.78 ± 0.90
Insulin, U/L	8.35 ± 1.45	10.11 ± 1.90	11.82 ± 2.58
HbA1c, %	6.97 ± 0.33	6.64 ± 0.58	6.51 ± 0.38
Total cholesterol, mmol/L	5.08 ± 0.52	5.24 ± 0.38	5.14 ± 0.46
HDL, mmol/L	1.29 ± 0.09	1.27 ± 0.10	1.26 ± 0.10
LDL, mmol/L	2.99 ± 0.46	3.07 ± 0.31	3.03 ± 0.37
Trigs , mmol/L	1.75 ± 0.23	1.97 ± 0.39	1.98 ± 0.63
Brachial SBP, mmHg	122 ± 8	122 ± 6	123 ± 6
Brachial DBP, mmHg	67 ± 3	68 ± 4	67 ± 3

SAC, ACU	0.12 ± 0.03	0.12 ± 0.03	0.13 ± 0.05
CVR – ACh, AFU	2237 ± 800	2296 ± 839	2366 ± 598
CVR – SNP, AFU	2364 ± 507	2088 ± 749	2512 ± 751

Values are mean ± SEM. FSH indicates follicle stimulating hormone; LH, leutinising hormone; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SAC, systemic arterial compliance; CVR, cutaneous vascular reactivity; ACh, acetylcholine; SNP, sodium nitroprusside.

Figure 7.1.



Flow mediated dilation of brachial artery at baseline, after 12 weeks of placebo and after 12 weeks of HT. *$p<0.05$.

7.4 DISCUSSION

The main findings of this study were that the addition of HT to rosiglitazone treatment reduces brachial artery diameter in response to ischaemia in postmenopausal women with T2D; whilst transdermal oestradiol and micronised progesterone provided no changes to other cardiovascular risk factors such as lipoprotein levels, arterial mechanical properties or small vessel reactivity. The addition of HT to rosiglitazone treatment did not adversely affect glycaemic control.

This is the first study to investigate the effects of combined treatment in a clinical setting. There has been no in vitro research, to the best of my knowledge, investigating the combined effects of these treatments; for this reason, it is difficult to determine what is occurring at a molecular level.

Epidemiological data has revealed that women with diabetes have a cardiovascular risk similar to that of non-diabetic men (Barrett-Connor 1991); suggesting that women with diabetes are denied cardioprotection afforded by oestrogen. This has been demonstrated in vitro, whereby aortic rings of ovariectomised streptozotocin mice treated with17 beta oestradiol showed attenuation in norepinephrine induced contractile response, in comparison to non-diabetic controls treated with oestradiol (Bolego 1999). Similar findings have been demonstrated in vivo, whereby non-diabetic postmenopausal women using HT had greater endothelium dependent dilation as opposed to postmenopausal women with T2D using HT (Lim 1999). The abolition of

the cardioprotective effects of oestrogens with diabetes does not, however, explain the reduction in brachial flow mediated dilation with the combination of rosiglitazone and HT.

Combined treatment reduced flow mediated dilation of the brachial artery to levels apparent in women prior to the commencement of rosiglitazone therapy (Chapter 6), indicating that HT attenuated the beneficial effect of rosiglitazone.

An interpretation for the possible molecular mechanism of this effect may be that oestrogen antagonises rosiglitazone binding to PPAR gamma in oestrogen target tissue such as the endothelium. Oestrogen alone does not function effectively as a ligand for PPAR gamma (Ma 1998), nor do PPARS and oestrogen receptors (ER) form a heterodimer in vivo (Keller 1995); therefore, the relationship between oestrogen and PPARs may involve indirect mechanisms. PPAR gammas are selectively activated by prostaglandins, specifically from the J2 series (Kliewer 1995). Oestrogen can regulate prostaglandin production in target tissue such as the uterus (Ham 1975, Pakrasi 1984, Wilson 1984, Tawfik 1987, Freyberger 1989, Chaud 1994), such that, oestradiol increases the release of arachidonate, a precursor of prostaglandins (Fayard 1994). These prostaglandins may compete for PPAR gamma binding with thiazolidinediones. Oestradiol treatment in ducks causes a decrease in prostaglandin D2 levels due to an increased conversion to a metabolite, which, appears to be extremely similar in structure and behaviour to prostaglandin ligands for PPAR gamma (^{12} –prostaglandin J_2) (Ma 1998). It may be speculated that this prostaglandin metabolite competitively binds to PPAR gammas and reduces the ligand binding of

rosiglitazone, subsequently, reducing endothelial function to levels recorded at baseline prior to rosiglitazone treatment.

