GIRLS' PUBERTY AND HEMODYNAMICS DURING EXERCISE IN THE HEAT

HEMODYNAMIC RESPONSES OF PRE/EARLY-PUBERTAL, MID-PUBERTAL AND LATE-PUBERTAL GIRLS TO EXERCISE IN THE HEAT

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ABSTRACT

During exercise in a hot climate, it has been reported that children respond with a greater shift in blood volume from the central to the peripheral circulation, compared with adults. This can lead to inadequate central circulation, and early cessation of activity in children. No studies have considered if a transition in this hemodynamic response occurs during puberty in females. This study measured hemodynamic responses in pre/early (PEP), mid- (MP), and late- (LP) pubertal girls. Twenty-seven 9- to 16- year-old healthy girls (n=9, each group) cycled in a climatic chamber set at 35 ± 1°C and 50 ± 5% relative humidity for two 20-min bouts (10-min rest in between and a 10-min rest at the end). Cardiac output was measured upon chamber entry, in the middle and at the end of each exercise bout. Forearm blood flow (FBF) was measured upon chamber entry, 3 minutes after exercise bout 1 (E1) and bout 2 (E2) and at the end of rest 1 (R1) and rest 2 (R2). Overall, there was no significant group difference in the central hemodynamic (e.g. cardiac index, stroke volume index) responses to exercise in the heat. However, FBF among the three groups at R1 was almost significantly different ($p=0.06$), such that it was lower in LP than in PEP and MP. FBF in LP also tended to decrease from E1 to R1 (7.91 ± 1.06 to 6.1 ± 0.83 $ml \bullet 100ml^{-1} \bullet min^{-1}$)

and from E2 to R2 (9.47±1.47 to 8.26±1.20 ml•100ml^{-1}•min^{-1}). In contrast, PEP and MP showed little change in FBF at all time points. These findings strengthen the notion that there is a maturity-related decrease in forearm blood flow from pre- to late-puberty. In conclusion, under the conditions of the present study, there were very few differences in the hemodynamic responses of pre-, mid- and late- pubertal girls. It is possible that the transition to an adult-like hemodynamic response does not occur until later in a girl's physical development.

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1.INTRODUCTION

1.1 Background and rationale

Exercise heat tolerance (EHT) is the ability to tolerate exercise in a hot environment (Armstrong, Maresh, 1995). It can be measured as the longest duration of a prescribed physical task that a person can sustain before reaching predetermined physiological or perceptual endpoints (Bar-Or, 1996). While some studies have shown that children cannot tolerate hot environments as well as adults (Wagner et al., 1972; Haymes et al., 1974; Drinkwater et al., 1977), others have shown that children have EHT that is actually similar to that of adults (Gullestad, 1975; Docherty et al., 1986). Closer examination of these studies reveals that in a thermoneutral environment (20-25°C) children are able to thermoregulate as effectively as adults by relying more on radiation and convection for heat dissipation, instead of on the evaporation of sweat. When ambient heat stress is extreme (ambient temperature exceeds skin temperature by 10°C), however, children's EHT is lower than that of adults.

Children, therefore, have a reduced time until the onset of syncope and fatigue during an exercise task in the extreme heat (Bar-Or et al., 1969 vs. Haymes et al., 1974), (Drinkwater et al., 1977; Drinkwater, Horvath, 1979). As reported by Armstrong & Maresh (1995), no scientific studies have observed

large exercise-induced differences between the rectal temperatures of children and adults. Research has suggested, instead, that children's reduced EHT is more likely due to the response of their cardiovascular system than to hyperthermia (Drinkwater et al., 1977). Children's heat tolerance is important to consider because they habitually spend more active hours outdoors than adults do. In particular, the question of what happens between childhood and adulthood to increase EHT has not been extensively studied.

Historically, thermoregulation studies have been conducted mostly with males. When gender comparisons were made, the physical and physiological differences between men and women were seldom considered. In particular, adult women tend to have a smaller body stature and body mass than adult men. Consequently, women tend to have a higher body surface area-to-mass ratio (BSA/M, $m^2 \bullet kg^{-1}$). As a group, females also have a lower maximal oxygen consumption ($\dot{V}O_{2max}$), and a higher percentage of body fat than adult males. A higher BSA/M increases absorption of heat at high ambient temperatures. As Bar-Or (1996) explained, since core temperature and cardiovascular strain are functions of the relative intensity of exercise, a lower $\dot{V}O_{2max}$ implies a greater metabolic demand at any absolute exercise intensity. Finally, adipose tissue has a lower heat content than lean tissue (conducts heat only 1/3 as readily as other tissues), so less heat can be stored before core temperature rises. Since these characteristics affect thermoregulatory responses, it is necessary to account for them when comparing genders, and even when comparing individuals within a

single gender. More recently, researchers have also recognized the need to control for the physiological variations associated with the menstrual cycle. In particular, a woman's core temperature is approximately 0.5°C higher in the luteal phase than in the follicular phase of the menstrual cycle (Harvey, Crocket, 1932). Therefore, studies that measure women's thermoregulatory responses should control for the phase of the cycle.

In the 1970s, a study by Haymes et al. (1974) examined the EHT pre-pubertal girls. By duplicating the protocol that Bar-Or et al. (1969) used with women, Haymes was able to make comparisons between the girls and the women. These comparisons led to an important conclusion that pre-pubertal girls had less tolerance for exercise in extreme heat than did women. A few years later, a study by Drinkwater et al. (1977) further confirmed these findings by comparing the physiological responses of pre-pubertal girls and college women to work in the heat. The subjects were matched in maximal aerobic power and walked on a treadmill at the same relative intensity (~30% $\dot{V}O_{2max}$) for two 50-min bouts at temperatures of 28°C (45% relative humidity, rh), 35°C (65% rh) and 48°C (10% rh). The pre-pubertal girls had a lower tolerance time than the adult women during exercise in the two warmer environments. Although post-exercise forearm blood flow was not significantly different among subjects, the authors suggested that the higher BSA/M of the girls required a larger proportion of the their total blood volume to maintain adequate peripheral flow. This would decrease venous return and contribute to the girls' lower stroke

volume index and cardiac output. This circulatory instability could have been the reason why the girls terminated their walks early.

There are several factors that could explain the differences in the thermoregulatory responses between children and adults when they exercise in the heat. The following will focus briefly on geometric and cardiovascular factors.

In general, children have a larger BSA/M than adults. BSA/M can be an indicator of heat injury susceptibility because heat dissipation is proportional to surface area, and heat production is proportional to mass. Children usually have a smaller mass than adults, so they have less heat-generating tissue per unit of skin surface area. Children's higher BSA/M allows them to rely more on dry heat loss (radiation + convection) than on sweat evaporation when ambient temperature (T_{amb}) < skin temperature (T_{sk}) (Gullestad, 1975; Davies, 1981). However, when $T_{amb} > T_{sk}$, children are at a disadvantage because they are capable of absorbing more heat.

When walking or running at the same absolute velocity, children have a higher oxygen cost than adults (MacDougall et al., 1983), and therefore produce more metabolic heat per unit mass. Factors such as stride frequency and inefficient running mechanics are believed to account for children's higher $\dot{V}O_2$ when performing the same locomotion task as an adult (Rowland, 1990). This places an added strain on the thermoregulatory system. At any given metabolic level, children also tend to have a lower cardiac output than adults (Bar-Or et al., 1971), but there is no data as to when this child-like pattern turns into an adult-

like pattern. If blood flow is limited to the muscles during exercise in the heat, exercise heat tolerance would likely be reduced in children. In addition, since children have a much smaller blood volume when expressed relative to surface area, Drinkwater et al. (1977) suggested that a larger proportion of their blood volume is diverted to the periphery. This combination of decreased cardiac output and pooling of blood in the peripheral vessels could further contribute to decreased tolerance time.

Because the differences above are altered by the growth and maturation that occur during puberty, it is possible that the transition to an adult-like thermoregulatory response during exercise in the heat occurs during puberty.

1.2 Objectives

This study was intended to measure the hemodynamic responses of pre/early-pubertal (PEP), mid-pubertal (MP) and late-pubertal (LP) girls to exercise in the heat by measuring cardiac output and forearm blood flow. We could then determine whether any observed differences during puberty in the cardiovascular responses among the three groups (PEP, MP, LP) demonstrated a transition from a child-like thermoregulatory response to an adult-like thermoregulatory response.

1.3 Hypotheses

It was hypothesized that during exercise in a hot climate, the MP and LP groups would have a higher stroke volume index, a lower post-exercise forearm blood flow, and fewer overt signs of cardiovascular difficulty (i.e. flushed faces, feelings of dizziness) than the PEP group. MP and LP would also have a higher cardiac output than PEP at any given oxygen uptake.

2. REVIEW OF THE LITERATURE

2.1 Temperature regulation

Everyday, the normal changes and extremes of the physical environment affect our physiological responses. Factors such as ambient heat, cold, light and pressure work in combination to challenge the systems of the human body. In spite of this, a human's core temperature range varies little from day to day (36.5-37.5°C). This is important because the metabolic processes that occur in the human body take place optimally within a narrow temperature range. Temperature does, however, vary considerably from one part of the body to another. In particular, the difference between the "shell" (skin) temperature and the "core" (deep central areas including heart, lungs, abdominal organs, brain) temperature can be as great as 20 °C (optimum difference ~ 4 °C) (Astrand, Rodahl, 1970). Several sites can be used for core temperature measurement, and it is the site that determines some of the recorded differences. For example, rectal temperature measurements are generally higher than those taken in the mouth, esophagus or tympanic membrane (Cooper, 1994). Skin temperatures are more variable because they rise and fall with the temperature of the surroundings. Skin temperatures are therefore important to consider when referring to the ability of the skin to lose to or gain heat from the surroundings.

2.2 Physical principles of temperature regulation

Heat production is one of the principal byproducts of metabolism. When heat production exceeds heat loss, core temperature rises and this heat must be dissipated. The rate at which this heat is lost is determined mainly by how fast heat can be conducted from the core to the skin, and how fast heat can then be transferred from the skin to the surroundings. Fortunately, the mechanisms that control and maintain a constant core temperature are intricate and delicately balanced.

Maintaining core temperature at rest requires a balance between heat gain and heat loss. The following equation, as reported by Folk (1966), was developed to quantify this relationship:

$M \pm R \pm C \pm W - E = S$

[all terms in $cal \bullet m^{-2} \bullet hr^{-1}$]

M = metabolic heat production
R = heat lost or gained by radiation
C = heat lost or gained by convection
W = heat lost or gained from water taken
E = heat lost by evaporation
S = stored heat ("+" if the body is storing heat, "-" if the body is losing heat)

Dissipation of heat from the skin is done by a combination of conduction, convection, radiation and evaporation. During rest at room temperature, the majority of heat is lost through radiation as humans give off infrared heat rays (electromagnetic waves) in all directions to the objects that surround them. Evaporation is also responsible for heat dissipation, of which 2/5 is from the

lungs and 3/5 is from the passive evaporation of water from the epidermis (insensible water loss). Conduction, the transfer of heat from one material to another through direct molecular contact, and convection, the transfer of heat by the motion of a gas or a liquid across a heated surface, are responsible for the remainder.

Depending on the temperature gradient between the skin and the environment, heat can be gained from or lost to the environment by conduction and convection. For example, when the surrounding air is warmer than the skin, heat will be absorbed by the body. Similarly, when the radiating sources surrounding a human are hotter than the absorbing surfaces (i.e. clothing, skin), the body will absorb heat. Evaporation of sweat becomes the only avenue for heat dissipation in a hot environment. Evaporation of sweat causes only heat loss, even when air temperature exceeds skin temperature.

2.3 Physiological principles of temperature regulation

Temperature regulation of the human body is not an isolated series of reactions. In particular, it is closely linked to cardiovascular regulation, which is responsible for transporting heat from the body core to the skin. In an exercise situation, the primary role of the cardiovascular system is to supply oxygen and nutrients to the active muscle. However, skeletal muscle activity is calorigenic, and therefore maintaining body temperature requires increased dissipation of heat to the environment. Adequate heat dissipation is made even more difficult

when exercise is taking place in a hot environment. The cardiovascular system is then faced with the difficult challenge of delivering enough blood flow to the active muscle and also to the skin for heat dissipation. The combined demands of the skeletal muscle and skin circulation could potentially exceed the pumping capacity of the heart. The cardiovascular system solves this problem by increasing cardiac output and by redistributing cardiac output among body organs and regions. The result is increased blood flow to the skin, which transports heat from the body core and allows for dry heat exchange to the environment (i.e. radiation and convection), and the evaporation of sweat. The following will address the two physiological processes that are activated for enhanced heat dissipation in humans: sweating to enhance evaporation from the skin and increased cutaneous circulation to enhance heat conduction from the core to the periphery.

Sweating and evaporation

The two main types of sweat glands are eccrine and apocrine. Eccrine glands are distributed all over the body, while apocrine glands are found mainly in the axillary and pubic areas. Since apocrine glands are sparsely distributed and their physiological function is not clear, the following will refer only to eccrine glands.

Thermoregulatory sweating closely parallels increases in core temperature (Gonzalez et al., 1974). Initially there is a recruitment of sweat glands and then

there is increased sweat volume per gland (Sato, Dobson, 1970). Stimulation of the anterior hypothalamus-preoptic area either electrically or by excess heat causes sweating. The impulses are transmitted in the autonomic pathways to the cord and then through the sympathetic outflow to the skin. Sweat glands are mainly innervated by sympathetic cholinergic nerve fibers. They can also be stimulated by epinephrine or norepinephrine circulating in the blood and acting on the α- and β-adrenergic receptors that are associated with eccrine sweat glands (Sato, 1977). However, most sweat glands do not have adrenergic innervation.

Sweat glands are made up of a deep subdermal coiled portion and a duct portion that passes outward through the dermis and epidermis of the skin. The secretory portion of the deep portion secretes a precursor fluid, and then the concentrations of the constituents in the fluid are modified as the fluid flows through the duct. Cholinergic sympathetic nerve fibers ending on or near the glandular cells elicit the secretion.

When ambient temperature exceeds that of the skin, evaporation of sweat becomes the only avenue for heat dissipation. However, sweat must evaporate if it is to provide cooling. Heat loss through the evaporation of sweat depends mainly on the humidity of the air close to the body surface. Humidity indicates the water vapor content of the air and when it is high, the air already contains many water molecules. Consequently, the concentration gradient is decreased and it is harder for the air to accept more water. This can have major

implications because sweat evaporation becomes limited and heat loss is compromised. Evaporation of sweat is also affected by wind, such that evaporation is enhanced with increased airflow. Finally, the amount of wetted skin available for exchange affects sweat evaporation, since the more skin surface area that is exposed, the greater the potential for evaporative heat loss.

Cutaneous circulation

The flow of blood to the skin is an effective mechanism of heat transfer from the body core to the periphery. This heat conductance is controlled by the degree of vasoconstriction of the arterioles, and to a lesser extent by arteriovenous anastomoses. The latter are large, thick-walled connections directly between arteries and veins, therefore lacking capillaries. They are not numerous in human skin and are found mainly on the digits, lips, ears, cheeks and palmar surfaces of the hands and feet (Braverman, 1989). As such, the following will focus only on blood flow in vessels with true capillaries.

Blood vessels penetrate the subcutaneous insulator tissues and are distributed profusely in the subpapillary portions of the skin. Immediately underneath the skin are venous plexuses that are supplied by an inflow of blood from skin capillaries. The rate of blood flow into the venous plexus can vary tremendously due to the large capacitance of the cutaneous veins. Therefore, the arterioles control heat elimination by setting the rate at which blood is delivered to the venous plexuses. The major site for heat exchange, however, is in these

plexuses. By venodilating, the thin-walled, wide venous channels slow the velocity of flow and allow increased time for heat transfer to the skin.

The cutaneous vascular response to heat stress is controlled mainly by core temperature, with a secondary role played by thermoreceptors in the skin, which affect the vascular smooth muscle directly (Benzinger, 1959; Wurster et al., 1966; Wyss et al., 1974; Wenger et al., 1975; Wenger et al., 1975; Brengelmann, 1983). Cutaneous vascular response is also controlled to a lesser extent by nonthermoregulatory factors such as the reflexes associated with exercise and blood pressure regulation (Johnson et al., 1986; Johnson, 1992).

Although venodilation enhances heat transfer to the environment, extensive peripheral venodilation can also precipitate a chain of cardiovascular consequences that are not beneficial. The cutaneous veins become more compliant with body heating, which increases the capacity for blood volume at a given venous pressure. In addition, the cutaneous arterioles are relaxed so there is a reduction in the pressure drop from artery to venule, which raises the cutaneous venous distending pressure and volume. Eventually, a large volume of blood pools in the cutaneous veins, reducing central venous pressure and thus reducing cardiac filling pressure. In turn, the reduced filling pressure decreases the cardiac stroke volume, so that heart rate must rise to deliver a given cardiac output during exercise.

Efferent control of the cutaneous circulation

The constriction and dilation of the cutaneous vessels is due almost entirely to the sympathetic nervous system, which responds mainly to changes in core temperature. In nonacral skin (e.g. arms, chest, legs), arterioles are innervated by two distinct sympathetic nerve types: sympathetic adrenergic vasoconstrictor nerves and sympathetic vasodilator nerves (Edholm et al., 1957; Fox, Edholm, 1963; Johnson, 1986). In contrast, the arterioles of acral skin (e.g. fingers, lips, ears) are innervated only by sympathetic adrenergic vasoconstrictor nerves. Therefore, vasodilation in these areas occurs only by the withdrawal of vasoconstrictor activity. The following will describe the cutaneous circulation in nonacral skin.

Adrenergic vasoconstrictor nerves are responsible for the subtle alterations in skin blood flow (SkBF) that occur during normal daily activities (Johnson et al., 1986). However, most of the increase in SkBF during exercise in a hot environment is obtained through sympathetic vasodilator nerves, whose response is termed "active vasodilation". Cutaneous active vasodilation relies on an intact sympathetic innervation, but is not adrenergic. The term "active" is used because the vasodilation depends on the action of neural signals in contrast to the passive relaxation of acral skin arterioles.

There is still much to learn about active vasodilation since the underlying mechanism is unknown. One theory links active vasodilation to sweat gland activity (Fox, Edholm, 1963; Brengelmann et al., 1981). When activated, sweat

glands produce a bradykinin-forming enzyme. This enzyme cleaves bradykinin, a potent vasodilator, from globulins in the interstitial space surrounding the glands and cutaneous arterioles. However, there are major objections to this proposed action. In particular, the timing of sweating and cutaneous vasodilation is not always coincident (Wyss et al., 1974; Johnson, Park, 1981), and the reflex control of sweat secretion, but not active vasodilation, is blocked by atropine (Roddie et al., 1957; Fox, Edholm, 1963; Kolka, Stephenson, 1987; Kellogg, Jr. et al., 1993).

More recently, it has been suggested that a co-transmitter might be released from cholinergic nerves (Kellogg, Jr. et al., 1994). In this case, acetylcholine and another transmitter would be co-released from sudomotor nerves in response to heat stress. While atropine would cause cholinergic blockade, the vasodilator action of the transmitter would persist. The transmitter evoking active vasodilation is currently unknown.

