MENSTRUAL STATUS AND LONG-TERM CARDIOVASCULAR EFFECTS OF INTENSE EXERCISE IN TOP ELITE ATHLETE WOMEN

Magnus Hagmar

Stockholm 2008
“Go placidly amid the noise and the haste,
And remember what peace there may be in silence.”

“Desiderata”, Max Ehrmann 1927

To all who strive to fulfill their dreams and aspire for higher goals
Menstrual status and long-term cardiovascular effects of intense exercise in top elite athlete women

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Abstract

Although physical exercise is generally beneficial for health, female athletes run an elevated risk of developing chronic energy deficiency, with ensuing severe consequences such as reproductive dysfunction, bone demineralization, more frequent injuries, impaired performance, and adverse cardiovascular effects. However, at present little is known about these issues with respect to sportswomen competing at the Olympic level, or about the long-term cardiovascular consequences of intense training for women.

Our aims were to characterize patterns of weight control in female and male Olympic athletes, as well as to assess the menstrual status, body composition, biomarkers of energy availability and circulating levels of sex hormones in Olympic sportswomen. In addition, cardiovascular function and the associated serum lipid levels and body composition of postmenopausal former elite athletes were compared to these same parameters in control subjects.

Among the 223 Swedish athletes who competed in the 2002 and 2004 Olympic Games, those participating in sports that emphasize leanness demonstrated less desirable strategies for weight and more frequent illness than competitors in other disciplines, particularly in the case of the male athletes. Among 90 of these sportswomen, and especially among the endurance athletes, menstrual dysfunction (MD) was frequent. The most common cause of MD was polycystic ovary syndrome (PCOS), rather than hypothalamic inhibition. Furthermore, no signs of chronic energy deficiency, as evaluated on the basis of body fat content and biomarkers of energy availability, were observed. Bone mineral density (BMD) was generally high and none of these athletes exhibited osteopenia or osteoporosis. Furthermore, among 20 postmenopausal former elite athletes we observed enhanced endothelial function in those not utilizing hormone replacement therapy, whereas the use of such therapy was associated with endothelial function similar to that of sedentary control subjects. Serum levels of cholesterol and low-density lipoprotein, body fat content and the frequency of ST-depressions in exercising electrocardiograms were lower in the former athletes; whereas the exercise capacity, dimensions of the left and right cardiac ventricles, and left atrial and stroke volumes were all significantly greater than in control subjects.

We conclude that the weight control practices employed by Olympic athletes participating in disciplines that emphasize leanness appear to be suboptimal, although female athletes may have adopted healthier nutritional practices than the men. Furthermore, our findings challenge the contemporary concept that reproductive dysfunction in sportswomen is typically a consequence of chronic energy deficiency. Here, the single most frequent underlying cause of menstrual disturbances in Olympic athletes was the hyperandrogenic disorder PCOS. Long-term strenuous exercise is associated with minor changes in cardiac structure, but overall beneficial effects on exercise capacity, vascular function and cardiovascular risk factors.

Key words: Elite athletes, female athlete triad, BMI, energy deficiency, amenorrhea, polycystic ovary syndrome, body composition, bone mineral density, postmenopausal women, endothelial function, athlete’s heart, echocardiography, exercise test.
LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


II. Magnus Hagmar, Bo Berglund, Kerstin Brismar, Angelica Lindén Hirschberg. The presence of hyperandrogenism and high bone mass in female Olympic athletes challenge the concept of chronic energy deficiency in elite sportswomen. Submitted.


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<table>
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<th>Full Form</th>
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<tbody>
<tr>
<td>A-wave</td>
<td>Peak late diastolic transmitral flow velocity</td>
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<td>A4</td>
<td>Androstendione</td>
</tr>
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<td>AH</td>
<td>Athlete’s Heart</td>
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<tr>
<td>BA</td>
<td>Brachial artery</td>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>BSA</td>
<td>Body surface area</td>
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<td>Chol</td>
<td>Cholesterol</td>
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<td>CV</td>
<td>Coefficient of variation</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<td>DE</td>
<td>Doppler echocardiography</td>
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<tr>
<td>DEXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone sulphate</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, ed 4</td>
</tr>
<tr>
<td>DT</td>
<td>Early filling deceleration time</td>
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<tr>
<td>DWR</td>
<td>Desired weight ratio</td>
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<tr>
<td>E-wave</td>
<td>Peak early diastolic transmitral flow velocity</td>
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<td>E2</td>
<td>Estradiol-17β</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EF</td>
<td>Ejection fraction</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>FMD</td>
<td>Flow-mediated vasodilatation</td>
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<td>FSH</td>
<td>Follicle-stimulating hormone</td>
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<tr>
<td>fT4</td>
<td>Free thyroxine</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
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<tr>
<td>HC</td>
<td>Hormonal contraceptives</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<td>HPG</td>
<td>Hypothalamic-pituitary-gonadal</td>
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<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
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<tr>
<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein-1</td>
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<td>IGF-I</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td>IRP</td>
<td>International Reference Preparations</td>
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<tr>
<td>IVRT</td>
<td>Isovolumetric relaxation time</td>
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<tr>
<td>LBM</td>
<td>Lean body mass</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<td>LH</td>
<td>Luteinizing hormone</td>
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<td>Abbreviation</td>
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<tr>
<td>LVOT</td>
<td>Left ventricular outflow tract</td>
</tr>
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<td>MD</td>
<td>Menstrual dysfunction</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>NTG</td>
<td>Nitroglycerine</td>
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<tr>
<td>PCO</td>
<td>Polycystic ovaries</td>
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<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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<tr>
<td>PRL</td>
<td>Prolactin</td>
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<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>RM</td>
<td>Regular menstruation</td>
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<tr>
<td>RPP</td>
<td>Rate-pressure product</td>
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<td>RWV</td>
<td>Relative weight variation</td>
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<td>T</td>
<td>Testosterone</td>
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<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
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<tr>
<td>17OHP</td>
<td>17α-hydroxyprogesterone</td>
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1 INTRODUCTION

Physical activity is well known to promote both physical and mental health for the majority of women engaged in competitive and recreational sports. However, some female athletes have been found to be at increased risk of eating disorders, reproductive dysfunction, bone demineralization, injuries and adverse cardiovascular effects.

1.1 ENERGY DEFICIENCY AND EATING DISORDERS

The intense physical training necessary for top athletic achievement requires a high energy output that many athletes do not match with a corresponding caloric intake. Hence, recent research has clarified that many sportswomen are chronically energy deficient (Loucks 2004).

The reasons for this common discrepancy between energy intake and output include the difficulties involved in eating and digesting large portions of food alongside a demanding training programme, and poor knowledge about nutritional requirements. Another problem for athletes is that biological feedback mechanisms for energy status are unreliable. For instance, appetite does not dependably reflect the caloric deficit induced by an intense training session (Truswell 2001; Loucks 2004).

A more frequent cause for energy deficiency, however, is an exaggerated strive for leanness, i.e., a low body fat mass in relation to a high muscle mass, which is a crucial component of athletic success in many disciplines. For instance, endurance athletes, such as long-distance runners or cycling athletes, and athletes participating in gravity opposing events such as the high jump, typically gain from having a low body mass to displace. Furthermore, athletes involved in events involving weight classes need to very strictly regulate their body fat content and body weight (Steen & Brownell 1990; Oppliger et al. 1996; Dale & Landers 1999).

Eating habits in athletes may be described along a continuous scale, ranging from restrictive behaviour, to severe conditions meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV diagnostic criteria for anorexia nervosa or bulimia nervosa. Symptoms of eating disorders in athletes not meeting the DSM-IV criteria for such conditions are often termed anorexia athletica (AA) (Sundgot-Borgen 2002). According to these criteria, a prevalences of eating disorders in female athletes has been reported between 1 and 62% (Smolak et al. 2000), and as high as 78% in sports where thinness is advantageous (Byrne & McLean 2001). This is to be compared to a prevalence of eating disorders of less than 1% for anorexia nervosa and about 1% for bulimia nervosa in young females in the general population (Faravelli et al. 2006; Hoek 2006).

Female athletes are particularly at risk for disturbed eating, although there is a paucity of literature concerning eating disorders in male athletes (Fogelholm & Hiilloskorpi 1999; Sansone & Sawyer 2005). However, in the general population, eating disturbances are far more frequent in women than in men (Andersen & Holman 1997). Besides optimizing body composition for performance, some female athletes are also under the pressure to attain an appealing appearance. This is particularly true for athletes in aesthetic disciplines, such as gymnastics and figure skating (Ziegler et al. 2005), but is becoming increasingly important in many other disciplines, due to an
intense media coverage and the importance of sponsorship. In summary, women athletes are more susceptible than men to harmful weight control practices and eating disorders (Sundgot-Borgen 1993; Sudi et al. 2004; Sundgot-Borgen & Torstveit 2004).

Even though regulation of body mass and nutritional status is such a critical issue for sportswomen, little is presently known concerning the weight control strategies employed by elite athletes in different disciplines, and in particular at the Olympic level (Brownell et al. 1987; Fogelholm & Hiilloskorpi 1999).

1.2 MENSTRUAL DISTURBANCES

In general, menstrual disturbances in athlete women are common, with prevalences ranging between 6 and 78% (O'Donnell & De Souza 2004; Goodman & Warren 2005; Redman & Loucks 2005; Torstveit & Sundgot-Borgen 2005) compared to 2-5% in the general population (Bachmann & Kemmann 1982; Munster et al. 1992). The actual prevalence is largely dependent on the definitions employed for diagnosing a menstrual disorder, since reproductive dysfunction ranges between mild luteal phase defects, through oligomenorrhea, to long-standing amenorrhea.