Another interpretation of these results may involve ERs, which are up-regulated with oestrogen administration, negatively regulating PPAR action on peroxisome proliferator response elements (PPRE) through competition for DNA binding. A similar molecular occurrence was apparent with thyroid hormone receptor alpha competitively binding with PPAR to PPREs; however, only PPAR mediated a transcriptional activation via PPRE (Miyamoto 1997). Thyroid hormone and oestrogens are both members of the steroid thyroid nuclear receptor superfamily, which form heterodimers with retinoid X receptors (RXR) to bind to hormone response elements; therefore, a similarity may exist in the relationship of these hormone receptors, with a reduction in PPAR activation and the subsequent reduction in endothelial function, as indicated by reduced brachial artery flow mediated dilation.

The effect of progesterone on rosiglitazone action has not been investigated, and although there is no evidence, it may be possible that progesterone negatively influences flow mediated dilation in postmenopausal women with T2D.

Neither of these interpretations takes into account the maintenance of glycaemic control and other cardiovascular risk factor such as SAC, blood pressure and lipoproteins. Further comprehensive in vitro investigations are required to substantiate these interpretations.

It is important to note that FMD measures conduit artery response as a measure of endothelial function; this correlates well with invasive testing of coronary endothelial function (Joannides 1995, Takase 1998), and the extent and severity of coronary atherosclerosis (Neunteufl 1997). CVR, on the other hand, examines skin microvascular endothelial cell function and smooth muscle function (Westerman 1988, Eneroth-Grimfors 1993, Morris 1995). The effects of combined treatment (rosiglitazone and HT) were apparent on forearm conduit vessels; however, these effects were not seen in microvessels. Although there is no evidence, PPAR gamma receptor concentrations may vary between tissues; supporting why we demonstrated a significant effect of treatment on conduit arteries but not on microvessels.

In our investigation women taking rosiglitazone at the commencement of the study recorded circulating glucose levels greater than 7 mmol/L, a level still regarded as high in comparison to recommended healthy ranges (4-7mmol/L). Alternate to the previous two interpretations of the reduced flow mediated dilation results, is a hypothesis that proposes in a postmenopausal T2D population experiencing hyperglycaemia, oestrogen may impair endothelial function; therefore, negating the beneficial cardiovascular effects of rosiglitazone. This hypothesis has been supported by one in vitro study that demonstrated high glucose levels decreased oestrogens ability to stimulate NO and VSMC proliferation (Ling In press).

The multiple end points measured within this study increased the risk of a Type I error. This should be taken into account when considering the outcomes of this study and are a potential limitation of this study.

Further in vitro research is required to determine the combined effects of HT and rosiglitazone on binding relationships and metabolite production.

8.0 The effects of rosiglitazone and hormonal therapy on bone turnover markers in postmenopausal women with Type 2 diabetes mellitus.

8.1 INTRODUCTION

There are a number of studies that have examined the glycaemic (Iwamoto 1996, Kumar 1996, Ghazzi 1997) and cardiovascular effects (Chapter 6) of thiazolidinediones in humans with T2D; however, no study, to the best of my knowledge, has investigated the effects of this drug on bone metabolism.

Menopause is associated with a 79-97% increase in bone resorption markers, whilst bone formation markers are increased by 37-52%, reflecting an overall increase in bone turnover (Garnero 1996). These alterations in bone turnover often precede changes in bone mass and density. Therefore, after menopause women need to consider their bone status as well as their cardiovascular and glycaemic profiles.