Cutaneous circulation during rest in the heat

The resting human body responds to heat stress with a marked increase in SkBF. Heat is therefore transferred via convection from the deep body tissues to the skin. Although not precisely known, a total maximal SkBF of approximately 8 liters•min^{-1} has been predicted (Johnson, Rowell, 1975; Taylor et al., 1984) in adults subjected to whole body heat stress. This high flow is due to the net vasodilation that occurs over the entire body surface (Fox, Edholm, 1963). The flow is mainly supplied by an elevated cardiac output, which

increases by 3-7 liters•min^{-1} during whole body heat stress (Koroxenidis et al., 1961; Rowell, 1974).

Cutaneous circulation during exercise in the heat

With exercise onset, humans actually show a reflex cutaneous vasoconstriction. Thermometry was the first method used to track this decreased SkBF. In short, a measurable fall in skin temperature was being recorded with the initiation of exercise. This fall was considered to be due to a decrease in SkBF. Although thermometry was easy to use, it was limited by its non-linear relationship to SkBF. Therefore, this conclusion has also been verified by measuring heat clearance and/or heat conductance, which demonstrates a linear relationship to SkBF. Heat conductance is the ability of the skin to conduct electric current. It is measured using a direct calorimeter, which determines the quantity of heat generated by the human body. This approach has confirmed a reduction in heat flow from the hand with the onset of leg exercise (Bishop et al., 1961; Hirata et al., 1983). This cutaneous vasoconstriction is probably due to reflexes associated with exercise since the response is too early to be attributed to a thermoregulatory drive.

As exercise continues for 5-10 minutes, metabolic heat production increases in proportion to the exercise intensity and quickly exceeds the rate of heat dissipation. The calorigenic nature of muscle contraction engages competition between the thermoregulatory vasodilator and the exercise-induced vasoconstrictor responses, resulting in a net cutaneous vasodilation. Beyond

this time, core temperature continues to rise and causes further vasodilation in the skin (Johnson, Rowell, 1975). The increased SkBF is achieved by an increase in cardiac output and by a reduction in splanchnic and renal blood flow. The reduction in blood flow to the splanchnic and renal regions shows a close inverse relationship to relative exercise intensity (Rowell, 1986), and is also increased by the addition of heat stress (Radigan, Robinson, 1949; Rowell et al., 1965). During exercise at a moderate intensity, the additional vasoconstriction can redistribute approximately 600-800 ml of blood to the skin each minute (Rowell, 1974). Blood flow through nonworking skeletal muscle is also reduced, but no inverse relationship to oxygen uptake has been observed.

When environmental temperature is high, internal temperature does not achieve steady-state, but instead continues to rise throughout exercise (Brengelmann et al., 1977). SkBF parallels this rise, but eventually reaches a plateau, after which there is little or no further vasodilation (Brengelmann et al., 1977). Plateau levels of SkBF are only 50-60% of maximum, which is relatively lower than the levels attained at rest under similar environmental conditions. This suppressive effect of exercise is through limiting active vasodilator outflow. It is not through exaggerated vasoconstrictor activity, since the local blockade of norepinephrine release does not reverse the plateau. Therefore, SkBF is not constantly driven upward by thermoregulatory drives to continually meet the demands for heat loss. A compromise is eventually met so that blood pressure is maintained.

Cutaneous circulation and the menstrual cycle

In the 1930s, Harvey and Crocket (1932) reported that women's resting core temperatures fluctuated during the menstrual cycle. In particular, there was an upward shift of 0.5°C following ovulation which persisted until the onset of menstruation (luteal phase).

The average reproductive cycle length is 30 days. The onset of menses until ovulation is the follicular phase (days 1-14), during which time all cyclic hormones are low. By day 13-14, estrogen secretion is at its peak, while progesterone is only slightly elevated. Follicule stimulating hormone and luteinizing hormone surge on day 14-15 and ovulation occurs soon after. The remaining 14 days are the luteal phase, when progesterone secretion continually increases. At the midluteal phase, progesterone peaks and is accompanied by a surge in estrogen. Progesterone levels then decrease throughout the rest of the luteal phase.

The regular temperature changes that occur throughout a cycle have sparked debate concerning the impact they have on thermoregulation during exercise and heat stress. Some studies have suggested that sweating and skin blood flow during rest or exercise are not affected by the different phases of the menstrual cycle (Cunningham, Cabanac, 1971; Candas et al., 1982). Others report that the increased core temperature associated with the luteal phase increases the temperature threshold for sweating and cutaneous vasodilation in both heat-exposed and exercising women (Hirata et al., 1986; Kolka,

Stephenson, 1989). It has also been reported that limb blood flow is higher during the luteal phase when compared with the follicular phase (Kenshalo, 1966; Keates, Fitzgerald, 1969; Bartelink et al., 1990). More recently, Charkoudian and Johnson (1997) reported that vasodilation is also initiated at higher internal temperatures during the high-hormone phase of oral contraceptive use (similar to the luteal phase, when both estrogen and progesterone are elevated).

Although it is generally accepted that the female reproductive steroid hormones, estrogen and progesterone, interact to exert their effects on the cutaneous circulation, the mechanisms remain unknown. It is thought that these hormones act through a common central mechanism rather than having peripheral action on cutaneous resistance vessels. In particular, it has been proposed that the hypothalamic thermoregulatory set-point temperature is shifted upward, therefore initiating heat-dissipating effector functions like cutaneous vasodilation and sweating at higher internal temperatures.

One major limitation of most studies in this area is that the menstrual phase of the subject is determined either by counting the days from the onset of menstrual flow or by following basal body temperature. These methods, although uninvasive and convenient, have been recognized as unreliable for timing ovulation (Frye, Kamon, 1981). The more sophisticated approach is to measure hormone levels to isolate the three distinct points in the hormone cycle 1) menstruation, when all the cyclic hormones are low, 2) the day of ovulation,

when estrogen, follicle-stimulating hormone, and luteinizing hormone are high, and 3) the midluteal progesterone peak, when estrogen is also high.

2.4 Hemodynamic responses to heat stress

The elevated cutaneous vascular conductance that occurs during exercise in a hot climate has the capacity to create an enormous demand for blood flow. There are two ways for the cardiovascular system to meet this demand: elevation of cardiac output and redistribution of blood flow from other tissues.

Heat stress can be environmental and metabolic, while heat strain is the physiological response to heat stress. The following section will describe the hemodynamic responses: to prolonged exercise in a thermoneutral environment (metabolic heat stress), to rest in a hot climate (environmental heat stress) and to exercise in a hot climate (environmental plus metabolic heat stress).

Responses to prolonged exercise in a thermoneutral environment

When working at a certain percentage of maximal oxygen consumption ($\dot{V}O_{2max}$), it is desirable to maximize cardiac output ($\dot{Q}$) and arteriovenous difference [$(a-\bar{v})O_2$] as per the Fick equation [$\dot{V}O_2=\dot{Q} \times (a-\bar{v})O_2$]. In general, as oxygen uptake ($\dot{V}O_2$) increases, $\dot{Q}$ and heart rate (HR) also increase. Stroke volume (SV), however, reaches a near-maximal value at a relatively low level of $\dot{V}O_2$. As $\dot{V}O_2$ increases, there is also a progressive increase in $(a-\bar{v})O_2$. In fact,

at $\dot{V}O_{2max}$, 80-85% of all available O_2 is extracted from arterial blood as it passes through the tissues (Rowell, 1974). This high extraction is due to an increase in the extraction of O_2 by the muscles and to a reduction in blood flow to non-exercising regions. In particular, the splanchnic and renal areas, which extract little of the O_2 from their high blood flow at rest, undergo the most active vasoconstriction. Non-exercising skeletal muscle and skin make up the other areas from which blood is redistributed. As such, a greater percentage of $\dot{Q}$ can perfuse the working muscle without compromising the $\dot{V}O_2$ of these non-exercising regions. The percentage reduction of splanchnic blood flow and renal blood flow is closely related to the relative $\dot{V}O_2$.

When exercise is prolonged in a neutral thermal environment, there is a "drift" in several of the cardiovascular responses. As exercise continues and regional vascular volume is reduced, progressive vasodilation of cutaneous resistance vessels may cause passive filling of the cutaneous veins. This can lead to a progressive reduction in cardiac filling pressure and SV. To compensate for this, HR shows a progressive increase with no significant change in cardiac output.

Responses to rest in a hot climate

The hemodynamic responses to environmental heat stress at rest depend on the intensity and duration of the stress. In general, there is a competition

between heat loss mechanisms and arterial blood pressure control. To increase blood flow to the skin, there is an increase in $\dot{Q}$ and a redistribution of blood flow from other regions. (Koroxenidis et al., 1961). In humans, approximately 2/3 of the demand for increased skin blood flow is met by an increased $\dot{Q}$, while the remaining 1/3 is met by redistribution (Rowell, 1983). The redistribution that occurs during whole body heat stress mainly involves a decrease in splanchnic circulation and in renal blood flow (Rowell, 1983), and is complemented by smaller changes in blood flow to other tissues.

The duration of the session is important to consider since humans subjected to mild heat stress do not show significant changes in cardiac output. Almost no change in $\dot{Q}$ was recorded after exposures to 38° to 46°C environments for over an hour (Sancetta et al., 1958; Carlsten et al., 1961; Damato et al., 1968). Increases in $\dot{Q}$ do occur as the duration of the exposure increases. For example, El Sherif (1970) reported a 95% increase in $\dot{Q}$ after a 2-hour exposure to an ambient temperature of 40°C. In these conditions, core temperature increased to 39.5°C.

The increase in $\dot{Q}$ is largely driven by increased HR, as SV changes little. SV during indirect heating is the net consequence of elevated sympathetic activity and reduced filling, therefore achieving a balance and remaining unchanged. In humans, HR increases approximately 30 beats•min^{-1} for each 1°C change in core temperature (Koroxenidis et al., 1961; El Sherif et al., 1970;

Wyss et al., 1974). This is mainly due to the action of temperature on the sinoatrial node. Secondary to this is parasympathetic withdrawal and elevated sympathetic activity. In seated or supine posture, blood pressure is well maintained in heat stress. Although blood pressure may fall by about 10% over periods of 30 min, it usually returns to the level seen during the prior period of normothermia (Damato et al., 1968; El Sherif et al., 1970). This is probably due to the major redistribution of blood flow and volume that accompany cutaneous vasodilation.

Responses to exercise in a hot climate

When considering the hemodynamic responses to exercise in hot environments, one must be aware of the experimental conditions. The severity of the environmental heat stress, the duration and intensity of the exercise, the subject's posture, core and skin temperatures, hydration status, and physical condition and degree of acclimatization will all have an effect on the observed responses.

The cutaneous vasodilation that accompanies exercise in a hot environment causes notable shifts in the distribution of blood volume. These shifts can have profound effects on the central circulation. As previously mentioned, exercise combined with heat stress introduces competition between skin and muscle for the available cardiac output. Cardiac output will eventually

be limited by a reduction in cardiac filling pressures, central blood volume, and stroke volume.

During prolonged mild exercise, $\dot{Q}$ can increase an additional 2-3 $l \bullet min^{-1}$ in response to environmental heat stress (Rowell, 1974). When the exercise is moderate to heavy, $\dot{Q}$ increases to facilitate heat dissipation, but does not continue to increase significantly with time. However, HR rises markedly and there is a progressive fall in SV (Nadel et al., 1979). Therefore, $\dot{Q}$ is maintained by an increased HR. Mean arterial pressure is well maintained, until hyperthermia becomes marked (core temperature > 39°-39.5°C). Once HR is maximal, $\dot{Q}$ declines due to a falling stroke volulme. After this, any increments in skin blood flow are provided exclusively by redistribution of blood flow away from visceral organs, and probably working and resting skeletal muscles as well.

Measuring cardiac output

Cardiac output in this study was measured using the single-breath constant exhalation method (acetylene uptake technique). This technique was performed using the Sensor Medics (Yorba Linda, California) V_{max} metabolic cart. This system integrates pneumatic, electronic, computer and analyzer components. The system was additionally modified for direct delivery of the acetylene mixture into a 3 litre rubber bag with a one-way valve. The rubber bag was directly attached to a two-way valve system, which let the investigator switch

the valve at the start of the measurement, allowing the subject to breathe in the test gas.

The technique is as follows: after exhaling to residual volume, the subject inhaled the gas mixture (0.3% C_2H_2, 0.3% methane, 21% oxygen, and balance nitrogen) to total lung capacity. The subject followed this with a 2-second breath-hold to allow for C_2H_2 tissue absorption and gas distribution equilibration (Zenger et al., 1993). The subject then exhaled at a constant rate of 200-500 $ml \bullet s^{-1}$. Acetylene diffuses rapidly from the lungs into the pulmonary capillaries, and it is a highly soluble gas. Therefore, its rate of absorption from the alveoli is proportional to the pulmonary capillary blood flow, which is equal to the total cardiac output. In addition to measuring gas volumes during the constant flow exhalation, the V_{max} system also had rapid-response infrared sensors for measuring gas concentrations throughout the manoeuvre (Zenger et al., 1993). Therefore, the volume of C_2H_2 expired reflected the volume uptake by the lungs, and the slope of the expiration curve (equaling the rate of uptake) was calculated by the system using linear regression (Sadeh et al., 1997). Methane was used as an indicator of mixing of the lung-bag system. Methane is an insoluble gas so that a constant level during exhalation reflected adequate mixing. Each time cardiac output was measured, the technique was performed three times (each measurement separated by 1 minute), therefore yielding three values.

Acetylene uptake is a relatively new technique that is proving to be useful in certain patient populations. This method would seem particularly

advantageous to use with children because it requires only a single inhalation and exhalation, making it easier for young subjects to learn and to perform. The validity of the acetylene (C_2H_2) uptake technique has been examined mostly at rest in patients with a variety of medical and surgical conditions (e.g. coronary artery disease) that necessitate the measurement of cardiac output. Elkayam et al. (1984) compared the single-breath technique with the thermodilution technique in 20 patients with cardiac disease. A close relationship was reported between methods with a mean difference in the estimation of $\dot{Q}$ of 0.03 ± 0.76 $l \bullet min^{-1}$. Ramage et al. (1987) compared the acetylene uptake technique with radionuclide angiography in 36 patients, and reported a good correlation (r=0.80) between the two methods. Zenger et al. (1993) reported even higher correlations between $\dot{Q}$ determined by the single-breath technique and that derived by both the thermodilution and the direct Fick methods (r=0.90 and r=0.92, respectively). With respect to the reliability of the single-breath C_2H_2 method, Thomas et al. (1997) recently reported that calculations of $\dot{Q}$ determinations over three repeat days revealed a coefficient of variation of 10, 2, 9 and 6%, respectively, at rest and during three incremental levels of exercise.

The single-breath C_2H_2 method has certain limitations, including the following examples. Some subjects may have difficulty exhaling at a constant flow rate. It was especially true in this study because the exhalation had to be performed while cycling. This constant exhalation is extremely important

because of simplifying assumptions made in the development of the equations used to calculate the slope. Also, since the V_{max} system used to measure cardiac output in this study does not allow the monitoring of residual C_2H_2 concentrations in the lungs, complete washout of this gas between measurements is not confirmed. In spite of these limitations, the single-breath method is accurate when compared with the direct Fick and thermodilution methods, and therefore offers a safe, affordable and noninvasive measurement of cardiac output.

3.METHODS

3.1 Subjects

Nine pre/early-pubertal (PEP), nine mid-pubertal (MP), and nine late-pubertal (LP) healthy females, between the ages of 9-16, were recruited for this study. Pubertal rating was determined by Tanner breast development staging (Tanner, 1962). PEP was stages 1 and 2 for breast development. MP included stage 3 and LP was stages 4 and 5. None of the PEP or MP subjects had reached menarche. However, all of the LP subjects were post-menarcheal. Subjects were habitually active in recreational activities, but were not competitive athletes. All subjects were non-smokers and none of them was using oral contraceptives. Experiments took place in the spring and summer months (May-August). Menstruating subjects were tested during the follicular phase (days 1-14).

A sample size calculation was done prior to subject recruitment. Assuming α=0.05, power = 0.80 and using forearm blood flow data from Falk et al. (1992), a sample size of 9 in each group was calculated.

3.2 Study protocol

There were 2 visits to the Children's Exercise and Nutrition Centre. This study was approved by the Ethics Committee of Health Sciences, McMaster University, Hamilton, ON, Canada.

Visit I

Visit I was for: 1.general orientation, 2.signing of consent, 3.medical clearance, 4.anthropometric measurements, 5.resting and submaximal cardiac output measurements, 6.resting and post-exercise forearm blood flow measurements and 7.peak oxygen consumption ($\dot{V}O_{2peak}$) testing. This all took place in a thermoneutral environment.

Once the study procedures had been explained and the subject had given verbal assent, a parent signed the informed consent (Appendix A). If the subject was 14 years or older, she also signed a consent form (Appendix B). A medical questionnaire (Appendix C) was then administered and anthropometric measurements were taken: height (Harpenden wall-mount Stadiometer 2109, accurate to 0.1cm), body weight (Ancaster electro-scale model UMC-600, accurate to 20g; Brantford, ON, Canada), and adiposity (bioelectrical impedance, BIA-101A RJL Systems, Clinton Twp, MN).

During this initial visit, cardiac output was measured by the acetylene uptake technique, as previously described. To practice, the subject was first

seated in front of a computer screen. The special breathing required for the acetylene uptake was introduced in a series of steps: 1. "Breathe everything out", 2. "Deep breath in", 3. "Hold", 4. "Breathe out constant". The subject was connected to an open circuit system by a mouth piece (adult size silicon rubber mouth piece, Hans Rudolph Inc., Kansas City Mo., USA) which fit onto a saliva trap and mass flow sensor (SensorMedics Corp., Yorba Linda, CA, USA). Using the V_{max} Enhanced Spirometry program, the subject was able to observe her breathing pattern on the screen and therefore ensure that each step was properly executed.

When the investigator was satisfied that the subject was competent in the breathing exercise, the cycle ergometer was properly adjusted for the subject. The settings were recorded so that they could be duplicated for the second visit. Forearm blood flow measurement was then introduced to the subject.

Forearm blood flow was measured by venous occlusion plethysmography, using a mercury-in-rubber strain gauge (Hokanson EC6 strain gauge plethysmograph, D.E. Hokanson Inc., Bellevue, WA). Forearm blood flow measurements are commonly used as an index of whole-body skin blood flow because blood flow to the forearm is controlled in a similar manner to that in most other parts of the body surface. In addition, arm muscle blood flow is not elevated during leg exercise (Johnson, Rowell, 1975), nor during body heating (Detry et al., 1972). Therefore, any increases in forearm blood flow during leg exercise in a hot climate should reflect increased blood flow to the skin alone.