Athletic amenorrhea is generally attributed to an inhibition of the hypothalamic-pituitary-gonadal (HPG) axis (Hirschberg & Hagenfeldt 1998). It was previously thought that such an inhibition was due to a low amount of adipose tissue, since fat mass was found to be associated with menstrual function. A relative fat content of at least 17% was suggested as necessary to achieve menarche, and 22% for maintaining regular periods (Frisch & McArthur 1974; Fleck 1983; Frisch 1987). This theory was supported by the observation that the highest occurrence of amenorrhea was found in the athletes with the lowest body fat content, e.g. women involved in endurance and aesthetic disciplines (De Souza 2004; Goodman & Warren 2005; Loucks & Nattiv 2005; O'Donnell & Redman & Loucks 2005; Torstveit & Sundgot-Borgen 2005).

It is now understood that the most important cause for inhibition of the HPG- axis is low energy availability, which leads to a disruption of the GnRH pulsatility (Loucks 2003). This in turn causes a reduced secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) resulting in a low production of sex steroids from the ovaries with ensuing anovulation and oligo/amenorrhea (Figure 1) (Goodman & Warren 2005; Redman & Loucks 2005).

Several mechanisms are involved in the inhibition of the HPG-axis in female athletes. Low energy availability and stress lead to an activation of the hypothalamic-pituitary-adrenal (HPA) axis with increased release of stress hormones, i.e. corticotropin-releasing hormone (CRH) and cortisol which inhibit reproductive function (Lindholm et al. 1995; Laughlin & Yen 1996; Miller et al. 2004). Endorphins, released in response to physical activity, have also been implicated in the CRH-induced inhibition of hypothalamic GnRH secretion (Barbarino et al. 1989). A hypometabolic state in athletes is furthermore reflected by high levels of IGF binding protein (IGFBP-1), low levels of insulin and insulin-like growth factor I (IGF-I), which in turn may decrease LH secretion (Laughlin & Yen 1996). Leptin, a hormone produced in adipocytes, has also been suggested to influence reproductive function and may thus be the link between a low fat mass and disruption of the menstrual cycle (Thong & Graham 1999, Miller et al. 2004). These hormonal pathways are summarized in figure 1.
Figure 1. Endocrine consequences of energy deficiency leading to amenorrhea and bone loss in athletic women. ACTH: adrenocorticotropic hormone; GH: growth hormone; GHRH: growth hormone releasing hormone. For abbreviations and explanations, see text.

Recent research has, however, identified essential hyperandrogenism, such as the polycystic ovary syndrome (PCOS), as an alternative etiology of menstrual dysfunction in endurance athlete women (Constantini & Warren 1995; Rickenlund et al. 2003; Rickenlund et al. 2004). PCOS is the most common hormonal aberration in women of fertile age and is associated with ovarian dysfunction, features of hyperandrogenism and polycystic ovarian morphology (Diamanti-Kandarakis 2008).

In contrast to inhibition of the HPG-axis, which is an acquired condition due to energy deficiency, PCOS requires a genetic predisposition even though it also is dependent on environmental factors. Hyperandrogenism in athletes appears to improve physical performance, as well as to provide protection from the metabolic consequences of estrogen deficiency (Rickenlund et al. 2003).

Despite the importance of endocrine balance for athletic performance, the prevalence of menstrual disorders and their underlying mechanisms have not been fully explored in Olympic athletes.
1.3 BONE LOSS

Bone density and quality are the result of the balance between bone formation and resorption. Mechanical loading of increasing intensity and frequency, promotes the formation of bone (Turner 1998; Meyer et al. 2004). A high lean body mass (LBM) is also related to a high bone mineral density (BMD) (Winters & Snow 2000), since with increasing LBM, the skeleton has to adapt to supporting a larger and stronger muscle mass. Therefore, physical activity and sports, which stimulate muscle growth and expose the skeleton to mechanical forces, promote a high bone density (Madsen et al. 1998; Kohrt 2001; Meyer et al. 2004).

Nutritional status is also an essential factor for bone health. Accordingly, low BMI, energy deficiency and eating disorders are all associated with low BMD and an increased risk of fractures (Wolfert & Mehler 2002). In women, estrogen status is furthermore a major determinant of BMD and involves specific estrogen receptor actions. Suppression of osteoclastic bone resorption and stimulation of osteoblastic bone formation are considered to form the basis for the bone preserving effects of estradiol (Nilsson et al. 2001; Manolagas et al. 2002; Syed & Khosla 2005). As a consequence, BMD, which decreases with advancing age, generally exhibits a distinct decline in connection with the decreased ovarian estradiol production following menopause. Together with other factors, this postmenopausal loss of bone mass contributes to the well known increased risk of fractures in elderly women (North American Menopause Society 2006). Moreover, bone loss is a frequent consequence of amenorrhea and an estrogen deficiency state (De Souza & Williams 2004).

Although athletic activity should promote a strong skeleton, athletes with amenorrhea often exhibit a low bone mass (Warren 1980; Drinkwater et al. 1984). This finding was initially considered as paradoxical. However, since then, numerous studies have lent support for the conclusion that the low BMD of amenorrheic athletes is due to the combined effects of low energy availability and its endocrine consequences (De Souza & Williams 2005). These are summarized in figure 1 and include hypoestrogenism, hypercortisolism with catabolic effects on the skeleton, and low levels of the anabolic hormone IGF-1 (Bennell et al. 1997; Otis et al. 1997; De Souza & Williams 2004; Gibson et al. 2004; Tauchmanova et al. 2007). Such hormonal alterations mainly affect trabecular bone, due to the higher turnover of this tissue compared to the cortical skeleton.

Women athletes in whom menstrual disturbances are instead caused by hyperandrogenism should be at lower risk for a harmful decline in BMD, since testosterone has anabolic effects on the skeleton (Notelevitz 2002). An indication that this assumption is correct was given in the study by Rickenlund and co-workers, who reported similar BMD in hyperandrogenic athletes with menstrual dysfunction compared to regularly menstruating athletes, and higher values than those of normoandrogenic women with oligo-/amenorrhea (Rickenlund et al. 2003).

Body composition including BMD has only been explored to a limited extent in young active athletes at the Olympic level. Also, to our knowledge, there have been no reports on BMD and body composition in postmenopausal, former top elite athlete women.
1.4 THE CONCEPT OF THE FEMALE ATHLETE TRIAD

Disordered eating, amenorrhea and loss of bone mass, together have been termed the "female athlete triad", first introduced in 1992 by the American College of Sports Medicine (ACSM) (Yeager et al. 1993). This triad is particularly prevalent among women involved in sports that emphasize leanness, and is often considered as one of contemporary female athletes’ most serious medical conditions (Otis et al. 1997; De Souza & Williams 2004; Torstveit & Sundgot-Borgen 2005).

The consequences of the Triad may include an increased risk of stress fractures, other musculoskeletal injuries, and reproductive failure, although the latter is reversible if energy availability is restored (Otis et al. 1997; De Souza & Williams 2004; Beals & Meyer 2007). Hence, huge efforts have been made from central sporting organizations to alert the athletic community to these issues, and to educate sportswomen in healthy training and eating habits in order to prevent the development of such symptoms (Nattiv et al. 2007).

Recently, Torstveit and Sundgot-Borgen demonstrated that the prevalence of the Triad in Norwegian female athletes was low and similar to that in a non-athletic control group (4.3% versus 3.4%) (Torstveit & Sundgot-Borgen 2005). These results have raised questions regarding the current clinical importance of the female athlete triad (DiPietro & Stachenfeld 2006).

1.5 THE CARDIOVASCULAR SYSTEM

Exercise generally improves cardiovascular function, promotes a favorable serum lipid profile and body fat content, and reduces blood pressure and levels of stress hormones. Exercise is therefore essential in the prevention of atherosclerotic cardiovascular disease (CVD) (Thompson 2003).

1.5.1 Endothelial function

The endothelium was long thought to constitute a passive part of the blood vessel wall, however, it is now understood that the endothelium is indispensable for vascular homeostasis. Pathologic alterations in endothelial function are induced by factors such as hypertension, hyperlipidemia, hyperglycemia, smoking, and age (Figure 2) (Bonetti et al. 2003; Endemann & Schiffrin 2004), and represent an early step in the complex development of atherosclerosis (Schachinger et al. 2000; Bonetti et al. 2003; Quyyumi 2003; Endemann & Schiffrin 2004).

The status of the endothelium can be assessed by measuring endothelium-dependent vasodilatation, i.e., the degree to which the endothelium induces dilatation of the blood vessel as a healthy response to various stimuli. An impaired endothelium-dependent vasodilatation constitutes an early risk marker for CVD.

Exercise directly improves endothelial function, mainly via an enhanced production of nitric oxide (NO), which is the foremost endothelium-derived vasodilator (Haram et al. 2008). Training also indirectly promotes vascular health by reducing the risk factors for CVD. The beneficial effects of exercise in this respect have been verified in several studies. For instance, improved endothelial function has been demonstrated in older male athletes, thus suggesting a reduction of cardiovascular morbidity in these
individuals (Jensen-Urstad et al. 1999; Rywik et al. 1999; Rinder et al. 2000; Taddei et al. 2000; Franzoni et al. 2005).