Only two investigations, to the best of my knowledge, have examined the effects of thiazolidinediones on bone tissue with conflicting results. One in vitro study has identified thiazolidinediones increase the expression of messenger ribonucleic acid (mRNA) levels of adipocyte specific genes, namely PPAR gammas, and subsequently increases adipocyte differentiation in bone marrow stromal lines (Gimble 1996). An increase in the differentiation of adipocytes in bone marrow may compromise the differentiation of progenitors into osteoblasts, predisposing an individual to the risk of developing osteopenia or osteoporosis; which are associated with increased bone marrow adipose tissue and reduced bone volume (Meunier 1971). In contrast, an in vivo study investigated thiazolidinedione administration on mouse whole bone marrow cell cultures, as assessed by pit formation assays. This study demonstrated a reduction in osteoclast-like cell formation and a dose dependent inhibition of bone resorption (Okazaki 1999). Research is required to determine the effects of thiazolidinediones in humans.

HT is a well-established intervention for the maintenance of skeletal integrity after menopause (Lindsay 1976, Horsman 1977, Recker 1977, Kiel 1987); however, there has been no clinical investigation, to the best of my knowledge, which assesses the combined effects of HT and thiazolidinediones in a T2D postmenopausal population.

The aim of this study was to determine the effects of rosiglitazone alone on bone turnover markers in postmenopausal women with T2D. It was also the aim of this study to investigate the effects of HT administered in conjunction with rosiglitazone on bone turnover markers.

8.2.1 Subjects

Twenty two women with established Type 2 diabetes mellitus were recruited from Melbourne diabetes clinics, diabetes support groups and the general public. All women had a glycated haemoglobin level of $\geq$ 7%; were postmenopause - confirmed by a serum FSH of $\geq$ 30 IU/L and were between the ages of 49 and 69 years. Subjects were excluded if they; were smokers; were taking insulin; had established cardiovascular disease; abnormalities of thyroid or hepatic function; or were taking medication that may influence bone metabolism.

8.2.2 Protocol

The study followed a randomised, double blind, parallel design. Subjects were randomly allocated to receive either rosiglitazone (n=15) (4mg /day, Glaxo-SmithKline, Victoria, Australia) or placebo (n=8) for a period of twelve weeks. Subjects were requested to visit the Baker Medical Research Institute, Menopause Clinic at baseline and after twelve weeks of treatment. At these time points subjects were assessed by a clinician to ensure that there were no contraindications to their involvement in the study and to monitor their diabetic control and general health. Testing was undertaken at baseline and after 12 weeks. Measurements included a morning serum sample taken between 10.00am and 12.00pm for analysis of oestradiol, FSH, LH and osteocalcin levels,

and a urine sample taken 2 hours post first morning void for analysis of pyridinoline and deoxypyridinoline.

After the initial 12 weeks, twelve subjects taking rosiglitazone continued in the study, whilst the placebo group was discontinued. The continuing study followed a randomised cross over design. Subjects continued rosiglitazone treatment and were randomly allocated to receive either HT (transdermal oestradiol, 50ug, 3M Pharmaceuticals and oral micronised progesterone 100mg, Ivesco Besnins) or placebo (patch and tablet) for a period of twelve weeks, after which subjects crossed over to the alternate treatment whilst continuing rosiglitazone treatment. Subjects were requested to visit the Baker Medical Research Institute, Menopause Clinic at baseline, after twelve weeks and twenty-four weeks of treatment. At these time points measurements of hormones and bone turnover markers were repeated.

8.2.3 Assays

Biochemical assay techniques for oestradiol, FSH, LH, DPD, PYR, and OC are described in Chapter 2.

8.2.4 Statistical analysis

Data were analysed using Sigma Stat. (2.0). Data reported are mean ± standard error of the mean. A Student's paired t-test was used to determine significance within groups. Significance between groups was determined using an un-paired t-test. Significance was reported at $p < 0.05$.

Rosiglitazone alone

There were no significant differences between the placebo or rosiglitazone groups at baseline for age, BMI, oestradiol, FSH, LH or bone turnover markers (Table 8.1).

There were no significant changes in hormone levels, PYR, DPD or OC with treatment (Table 8.1).