The basic concept behind venous occlusion plethysmography is that by obstructing venous return from the forearm, but allowing arterial inflow to continue unimpeded, the forearm swells at a rate proportional to the rate of arterial inflow (Whitney, 1953). Therefore, a cuff was placed around the upper left arm and inflated to 50 mmHg for 6 seconds. Another cuff was placed around the wrist and inflated to suprasystolic pressure, inhibiting blood flow to the hand. The rate of swelling of the forearm in ml•100 ml^{-1} of tissue•min^{-1} (% per minute) was calculated by changes in forearm circumference by means of a strain gauge placed around the largest circumference of the forearm. The gauge consists of a thin elastic rubber tube filled with mercury. The electrical resistance of the strain gauge increases when the gauge is stretched because the mercury column is both longer and thinner. Therefore, the volume of mercury in the gauge remains constant, but the resistance increases. Strain-gauge plethysmography, therefore, estimates total flow in the forearm from wrist cuff to collecting cuff.

Throughout the measurement, the left forearm rested on an adjustable support at about the level of the right atrium to facilitate venous drainage. Each time a measurement was taken, the arm cuff was inflated 6 seconds in every 15 seconds (allowing 9 seconds for venous drainage), for a total of 1 minute, therefore giving four values. Due to the high sensitivity of the gauge, the lead wire was taped to the forearm so that small movements of the cable would not perturb the gauge. Subjects also had to remain completely still during these 6 seconds (sampling period) to avoid any artifact caused by even slight body

movements. Therefore, the fingers of the left hand were tucked underneath an elastic bandage that was attached to the support to prevent even small movements of these digits. The subject was also asked to not breathe during each sampling period. At the end of the minute, the wrist cuff was then deflated and the subject was asked to wiggle her fingers to aid recirculation to the left hand.

Once the techniques were introduced, *resting* cardiac output and forearm blood flow were measured. The subject then performed three individual 8-min submaximal loads based on her heart rate (bout 1 HR=105-115, bout 2 HR=120-130, bout 3 HR=135-145). Each 8-min bout was separated by a 5-min rest. Submaximal cardiac output was measured three times (each measurement separated by one minute) in the middle of each bout. Post-exercise forearm blood flow was taken after each bout: four times during min 1-2 and four times during min 3-4, with time 0 being when the subject stopped cycling. By the end of the first visit to the laboratory, each subject was familiar with the equipment that would be used during the second visit. More importantly, each subject had extensive practice with the procedures required for the cardiac output and forearm blood flow measurements.

Finally, peak aerobic power was assessed by an open-circuit system ($\dot{V}_{max}$ Series/V6200, SensorMedics, Yorba Linda, CA). The protocol was an all-out continuous progressive cycle ergometer (Fleisch-Ergostat Universal, Metabo) test, 2-min per stage (50 revolutions$\bullet$min^{-1}). The test was terminated on the

subject's volitional exhaustion or when she could not maintain the cadence in spite of the encouragement given by the investigator. Tests were considered maximal if at least two of the following criteria were reached: 1) failure to maintain the cadence in spite of encouragement, 2) RER ≥1.10, 3) maximal HR ≥190 beats per minute. However, because of an apparent malfunction of the O_2 sensor, $\dot{V}O_{2peak}$ data might not be valid and will not be reported. Instead, peak mechanical power was determined as the highest power ($\dot{W}$, watts) that the subject could sustain for 2 minutes. When the last stage was less than 2 minutes, the calculated maximal power was prorated (Bar-Or, 1983).

Visit II

Visit II took place in the climatic chamber at the Children's Exercise and Nutrition Centre. The environmental conditions were 35°C and 45-50% rh. Upon entry in the chamber, each subject performed two 20-min cycling tasks, with a 15-min pre-exercise rest period, a 10-min rest period in between bouts, and a 10-min rest period after the last exercise bout (for protocol details see APPENDIX D). The chosen intensity was 50% $\dot{V}O_{2peak}$, which corresponded to 0.74±0.09, 0.84±0.12, 0.99±0.11 watts•kg^{-1} (28.29±2.06, 31.61±3.37, 36.08±2.85% $power_{peak}$) for PEP, MP and LP, respectively. Skin temperatures, heart rate, forearm blood flow, cardiac output, blood pressure and body weight were measured throughout each session (Appendix D). Thermal sensation (-4-very

cold to +4-very hot), general comfort (1-comfortable to 4-very uncomfortable), and rating of perceived exertion (RPE) were also recorded (Appendix D). Skin temperatures were determined on the subscapular region, the forearm, and the thigh (representing trunk and peripheral skin temperatures) by a Micron portable digital infrared thermometer (Model M80G-OCH±0.1°C accuracy). Heart rate was monitored by a heart rate monitor (Polar Vantage XL, Polar CIC, Port Washington, New York). Forearm blood flow was measured by venous occlusion plethysmography. Cardiac output was measured by the acetylene uptake technique. Oxygen consumption was measured using an open-circuit system. Blood pressure was measured by a Tango™ Stress BP Monitor (Suntech Medical, Scarborough, ON). Blood pressure was measured two times (three times if the difference in systolic pressures was greater than 6 mmHg), just prior to each set of three cardiac output measurements.

Subjects were encouraged to drink chilled water to maintain body weight. Based on previous studies conducted in this laboratory, it is estimated that 1% of body weight is lost per hour in children exercising in similar climatic conditions. Since this session lasted just over 1 hour, 1% of body weight was calculated for each subject, and this amount of water was divided equally between two water bottles, with one being presented at the beginning of each exercise bout. The session lasted approximately 75 minutes or until termination criteria were reached. These criteria included heart rate $\geq$ 195 beats$\cdot$min^{-1} (objective criterion), nausea, dizziness, chills, exhaustion, or headache (subjective criteria).

4. CALCULATIONS AND DATA REDUCTION

Calculations

Body surface area (BSA) was calculated from height and weight, according to DuBois and DuBois (1916). Whole body sweating rate (SR) was calculated from the net change in body weight corrected for fluid intake, respiratory water loss (Mitchell et al., 1972), urine output, and the change in weight of clothes and electrodes.

Stroke volume (SV, ml) was calculated as $\dot{Q}(ml \bullet min^{-1})/HR\ (beats \bullet min^{-1})$. Stroke volume index (SVI, $ml \bullet beat^{-1} \bullet m^{-2}$) was calculated as SV/BSA. Cardiac index (CI, $l \bullet min^{-1} \bullet m^{-2}$) was calculated as $\dot{Q}$/BSA. Mean blood pressure (MBP, mmHg) was determined as [systolic blood pressure + 2 • diastolic blood pressure]/3 (MacDougall et al., 1999). Total peripheral resistance (TPR, $mmHg \bullet l^{-1} \bullet min^{-1}$) was calculated as MBP/$\dot{Q}$.

Data reduction

Cardiac output

Editing was necessary to choose the linear region of the constant exhalation phase, after dead space washout and before closing volume

(Appendix E). A sampling volume of 0.7 litre was chosen after discarding the first 0.7 litre. When all three values were successfully edited, their median was recorded as the cardiac output. If only two values were available, the average was taken.

The cardiac output response to exercise in the heat, factoring out differences in work intensity, was represented as $\Delta\dot{Q}/\dot{W}$. To calculate this variable, each subject's resting cardiac output was subtracted from her average exercise cardiac output (determined from the four exercise cardiac output measurements) and divided by her mechanical power at 50% $\dot{V}O_{2peak}$. $\dot{Q}/\dot{V}O_2$ was calculated as a subject's average exercise cardiac output divided by her average exercise oxygen uptake.

Forearm blood flow

Although forearm blood flow was always taken during min 1-2 and min 3-4 post-exercise, the yield of values for min 1-2 was considered too low due to subjects being unable to stay completely still so soon after exercise. Therefore, these time point values were omitted from analysis.

The arterial inflow slopes chosen by the NIVP3 program were not accurate and required editing. On the editing screen, two vertical lines intersected the volume flow curve and a slope was drawn between these intersection points (Appendix F). The "first point" was chosen to coincide with the first good peak after the "cuff artifact". This artifact is an abrupt rise in limb volume that occurs when the cuff is inflated. It is due to the reflux of blood due to the cuff inflation

and should be ignored. The second point was placed two pulse-peaks to the right of the starting point. When all four forearm blood flow measurements were obtained in one minute, the highest and lowest values were excluded and forearm blood flow was recorded as the mean of the remaining two measures. When three values were available, the median was recorded. With two values, the average was taken, but only if there was a 15% difference or less between them.

To examine the effects of exercise on forearm blood flow (ΔFBF), each subject's resting forearm blood flow was subtracted from her average post-exercise forearm blood flow (calculated as the average of min 3-4 after bout 1 and bout 2).

Blood pressure

When three blood pressure measurements were available, the median was selected. When there were two measurements, the average of both the systolic and diastolic blood pressures was recorded. Mean blood pressure was then calculated.

Heart rate

Heart rate was recorded every 5 seconds throughout the session. To determine the average heart rate during a cardiac output measurement, the running time on the Polar watch was recorded at the beginning of each cardiac output measurement. Assuming that a cardiac output measurement took

approximately 1 minute, the heart rate was calculated as the average of the 12 values recorded during that minute.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine any inter-group differences in subjects' physical characteristics. A two factor (group and time) ANOVA with repeated measures for time was used to analyze the physiological and perceptual variables that had complete data sets (e.g. mean blood pressure, RPE, thermal sensation, general comfort). All other physiological variables were analyzed with the General Linear Model (GLM), using Minitab 8.2. This approach allows unequal sample sizes at different time points, so that the maximum number of observations can be used in the analysis. As such, missing data at a given time point did not cause the other observations for that subject to be excluded from analysis. GLM was appropriate for cardiac output and forearm blood flow due to the missing data. This was mostly due to the difficulty that the subjects had in executing these two techniques.

Because all of the PEP and MP subjects were pre-menarcheal, and all of the LP subjects were post-menarcheal, it was also possible to divide the subjects into a pre-menarcheal (PreM) group and a post-menarcheal (PostM) group. This increased the sample size in the less mature group and allowed the use of a two factor (group and time) ANOVA with repeated measures for time for the physiological variables.

Test-retest reliability of the cardiac output measurements, and inter/intra-tester reliability for the editing of forearm blood flow measurements were determined by the intraclass correlation R. This is the estimated variation due only to the subjects, divided by the total variation in the data. It was calculated as:

Reliability = $\sigma^2_{subj} / \sigma^2_{subj} + \sigma^2_{error}$.

When appropriate, *post-hoc* analyses were performed using a Tukey test. Statistical significance was set at $p < 0.05$ for all statistical tests. Data are reported as the mean ± SEM, unless otherwise specified.

5. RESULTS

The $\dot{V}O_2$ values may have had a systematic error, possible due to a faulty O_2 sensor. This error was detected from RER values at submaximal levels that were too high (range ~ 0.95 to 1.50). Because of the above error, aerobic performance is described as peak mechanical power, rather than as $\dot{V}O_{2peak}$.

The subjects' characteristics are presented in Table 1. Age, height and body surface area were significantly different among all three groups. Weight was not significantly different between PEP and MP, but those two groups were significantly lighter than the LP group. Body surface area per mass was significantly higher in PEP than in LP. Body fat percentage and peak mechanical power ($watts \bullet kg^{-1}$) were not significantly different among the groups.

All subjects completed the heat plus exercise session. No significant differences were observed in the percent change of initial body weight among the three groups by the end of the session (PEP= -0.30 ± 0.14, MP= -0.17 ± 0.12, LP= -0.15 ± 0.10 % initial weight).

Responses to exercise in a thermoneutral environment – Visit I

Pre-menarcheal vs post-menarcheal

Even though we were not confident in the $\dot{V}O_2$ data, it was consistent with current data in the literature (for further details see DISCUSSION, p.47), and could therefore be used for comparison purposes. Overall, as oxygen consumption increased, cardiac output increased (Figure 1). There was no significant difference between the two groups with respect to cardiac output at a given oxygen uptake. There was no significant difference in the slopes between the two groups (Figure 2). Because of the above methodological concern regarding $\dot{V}O_2$, the absolute $\dot{Q}$ vs. $\dot{V}O_2$ relationship may not be correct. It is unlikely, however, that this error created a bias in one group and not in the others.

Responses to exercise in a hot climate - Visit II

Comparing the three maturational groups

Cardiac output in each group increased significantly from rest to exercise, but did not show any significant change throughout exercise (Figure 3). Overall, exercise cardiac output was significantly different among the three groups. However, there was no interaction, and there was no significant difference at any specific time points. Cardiac index also showed a significant increase from rest to exercise, but there were no group differences (Figure 4). PEP and MP tended ($p=0.12$) to have a greater increase from rest in cardiac output per watt than LP

(PEP= 0.15 ± 0.02, MP= 0.15 ± 0.03, LP= 0.10 ± 0.01 $l \bullet min^{-1} \bullet watt^{-1}$) (Figure 5). There was no significant difference among the groups when the average exercise cardiac output was expressed over the average exercise oxygen consumption (Figure 6).

Forearm blood flow had a main effect for time such that the two values taken "pre-exercise" were significantly lower than the remaining post-exercise measures (Figure 7). Although not significant, after bout 1, the LP group had a lower forearm blood flow than PEP and MP in minutes 3-4 post-exercise and showed a further decrease in the following measurement ($p=0.06$), taken at the end of the rest period. The pattern was similar after both exercise bouts (bout 1= 7.91 ± 1.06 to 6.1 ± 0.83, bout 2= 9.47 ± 1.47 to 8.26 ± 1.20 $ml \bullet 100ml^{-1} \bullet min^{-1}$). In contrast, the PEP and LP girls showed little change in forearm blood flow post-exercise and at the end of rest (Table 2). PEP had the highest change in FBF from rest to post-exercise, but this difference was not significant among the groups (Figure 8).

Heart rate increased significantly from rest to exercise and then had a significant increase throughout exercise (Figure 9). There was no group effect for heart rate. Mean blood pressure at rest was significantly lower than exercise mean blood pressure. Although not significant, PEP had a lower resting mean blood pressure than MP and LP (PEP= 74 ± 3, MP= 79 ± 2, LP= 81 ± 2 mmHg). PEP had a significantly lower mean blood pressure than MP at the second measurement of the first exercise bout (PEP= 82 ± 2, MP= 91 ± 3 mmHg).

Stroke volume increased significantly from rest to exercise, but there were no significant differences throughout exercise (Figure 10). Overall, there was a significant difference among the three groups, but similar to cardiac output, there was no interaction and no significant differences at any specific time points. Stroke volume index also increased significantly from rest to exercise, but there were no group differences (Figure 11). Total peripheral resistance decreased significantly from rest to exercise, but remained stable throughout exercise among the three groups (Figure 12).

RPE, although not significantly different, was higher in the PEP group in both exercise bouts than in the MP and LP groups. The increase in RPE from the first to the last exercise bout was not significantly different among the three groups (Figure 13). When RPE was divided by HR and expressed over time, the pattern changed (Figure 14). The three groups were similar during the first bout, but the PEP group tended to have a lower RPE during the second bout. The general comfort score was similar among the three groups, and showed little change from the first exercise bout to the second (Figure 15). The increase in the thermal sensation score tended to be greater in the PEP and MP groups when compared to the LP group (PEP= 0.8 ± 0.4, MP= 1.0 ± 0.3, LP= 0.3 ± 0.3).

Forearm skin temperature of the LP group tended to be lower than the PEP or MP groups beginning at Time 32-min (end of first exercise bout) and continuing throughout the session, but an ANOVA for repeated measures revealed a significant effect for time, but no group effect (Figure 16). Sweating

rate was not significantly different among PEP, MP and LP (PEP= 223 ± 24, MP= 236 ± 28, LP= 232 ± 24 $g \bullet m^{-2} \bullet hr^{-1}$).

Pre-menarcheal vs Post-menarcheal

The PostM group had a higher cardiac output throughout exercise, but this did not reach a level of significance (Figure 17). Cardiac index tended to be higher in the PreM group, but again there was no significance. PreM had a significantly higher $\Delta\dot{Q}/\dot{W}$ than PostM (PreM= 0.15 ± 0.02, PostM= 0.10 ± 0.01 $l \bullet min^{-1} \bullet watt^{-1}$) (Figure 18). When average exercise cardiac output was expressed over average exercise oxygen consumption, the two groups were almost identical (Figure 19). Analysis of stroke volume, stroke volume index, heart rate, mean blood pressure and total peripheral resistance showed the same time effects as the PEP, MP and LP groups, but there were no group effects and no interactions.

Although not significant, after bout 1, the PostM group had a lower forearm blood flow than PreM in minutes 3-4 post-exercise and showed a further decrease in the following measurement (p=0.17), taken at the end of the rest period (Figure 20). This pattern was the same after both exercise bouts. In contrast, the PreM group showed little change in forearm blood flow post-exercise and at the end of rest. PreM was not significantly different from PostM with respect to ΔFBF (Figure 21).

The reliability coefficient for the cardiac output measurement over the 4 exercise time points was R=0.88. The intra-observer reliability coefficient for

editing of the forearm blood flow measurements was R=0.89. The inter-observer reliability coefficient was R=0.89.

6. DISCUSSION

This is the first study to compare hemodynamic responses to exercise in the heat in females who are at different stages of puberty. Previous studies on thermoregulatory responses to heat stress have compared prebuscents with young adult women or men, therefore leaving a gap as to what is happening in mid and late puberty.

The main finding of this study is that although there was no significant group difference in the central hemodynamic (e.g. cardiac index, stroke volume index) response to exercise in the heat, there were strong trends in the cutaneous blood flow response. Specifically, while forearm blood flow (FBF) did not differ between PEP and MP, the LP girls had a consistently, albeit not significantly, lower FBF than the less mature ones. LP also seemed to have a faster "recovery" FBF, as evidenced by the drop in FBF seen from the beginning to the end of each rest period (Figure 7). In contrast, PEP and MP maintained a relatively constant FBF throughout these same time periods.

Responses to exercise in a thermoneutral environment

Due to previously described problems with the metabolic cart, it was decided to approach $\dot{V}O_2$ as follows. During visit I, subjects cycled at three different intensities, therefore allowing us to examine $\dot{Q}$ over a range of $\dot{V}O_2$ values. We could then compare the results to data in the literature that were also collected in a thermoneutral environment, using a cycling protocol. The subjects were separated into PreM and PostM because the sample size was considered too low for the less mature groups if they were divided into PEP and MP.

When comparing the regression lines for each group (Figure 2), the slopes were similar between the PreM and PostM groups and the slope calculated by Turley and Wilmore (1997). Their study involved 7-9 year old girls who cycled at 20, 40 and 60 watts. This indicates that any error in the $\dot{V}O_2$ data was probably random and should not affect the overall results.

At any given metabolic level, children's cardiac output is somewhat lower than in adults (Bar-Or et al., 1971; Eriksson et al., 1971; Turley, Wilmore, 1997). Figures 1 and 2, however, did not show a separation between the groups in the $\dot{Q}$ response at a given $\dot{V}O_2$. Figures 6 and 19 (data from visit II) added further confirmation because the ratio of $\dot{Q}$ to $\dot{V}O_2$ was not significantly different whether the subjects were divided into PEP, MP and LP, or into PreM and PostM. Unlike previous studies, there was no "adult group" in this study. In fact, only one subject in the PostM group rated herself as Tanner stage 5 (full maturity).

Consequently, the majority of subjects in the most mature group had probably not attained full physical maturity. Therefore, it seems that a change in the $\dot{Q}$ response at a given metabolic level is not occurring from pre- to late- puberty, but instead might occur in later years.