**Figure 2.** Cardiovascular risk factors affecting endothelial function. Enhancement is indicated by +, and impairment by –.

In women, estrogen status is one of the major determinants of endothelial function. In this context estrogen promotes rapid vasodilatation, primarily by directly activating the production of NO in endothelial cells, as well as by causing long-term, receptor-mediated up-regulation of NO synthase (Cid et al. 2002; Mendelsohn 2002). Estrogen also exerts indirect beneficial effects on endothelium-dependent vasodilatation by favorably influencing the serum lipid profile (Schnaper et al. 2000; Mendelsohn 2002). Hence, while in men progressive deterioration of endothelial function is associated with advancing age, in women similar deterioration is observed in connection with menopause (Sarabi et al. 1999), causing the well-documented increase in morbidity and mortality due to CVD following menopause (Kannel et al. 1976). There are indications that endothelial dysfunction in postmenopausal women can be reversed by hormone replacement therapy (HRT) involving estrogen alone or in combination with a progestin (Herrington et al. 2001; Wild & Reis 2001; Davis et al. 2002).

Furthermore, recent investigations on amenorrheic athletes have detected impaired endothelial function in such individuals similar to that observed in postmenopausal women (O’Donnell & De Souza 2004; Rickenlund et al. 2005; Hoch et al. 2007). It is thus possible that the deleterious cardiovascular consequences of athletic amenorrhea may, with advancing age, outweigh the protective effects of exercise on the vascular
In this context, it has also been suggested that endothelial dysfunction could be regarded as a fourth component of the athlete triad (Rickenlund 2004; Hoch et al. 2007).

Only a few investigations regarding exercise and endothelial function have been carried out on older women, indicating favorable effects of physical activity in such individuals (Harvey et al. 2005a,b; McKechnie et al. 2001). Yet, to our knowledge, there have been no previous reports concerning endothelial function in senior female athletes.

1.5.2 Cardiac structure and function

The concept of that the cardiovascular system of trained athletes differs structurally and functionally from the normal general population has existed over a century. Henschen is credited with the first description in 1899 of what we today recognise as the “Athlete’s heart” (AH), using only a basic physical examination with careful percussion to recognize enlargement of the heart caused by athletic activity in cross-country skiers (Henschen 1899). Henschen concluded that dilatation as well as hypertrophy were present, involving both the left and right side of the heart, and that these changes were normal and favorable: “Skiing causes an enlargement of the heart which can perform more work than a normal heart” (Henschen 1899).

Indeed, subsequent studies showed that long-term athletic training involving large muscle groups is associated with dimensional and structural changes of the heart, such as ventricular dilatation, increased ventricular wall thickness and mass (Mumford & Prakash 1981; Fagard 1996; Erol & Karakelleoglu 2002), and functional changes, such as increased stroke volume and decreased heart rate (Blomqvist & Saltin 1983). These cardiac features, termed “athlete’s heart” (AH), are generally regarded as benign, physiological adaptations to the demands of strenuous exercise (Pelliccia et al. 1999) and have been well-described in both young male (Urhausen et al. 1996; Scharhag et al. 2002) and young female athletes (Pelliccia et al. 1996; Urhausen et al. 1996; Pelliccia & Maron 1997; Stolt et al. 2000).

Only a few studies with small number of subjects have assessed cardiac structure and function in senior sportsmen (Di Bello et al. 1993; Seals et al. 1994; Giada et al. 1998; Hood & Northcote 1999; Bouvier et al. 2001). These studies have demonstrated larger cardiac dimensions (Seals et al. 1994; Giada et al. 1998), increased posterior wall thickness and interventricular septum thickness (Giada et al. 1998; Bouvier et al. 2001), and increased left ventricular mass (Di Bello et al. 1993; Giada et al. 1998) than in age-matched controls. Also, improved systolic and diastolic left ventricular function have been described in senior male sportsmen after life-long training (Bouvier et al. 2001).

There is a relative paucity of echocardiographic studies on cardiac effects of training in elderly women. In a recent study, Macchi et al. found no differences in cardiac measurements between sedentary and non-sedentary elderly women (Macchi et al. 2003). Spina et al. (1993) could not demonstrate any change in left ventricular size in older women after a 9- to 12-months training program. None of these investigations included athletes. Furthermore, there have been no investigations of the effect of long-term endurance training on cardiac dimensions and functional capacity in postmenopausal sportswomen.
2 AIMS OF THE THESIS

The general aim of this thesis was to investigate the effects of intense exercise on metabolic, endocrine and cardiovascular parameters in top elite athlete women of fertile and postmenopausal age.

The specific aims of the thesis were:

- To characterize patterns of weight control used by female and male Olympic athletes competing in different disciplines.

- To examine menstrual status, body composition, biomarkers of energy availability and circulating levels of sex hormones in female Olympic athletes participating in different disciplines.

- To investigate endothelial function in relation to serum lipids and body composition in postmenopausal former top endurance athletes in comparison to sedentary control subjects.

- To assess cardiac structure and function in postmenopausal former top endurance athletes compared with age-matched sedentary controls.
3 MATERIALS AND METHODS

3.1 SUBJECTS AND STUDY DESIGN

3.1.1 Paper I

3.1.1.1 Subjects

All of the 224 Swedish Olympic athletes who participated either in the Winter Olympic Games in Salt Lake City in 2002 (n=106) or in the Summer Olympic Games in Athens in 2004 (n=117) were asked by the chief physician of the Swedish Olympic Team, Dr. B. Berglund, to complete a questionnaire regarding health issues, within two weeks prior to the respective Olympic game. Two hundred and twenty-three of these athletes (125 men and 98 women, only 1 drop-out, i.e., 99.6% participation) volunteered for the study.

In connection with the Olympic Games of 2002, 24 of these athletes won a total of 7 medals (2 silver and 5 bronze, with one of the bronze medals being awarded collectively to the 20 members of the women’s ice hockey team). In 2004, 16 of these Swedish athletes won a total of 11 medals (4 gold, 2 silver and 5 bronze).

This study was approved by the Committee of Ethics at the Karolinska University Hospital.

3.1.1.2 Study design and investigational setting

The following information was obtained from the study questionnaire: sporting discipline; gender; present body weight and height, as well as maximal and minimal weights during the preceding 12 months; desired body weight; eating strategies (i.e., “Have you attempted to lose weight? Y/N” (during the past 12 months), “Have you attempted to gain weight? Y/N” (during the past 12 months), “Have you previously had an eating disorder? Y/N”, “Are you currently suffering from an eating disorder? Y/N”, “Have you consulted a dietician? Y/N”); exercise time (h) and exercise load (on a subjective scale ranging from 2 = very, very light, 4 = very light, 6 = light, 8 = medium, 10 = heavy, 12 = very heavy and 14 = very, very heavy) during the preceding 12 months; medical conditions resulting in interruption of training (“Have you been ill for one week or more during the past 3 months?”, and injuries (“Have you suffered an injury that caused you to stop training for longer than 1 week during the past 3 months?”).

This information was utilized to calculate body mass index (BMI) (weight (kg)/height (m)²); weight variation during the 12 months immediately prior to Olympic competition (Relative Weight Variation (RWV) = ((maximum weight - minimum weight)/(maximum weight + minimum weight)/2) x 100); as well as a ratio between the desired and actual weights (Desired Weight Ratio (DWR) = ((actual weight - desired weight)/actual weight) x 100). A positive DWR indicates that the athlete weighed more than desired, a negative DWR that he/she weighed less than desired and a value close to zero indicates satisfaction with his/her actual weight.

In order to compare athletes who typically gain from attaining a low body fat mass, i.e., being lean, to those who do not, we used a classification that has been employed previously (Sundgot-Borgen 1993; Torstveit & Sundgot-Borgen 2005). According to
this classification, the 37 sports that Sweden represented in the Olympic Games of 2002 and 2004 were grouped into leanness and non-leaness groups (Table 1).

Table 1. Classification of the Olympic athletic events into two groups, based on whether the sporting discipline emphasizes leanness or not.

<table>
<thead>
<tr>
<th>Leanness disciplines</th>
<th>Non-leaness disciplines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-country skiing/biathlon</td>
<td>Alpine skiing</td>
</tr>
<tr>
<td>Speed skating</td>
<td>Snowboarding</td>
</tr>
<tr>
<td>Middle/long-distance running, Cycling</td>
<td>Ice hockey</td>
</tr>
<tr>
<td>Canoeing/rowing, Gymnastics</td>
<td>Bob/luge</td>
</tr>
<tr>
<td>Equestrian-jumping, Wrestling/judo</td>
<td>Soccer</td>
</tr>
<tr>
<td>Diving</td>
<td>Raquet sports</td>
</tr>
<tr>
<td>Technical athletics, Boxing</td>
<td>Swimming</td>
</tr>
<tr>
<td>Modern pentathlon</td>
<td>Athletic sprint</td>
</tr>
<tr>
<td></td>
<td>Beach volleyball</td>
</tr>
<tr>
<td></td>
<td>Curling</td>
</tr>
<tr>
<td></td>
<td>Equestrian-dressage</td>
</tr>
<tr>
<td></td>
<td>Sailing</td>
</tr>
<tr>
<td></td>
<td>Shooting/archery</td>
</tr>
</tbody>
</table>

3.1.2 Paper II

3.1.2.1 Subjects

This study was performed as a collaboration between the Swedish Olympic Committee and their chief physician (Dr B. Berglund) and official gynecologist (Dr A Lindén Hirschberg). The recruitment of subjects was initiated at the Swedish pre-Olympic training camp in 2001, where all of the female athletes received written information concerning this study. Oral information in connection with competition events and training camps was also given by the authors on several occasions. Subsequently, a number of the athletes of at least 18 years of age and considered to be representative of the Swedish participation in the summer and winter Olympic Games were approached in person. Of those invited to participate, 22 (20%) declined, giving a busy schedule, together with living, training and competing at distant locations as the primary reasons. In the end, 90 of Sweden’s top elite female athletes were included in this study.