Rosiglitazone and HT

There was a trend for serum oestradiol levels to increases with the administration of HT ($p=0.066$), whilst circulating FSH ($p=0.01$) and LH ($p=0.02$) were significantly reduced (Table 8.2). There was a significant reduction in DPD ($p=0.01$) from baseline levels with HT. No significant alterations in PYR or OC levels occurred as a result of adding HT to rosiglitazone treatment. There were no changes in any parameter measured as a result of placebo.

Table 8.1. Group characteristics, rosiglitazone

Variable	Placebo	Placebo 12 weeks	Rosiglitazone	Rosiglitazone 12 weeks
Age, years	58 ± 3		56 ± 2	
BMI, $kg.m^{-2}$	30.6 ± 3.4	30.9 ± 3.6	31.5 ± 3.1	31.7 ± 3.1
Oestradiol, pmol/L	58.8 ± 18.2	65.1 ± 20.6	83.9 ± 17.7	86.3 ± 19.8
FSH, pmol/L	57.1 ± 10.5	61.2 ± 9.7	57.1 ± 9.8	54.9 ± 9.7
LH, pmol/L	31.2 ± 4.3	29.1 ± 4.5	35.4 ± 7.0	33.1 ± 6.6
OC U/L	9.2 ± 2.3	10.0 ± 1.8	13.1 ± 4.1	13.1 ± 2.7
PYR, mmol/L/Cr	62.2 ± 10.8	65.8 ± 9.9	66.3 ± 10.2	67.7 ± 10.0
DPD, mmol/L/Cr	14.4 ± 2.4	14.0 ± 1.8	15.0 ± 2.1	15.0 ± 2.4

Values are mean ± SEM. BMI indicates body mass index; FSH, follicle stimulating hormone; LH; luteinising hormone; OC, osteocalcin; PYR, pyridinoline; DPD, deoxypyridinoline.

Table 8.2. Group characteristics rosiglitazone and HT

Variable	Baseline	Placebo	Hormonal therapy
Oestradiol, pmol/L	78.45 ± 23.25	76.42 ± 16.57	125.36 ± 33.01
FSH,pmol/L	55.28 ± 9.31	63.54 ± 11.23	40.26 ± 7.47*
LH, pmol/L	34.37 ± 7.24	35.97 ± 7.31	26.85 ± 8.45*
OC, U/L	13.4 ± 2.74	12.28 ± 4.11	12.13 ± 3.05
PYR, mmol/L/Cr	68.8 ± 9.9	65.8 ± 11.1	63.1 ± 8.7
DPD, mmol/L/Cr	15.0 ± 2.7	14.2 ± 2.4	12.2 ± 1.5*

Values are mean ± SEM. * indicates significantly different from baseline levels ($p<0.05$). BMI indicates body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; OC, osteocalcin; PYR, pyridinoline; DPD, deoxypyridinoline.

8.4 Discussion

The main findings of this study were that rosiglitazone treatment had no significant effect on bone turnover markers in postmenopausal women with T2D. The addition of HT to rosiglitazone treatment significantly reduced bone resorption.

This is the first study to investigate the effects of rosiglitazone alone and combined with HT in a clinical setting on bone turnover markers. As previously mentioned, only two animal and in vitro studies have assessed the effects of rosiglitazone on bone, with conflicting results. The lack of change in bone turnover markers as a result of rosiglitazone administration in this study indicates no significant effects of this drug on bone metabolism in postmenopausal women with T2D.

HT, when administered alone, reduces bone turnover in postmenopausal women (Prestwood 1994, Prior 1997). Similarly, when HT is co-administered with rosiglitazone the beneficial effect of HT on bone turnover is not compromised. Therefore the use of rosiglitazone for the amelioration of altered glycaemic control and cardiovascular function will not adversely affect bone metabolism in postmenopausal women with T2D.

9.0 Conclusions

The baby boom after the Second World War, from the forties through to the sixties, has exerted considerable power in altering many health and health care aspects, including the changing patterns of need concerning health care delivery. Female baby boomers are now contending with the entangled physiological and psychological changes associated with menopause and ageing. The complex and diverse issues related to these changes, previously not widely discussed, are now experiencing considerably greater exposure. Women are faced with a greater variety of health information as new research is undertaken and prophylactic and intervention treatments become available. The amount of information concerning menopause has significantly increased. To enable women to make informed choices about their own health and well being throughout, and after menopausal years, information is required to be presented in clinically relevant formats. This chapter will discuss the results of studies undertaken as part of this thesis, relate how they apply to women in a clinical setting and suggest further research required.