Responses to exercise in a hot climate

It was not surprising that all of the subjects completed the prescribed task. When the dry bulb temperature is below, and not more than 5° to 7°C above skin temperature, children seem to dissipate body heat as effectively as adults (Haymes et al., 1974; Drinkwater et al., 1977; Inbar, 1978; Docherty et al., 1986; Blanchard, 1987). The dry bulb temperature in this study was 35°C, and none of the forearm skin temperatures exceeded 35.5°C. BSA/M was significantly higher in PEP compared to LP. However, since the air to skin temperature gradient was small (gradient range ~ –0.5°C to 3°C), it is unlikely that the PEP group was subjected to greater environmental heat stress due to their higher BSA/M. It is possible that if the climatic stress had been greater (e.g. ≥42°C), there might have been more separation in the hemodynamic responses among the three groups. Physiological responses to heat are also affected by relative work intensity. Although each of the three pubertal groups was working at a different % $power_{peak}$, these differences were not significant.

Absolute cardiac output tended to be higher in MP and LP, but these two groups were also working at a higher absolute intensity than PEP. Since there was a wide age range among the groups, it was important to account for body size. When accounting for body size (e.g. cardiac index, stroke volume index), all differences among the groups were eliminated. To account for the different absolute intensities, $\Delta\dot{Q}/\dot{W}$ was calculated, and PEP and MP tended to have a greater increase from rest to exercise in cardiac output per watt than did LP (Figure 5). Although this would seem contradictory to our hypothesis, it could be that the less mature individuals required this higher cardiac output to supply the cutaneous vessels.

To examine this relationship between central and peripheral circulation, FBF was measured as soon after exercise as possible. Consistent with previous studies, forearm blood flow tended to be higher in the less mature subjects (Drinkwater et al., 1977; Falk et al., 1992), a difference that was nearly significant (Figure7). Of particular interest were the different FBF patterns between PEP and MP vs. LP from the beginning to the end of each rest period. It is difficult to speculate on the mechanism controlling this pattern. However, it does give further strength to the idea that when pre-pubertal girls exercise in the heat, a greater proportion of their blood volume shifts from the central to the peripheral circulation. If this peripheral blood flow is crucial for thermoregulation, then the less mature subjects may need to maintain a high FBF even during recovery. In

contrast, the more mature subjects can redistribute blood volume to other areas, without compromising heat dissipation.

Because menarche is a clear biological marker, and due to the low yield of data for the main hemodynamic responses, it seemed appropriate to also divide the subjects into a pre- and post menarcheal group and proceed with analysis. However, higher blood flow in the less mature group still did not reach significance. There was a significant difference in the $\Delta\dot{Q}/\dot{W}$ such that the pre-menarcheal subjects had a higher change in cardiac output per watt than did the post-menarcheal subjects (Figure 18). As previously mentioned, the higher values in the less mature subjects was unexpected and it is difficult to speculate on the mechanism.

Davies (1981) reported that skin temperature was higher in children compared with adults during exercise in the heat. This can either reflect lower evaporative cooling or higher skin blood flow and vasodilation in the children. Although not significant, PEP and MP did have a consistently higher forearm skin temperature than LP from the middle of the first bout to the end of the session (Figure 16).

The whole body sweating rates of the three groups were not significantly different and therefore cannot explain the tendency for higher forearm skin temperatures in the less mature groups. Sweating rate (when expressed per unit body surface area) of boys has repeatedly been shown as lower than that of men (Kawahata, 1960; Wagner et al., 1972; Rees, Shuster, 1981). Controversy exists

when comparing the sweating rate of girls and women. Earlier studies reported little difference exists between the sweating rate of girls and women (Kawahata, 1960; Drinkwater et al., 1977; Rees, Shuster, 1981). However, a study that compared prepubescent, pubescent and young adult females who were matched for $\dot{V}O_{2max}$ and exercised at 50% $\dot{V}O_{2max}$ in 42°C, 18% rh reported that the sweating rate of the adult women was in fact 50-70% higher than in the younger groups (Meyer et al., 1992). These findings suggest that large differences in sweating rate may not be seen among females until the latter stages of puberty. Therefore, we might have seen a significant difference in sweating rates and consequently skin temperatures if our LP group had been older. Also, since overall sweating rate was calculated, sweating pattern was not specific to the forearm. It has been suggested that children sweat more profusely on the trunk and less on the limbs than adults do (Kuno, 1956). If there was a lower sweating rate of the forearm, this could explain the tendency for higher forearm skin temperatures in the less mature groups. Although most of the SkBF responses to heat stress arise through neurogenic vasoconstrictor and vasodilator systems, local warming of the skin can vasodilate the underlying resistance vessels. Forearm skin blood flow shows a fairly small increase per °C increase in the local surface temperature between 5°C and 35°C (Barcroft, Edholm, 1943). When skin temperature exceeds 37°-38°C, SkBF rises more steeply. However, forearm skin temperatures in this study were similar among groups and did not exceed 35.5°C in any of our subjects. As such, it is not likely

that evaporative heat loss was significantly lower on the forearm of the PEP and MP groups, nor is it likely, therefore, that local skin temperature had a notable effect on forearm blood flow.

Unlike Drinkwater et al. (Drinkwater et al., 1977), who studied prepubescent and young college women, the PEP girls in our study did not maintain a significantly higher heart rate and lower stroke volume index during exercise than the more mature subjects. As previously stated, at any given metabolic level, children's cardiac output is somewhat lower than in adults (Eriksson et al., 1971; Turley, Wilmore, 1997). This response is marked by a lower SV, which is not entirely compensated for by a higher HR at a given absolute $\dot{V}O_2$. Drinkwater et al. (1977) suggested that the greater increase in FBF in girls compared to young college women, combined with a lower ratio of $\dot{Q}$ to $\dot{V}O_2$ provided evidence that a greater proportion of the girls' blood volume (and therefore $\dot{Q}$) was diverted to the skin, leaving less blood available for the central circulation and resulting in a lower heat tolerance. Unfortunately the $\dot{V}O_2$ data for the second visit in the present study were not accurate, so it is not possible to comment on the $\dot{Q}$:$\dot{V}O_2$ relationship during exercise in the heat. However, expressing $\Delta\dot{Q}/\dot{W}$ provided an unexpected tendency, such that PEP and MP had a higher change in $\dot{Q}$ per watt than did the LP.

Blood pressure, as previously stated, is usually well maintained in heat stress. In spite of the redistribution of blood flow and volume that accompanies cutaneous vasodilation, peripheral resistance and cardiac output are well matched and favor the maintenance of blood pressure. This was also the pattern among the three groups in this study. Consequently, as cardiac output increased to meet the metabolic and thermoregulatory demands, total peripheral resistance decreased and blood pressure remained constant.

The tendency for a higher rating of perceived exertion (RPE) in the PEP group is consistent with a similar study conducted with pre-, mid- and late-pubertal boys (Falk et al., 1992). This previous study took place under hotter conditions (42°C, 50% rh) and included three 20-min bouts of cycling. Those who did not complete the session in the pre-pubertal group stopped only because of subjective distress and not because they had reached the objective termination criteria (Falk et al., 1992). It was suggested that the response of the pre-pubertal boys was consistent with their somewhat greater RPE toward the end of the session. As previously mentioned, all of the subjects in the current study completed the protocol, so the higher RPE in the PEP group was not enough to limit exercise. In addition, thermal sensation scores for PEP and MP suggested that they felt subjectively warmer than LP by the end of the session, but general comfort remained similar among the three groups and changed very little from the first to the second exercise bout. Therefore, although RPE values

were higher in the less mature subjects, they did not seem to affect their overall comfort and therefore did not cause overwhelming subjective distress.

It is interesting that when RPE was expressed relative to heart rate, the subjects were very similar in the first bout, but the PEP subjects tended to have lower ratio than MP and LP during the second bout (Figure 14). Therefore, for a given HR, the PEP subjects actually reported a lower rating of perceived exertion. Earlier data (Bar-Or, 1977) represented children's (7-17 years) RPEs during continuous, incremental cycle ergometry over an exercise range of 50-200 watts. With the exception of the 7-9 year old group, the children gave lower ratings of effort than adults at the same relative exercise intensity. Although all of the subjects in the current study were younger than 17 years, it is possible that this lower rating of effort starts to become evident even when comparing pre/early-pubertal subjects to mid- and late- pubertal subjects.

7. CONCLUSIONS

Under the conditions of the present study, and using a sample of healthy girls ranging from 9-16 years, the following conclusions are suggested.

1. Physical maturity does not seem to affect the central hemodynamic responses (e.g. cardiac index, stroke volume index) of pre- to late- pubertal girls to exercise in the heat.

2. There was a consistent trend among the three groups with respect to peripheral blood flow: while forearm blood flow did not differ between the pre- and mid-pubertal girls, the late-pubertal girls had a lower forearm blood flow than the less mature ones. The late-pubertal girls also had a faster recovery forearm blood flow than the less mature groups, as seen by the decrease in forearm blood flow from immediately post-exercise to the end of rest. This strengthens the notion that there is a maturity-related change in forearm blood flow from pre- to late- puberty.

3. Overall, the hemodynamic responses of the pre-, mid- and late-pubertal girls were similar and did not show a clear transition from a child-like to an adult-like response.

4. There were also no significant differences in the hemodynamic responses when the subjects were divided into pre- and post- menarche groups. This would

also suggest that a transition is not occurring until later in girls' physical development.

5. In a thermoneutral environment, there was no difference in cardiac output at a given oxygen uptake from early to late puberty.

6. The above conclusions are specific to an environmental temperature of 35°C and 50% rh. As such, the skin to air temperature gradient was minimal. It is possible that hotter or more humid conditions could have elicited significant differences in the hemodynamic responses among the three pubertal groups.

Study Limitations

1.Sample size: One of the major limitations of this study was the low data yield from both the forearm blood flow and the cardiac output measurements. Since there were only 9 subjects in each group, it was important to have as few missing data points as possible. A higher sample size is necessary for challenging protocols since one can presume that not all subjects will be successful in each measurement.

2. Oxygen uptake: The $\dot{V}O_2$ values collected in this study were suspected of random error. This represented the loss of valuable data, since it would have allowed a comparison of the physiological variables among the groups at any given oxygen uptake, regardless of differences in absolute work intensity.

3. Core temperature: Due to some difficulty in recruiting subjects, it was decided not to include rectal temperature measurements. Core temperature data are an indication of the temperature information being sent to the central thermoregulatory sytem from thermosensitive neurons throughout the body. An increase in core temperature, as detected by the central thermoregulatory system, is responsible for activating the physiological responses that enhance heat dissipation. Therefore, it would have been beneficial to monitor core temperature throughout the session.

4.Pubertal stages: Puberty involves a series of anatomic, physiologic and emotional changes, the end result of which is full sexual maturity. Pubertal changes progress in a continuum, but for clinical purposes these changes have

been divided into a series of physical stages based on identifiable secondary sexual characteristics. The present study employed the rating system for breast development devised by Tanner (Tanner, 1962). Self-report using Tanner staging photographs was used to estimate the level of sexual maturation of the subjects. Although this method provides an adequate estimate of pubertal status (Neinstein, 1982), it is still highly subjective, and could therefore have influenced the grouping of the subjects in the current study. In addition, because girls begin puberty earlier than boys, a "truly" pre-pubertal female subject is often young in age and small in size. Due to the complex and demanding protocol of the present study, it was not feasible to recruit subjects younger than 9 years old. Therefore, it was necessary to call the least mature group "pre/early" since several of the subjects were already exhibiting secondary sexual characteristics. More separation in the hemodynamic responses of the subjects might have occurred if it had been possible to have a purely "pre-pubertal" group.

5. Menstrual cycle phase: The post-menarcheal subjects were tested during the follicular phase of their cycle (days 1-14) because female reproductive steroid hormones can influence cardiovascular function (Stephenson, Kolka, 1993). However, not all of the subjects had regular menses. Therefore, without direct hormone measurements, it is possible that some post-menarcheal subjects were not tested at the follicular phase, when all cyclic hormones are low.

6. Heat acclimatization: It was originally planned that data collection would take place during the months March-May to reduce the possibility that the subjects

were heat-acclimatized. Heat acclimatization includes the physiological and perceptual changes that occur from natural exposure to hot climates, while heat acclimation includes repeated exposure to artificially produced climatic heat stress (Bar-Or, 1989). In adults, the changes that accompany acclimatization to exercise in hot environments include reductions in heart rate, core temperature, skin temperature, and the threshold core and skin temperatures at which sweating begins. Sweating rate is also increased at a given core temperature. Due to equipment modifications, the months of testing were actually May-August. There is limited information on acclimatization and the young female. In general, however, the heat acclimation process is similar in adults, children and adolescents. The only difference is in the rate of acclimation (and probably natural acclimatization), such that the process seems to be slower in children (Inbar, 1978). It is possible, therefore, that some subjects were already heat acclimatized when they participated in this study. This could alter their hemodynamic responses. In an attempt to account for this, the number of subjects in each group that were tested each month was counterbalanced.

7. Difficulty of protocol: The protocol in the current study was demanding and involved superior coordination on the part of the subject. It became evident that data yield was much higher in the two more mature groups. For example, after editing, the cardiac output yield was 67%, 84%, and 93% for the pre/early-, mid-, and late-pubertal groups, respectively. It appears that the use of the acetylene uptake technique in subjects younger than 10 years may not be appropriate,

particularly if it must be coordinated with cycling. Alternately, more visits may be necessary to ensure that the younger subjects can perform the techniques properly and consistently.

8. Heat stress: When comparing the cardiovascular responses of individuals who vary in size, it is important to provide a comparable challenge to all subjects. Heat stress is comprised of the environmental conditions and the metabolic load. In this study, all subjects exercised in a similar environment (35°C, 45-50% rh). However, the less mature groups were working at a lower, albeit not significantly different, percentage of their $power_{peak}$. Consequently, the metabolic heat stress could have been less for some subjects than for others.

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Table 1. Subject characteristics (means±SD)

	Pre/early-pubertal (PEP)	Mid-Pubertal (MP)	Late-Pubertal (LP)
N	9	9	9
Age, yr [a]	10.5 1.3	12.6 1.6	14.5 1.2
Height, cm [a]	142.1 8.5	151.0 7.7	164.7 5.9
Weight, kg [b]	38.3 10.5	46.7 6.1	58.6 7.3
Surface area, m^2 [a]	1.22 0.18	1.39 0.12	1.64 0.09
Surface area per mass, $cm^2 \bullet kg^{-1}$ [c]	328.4 38.6	300.6 18.4	281.3 21.4
Fat, %	21.5 7.1	22.0 4.7	25.7 4.9
$Power_{peak}$, $watts \bullet kg^{-1}$	2.59 0.49	2.61 0.82	2.70 0.75

Significant difference ($p<0.05$): [a] PEP vs. MP vs. LP
[b]PEP and MP vs. LP, [c]PEP vs. LP

Table 2. Forearm blood flow $ml \bullet 100ml^{-1} \bullet min^{-1}$ (means ± SEM)

	Upon chamber entry Time 0	Before 1st bout Time 15	Minute 3-4 after 1st bout Time 38	End of 10-min rest Time 43	Minute 3-4 after 2nd bout Time 68	Before exiting chamber Time 75
PEP	4.93 0.65	5.61 0.65	9.60 0.83	9.75 0.66	10.00 1.27	9.59 1.56
MP	5.16 0.41	7.12 0.83	10.03 1.51	10.29 1.94	9.93 1.27	9.91 1.65
LP	4.68 0.41	5.56 0.69	7.91 1.06	6.10 0.83	9.47 1.27	8.26 1.20

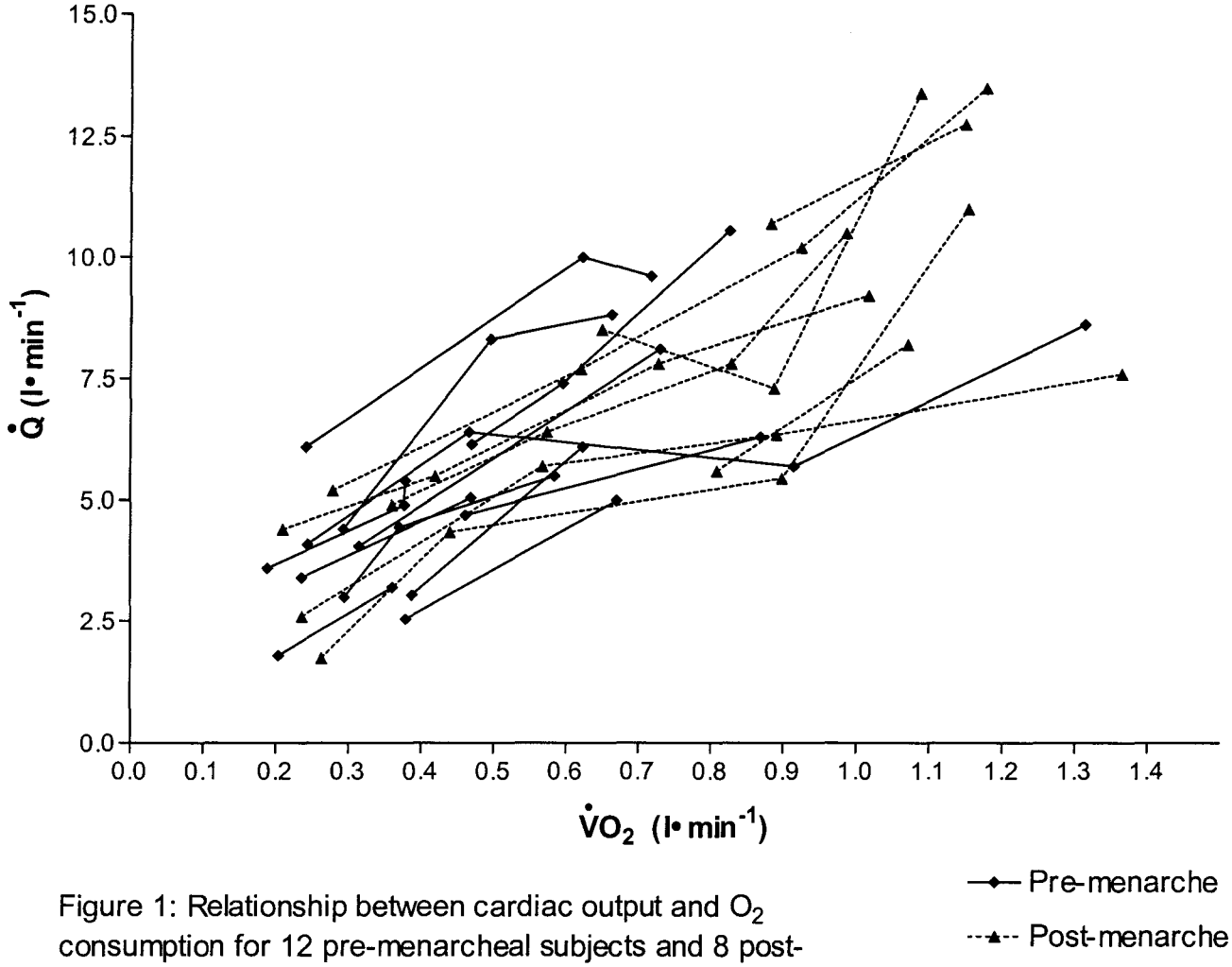


Figure 1: Relationship between cardiac output and O_2 consumption for 12 pre-menarcheal subjects and 8 post-menarcheal subjects.