The athletes represented a total of 27 disciplines, and were divided into three sport groups: Power (i.e., disciplines involving high mechanical load and short bouts of intense exertion), Endurance (i.e., disciplines involving prolonged periods of submaximal exertion and low mechanical load, and Technical (i.e., disciplines that emphasize technical skills and demanding low physical exertion and low mechanical load).

All of these athletes were subjected to urine tests for doping at several random times each year, in accordance with the initiative and decisions made by the WADA, IOC.
International Sports Federation and the organization Antidoping Sweden. None had tested positively for androgens or other unallowed substances.

The study protocol was approved by the local ethics committee of the Karolinska University Hospital, and informed consent was given by all the subjects.

3.1.2.2 Study design and investigational setting

In connection with a visit to the Women’s Health Clinical Research Unit at the Karolinska University Hospital in the morning following an overnight fast, body weight and height were recorded and a detailed medical and gynecological history obtained. Menstrual status was categorized as: amenorrhea (absence of menstruation during at least the previous three months), oligomenorrhea (5-9 periods during the past year occurring at intervals greater than six weeks) or regular menstruation (RM) (consistent periods at an interval of 22-34 days). Subjects in the first two categories were defined as exhibiting menstrual dysfunction (MD). In addition, all of the subjects estimated the degree of their physical training (on a subjective scale ranging from 2 = very, very light, 4 = very light, 6 = light, 8 = medium, 10 = heavy, 12 = very heavy and 14 = very, very heavy) during the week preceding the visit.

A gynecological examination (including ultrasonography with the Siemens Sonoline SI-250 equipped with a transvaginal transducer operating between 5.0 – 7.5 MHz) was performed. The ovarian parameters assessed were the maximum number of follicles present in a single plane and total volume (calculated as described previously (Goswamy et al. 1983)). Criteria for polycystic ovaries (PCO) were findings of at least one ovary demonstrating 12 or more follicles in one plane and/or a volume of >10 ml, in accordance with the Rotterdam criteria (Rotterdam Consensus 2004) (Figure 3).

![Endovaginal ultrasound scan, showing an ovary with typical PCO-morphology.](image.png)

A fasting peripheral venous blood sample was collected from each woman in the morning, while resting. In women not utilizing hormonal contraceptives, blood sampling was performed early in the follicular phase of the menstrual cycle (days 1-5) if they were menstruating, or otherwise, on an arbitrary day. Blood was drawn from women using hormonal contraceptives (HC, defined as any contraceptive regimen involving sex steroids, i.e., preparations of ethinyl estradiol and gestagen or gestagen...
only methods) either at the end of an active period of treatment, if the HC was used cyclically, or on an arbitrary day, if use was continuous. Sera, obtained by centrifugation, were stored at -20°C until being assayed.

In accordance with the Rotterdam consensus, a diagnosis of PCOS was made when at least two of the following phenomena were present: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and/or 3) detection of PCO by ultrasound (Rotterdam Consensus 2004).

Following clinical examination and collection of serum, body composition in terms of fat mass, lean body mass and bone mineral density (BMD, g/cm²) was determined by dual energy X-ray absorptiometry (DEXA) (see below).

3.1.3 Papers III and IV

3.1.3.1 Subjects

Twenty healthy, postmenopausal endurance female athletes were included in this study. Possible candidates living in the Stockholm area were identified using the membership records of Sweden’s athletic societies, which list a total of approximately three million members of all ages and both sexes. Thirty-seven women fulfilled the inclusion criteria of being postmenopausal former top elite athletes, and were subsequently invited to participate in the study.

Of the 20 subjects thus recruited, 19 were former endurance athletes (17 runners, one swimmer and one cross-country skier) and one a downhill skier. This group constituted a sample of Sweden’s former top elite female athletes, including 9 women (50%) who had won one or more medals in international competitions. One of the participants was a former world record-holder, and another had won two Olympic gold medals. At the peak of their careers, these women performed aerobic training for 8 h each week (median; range= 5 –24 h/week). At the time of our investigation, all but two were still training regularly at a recreational level in the same disciplines in which they had competed, i.e. they did primarily aerobic endurance training (median = 6 h/week; range = 1.7-10 h/week).

An age-matched control group of 19 healthy non-athletic postmenopausal women was recruited using local advertising at the Karolinska University Hospital. All of these subjects, none of whom performed more than two hours of light aerobic exercise each week, were healthy, with no previous history of cardiovascular disease.

In both groups of athletes and controls, half of the women were on HRT of combined estrogen and gestagen type. None of the subjects were taking any other medication and all were non-smokers.

The study was approved by the Local Ethics Committee at the Karolinska University Hospital, and informed consent was given by the subjects.

3.1.3.2 Study design

All women were investigated in the morning at the Women’s Health Research Unit at the Karolinska University Hospital. Detailed medical and gynecological histories were taken and the subjects were considered to have had amenorrhea if they had experienced
one or more absences of menstruation for a period of three months or longer. In connection with a routine physical examination, the subjects’ heights and weights were measured. Body surface area (BSA) was calculated since heart dimensions correlate to this value. Blood samples for analysis of lipids were collected from a peripheral vein in a fasting, resting state and the sera, obtained by centrifugation, stored at -20°C until being assayed.

Approximately two hours after a low-fat breakfast, endothelial function was assessed by brachial artery flow-mediated vasodilatation (FMD), a Doppler echocardiography, and a standard 12-lead electrocardiogram (ECG) at rest and during a bicycle exercise test (see below).

For analysis of parameters dependent on HRT, the athlete and control groups were each subdivided into two groups on the basis of utilization or non-utilization of HRT.

3.2 BODY COMPOSITION

Body composition in terms of fat mass, LBM and BMD was determined by DEXA using the Lunar Prodigy (Lunar Corp., Madison, WI) for paper II and the Hologic® QDR/4500A, scanner ID 45022 (Hologic®, Bedford, MA) for Paper III.

The softwares of these scanners automatically calculate the amount of fat in the trunk and legs, (the limit between these regions being defined by the line drawn from the upper margin of the iliac crest to the neck of the femur. The ratio of trunk/leg fat mass was taken as an estimate of the ratio of upper/lower body fat mass.

From the whole body DEXA, spinal BMD was also determined. The spinal subregion consisted of the lower portion of the cervical spine, together with the thoracic and most of the lumbar spine (approximately L1 - L4). T- and Z-scores were calculated from the mean BMD and their standard deviations (SD) values supplied by the manufacturer of the scanners. The reproducibility in determination of whole body BMD in this manner has been calculated to be <0.01g/cm² or 0.1 x SD (Nuti et al. 1991; Brismar & Ringertz 1996).

3.3 ENDOTHELIAL FUNCTION

Examinations of endothelial function were performed at the Department of Clinical Physiology at Karolinska University Hospital. Flow velocity in the brachial artery (BA), endothelium-dependent FMD and endothelium-independent nitroglycerin (NTG)- induced dilatation were examined according to the non-invasive procedure first described by Celermayer et al. (Celermajer et al. 1992; Corretti et al. 2002), employing a high-resolution scanner (Acuson 128 XP/10c, Acuson, Mountain View, California, USA) equipped with a 7-MHz linear array transducer.

The left BA was scanned longitudinally 1-10 cm above the elbow, where a clear image was obtained with the artery placed horizontally across the screen. Baseline measurements of blood flow and the inner diameter of the BA were performed at rest. For examination of reactive hyperemia, blood velocity was measured immediately after release of distal forearm artery occlusion (with a 12.5-cm cuff inflated to a pressure of
300 mm Hg for 4.5 minutes) and the diameter of the artery determined 50 to 60 s following cuff deflation. The BA diameter was measured once again after a 10-minute rest, followed by administration of 0.4 mg nitroglycerin (NTG) sublingually. Four minutes later, blood velocity and the diameter measurements were repeated (Figure 4). In order to minimize variability, one experienced investigator performed all of these procedures.

![Figure 4. Assessment of flow-mediated vasodilatation. The brachial artery inner diameter is measured before and after distal forearm occlusion (Reactive Hyperemia), and after administration of nitroglycerin (NTG).](image)

All analyses of the BA diameters were performed off-line and “blind” by one investigator. Three consecutive late-diastolic frames taken at the R-wave of the electrocardiogram were analyzed at rest (baseline) and following different forms of stimulation and the mean diameter calculated. Blood flow was calculated on the basis of Doppler velocity, vessel diameter and heart rate. The increase in blood flow associated with reactive hyperemia is expressed as a percentage of the basal flow value. The intra-individual variations between two separate determinations of FMD performed on the same or different days were 0.88 ± 0.82% and 3.3 ± 2.7%, respectively, as previously reported (Lundman et al. 2001).