It was hypothesised that interventions such as exercise, HT and rosiglitazone would independently improve cardiovascular risk markers, glucose and insulin levels and bone turnover markers in postmenopausal women, whilst combined interventions would provide added benefit.

Exercise is often advocated for women after menopause to reduce risks associated with cardiovascular disease, diabetes and osteoporosis. The type, frequency and duration of exercise required to elicit these reductions in risks are of paramount importance. Walking is a safe, accessible and cost effective exercise that can be easily employed. However, moderate walking was not effective in reducing any of the measured risk markers for cardiovascular disease, diabetes or osteoporosis in previously sedentary women with no history of hypertension, hypercholesterolaemia, glucose intolerance or low bone density (Chapter 3). Therefore, our hypothesis was not supported. However, the low statistical power reached for the above mentioned parameters should be taken into consideration when interpreting these results. The prescribed intensity of exercise generated by walking in this thesis may be insufficient to induce alterations in these risk markers when women are not at imminent threat of developing these diseases. Further research that investigates the effects of moderate exercise, such as the walking program undertaken by subjects in Chapter 3, on measures of serum lipid concentrations, SAC, glucose and insulin metabolism and bone turnover markers in older women and women with established cardiovascular disease, altered glucose and insulin metabolism, diabetes and osteoporosis is required.

Chapter 4 demonstrated that body mass index and blood pressure were significantly lower and aerobic capacity and bone formation were significantly higher in early postmenopausal women who regularly participate in vigorous weight bearing exercise, compared with early postmenopausal women with a sedentary lifestyle. There were no significant differences in cholesterol, cholesterol sub-fractions, SAC or glucose and insulin levels between women. These observational results suggest that a greater intensity and duration of weight bearing exercise, as

opposed to walking alone, may provide greater benefit to bone turnover markers in early postmenopausal women. Valuable further research would involve a large scale, prospective study identifying the effects of various types – including resistance training, intensities and durations of exercise on healthy postmenopausal women and women with established cardiovascular disease, diabetes or osteopenia. This would assist in the prescription of the most appropriate exercise to women.

HT is often prescribed to alleviate menopausal symptoms and possibly reduce the risk of cardiovascular disease after menopause. In Chapter 3, however, transdermal HT use in sedentary, early postmenopausal women with no history of CVD produced no alterations in cardiovascular risk factors; nor did HT influence the cardiovascular or diabetic risk profiles of athletic postmenopausal women (Chapter 4). The cardiovascular effects of combined walking and HT did not differ from those of either treatment alone. The results of these studies, therefore, did not support the hypothesis. Future directions of research should assess the administration of a variety of HT preparations, such as conjugated equine oestrogens compared with 17 beta oestradiol, MPA compared with micronised progesterone and varying concentrations of HT on cardiovascular risk factors, bone turnover markers and glucose and insulin metabolism in the above populations of postmenopausal women. Future studies should also involve populations of postmenopausal women at varying levels of risk for cardiovascular disease, diabetes and glucose intolerance to determine whether the effects of HT are greater on the above parameters when women have an established risk profile.

HT reduced bone turnover in sedentary women, whilst the combination of HT and exercise provided no additional benefit (Chapter 3), partially supporting the initial hypothesis. HT was also beneficial in attenuating bone turnover markers in athletically trained women (Chapter 4), reducing the risk of osteoporosis in these population groups without affecting aerobic capacity.

There has been a rapid rise in the interest of natural therapies, such as progesterone supplementation for postmenopausal women to alleviate menopausal symptoms. Progesterone is also commonly administered in combination with oestrogen in a HT regimen. The use of micronised progesterone alone on oestrogen unprimed postmenopausal women, however, had no advantageous or deleterious affect on cardiovascular risk profiles (Chapter 5). Therefore, physiological doses of progesterone may be superfluous in this situation. Future research that assesses the effectiveness of progesterone administration on premenopausal women may provide valuable information about the effects of progesterone in an environment whereby progesterone receptor expression, and therefore action, is not being compromised by physiologically low oestrogen levels.