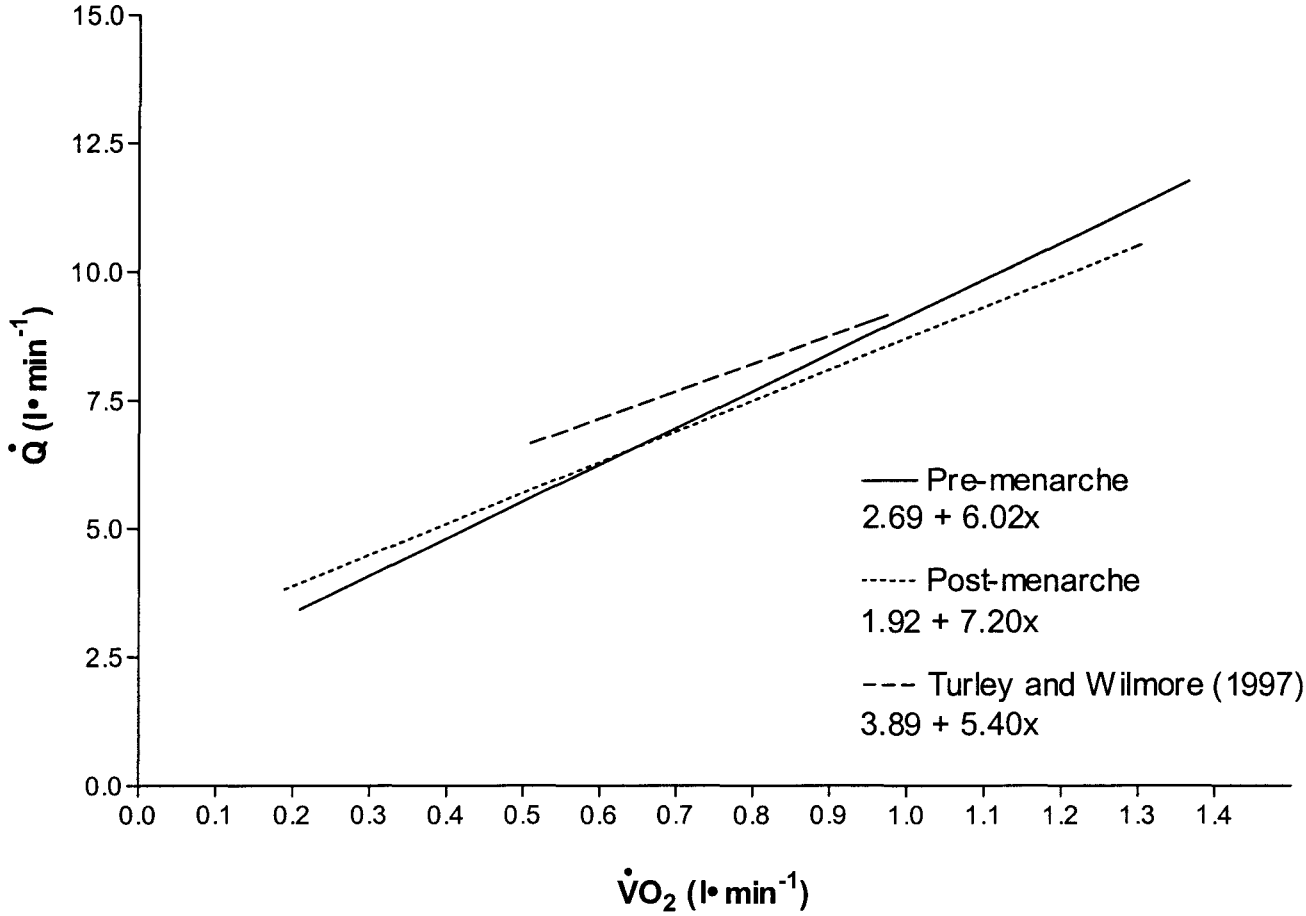


Figure 2: Regression lines of cardiac output on O_2 consumption for the pre-menarcheal group, the post-menarcheal group, and the pre-pubertal girls (7-9 yrs) studied in Turley and Wilmore (1997). *Insets:* regression equations for each group.

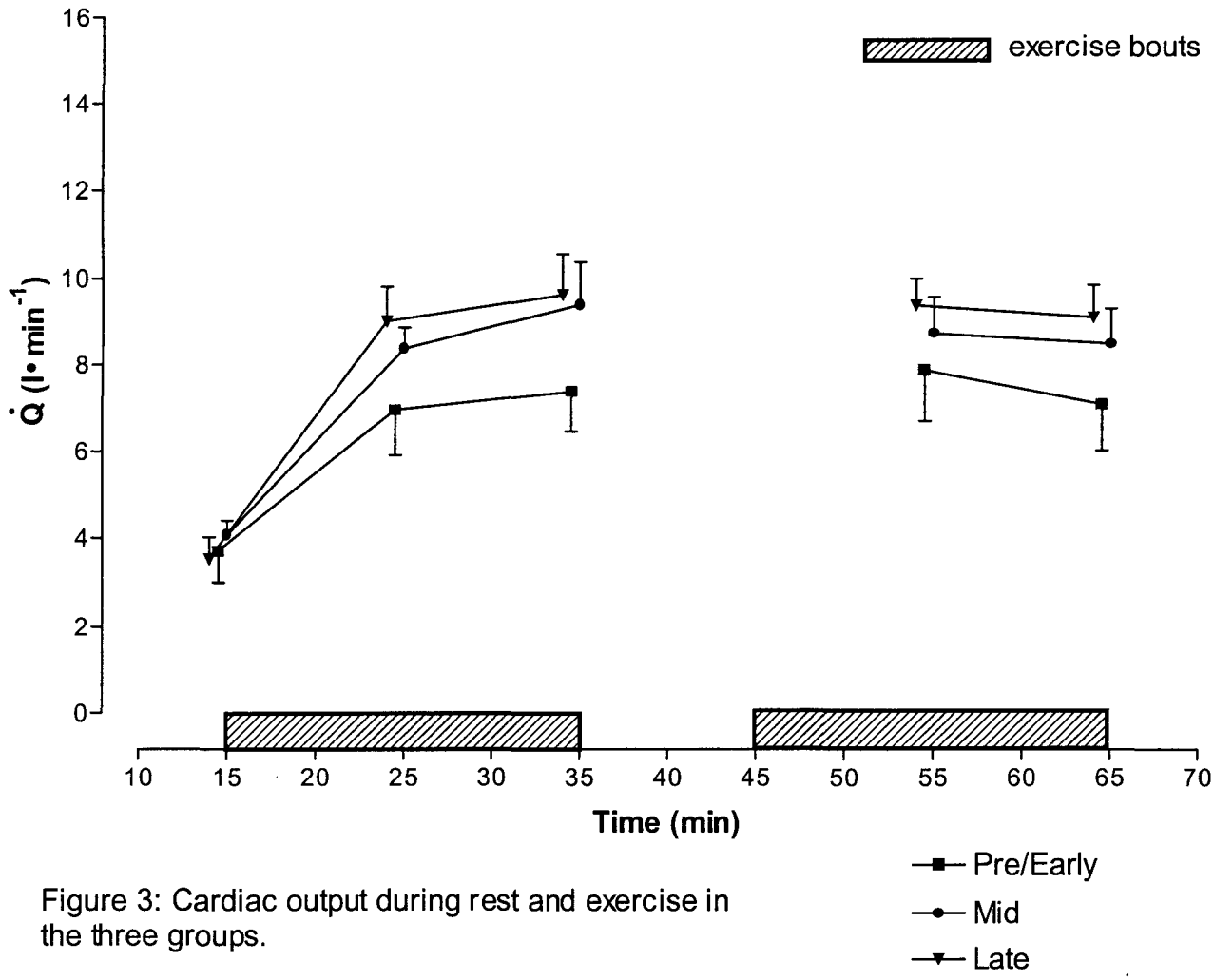


Figure 3: Cardiac output during rest and exercise in the three groups.

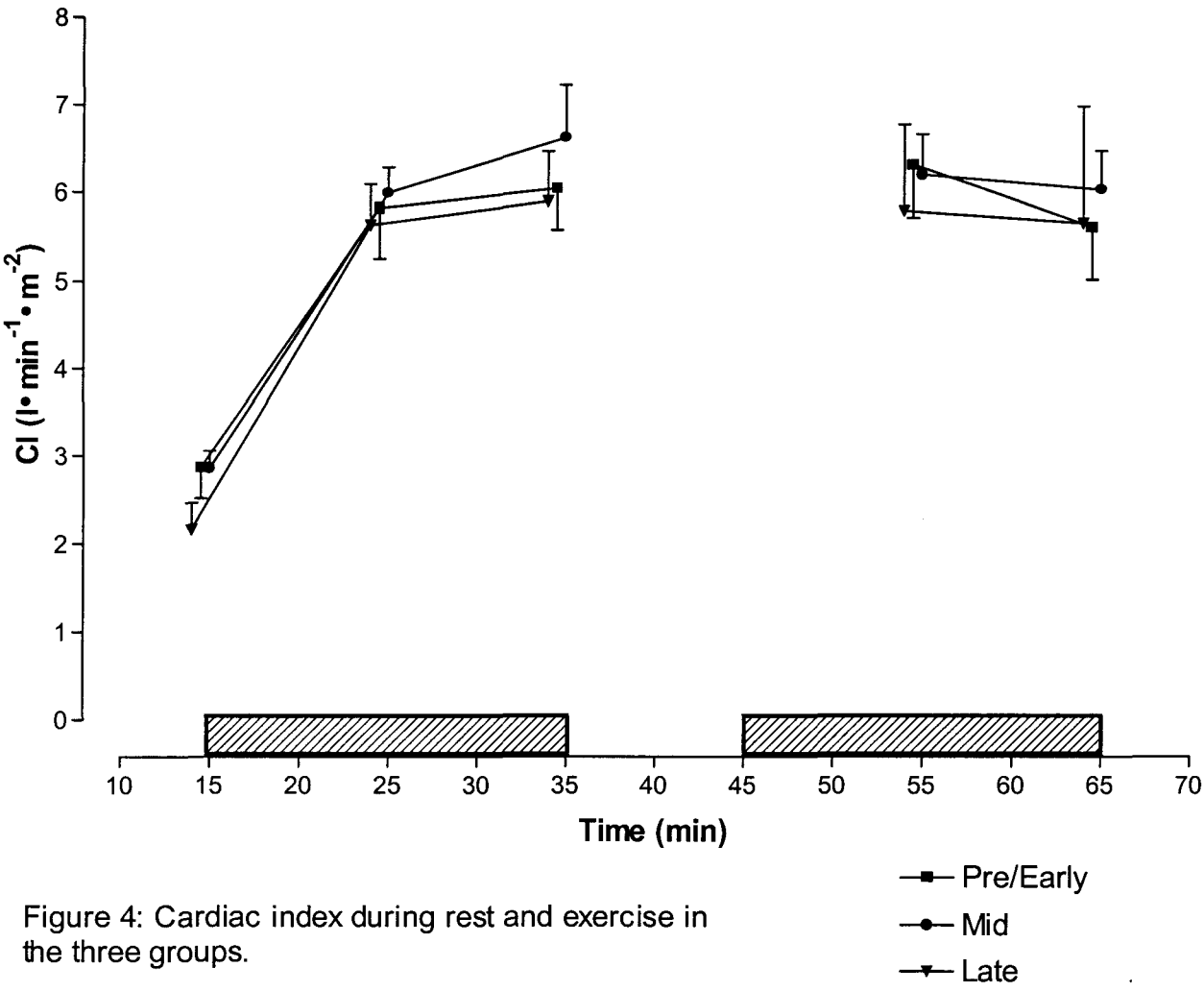


Figure 4: Cardiac index during rest and exercise in the three groups.

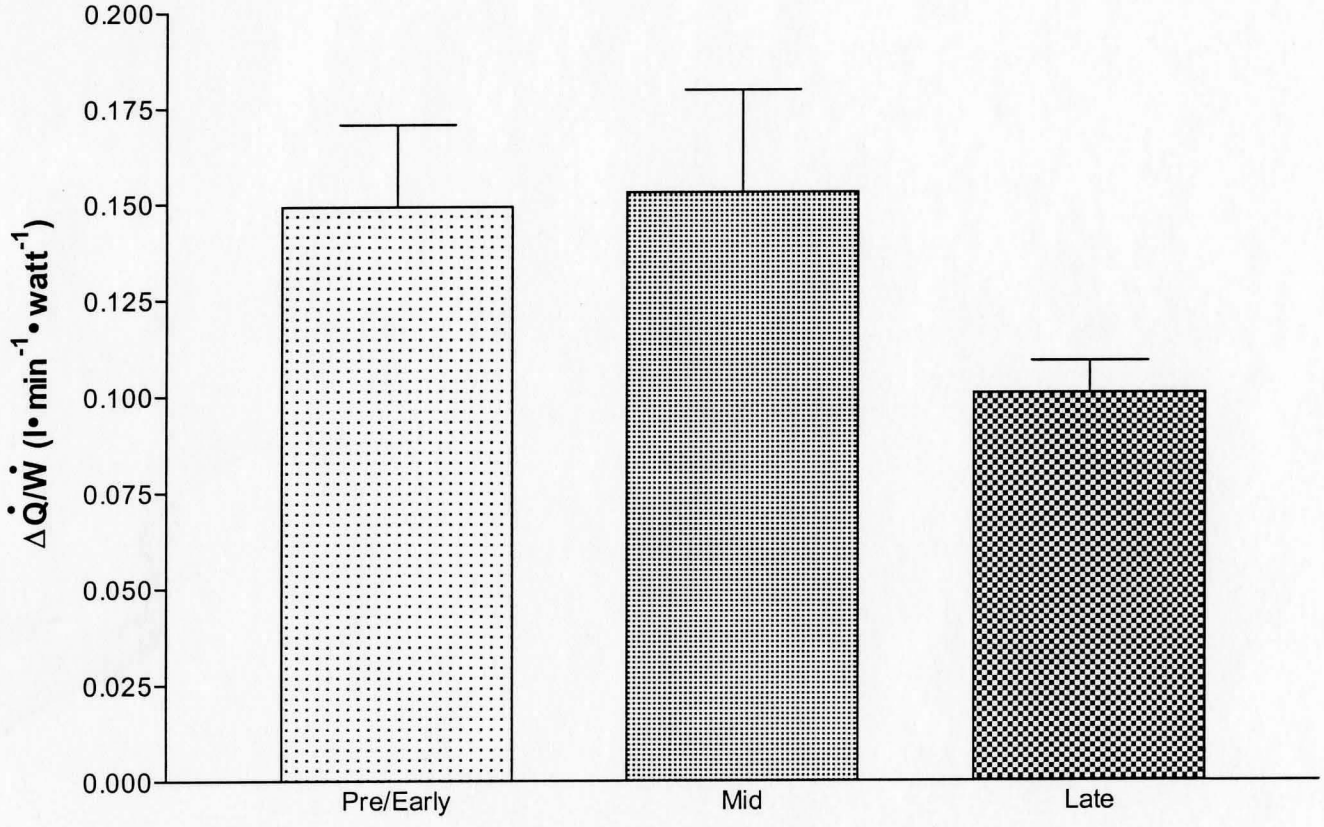


Figure 5: Net exercise cardiac output per watt in the three groups.
Δ denotes exercise minus rest.

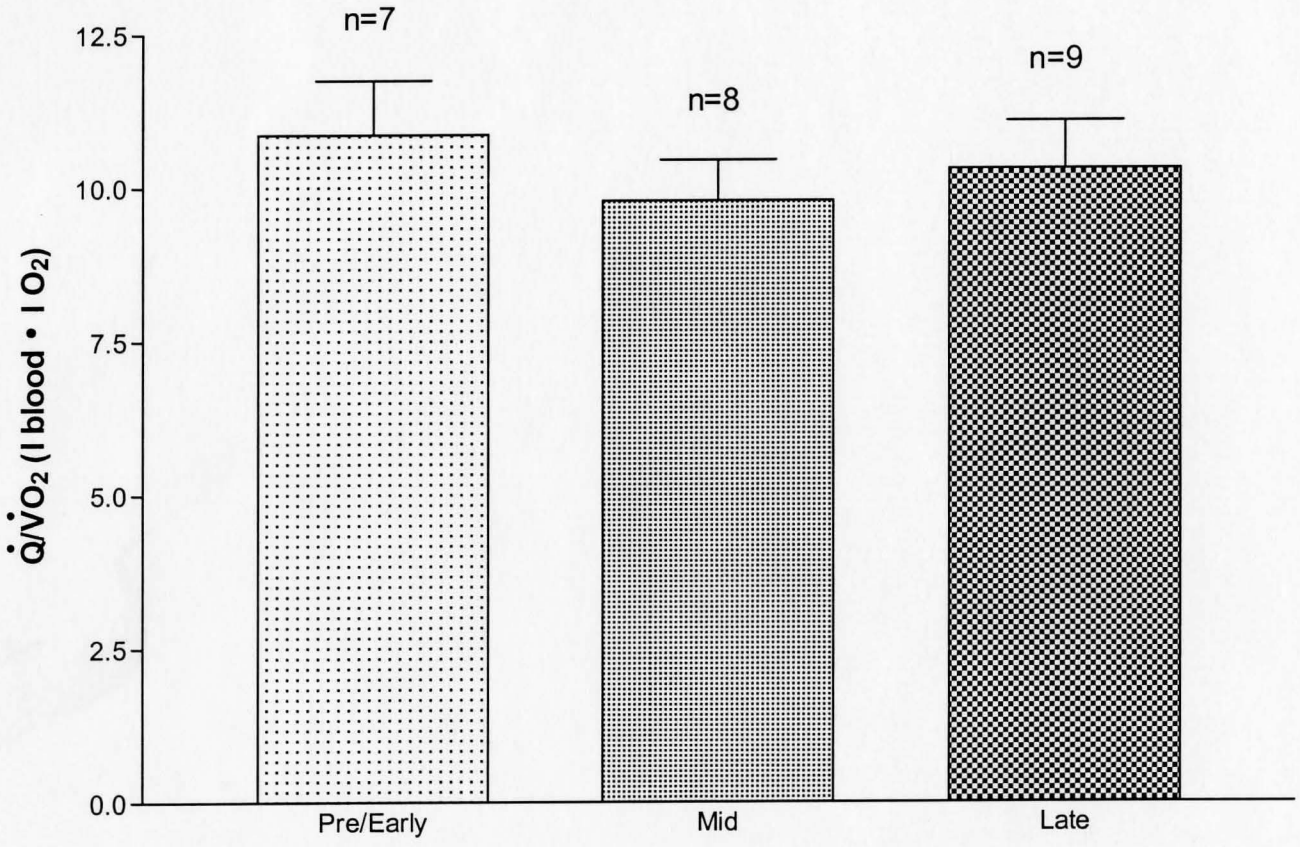


Figure 6: Cardiac output / oxygen consumption for the three pubertal groups.