### 3.4 DOPPLER ECHOCARDIOGRAPHY

Transthoracic Doppler echocardiography (DE) was performed by one experienced operator, unaware of the subjects’ study group, using Acuson 128 XP/10 ultrasound equipment (Acuson, currently Siemens, Mountain View, California, USA) with a 2.5-4.0 MHz imaging transducer (Figure 5). Complete colour, pulse-wave and continuous-
wave Doppler measurements were carried out. M-mode measurements of the left ventricular dimensions and wall thickness were performed. Left ventricular mass was calculated from M-mode measurements according to the recommendations of the American Society of Echocardiography (Sahn et al. 1978; Devereux et al. 1986). Left ventricular systolic function was evaluated from the two-dimensional images and ejection fraction (EF) calculated using Simpson’s’ rule (Schiller et al. 1989). The diameter of the left ventricular outflow tract (LVOT) was measured from the parasternal long-axis view in early systole. The left atrial volume was calculated using the “length-diameter ellipsoid” method (Weyman 1994). All cardiac dimensions and volumes were also normalized for BSA. Left ventricular diastolic function was assessed from the left ventricular filling pattern using pulsed Doppler recordings of the mitral flow velocity.

The following variables were measured: peak early diastolic transmitral flow velocity (E-wave), peak late diastolic transmitral flow velocity (A-wave), early filling deceleration time (DT) and isovolumetric relaxation time (IVRT). A ratio between E-wave and A-wave (E/A ratio) was calculated. Pulmonary venous flow velocities at systole and diastole were obtained by placing a pulsed Doppler sample volume in the right paraseptal vein from the apical chamber view. A systolic to diastolic pulmonary venous flow velocity ratio was calculated. The flow velocity in the LVOT was recorded with pulsed Doppler, approximately 0.5 to 1 cm proximal to the aortic valve. Velocity time integral in the LVOT, together with two-dimensional measurements of the LVOT area were used to calculate stroke volume (Weyman 1994). All measurements are presented as the average of three consecutive cardiac cycles. Calculations were made off-line, with the operator unaware of the subjects’ study group.

**Figure 5.** Echocardiographic image showing parasternal long axis view (A) and apical four chamber view (B) of the normal heart. AO - aorta, LA – left atrium, LV – left ventricle, RA – right atrium, RV – right ventricle. LV diastolic diameter is indicated by yellow arrow, red arrows indicate thickness of interventricular septum and posterior wall.
3.5 RESTING ELECTROCARDIOGRAM AND EXERCISE STRESS TEST

A symptom-limited stress test was performed on an electrically braked bicycle (Megacart 840, Siemens-Elema, Solna, Sweden), increasing the workload stepwise by 10 W/min from the starting load of 30 W/min for all but one subject. In this subject, a former athlete, the working load was increased by 20 W/min from a starting load of 50 W/min. The exercise test was interrupted when maximal perceived exertion, graded with psycho-physical scaling (Borg 1982), occurred. Predicted maximal heart rate was defined by 220 minus age.

A continuous signal-averaged 12-lead ECG was recorded before, during and until 10 minutes after exercise. The systolic and diastolic blood pressures were measured every 2 min in the right arm. The maximum systolic blood pressure refers to the blood pressure taken during the last minute of cycling. The same investigator performed all the exercise tests, unaware of the subjects study group.

Resting ECG was analyzed for rhythm and presence or absence of left ventricular hypertrophy using the Sokolow-Lyon index (Sokolow & Lyon 2001). Exercise-induced ST-segment depressions were considered as pathologic if they were ≥ 1 mm J point depression, horizontal or downsloping and occurred in at least two adjacent ECG leads. All ECG recordings were analysed by one experienced investigator unaware of the subjects’ group. Results with unclear interpretation were evaluated by other experienced investigators to reach consensus.

Rate-pressure product (RPP = heart rate (bpm) x systolic blood pressure (mm Hg)) was calculated at rest and peak exercise. The change in RPP was expressed as the difference between RPP at peak exercise and RPP at rest. The maximum workload attained during exercise was compared to the references values for physical work capacity on a bicycle ergometer for healthy Swedish women (Farazdaghi & Wohlfart 2001). The expected maximum work-load was calculated for each subject according to the following formula: Max load expect (W) = (137.7 * Height (m) – 23.1) / (1 + exp (0.064 * (Age (years) –75.9)) (Farazdaghi & Wohlfart 2001). For each group the attained load was also expressed as a percentage of the expected maximum load.

3.6 ANALYTICAL METHODS

3.6.1 Pituitary, gonadal, adrenal and thyroid hormones

Serum levels of FSH, LH, sex hormone-binding globulin (SHBG), prolactin (PRL), thyroid-stimulating hormone (TSH), free thyroxine (T4) and dehydroepiandrosterone sulfate (DHEAS) were determined by enzyme immunoassays involving direct chemiluminescence, using commercial kits procured from Siemens Medical Solutions Diagnostics (Immulite®; Los Angeles, CA) in all cases except DHEAS, where the kit was obtained from Roche Diagnostics GmbH (Modular®; DHEAS; Mannheim, Germany). The concentrations of FSH, LH, PRL and TSH were determined as units per liter (on the basis of comparison to the 2nd FSH International Reference Preparations (IRP) 78/549 and 1:st IRP LH 68/40), as micrograms per liter (using the 3rd PRL IRP 84/500) and as milliunits per liter (with the 2nd TSH IRP 80/558), respectively. Serum levels of testosterone (T), androstenedione (A4), 17α-hydroxyprogesterone (17OHP) and estradiol-17β (E2) were determined by radioimmunoassay (RIA) using commercial kits purchased from Siemens Medical Solutions Diagnostics (T and A4; “Coat-a-
The ratio between total serum T and SHBG (the T/SHBG ratio or ‘free androgen index’) is considered to be a useful indicator of T activity in women (Kapoor et al. 1993; Rotterdam Consensus 2004) and was therefore calculated here. A T/SHBG ratio > 0.050 was classified as biochemical hyperandrogenism. LH values of < 2 U/l were considered to indicate inhibition of the HPG axis.

### 3.6.2 Biomarkers of energy availability

The biomarkers of energy availability analyzed here were the serum levels of insulin, IGF-I, IGFBP-1, cortisol and glucose. Insulin was measured with a commercial enzyme-linked immunosorbent assay (ELISA) (DakoCytomation Ltd, Cambridgeshire, UK) and expressed as milliunits per liter (in comparison to IRP 66/304). Following separation from IGFBPs by acidic ethanol extraction and cryoprecipitation, IGF-I was determined by RIA, utilizing des (1-3) IGF-I as the radioligand in order to minimize interference from any remaining IGFBPs (Bang et al. 1991). The concentrations of IGFBP-1 in serum samples were determined by RIA according to Póvoa and co-workers (Póvoa et al. 1984). Serum cortisol was quantified by enzyme immunoassay involving direct chemiluminescence, using a kit obtained from Siemens Medical Solutions Diagnostics (Immuliite®; Los Angeles, CA). Finally, glucose was determined utilizing a glucose analyzer (YSI2300 Stat plus, Yellow Springs, OH) based on an enzymatic electrochemical procedure.

The lower limits of detection, and intra- and interassay coefficients of variation for these procedures are presented in Table 2.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Manufacturer</th>
<th>Detection limit</th>
<th>Within assay CV</th>
<th>Between assay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>CEIA</td>
<td>Siemens</td>
<td>0.1 U/L</td>
<td>8.0%</td>
<td>8.0%</td>
</tr>
<tr>
<td>LH</td>
<td>CEIA</td>
<td>Siemens</td>
<td>0.7 IU/L</td>
<td>6.0%</td>
<td>9.0%</td>
</tr>
<tr>
<td>T</td>
<td>RIA</td>
<td>Siemens</td>
<td>0.1 nmol/L</td>
<td>6.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>SHBG</td>
<td>CEIA</td>
<td>Siemens</td>
<td>0.2 nmol/L</td>
<td>6.5%</td>
<td>8.7%</td>
</tr>
<tr>
<td>A4</td>
<td>RIA</td>
<td>Siemens</td>
<td>0.14 nmol/L</td>
<td>3.2%</td>
<td>5.6%</td>
</tr>
<tr>
<td>DHEAS</td>
<td>CEIA</td>
<td>Roche</td>
<td>0.003 µmol/L</td>
<td>2.4%</td>
<td>3.3%</td>
</tr>
<tr>
<td>T(10HP)</td>
<td>RIA</td>
<td>CIS Bio</td>
<td>0.9 nmol/L</td>
<td>7.8%</td>
<td>12.0%</td>
</tr>
<tr>
<td>E2</td>
<td>RIA</td>
<td>Orion</td>
<td>5 pmol/L</td>
<td>3.0%</td>
<td>6.0%</td>
</tr>
<tr>
<td>PRL</td>
<td>CEIA</td>
<td>Siemens</td>
<td>0.2 µg/L</td>
<td>2.6%</td>
<td>7.2%</td>
</tr>
<tr>
<td>TSH</td>
<td>CEIA</td>
<td>Siemens</td>
<td>0.002 mIU/L</td>
<td>5.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>fT4</td>
<td>CEIA</td>
<td>Siemens</td>
<td>1.9 pmol/L</td>
<td>4.5%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Insulin</td>
<td>ELISA</td>
<td>Dako</td>
<td>3 pmol/L</td>
<td>6.7%</td>
<td>7.5%</td>
</tr>
<tr>
<td>IGF-I</td>
<td>RIA</td>
<td>In house</td>
<td>6 µg/L</td>
<td>4.0%</td>
<td>11.0%</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>RIA</td>
<td>In house</td>
<td>3 µg/L</td>
<td>3.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Cortisol</td>
<td>CEIA</td>
<td>Siemens</td>
<td>5.5 nmol/L</td>
<td>5.8%</td>
<td>6.3%</td>
</tr>
</tbody>
</table>
3.6.3 Serum lipids

Serum triglycerides (TG), total cholesterol (Chol) and high-density lipoprotein (HDL) were determined enzymatically employing kits from Beckman Coulter, Inc., Fullerton, CA (SYNCHRON LX® Systems). Low-density lipoprotein (LDL) was then calculated using the Friedewald formula (Friedewald et al. 1972). The limits of detection and coefficients of variation within and between assays were 0.1 mmol/L, 2.3% and 3.1% for TG; 0.13 mmol/L, 1.1% and 1.6% for Chol; and 0.13 mmol/L, 3% and 4.5% for HDL.