Hyperglycemia and hyperinsulinaemia, which accompany T2D, are associated with an increase in the risk of CVD. Rosiglitazone reduced fasting glucose and insulin levels and improved glycaemic control in postmenopausal women with T2D, thereby supporting our hypothesis by reducing cardiovascular risk factors. Rosiglitazone also attenuated cardiovascular risk factors, such as SAC and endothelial function - as measured by FMD, independently of reductions in fasting glucose and insulin levels. Accordingly, this is an effective drug for the

treatment of T2D and reducing surrogate measures of cardiovascular risk in the short term; however, further studies are required to confirm that the effects of rosiglitazone persist over longer periods of time, and reduce cardiovascular events and mortality.

Interventions such as exercise, HT, progesterone, rosiglitazone and combined treatments did not significantly affect the body weight of subjects involved. Therefore, this enabled the examination of metabolic and cardiovascular parameters independent of this variable.

Rosiglitazone had neither a beneficial nor a deleterious effect on bone turnover markers in postmenopausal women with T2D. The addition of HT to rosiglitazone treatment reduced bone turnover, an effect speculated to be afforded primarily by HT. The addition of HT to rosiglitazone treatment had no effect on glucose and insulin metabolism; however, combined treatment adversely affected cardiovascular risk factors, reducing endothelial function to levels apparent prior to rosiglitazone treatment. These results are unsupportive of the proposed hypothesis and indicate that care should be taken when co-administering these treatments. Further in vitro research is required to establish the effects of combined treatment on PPAR receptor binding and metabolite production, and the subsequent effects on vascular endothelial functioning.

10.0 Appendix

Appendix 1A

Victoria University

UR No. | | | | | | | NAME .....

DATE OF BIRTH / / ADDRESS.....

CONSENT FORM FOR RESEARCH PROJECT FOR COMPETENT SUBJECTS

PROJECT No. ..... TITLE:

CHIEF RESEARCHER

1. I, the undersigned ..... hereby consent to my involvement in the above research project.
2. I acknowledge that the nature, purpose and contemplated effects of the project so far as it affects me have been fully explained to my satisfaction by the researcher and my consent is given voluntarily.
3. I have received the Explanatory Statement and am familiar with the nature of the study including the anticipated length of time the study will take, the frequency with which visits and tests will be performed, and an indication of any discomfort which may be expected.
4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.
5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
6. I have been informed that no information regarding my medical history will be divulged to unauthorised persons and that the results of any tests involving me will not be published in such a way as to reveal my identity.
7. I understand that my involvement in the project will not affect my relationship with my medical advisers in their management of my health. I also understand that I am free to withdraw from the project at any stage.
8. I confirm that it has been explained to me that the
   (a) has approved the above project
   (b) ensures that explanations such as I have received conform to ethical standards which this Hospital is required to observe, and
   (c) has officers who may be authorised to contact me to check whether the proper standards are being observed and who are pledged to preserve the confidentiality of my involvement.

Signed ..... this day ...../...../.....

Witness Name.....Signature..... this day ...../...../....

Address.....

Researcher .....Signature.....

Copy - to be filed in patient record

Original: to be kept by principal researcher

Appendix 1B

THE ALFRED HEALTHCARE GROUP

A Member of the Inner & Eastern Health Care Network
Commercial Road, Prahran Victoria 3181

UR No. | | | | | | | NAME .....

DATE OF BIRTH / / ADDRESS.....

CONSENT FORM FOR RESEARCH PROJECT FOR COMPETENT SUBJECTS

PROJECT No. ..... TITLE:

CHIEF RESEARCHER

1. I, the undersigned ..... hereby consent to my involvement in the above research project.
2. I acknowledge that the nature, purpose and contemplated effects of the project so far as it affects me have been fully explained to my satisfaction by the researcher and my consent is given voluntarily.
3. I have received the Explanatory Statement and am familiar with the nature of the study including the anticipated length of time the study will take, the frequency with which visits and tests will be performed, and an indication of any discomfort which may be expected.
4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.
5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
6. I have been informed that no information regarding my medical history will be divulged to unauthorised persons and that the results of any tests involving me will not be published in such a way as to reveal my identity.
7. I understand that my involvement in the project will not affect my relationship with my medical advisers in their management of my health. I also understand that I am free to withdraw from the project at any stage.
8. I confirm that it has been explained to me that the hospital has an Ethics Committee which:

   (a) has approved the above project

   (b) ensures that explanations such as I have received conform to ethical standards which this Hospital is required to observe, and

   (c) has officers who may be authorised to contact me to check whether the proper standards are being observed and who are pledged to preserve the confidentiality of my involvement.