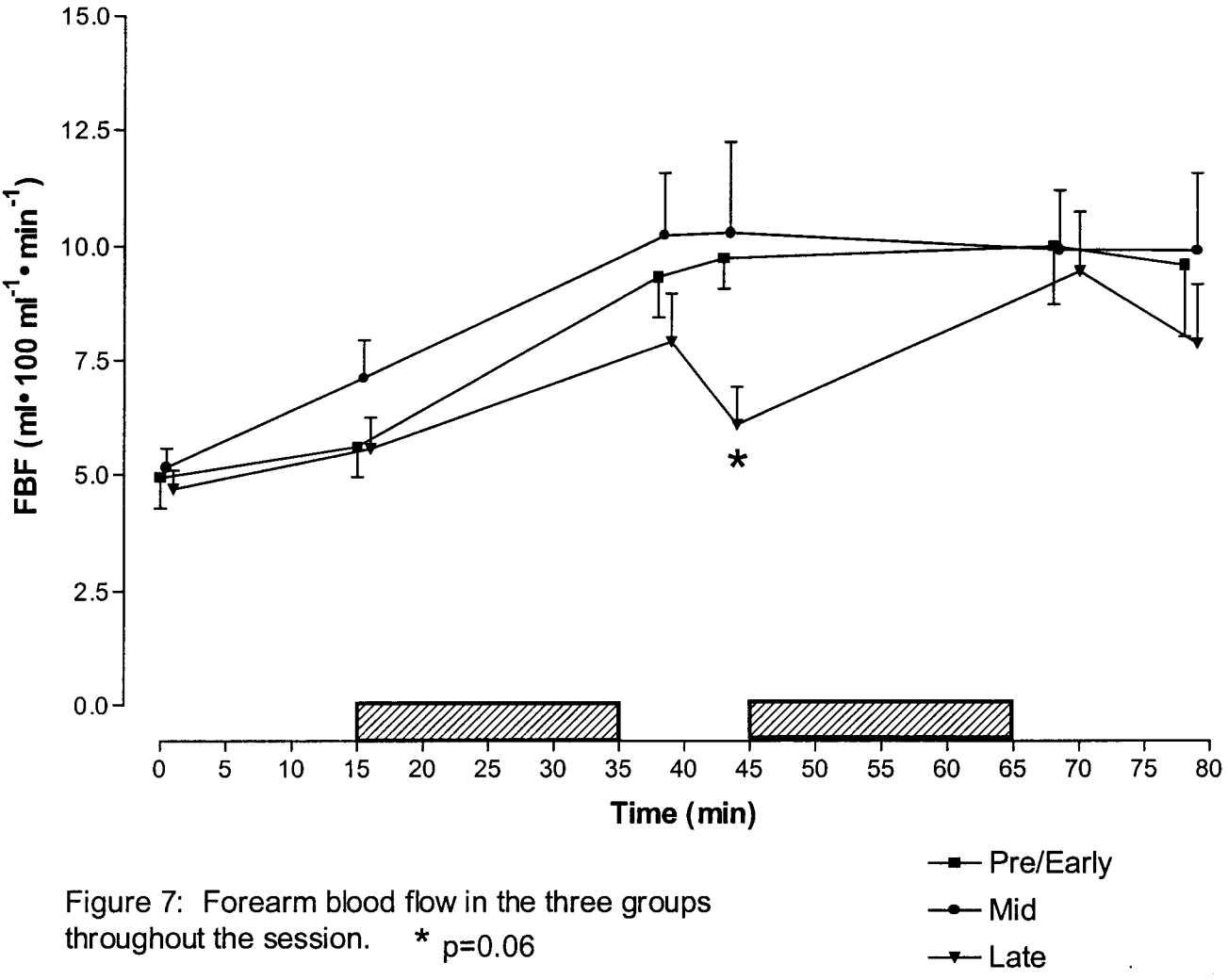


Figure 7: Forearm blood flow in the three groups throughout the session. * p=0.06

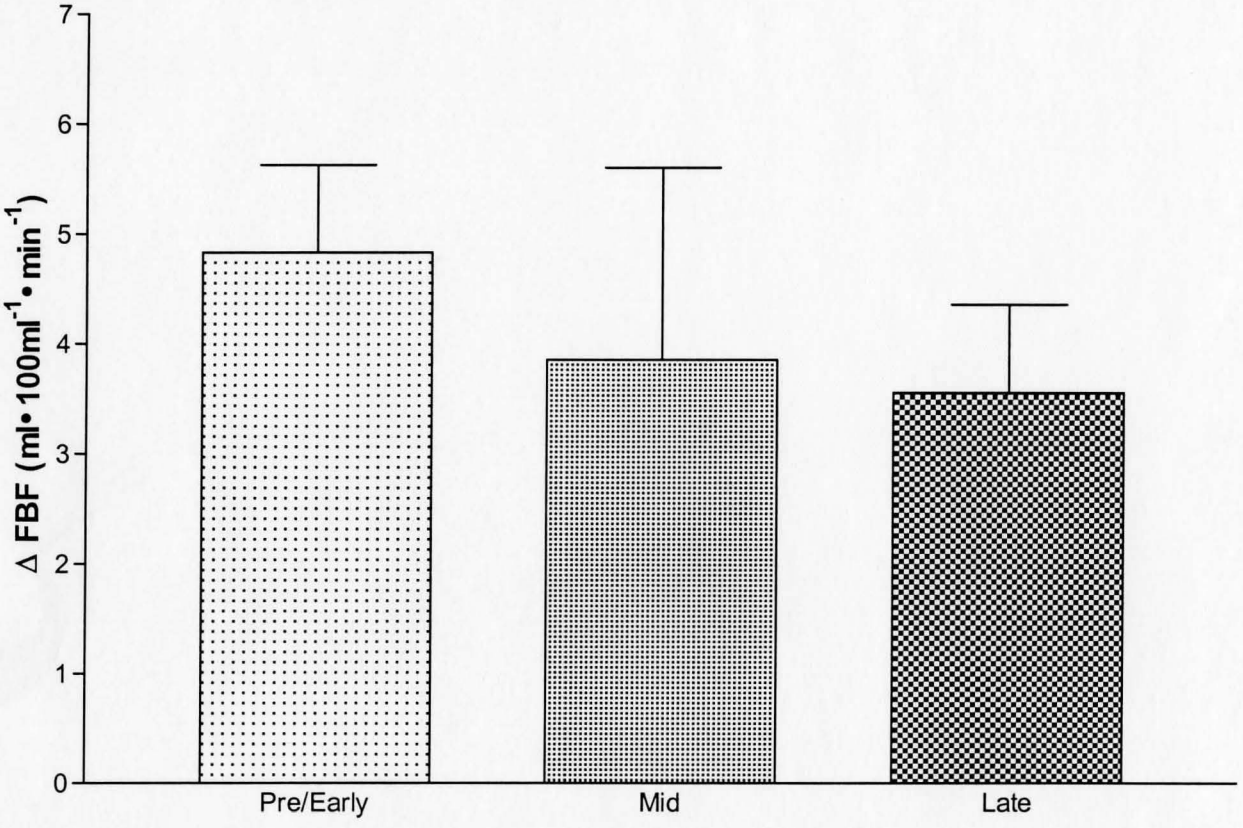


Figure 8: Post-exercise forearm blood flow minus resting forearm blood flow in the three groups

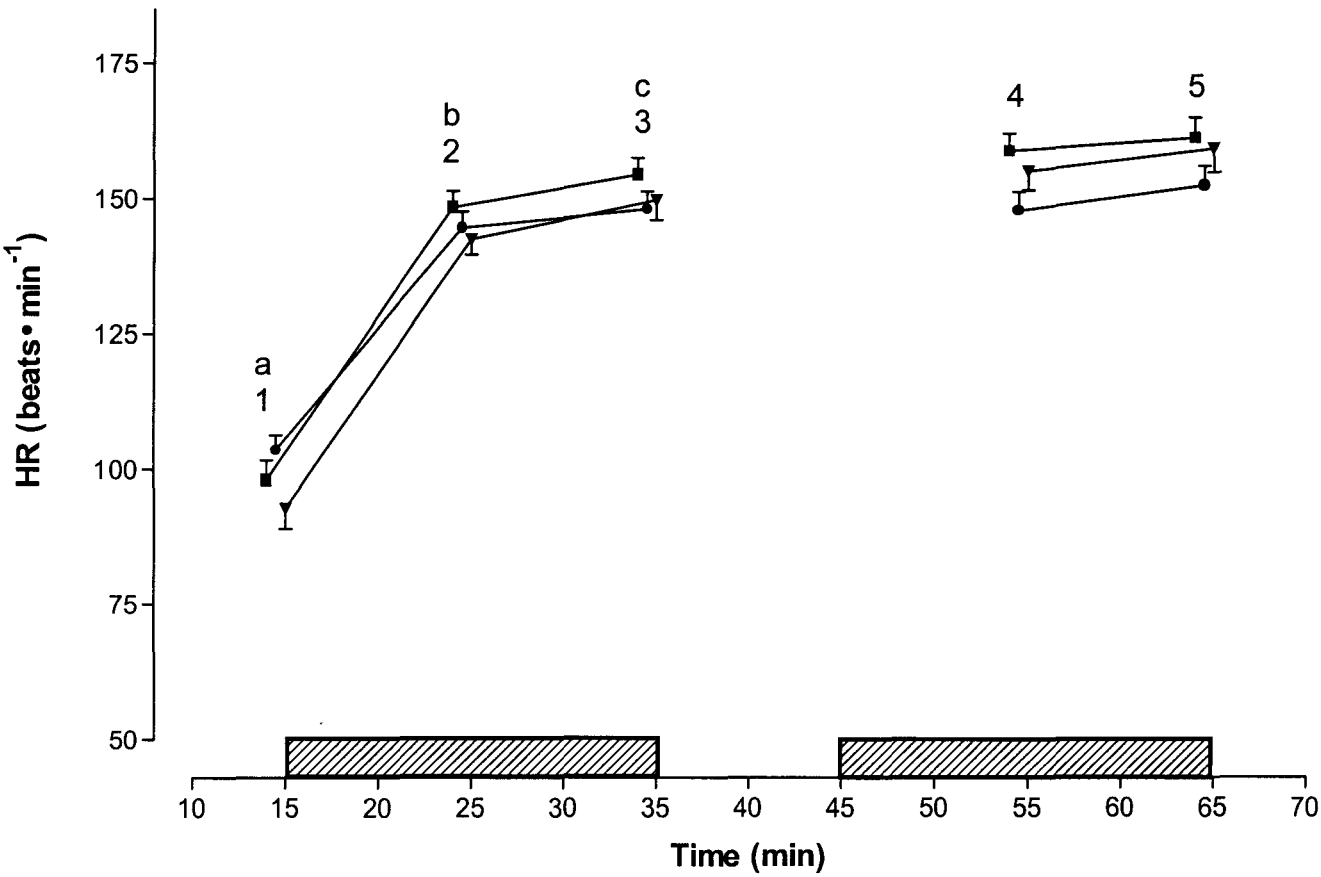


Figure 9: Heart rate during rest and exercise in the three groups.

a 1 < 2,3,4,5
b 2 < 3,4,5
c 3 < 5

} p<0.05

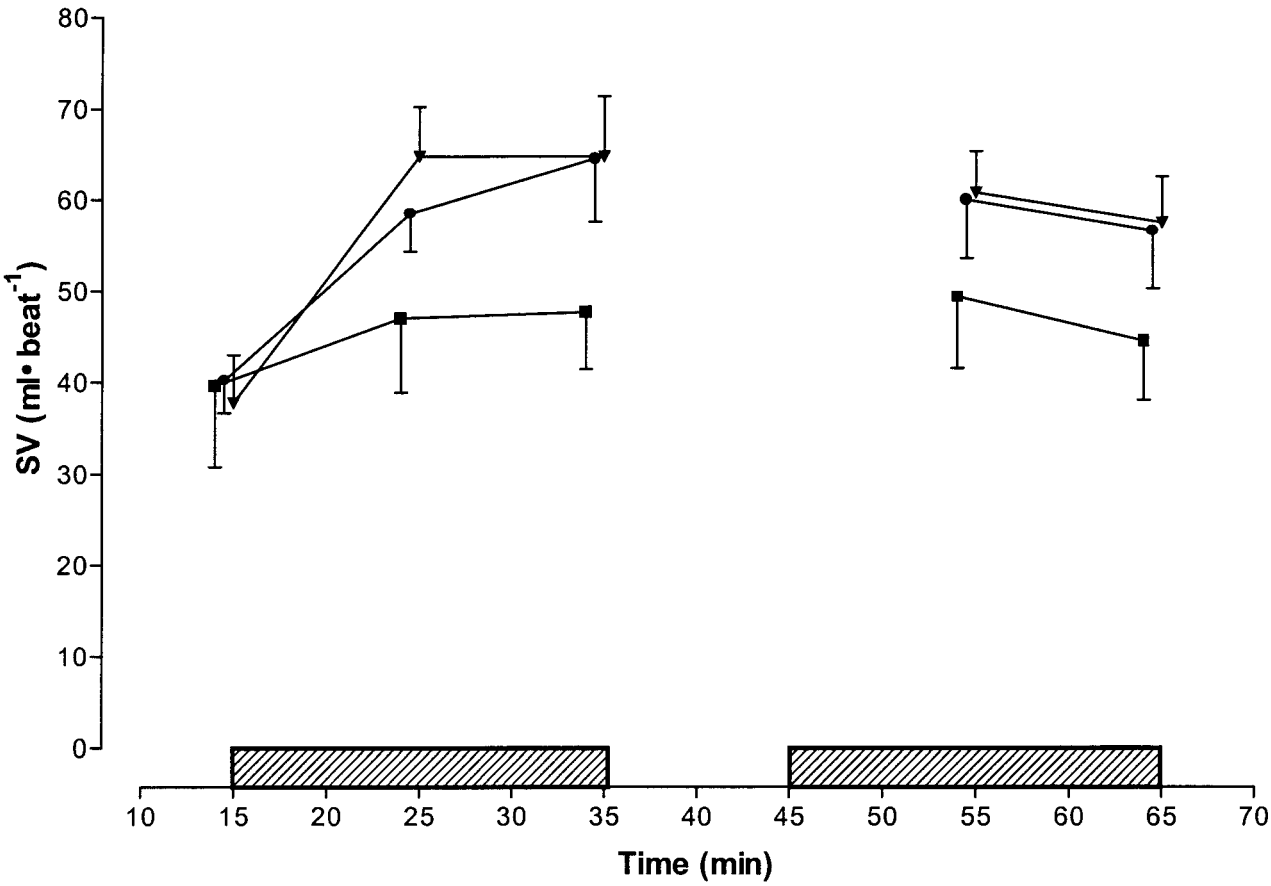


Figure 10: Stroke volume during rest and exercise in the three groups.

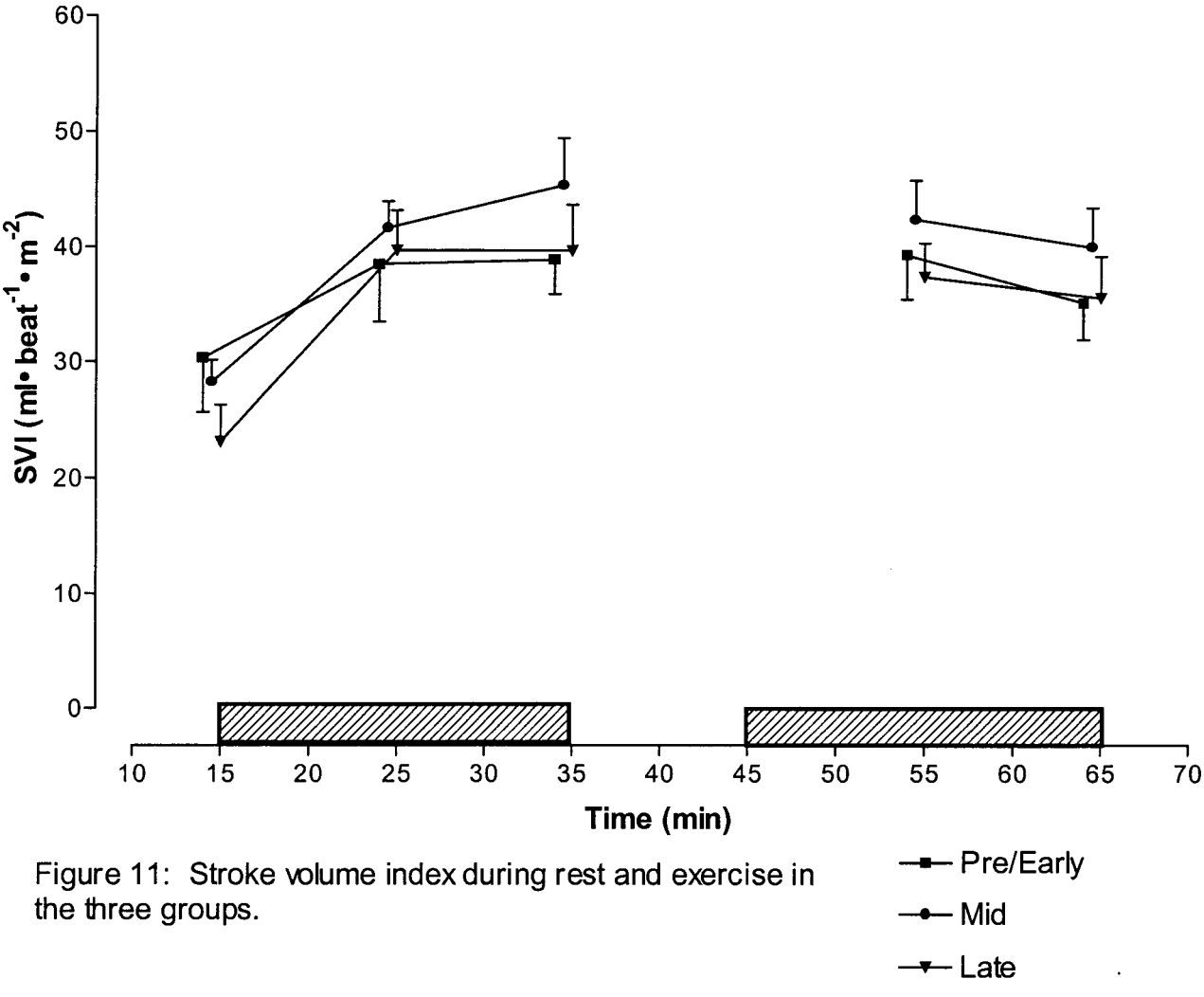


Figure 11: Stroke volume index during rest and exercise in the three groups.