3.7 STATISTICAL ANALYSES

The data were analyzed utilizing the Statistica™ 6.1 and 8.0 softwares (StatSoft® Inc., Tulsa, OK, USA). Groups were compared employing the unpaired t-test (I-IV) or Mann-Whitney U-test (I-IV) depending on the statistical distribution. Categorical variables were compared utilizing the Fischer exact test (I-IV). Where appropriate, the values for groups and subgroups were compared employing factorial ANOVA (I-IV) and ANCOVA (II, III), and Fischer LSD post-hoc analyses. Correlations between variables were assessed with Spearman’s rank-order correlation. A multiple regression model was designed for determination of factors that correlated to BMD (II). Regression coefficients are expressed as Standardized b (b_1). Analysis of covariance was used to adjust for the effect of BSA (IV). Variables exhibiting significant skewness were transformed to logarithms prior to analysis, where appropriate. P-values of < 0.05 were considered to be statistically significant.
4 RESULTS AND DISCUSSION

4.1 WEIGHT CONTROL PRACTICES AND NUTRITIONAL STATUS

4.1.1 Weight control practices

Female and male Olympic athletes participating in leanness disciplines demonstrated lower BMI than those in non-leanness disciplines (22.7 ± 2.7 versus 23.7 ± 2.3, p<0.05) (I), which is an obvious consequence of their conscious efforts to gain a competitive advantage, as has been described previously (Sundgot-Borgen 1993). Leanness athletes also had larger variations in weight (5.3% versus 4.7%, p<0.05), more time spent training (p<0.001) and a heavier reported training load than did those in non-leanness disciplines (p<0.05). In addition, more of the athletes involved in leanness disciplines had attempted to lose weight during the 12 months prior to competition (p<0.001), but still appeared to perceive their efforts as insufficient, since more of them were heavier than they desired at the time of the Olympic Games in comparison to athletes participating in non-leanness sports (p<0.05).

These findings may reflect a less than optimal eating behavior in these leanness athletes of both sexes. In support of such an assumption, a significantly higher proportion of the female and male leanness athletes had previously suffered from an eating disorder (p<0.05). Although none of our subjects reported an on-going eating disorder, we believe that athletes with such a disorder might well have refrained from stating so. It is also noteworthy that the athletes in our study who were involved in leanness sports had consulted a dietician to a lesser extent than did the other athletes (54.7% versus 73.5%, p<0.01), which might possibly reflect an unwillingness to be questioned concerning inadequate eating habits, which is typical for individuals with anorectic tendencies.

Our findings are consistent with the numerous previous investigations that have concluded that eating disorders are common among participants in sports in which leanness improves performance (Byrne & McLean 2002; Sundgot-Borgen & Torstveit 2004). Surprisingly, however, the differences between athletes participating in leanness and non-leanness disciplines with respect to BMI, variations in weight, attempts to lose weight, exercise load and frequency of illness were statistically significant only for the men. However, with the exception of weight variation over the past 12 months, the women demonstrated similar tendencies.

One obvious interpretation is that the smaller size of our female study population may have prevented these differences from reaching statistical significance. At the same time, although the negative impact of exaggerated weight control on women has been well characterized, the vulnerability of male athletes to harmful dietary and training habits has not received the same degree of attention (Fogelholm & Hiilloskorpi 1999; Sansone & Sawyer 2005).

Thus, another possibility is that the female athletes involved in the present study had adopted healthier nutritional practices than the men. We therefore conclude that while there still is a need for attention to the weight control strategies of athletes in general, these habits may have improved in women.
4.1.2 Indices of nutritional status

Among female Olympic sportswomen, the lowest body fat content was seen in endurance athletes (II). The normal range for body fat content in sedentary, healthy women is considered as being 20-25% of the total body mass (Frisch 1987). This was the case for 39% of the athletes in our study, whereas 33% had values below and 28% above this interval. Also, BMI ranged between 18.8 and 26.6 kg/m², which is very close to the normal range as defined by the World Health Organization (18.5 to 24.9 kg/m²) (World Health Organisation 2000). Athletes with menstrual disturbances did not differ in body fat content compared to their eumenorrheic counterparts (Figure 6).

![Figure 6. Body fat content of Olympic athletes with regular menstruation (RM) and menstrual dysfunction (MD). The difference in median values between the two groups was not significant (p=0.21).](image)

In the case of the postmenopausal endurance athletes, body fat content ranged between 18.1% and 41.8% and averaged 28.3%, which was significantly lower than in controls (p<0.05) (III). Furthermore, their BMI ranged between 18.6 and 27.6 kg/m² versus 20.4 and 31.3 kg/m² in controls (p<0.05). Thus, the postmenopausal athletes had a healthier BMI and body fat content than the sedentary women.

Interestingly, our findings concerning body fat content in young, active female athletes (II) differed from those obtained in previous studies of Olympic athletes. In an investigation from 1983 based on two hundred and ninety-eight women participating in 15 Olympic events, the average body fat content (as assessed by hydrostatic weighing) was 16.3%. Sparling et al. found an average body fat content of 17.6% (DEXA) in the United States Olympic women's field hockey team in 1998 (Sparling et al. 1998), and, in 2004, Meyer et al. recorded a body fat content of 17.5 ± 3.8% in 14 Olympic level female winter sport athletes (Meyer et al. 2004). Although no direct comparison can be made, average body fat content in our study seems to be notably higher than in these previous investigations.
In addition, the analysis of biomarkers of energy availability (insulin, IGF-I, IGFBP-1, cortisol and glucose) in young Olympic sportswomen did not give any indication of caloric deficit (II). The median value of IGF-I was significantly lower in technical athletes compared to the corresponding values in women involved in power and technical disciplines. However, this difference was abolished after adjustment for age. Moreover, there were no differences in these parameters between regularly menstruating athletes and those with oligo-amenorrhea, or between different sport disciplines, i.e. power, endurance and technical events.

In our investigation of the 223 men and women who competed in the 2002 and 2004 Olympic Games (I), we found a higher frequency of illness, primarily in the form of upper respiratory tract infections, among leaness compared to non-leanness athletes (38.5% versus 21.6%, p<0.05). Chronic energy deficiency in combination with intense physical exercise is associated with an increased susceptibility to upper respiratory tract infections.(Mackinnon 2000; Venkatraman & Pendergast 2002). Therefore, this finding may indicate poor nutritional habits in the leaness athletes. However, again, this difference between leanness and non-leanness athletes was only present in the men.

In summary, we did not record any signs of chronic energy deficiency in terms of body fat mass (II, III), biomarkers of energy availability (II) or disease (I) in female Olympic athletes.

4.2 MENSTRUAL DISTURBANCES IN OLYMPIC ATHLETES

Among the Olympic athletes, 47% were using HC. The majority of the women were taking HC for birth control, whereas only one was taking such medication to regularize her periods (II). In athletes not utilizing HC, the overall prevalence of menstrual disturbances was 27%, and highest in endurance athletes (67%) (Figure 7). However, reproductive dysfunction in these women was not related to an exceedingly low body fat content or a catabolic hormonal status, as described above.

The group of athletes with MD demonstrated a higher mean free androgen index (p<0.05) and lower serum levels of FSH (p<0.05) than those who menstruated regularly. More specifically, 6 out of these 13 athletes were diagnosed with PCOS (Table 3), which was found to explain the higher androgen levels in the group of athletes with reproductive dysfunction. Only one out of the 13 athletes was diagnosed with hypothalamic inhibition with a typical hypogonadotropic state. The remaining 6 women had hormone levels (gonadotropins, androgens, PRL, thyroid hormones) within normal ranges, and were hence considered as idiopathic.

<table>
<thead>
<tr>
<th>N Total=13</th>
<th>Endocrine disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>1</td>
<td>Hypothalamic amenorrhea</td>
</tr>
<tr>
<td>6</td>
<td>Idiopathic</td>
</tr>
</tbody>
</table>

Table 3. Endocrine diagnoses of Olympic athletes with menstrual dysfunction.
The important finding that PCOS was the predominant cause of MD in Olympic athletes is supported by an earlier observation of essential hyperandrogenism in a subgroup of endurance athletes competing at a lower level than the athletes in the present investigation (Rickenlund et al. 2003; Rickenlund et al. 2004).