Signed ..... this day ...../...../.....

Witness Name.....Signature..... this day ...../...../....

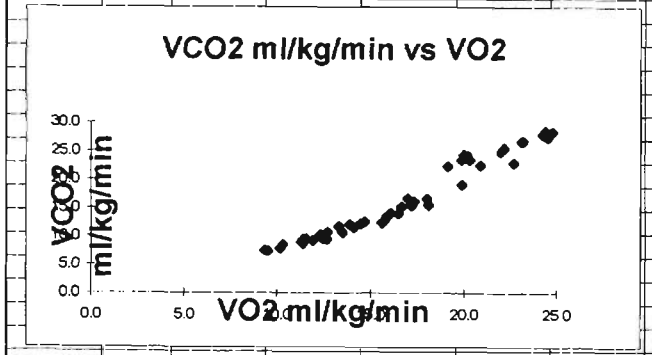
Address.....

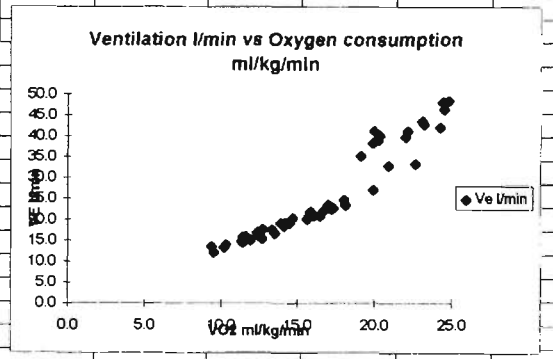
Researcher .....Signature.....

Copy - to be filed in patient record

Original: to be kept by principal researcher

Name:
Date:





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Group data

Time	Work	HR	VO2	VCO2	RER	Ve	Ve/VO2	Ve/VCO2
min	watts	bpm	ml/kg/min	ml/kg/min	units	l/min	units	units
0.25	25		9.3	7.5	0.81	13.8	23.4	28.9
0.5	25		10.2	7.9	0.78	13.5	21.1	27.1
0.75	25		9.5	7.5	0.79	12.3	20.5	26.1
1	25	99	11.4	8.7	0.77	14.7	20.5	26.6
1.25	35		12.7	9.6	0.75	15.6	19.6	25.9
1.5	35		12.5	9.6	0.77	16.1	20.5	26.5
1.75	35		11.9	9.4	0.79	15.3	20.4	25.9
2	35	104	13.5	10.6	0.79	16.9	19.9	25.2
2.25	45		11.3	9.0	0.80	15.0	21.0	26.3
2.5	45		15.6	12.5	0.80	20.3	20.6	25.7
2.75	45		11.6	9.6	0.83	16.1	22.1	26.5
3	45	103	11.4	9.5	0.83	16.0	22.3	26.7
3.25	55		14.1	11.6	0.82	19.3	21.7	26.3
3.5	55		14.1	11.7	0.83	18.7	21.1	25.4