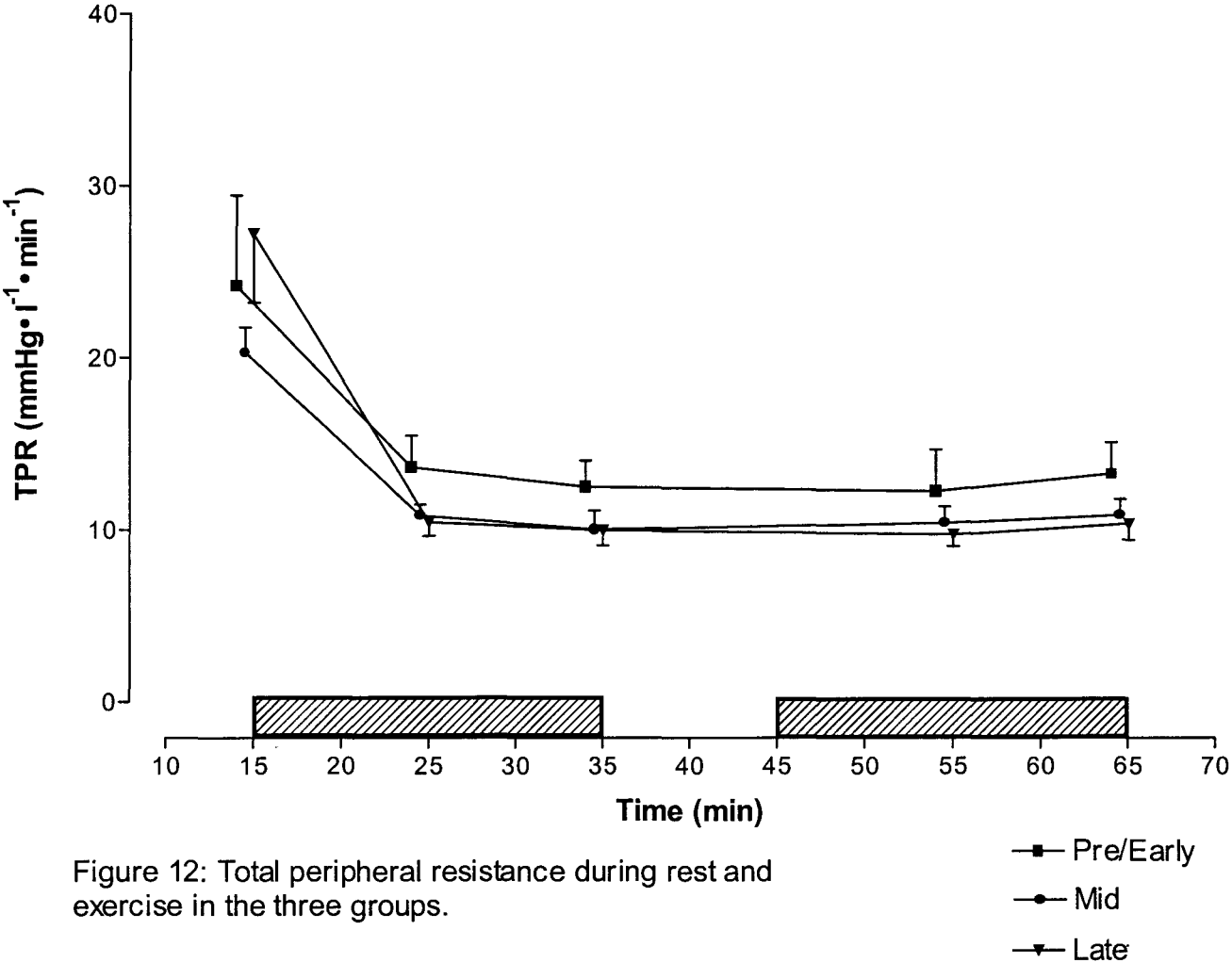


Figure 12: Total peripheral resistance during rest and exercise in the three groups.

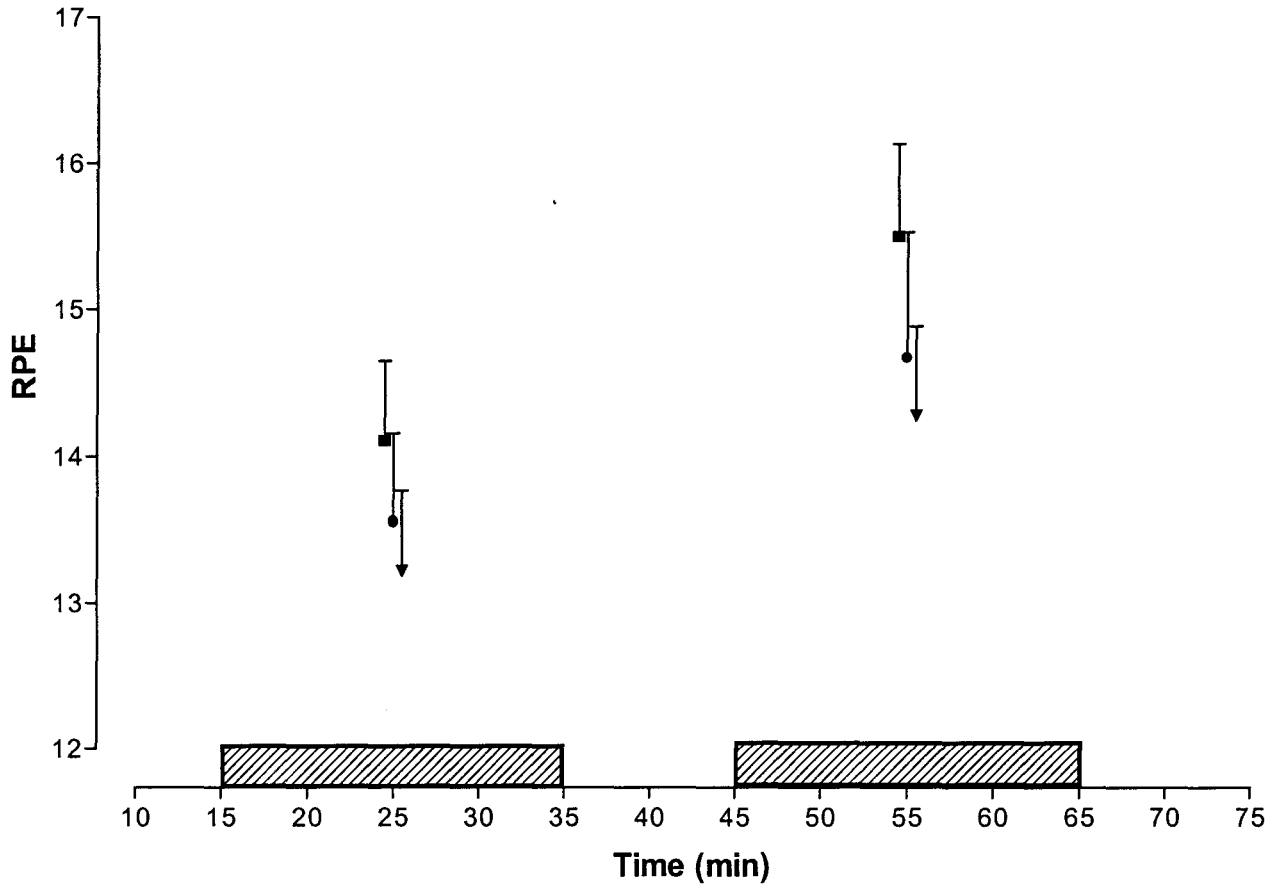


Figure 13: Rating of perceived exertion in the three groups in the middle of the first and second exercise bouts.

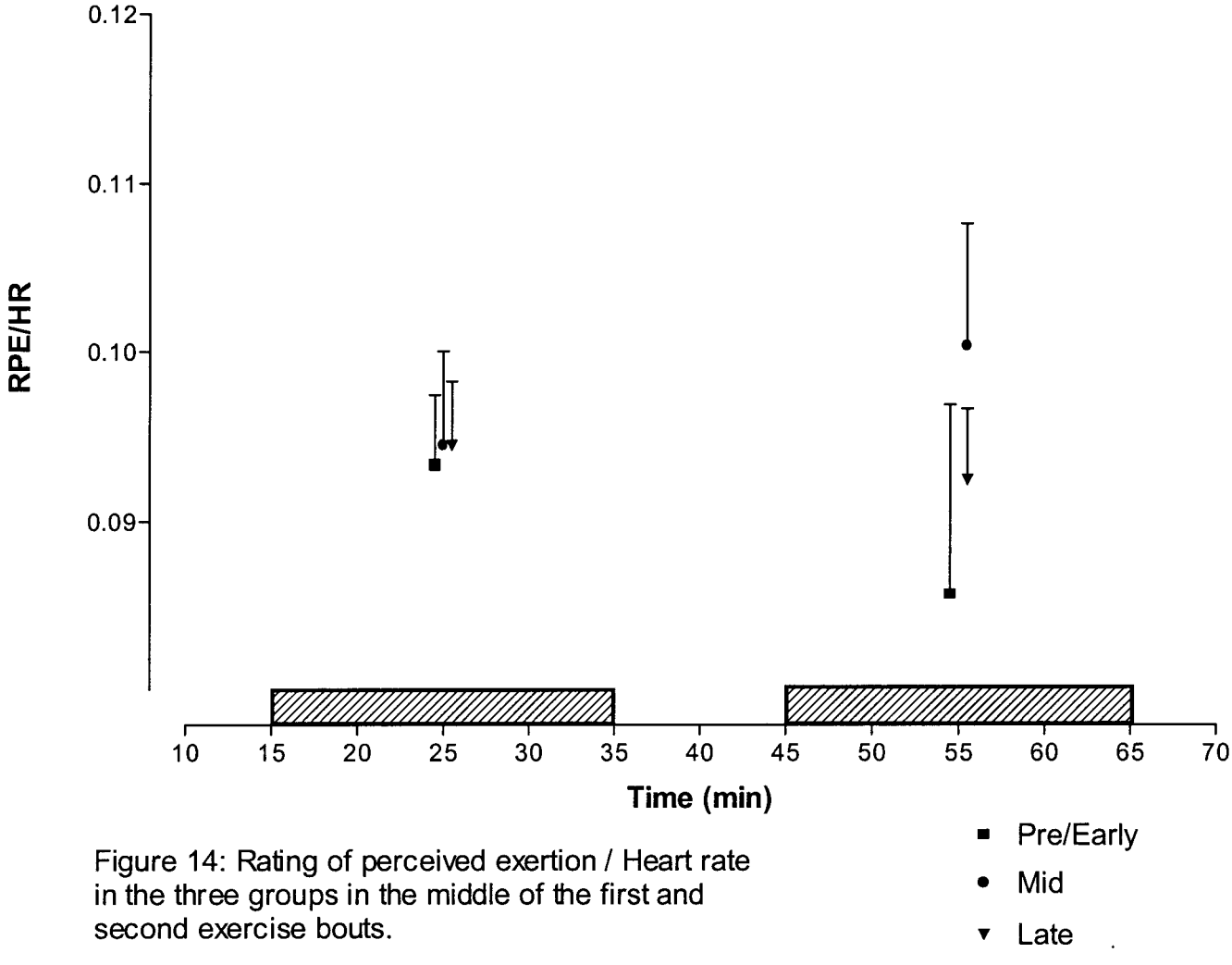


Figure 14: Rating of perceived exertion / Heart rate in the three groups in the middle of the first and second exercise bouts.

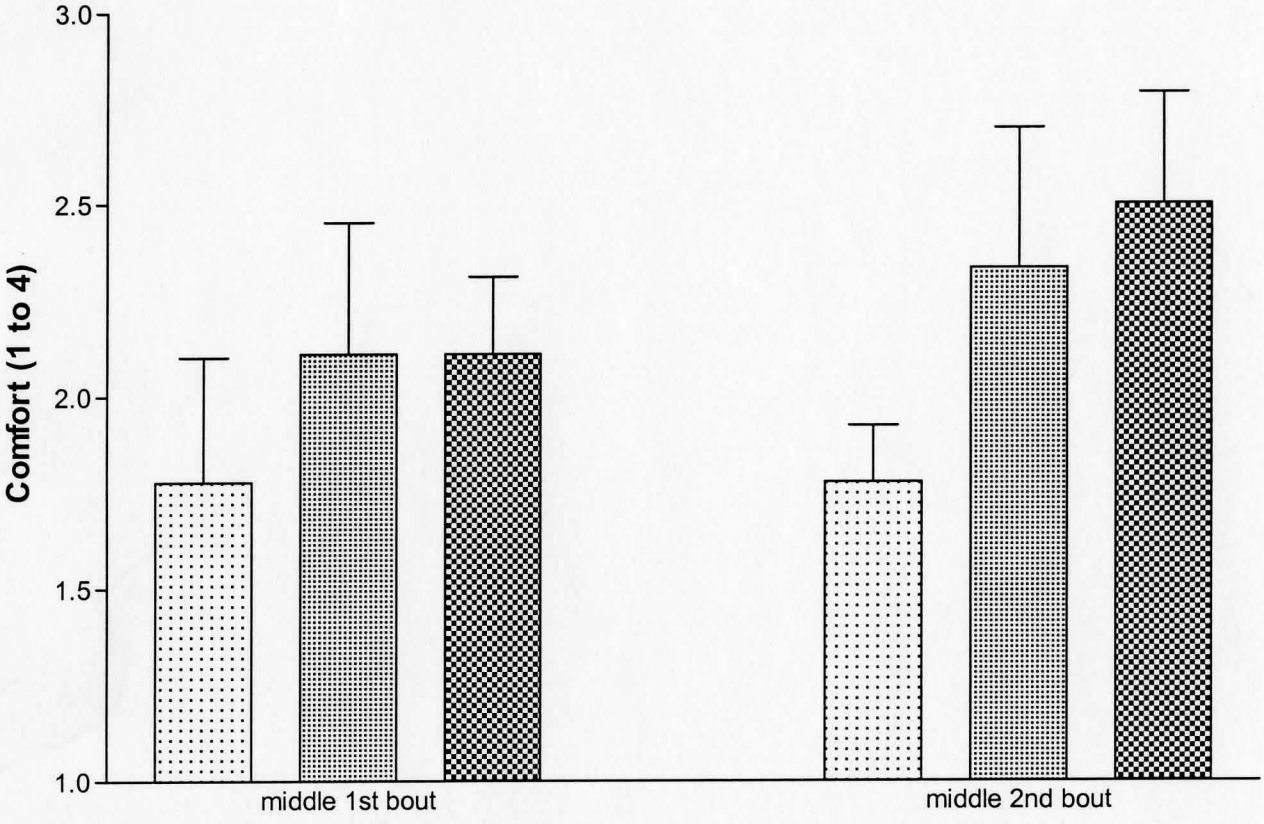


Figure 15: General comfort score in the three groups in the middle of the first and second exercise bouts. 1=comfortable 4=very uncomfortable

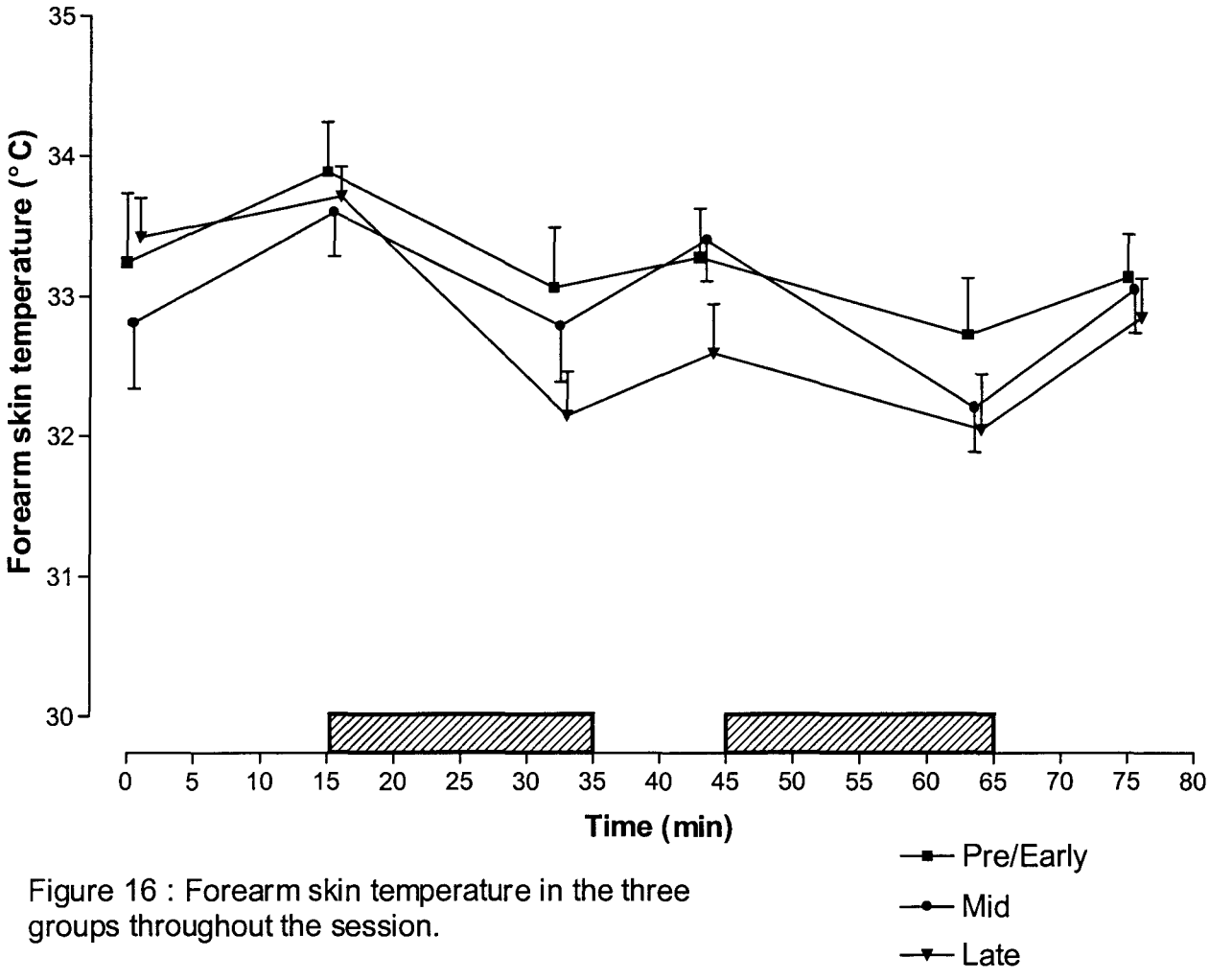


Figure 16 : Forearm skin temperature in the three groups throughout the session.