In previous reports (Rickenlund et al. 2003; Rickenlund et al. 2004), it was demonstrated that athletic amenorrhea was associated with a hormonal profile consistent with a hypometabolic state, e.g., attenuated diurnal secretion of LH, low serum levels of insulin and enhanced diurnal secretion of growth hormone (GH) and cortisol. In contrast, the oligomenorrheic athletes displayed elevated diurnal secretion of both LH and testosterone and a high incidence of PCO. Thus, the hormonal pattern of amenorrheic athletes was consistent with hypothalamic inhibition as a consequence of energy deficiency, whereas oligomenorrhea in the athletes was a reflection of PCOS. Our present investigation also reveals that oligomenorrhea can be a symptom of hyperandrogenism, i.e., five of the eight Olympic athletes who exhibited oligomenorrhea were diagnosed with PCOS.

Polycystic ovaries are a well-recognized feature of a hyperandrogenic condition, such as PCOS. The prevalence of PCO observed with ultrasound in the Olympic athletes who were not using HC was high (37%) (II) compared to the corresponding estimated prevalence of approximately 20% in the general population (Lowe et al. 2005). PCO was most common among the power athletes, and least frequent among those participating in technical sports (Figure 7). This condition was also significantly more prevalent in women who were not using HC compared to those who were (37.0% versus 12.5%; p<0.01).

**Figure 7.** The proportion of athletes not using hormonal contraceptives who had menstrual dysfunction (MD) and polycystic ovaries (PCO). Values are presented as % of all athletes in each sport group. A significantly larger proportion of the endurance athletes than of those participating in power disciplines exhibited menstrual disturbances.
It has previously been demonstrated that athletes with PCOS performed better than normoandrogenic athletes in physical tests, e.g., exhibiting higher maximal oxygen uptake (Rickenlund et al. 2003). Androgens are well known to increase muscle strength in a dose-dependent manner and enhance physical performance in both men and women (Storer et al. 2003; Cardinale & Stone 2006). Therefore, we speculate that the presence of PCO/PCOS may reflect an anabolic state that could be advantageous for physical performance and may thereby play a key role in the achievement of a high competitive standard by female athletes.

We conclude that PCOS is a major cause of menstrual dysfunction in Olympic athletes. However, other mechanisms such as hypothalamic inhibition of the HPG axis might also be present. While women suffering from hypothalamic amenorrhea are at risk for bone loss, injuries, and subsequently decreased performance (Nattiv et al. 2007), hyperandrogenic women might have a competitive advantage due to the anabolic effects of androgens. On the other hand, women with PCOS might suffer long-term metabolic complications such as type-2 diabetes and cardiovascular disease (Diamanti-Kandarakis 2008). Menstrual disturbances in athletes should therefore be carefully assessed, in order to provide adequate medical counselling and treatment.

### 4.3 Bone Mineral Density

Bone mineral density was generally high in the female Olympic athletes (II). Physical activity is well known to promote bone formation (Madsen et al. 1998; Kohrt 2001; Meyer et al. 2004), and in particular, mechanical loading of high frequency and intensity is recognized as a strong determinant of BMD (Turner 1998; Meyer et al. 2004). Here, we found very high BMD values in power athletes (mean Z-score +2.2 SD of the age-matched mean), which might be explained by the far higher impact forces these athletes are subjected to, compared to endurance and technical athletes.

Although menstrual disturbances were common, not a single one of the 90 Olympic athletes was diagnosed with osteopenia or osteoporosis. This finding might reflect the fact that the cases of MD were due primarily to PCOS, which should instead exert an anabolic impact on the BMD (Good et al. 1999). Athletes with MD did have significantly lower total BMD than athletes with RM and lower spinal BMD than those using HC (Figure 8). A stepwise multiple regression analysis, however, revealed that participation in power sports was the strongest determinant of BMD (total BMD: $b_s$ = +0.54, p<0.001; spinal BMD: $b_s$ = +0.32, p<0.05), whereas a diagnosis of MD was not a significant independent predictor of bone density.

Some previous studies have in fact also documented high BMD in elite athletes despite menstrual disturbances (Wolman et al. 1991; Fehling et al. 1995; Robinson et al. 1995; Sparling et al. 1998; Meyer et al. 2004). Hormonal status was however not assessed in these investigations.

In the postmenopausal athletes (III), BMD and T-scores were comparable to controls (T-score: +0.04 ± 1.24 versus -0.50 ± 1.14, n.s.), also after adjustment for BMI. 3/20 athletes versus 7/19 controls had osteopenia (T-score < -1). This difference however did not reach statistical significance.
In summary, female Olympic athletes had in general very high BMD although MD was common. This important finding is in contrast to previous findings of low bone mass in female athletes with menstrual disturbances (De Souza & Williams 2005). Furthermore, the postmenopausal former top elite athletes had BMD values similar to those of controls.

Figure 8. Total and spinal BMD in athletes with regular menstruation (RM), menstrual dysfunction (MD) and in those using hormonal contraceptives (HC). Values are mean, ±SD and min-max. Athletes with MD had significantly lower total BMD than athletes with RM and significantly lower spinal BMD than those using HC.

4.4 THE FEMALE ATHLETE TRIAD

Our findings challenge the contemporary concept of the female athlete triad. Although the purpose of this thesis was not to investigate the prevalence of the Triad, this symptom combination was not found in a single of the Olympic athletes (II), since none of them had osteopenia/osteoporosis. In fact, Torstveit and Sundgot-Borgen recently demonstrated that the prevalence of the Triad among elite female Norwegian athletes was low (4.3%) and similar to that in a non-athletic control group (3.4%) (Torstveit & Sundgot-Borgen 2005). These results have raised the question whether the Triad is an issue in a clinical setting of athletes in mixed disciplines (DiPietro & Stachenfeld 2006), which however has been refuted (Loucks 2007). The data documented in our studies further strengthen the challenge to the concept of chronic energy deficiency in elite women athletes.

4.5 LONG-TERM CARDIOVASCULAR EFFECTS OF INTENSE EXERCISE

4.5.1 Endothelial function

Endothelial function in the study group of postmenopausal former elite endurance athletes not utilizing HRT was improved in comparison to sedentary controls (p<0.05),
whereas no difference in this function could be observed between athletes and control subjects taking HRT (III) (Figure 9).

The more favorable endothelial function observed here can be explained by the direct positive effects of exercise on the endothelium, including enhanced bioavailability of NO as a consequence of increased production and decreased inactivation of this signal substance (Green et al. 2004; Higashi & Yoshizumi 2004) as well as reduced oxidation of LDL (Shern-Brewer et al. 1998).

Furthermore, the postmenopausal athletes also had lower relative fat mass, (p < 0.05), circulating LDL (p<0.01) and Chol (p<0.05), and higher lean mass than controls (p<0.05). Elevated serum levels of Chol, LDL, and TG have been found to be associated with impaired endothelial function (Landman et al. 2001; Laroia et al. 2003), whereas HDL is thought to improve endothelial function, counteracting the effects of LDL (O’Connell & Genest 2001; O’Donnell & De Souza 2004). Moreover, larger adipose tissue mass has been shown to exacerbate pathological processes, leading to endothelial dysfunction through increased secretion of proinflammatory adipokines and attenuated secretion of adiponectin (Sonnenberg et al. 2004).

Even though FMD did not correlate to either serum levels of lipids or the various parameters of body composition in our study, the leaner body composition and lower circulating levels of lipids in the athletes still may have contributed to an enhancement of FMD.

We found no significant effect of HRT on the FMD of either former athletes or control subjects. However, there was some indication of a higher mean FMD in the subgroup of control subjects utilizing HRT in comparison to the other control subjects (Figure 9). It can therefore not be excluded that the sample size might have been too small to detect differences between the subgroups, as improvement of endothelial function is well known in postmenopausal women that receive HRT treatment (Herrington et al. 2001; Wild & Reis 2001; Davis et al. 2002).

The athletes involved in the present study, however, seem not to have had advantageous effects of HRT on FMD. Our results might therefore be in agreement with the report by Harvey and co-workers (Harvey et al. 2005b), who demonstrated that acute exercise and estrogen alone each enhanced FMD in postmenopausal women, but that these effects were not additive.

The former athletes in our study reported more episodes of amenorrhea during their reproductive period than did the control subjects, although this difference was of borderline significance (p = 0.053). However, no impact of previous amenorrhea on FMD could be detected. We have no data concerning the mechanisms underlying amenorrhea in the postmenopausal athletes, therefore, it is not possible to conclude whether these women had been hypoestrogenic or not at a younger age. There are some data indicating the reversal of endothelial dysfunction in amenorrheic athletes following resumption of regular menstruation (Yoshida et al. 2006; Hoch et al. 2007), and we can only speculate that this might have been the case with our former athletes as well.
Figure 9. Flow-mediated vasodilatation (FMD) in athletes and controls, subdivided according to utilization of hormone replacement therapy (HRT) or not. Values are mean ± SD. Athletes not utilizing HRT had significantly higher FMD than controls.

In summary, our results indicate that long-term intense training has beneficial effects on endothelial function and other cardiovascular risk factors in elderly women, despite the fact that some of the athletes had experienced episodes of amenorrhea at a younger age.