Time	Work	HR	VO2	VCO2	RER	Ve	Ve/VO2	Ve/VCO2
min	watts	bpm	ml/kg/min	ml/kg/min	units	l/min	units	units
3.75	55		10.3	8.7	0.84	14.2	21.8	25.9
4	55	103	12.3	10.3	0.84	17.2	22.1	26.3
4.25	65		15.8	13.1	0.83	21.1	21.2	25.6
4.5	65		12.7	10.9	0.85	17.9	22.4	26.2
4.75	65		14.7	12.7	0.86	20.5	22.2	25.7
5	65	109	14.5	12.3	0.85	19.3	21.1	25.0
5.25	75		15.9	13.6	0.86	22.1	22.2	25.8
5.5	75		13.9	12.2	0.88	19.3	22.0	25.1
5.75	75		13.3	11.8	0.88	17.8	21.2	24.0
6	75	118	16.5	14.2	0.86	21.0	20.2	23.5
6.25	85		18.1	15.6	0.86	23.6	20.7	24.1
6.5	85		16.1	14.2	0.89	21.1	20.9	23.6
6.75	85		17.2	15.5	0.90	22.7	20.9	23.2
7	85	130	16.7	15.2	0.91	22.2	21.2	23.2
7.25	95		18.0	16.8	0.93	24.9	22.0	23.6
7.5	95		17.3	16.2	0.94	23.0	21.0	22.5
7.75	95		19.9	19.1	0.96	27.3	21.7	22.6
8	95	141	17.0	16.8	0.99	23.8	22.2	22.4
8.25	105		22.7	23.0	1.01	33.6	23.5	23.2
8.5	105		20.9	22.6	1.08	33.1	25.1	23.3
8.75	105		24.3	28.0	1.15	42.2	27.6	24.0
9	105	153	20.2	24.3	1.20	39.2	30.8	25.6
9.25	115		19.1	22.4	1.17	35.3	29.3	25.0
9.5	115		23.2	26.8	1.16	42.9	29.4	25.4
9.75	115		20.0	24.5	1.23	41.3	32.8	26.8
10	115	156	19.9	23.5	1.18	38.5	30.8	26.0
10.25	125		22.0	25.0	1.13	39.9	28.8	25.4
10.5	125		22.1	25.6	1.16	41.3	29.6	25.6
10.75	125		23.1	26.8	1.16	43.8	30.1	25.9
11	125	163	24.4	28.5	1.17	48.2	31.4	26.8
11.25	135		20.3	23.5	1.16	40.4	31.5	27.2
11.5	135		24.5	27.4	1.12	46.5	30.1	26.9
11.75	135		24.8	28.4	1.15	48.6	31.1	27.1
12	135	168						
12.25	145							
12.5	145							
12.75	145							

Appendix 3

Week number	Training heart rate (% of AT threshold)	Duration minutes	Frequency Sessions/week
9	70	15	3
10	70	15	3
11	75	15	3
12	75	20	3
13	80	20	4
14	80	25	4
15	85	25	4
16	85	30	4
17	90	30	4
18	90	30	4
19	95	30	4
20	95	30	4

Expressed as a percentage of heart rate at anaerobic threshold (AT) determined at the first treadmill test. * Total time spent training is made up of the duration of exercise at target heart rate plus 5 minutes warm up and cool down period.

Appendix 4

2 DAY RECALL DIET

DAY 1

FOOD ITEM	AMOUNT	COMMENTS
BREAKFAST	cup/slice/grams/spoons	specifications of foods e.g. types,brands
Bread		
Cereal		
Juice		
Milk		
Tea/Coffee		
Eggs		
Other		
LUNCH		
Bread		
Meat		
Cheese		
Salad		
Juice		
Tea/Coffee		
Soup		
Fruit		
Other		
DINNER		
Meat		
Vegetables		
Bread		
Eggs		
Cheese		
Pasta		
Chicken		
Seafood		
Other		
SNACKS		
Yoghurt		
Cheese		
Bread		
Tea/Coffee		
Juice		
Cake		
OTHER FOODS		
VITAMINS		
MINERALS		

Appendix 4

DAY 2

FOOD ITEM	AMOUNT	COMMENTS
BREAKFAST	cup/slice/grams/spoons	specifications of foods e.g. types,brands
Bread		
Cereal		
Juice		
Milk		
Tea/Coffee		
Eggs		
Other		
LUNCH		
Bread		
Meat		
Cheese		
Salad		
Juice		
Tea/Coffee		
Soup		
Fruit		
Other		
DINNER		
Meat		
Vegetables		
Bread		
Eggs		
Cheese		
Pasta		
Chicken		
Seafood		
Other		
SNACKS		
Yoghurt		
Cheese		
Bread		
Tea/Coffee		
Juice		
Cake		
OTHER		
VITAMINS		
MINERALS		

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