4.5.2 Cardiac structure and function

Cardiovascular adaptations to endurance training such as long-distance running, include cardiac remodelling (“athlete’s heart”), which consists of alterations in ventricular chamber dimensions, e.g. increased left and right ventricular and left atrial cavity size, associated with normal systolic and diastolic function (Pelliccia et al. 1999). Indeed, echocardiographic assessment in our study demonstrated that left and right ventricular dimensions, left atrial volume and stroke volume normalized for BSA were 11 %, 14%, 25% and 22% larger, respectively, in comparison with age-matched inactive controls (Figure 10). In contrast to senior male athletes, we found no increase in wall thickness, relative wall thickness or left ventricular mass in the postmenopausal sportswomen.

It has been suggested that the persistency of “athlete’s heart” features seen in the studies of senior male athletes can be due to maintained training or increased body weight (Pelliccia et al. 2002), however, the possibility that these changes might be irreversible has also been discussed (Pelliccia et al. 2002). Differences in body weight cannot explain the findings in the present study, since the athletes had lower body weight than controls and, furthermore, all echocardiographic measurements were corrected for BSA.

The postmenopausal athletes were still involved in regular training at the time of this study, although not to the same extent as at their career peak. The persistent cardiac enlargement without increased myocardial thickness in these women may consequently be a result of a high degree of recreational training after the end of the sporting career,
or may indicate irreversible cardiac remodelling after adaptation to long-lasting volume overload. Despite the increase in left ventricular size, there were no signs of impairment of systolic or diastolic left ventricular function and therefore AH in this group of postmenopausal women seems benign.

The reason for the absence of increased myocardial thickness in the postmenopausal athletes, in contrast to findings reported in senior males, though, is unclear. AH features are known to recede to some degree upon detraining. A study by Pelliccia et al. in 2002 assessing cardiac structure in 40 elite male athletes who had shown left ventricular cavity enlargement and increased wall thickness, at peak training and subsequently after long-term deconditioning, revealed an incomplete resolution of cavity enlargement and normalization of wall thickness (Pelliccia et al. 2002).

Thus, one possible explanation for the absence of increased myocardial thickness in the former athletes is that cardiac hypertrophy, present at a younger age, had receded at the time of the investigation, due to a reduced amount of exercise. Yet, as previously mentioned, our athletes were still highly active at the time of the investigation, and increased posterior wall thickness, interventricular septum thickness and left ventricular mass have been shown to persist in older, still active males (Di Bello et al. 1993; Seals et al. 1994; Giada et al. 1998; Hood & Northcote 1999; Bouvier et al. 2001).

![Figure 10. Echocardiographic variables that were significantly different between athletes and controls. BSA: body surface area.](image-url)

Therefore, detraining alone seems unlikely to be the cause for the lack of hypertrophy in our athletes. One can only speculate that other factors such as gender, hormonal
differences, genetic factors, or different styles and intensities of sporting activities may play a role in different patterns of AH in older former male and female athletes. It is also known that absolute cardiac output and systolic pressure response during exercise is lower in women than in men and may therefore be an insufficient stimulus for development of increased wall thickness. Furthermore, animal studies have shown that testosterone plays an important role in generating left ventricular hypertrophic response to exercise (Koenig et al. 1982).

The athletes had a longer exercise time during the stress test (p<0.01), and also achieved 117 ± 14%, while controls reached on average 91 ± 19% of the Swedish reference values for expected maximum work load (p<0.001). Fewer postmenopausal athletes than controls had pathological ST-depressions during exercise (Figure 11). A criterion of ST-depression > 1 mm in two adjacent ECG leads as an indicator of a pathological stress test was set according to a common clinical practice.

Studies have demonstrated that asymptomatic subjects showing exercise-induced ST-segment depressions are at higher risk for future cardiac events than those with normal exercise ECG:s (Rywik et al. 2002). Still, the significance of ST-depressions in healthy, asymptomatic women is uncertain, mainly due to paucity of data on large series and a high prevalence of an abnormal exercise ECG in the female population. In middle-aged asymptomatic women abnormal exercise-induced ECG ranges from 20-30% (Braunwald 1997). The poor prognostic value of an abnormal exercise ECG in an asymptomatic female population (Manca et al. 1978), and the low ability of exercise testing to predict cardiovascular disease in women (Al-Khalili et al. 2000; Mora et al. 2003) are well known. Therefore, the significance of our results should be judged with caution. Besides a slightly larger BMI in the controls, no risk factors for coronary heart disease were found in the study group, and none of the subjects had any symptoms suggestive of myocardial ischemia during exercise. All subjects achieved high work-load consistent with normal or supra-normal exercise capacity. It is possible therefore that the high number of individuals with ST-depressions in our control group does not...
represent a coronary pathology. The lesser proportion of ST-depressions in the athletes could possibly be explained by a lower sympathetic activity during exercise, also reflected in the lower heart rate increment in this group. This, in turn, could be of benefit to long-term cardiac health.

In conclusion, there were no signs of adverse long-term cardiac effects of intense training in the postmenopausal athletes. On the contrary, the results indicate an enhancement of cardiac function in these women compared to the controls.
5 CRITICAL ASSESSMENT AND FUTURE PROSPECTS

Data in paper I were collected from a health questionnaire in connection with the Olympic Games of 2002 and 2004. Although this questionnaire is routinely used by the Swedish Olympic Committee, it has not been validated for research purposes. Also, data were self-reported. For instance, athletes may well have refrained from stating to have a current eating disorder. There may also be some recollection bias concerning other questions. Furthermore, body weight alone does not provide a sufficient basis for assessment of health or energy balance. A personal interview with each athlete, combined with assessment of nutritional intake, body composition and endocrine balance, could further have improved the study. Nevertheless, the use of a questionnaire gave a reasonably good data collection for the purposes of the investigation. More importantly, the questionnaire was answered by almost all of the athletes participating in these Olympic Games, giving a unique insight into the habits of such a population of athletes.

We used different methods for the classification of athletic disciplines in papers I and II. In the case of paper I, our aim was to identify athletes at risk for an exaggerated strive for a low body fat content. In paper II, on the other hand, it was important to choose a classification that would most accurately reflect the impact of different sports on body composition. Also, it was central in this paper to specifically identify the characteristics of endurance athletes, who are well known to be prone to menstrual disturbances.

One of the aims of paper II was to evaluate menstrual status in Olympic athletes. However, since almost half of the women were using HC, the number of athletes in whom hormonal balance could be assessed was small. This limited in particular our ability to detect differences between sport disciplines and women with different endocrine diagnoses. In this study, we chose not to include a control group, since reliable reference values exists for all parameters that were assessed. To some extent, the technical athletes also served as controls. It should be acknowledged that this investigation includes a unique population of Olympic athletes that is difficult to access for research purposes.

Blood hormone levels in paper II were assessed from single morning samples. This could be another limitation of our study, since there is a well recognized circadian rhythm and pulsatility in the secretion of several hormones and binding proteins. Furthermore, it is possible that stress in the experimental situation could have affected the levels of some hormones. To minimize the interference of such variations, all subjects underwent a 30 minutes rest before blood was drawn and the blood sampling was performed in a standardized manner, at the same time in the morning, and during the early follicular phase of the menstrual cycle in regularly menstruating subjects.

The small sample size in the papers III and IV may have limited the power to detect differences between subgroups of athletes and controls using and not using HRT. However, the study comprises a well-characterized and unique sample of Sweden’s former elite female athletes, and is the first report on cardiovascular function in such women of postmenopausal age. It should be recognized that assessment of FMD only represents a surrogate estimation of the risk of developing CVD, as well as BMD is for
the risk of fracture. The clinical implications of our findings can only be addressed in long-term studies of fracture incidence and cardiovascular morbidity.

The thesis may indicate that we are witnessing an ongoing trend towards healthier training and eating practices in female elite athletes. Further studies are needed to verify this assumption. There is also a call for clarification as to whether or not an excessive pursuit of leanness may be a limitation for the performance of male athletes.

A very interesting future prospect is to investigate the role of essential hyperandrogenism for Olympic athletic achievement, which would require large materials.

Future studies could also aim at investigating the long-term cardiovascular consequences of energy deficiency and/or amenorrhea in elite athletes, as impaired endothelial function has been suggested as the fourth component of the female athlete triad.
6 CONCLUSIONS

Major conclusions based on the results of the studies described in the papers I to IV:

- The weight control practices employed by Olympic athletes participating in disciplines that emphasize leanness appear to be suboptimal, although female athletes may have adopted healthier nutritional practices than the men. Counseling concerning weight control could be used as a tool to prevent illness and enhance performance.

- Menstrual disturbances are common in Olympic athletes, especially in women involved in endurance disciplines. Polycystic ovary syndrome seems to be a common cause for reproductive dysfunction in top athletes, rather than hypothalamic inhibition due to low caloric intake. Female Olympic athletes participating in different events appear to have an anabolic body composition and biomarkers of energy availability within the normal ranges. Hence, our findings challenge the contemporary concept that reproductive dysfunction in sportswomen is typically a consequence of chronic energy deficiency.

- Postmenopausal former elite endurance athletes not utilizing hormone replacement therapy exhibit more favorable endothelial function, serum lipid profile, and body composition than sedentary, age-matched control subjects. Our results indicate that long term elite physical training has overall beneficial effects on cardiovascular risk factors in women.

- Former top endurance athletes of postmenopausal age demonstrate cardiac enlargement without increased wall thickness or left ventricular mass. Our results indicate that there is a gender difference in long-term consequences of elite endurance training on cardiac structure. The lower occurrence of ST-depressions during exercise in combination with greater exercise performance in the athletes, suggests that intense training enhances cardiovascular performance in the aging female athlete.
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