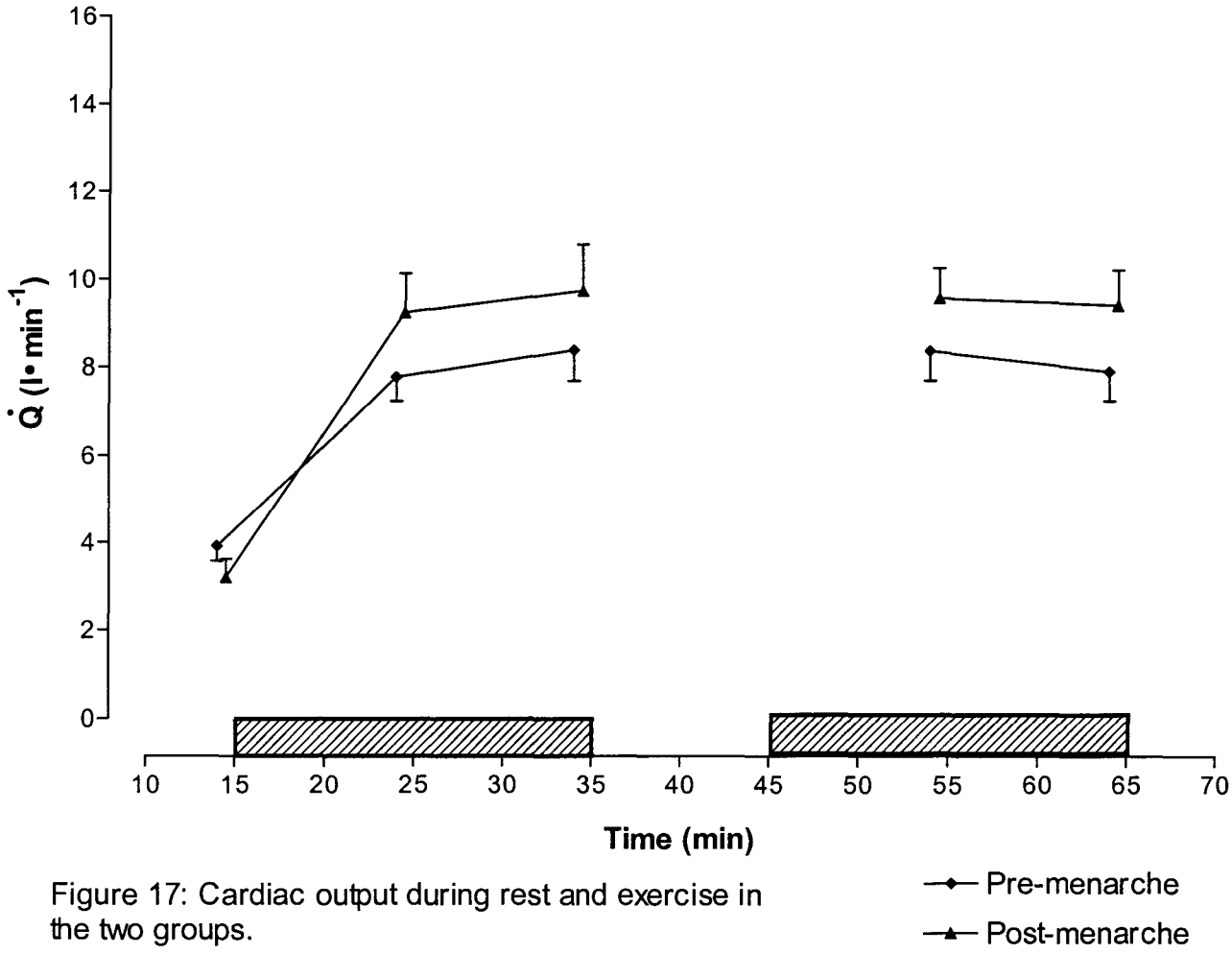


Figure 17: Cardiac output during rest and exercise in the two groups.

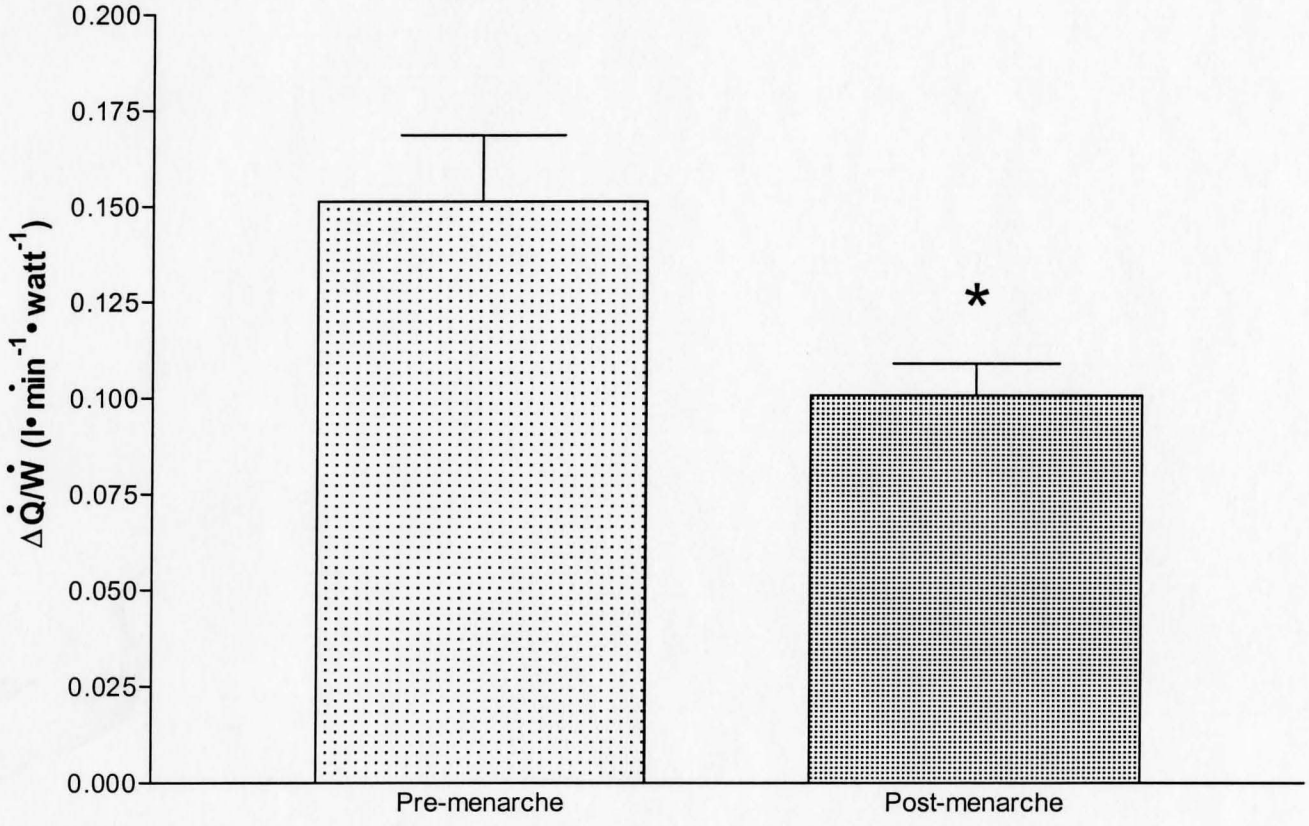


Figure 18: Net exercise cardiac output per watt in the two groups.
Δ denotes exercise minus rest. *=p<0.05

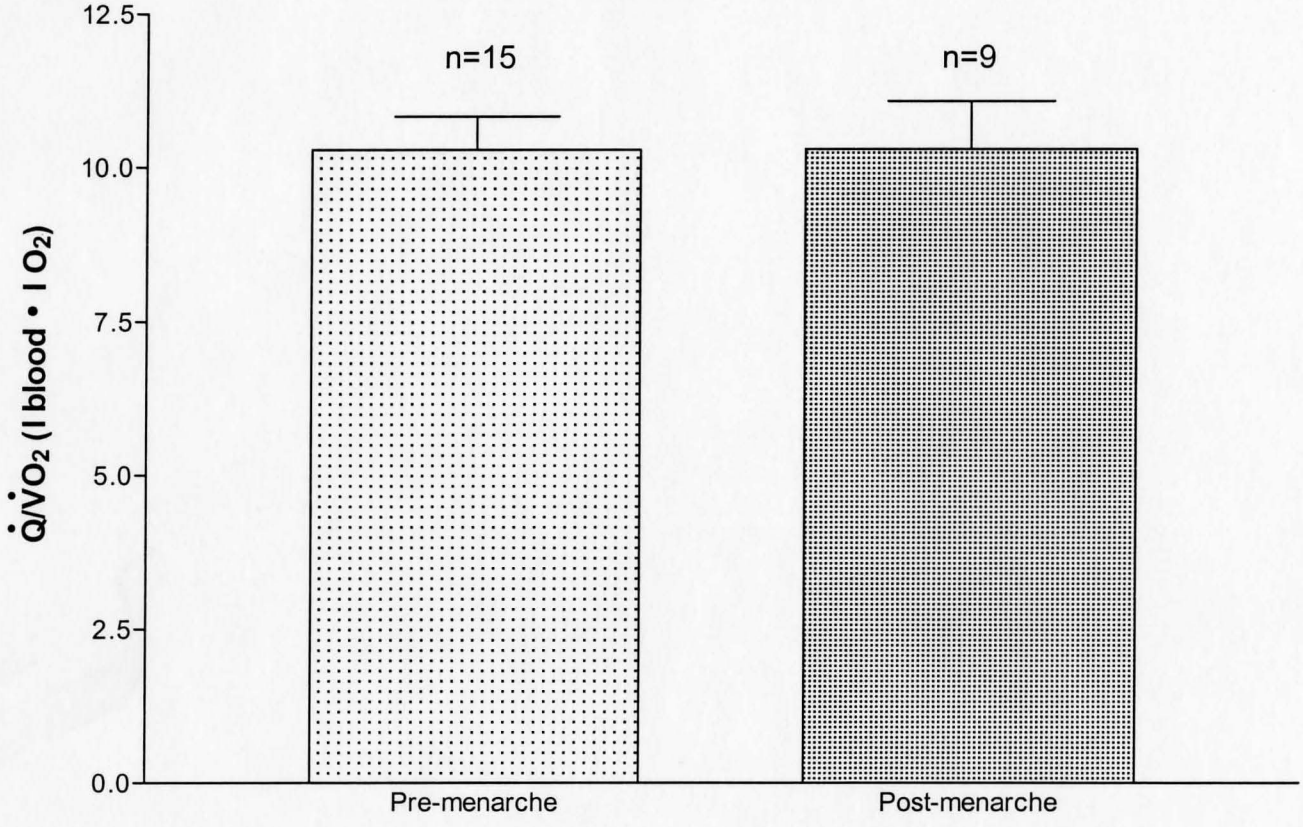


Figure 19: Cardiac output / oxygen consumption for the pre-menarcheal and post-menarcheal groups.

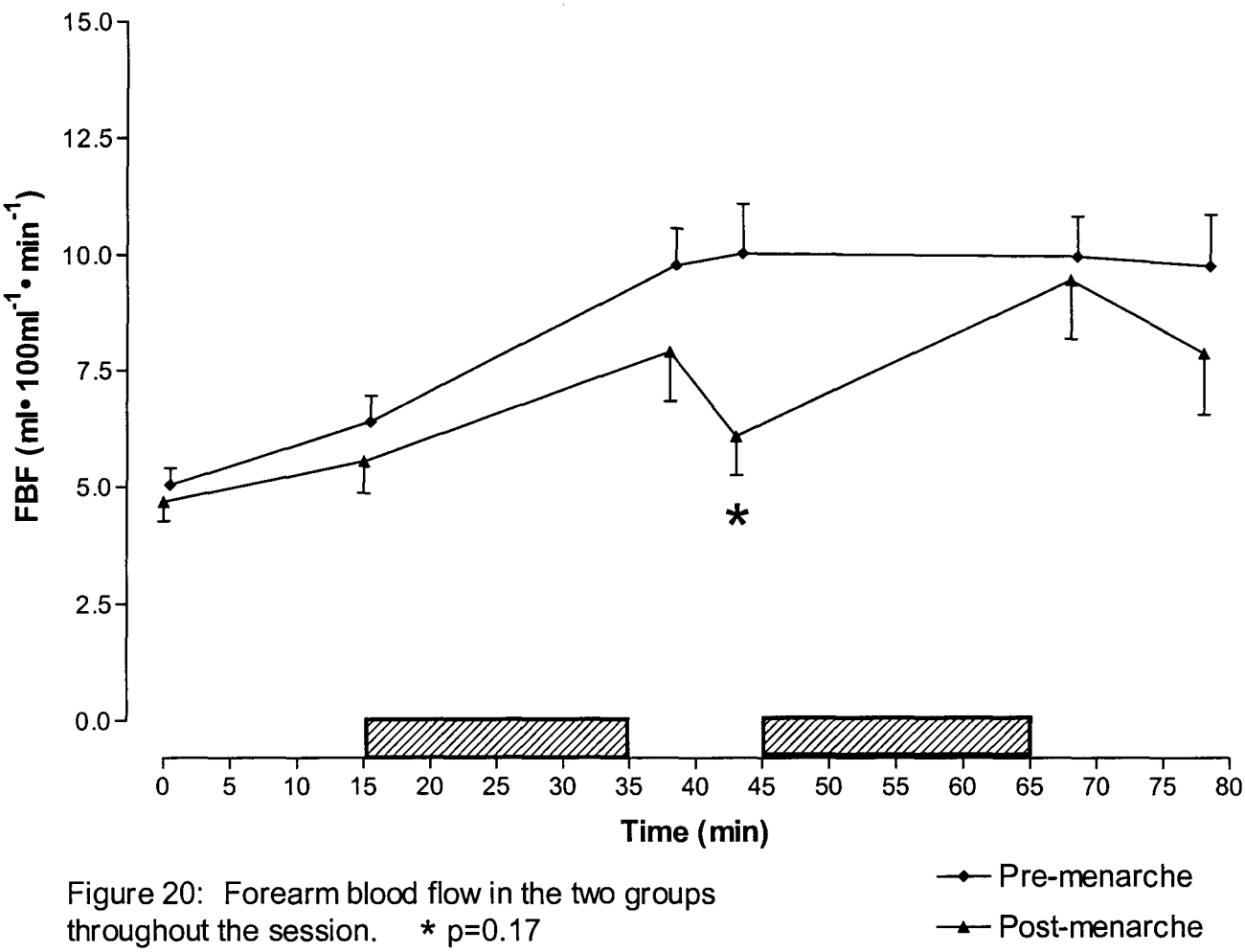


Figure 20: Forearm blood flow in the two groups throughout the session. * p=0.17

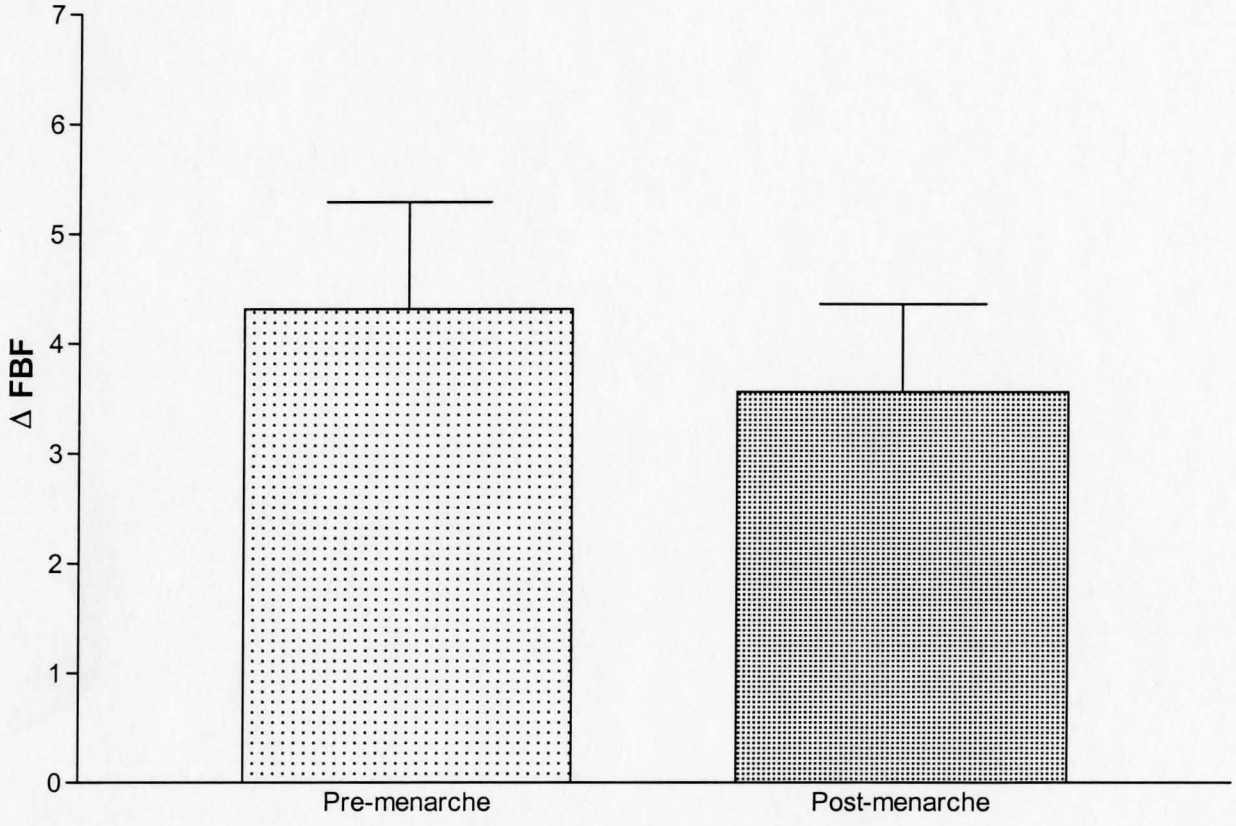


Figure 21: Post-exercise forearm blood flow minus Resting forearm blood flow in the two groups.

9. APPENDICES

Appendix A Parent Consent Form

I, _____, consent to allow my daughter _____, to participate in a study designed to test her physiologic responses while exercising in a warm environment. Dr. Bar-Or (521-2100 ext 77615), the principal investigator, or Erin Brien (ext 77259), the co-investigator, has explained that my child will be invited to the laboratory for 2 visits, as outlined in the information sheet overleaf.

I understand that no known harmful effects will occur during or following the above observations. However, my child may feel tired and hot for 1-2 hours after the session. I understand that there are no direct benefits to my child for taking part in this study. I further understand that my child can withdraw at any time from participation in the study, even after I have signed this form. Any information that is collected will be kept confidential, and will not identify my child in any way. This is also true if the results are published.

_____	_____	_____
NAME (print)	SIGNATURE	DATE

_____
RELATIONSHIP TO CHILD

_____	_____	_____
WITNESS (print)	SIGNATURE	DATE

I have explained the nature of this study to the child's parent (relative) and believe it to be understood.

_____	_____	_____
INVESTIGATOR (print)	SIGNATURE	DATE

Appendix B Consent Form

I, _____, consent to participate in a study designed to test my physiologic responses while exercising in a warm environment. Dr. Bar-Or (521-2100 ext 77615), the principal investigator, or Erin Brien (ext 77259), the co-investigator, has explained that I will be invited to the laboratory for 2 visits, as outlined in the information sheet overleaf.

I understand that no known harmful effects will occur during or following the above observations. However, I may feel tired and hot for 1-2 hours after the session. I understand that there are no direct benefits for taking part in this study. I further understand that I can withdraw at any time from participation in the study, even after I have signed this form. Any information that is collected will be kept confidential, and will not identify me in any way. This is also true if the results are published.

_____	_____	_____
NAME (print)	SIGNATURE	DATE
_____	_____	_____
WITNESS (print)	SIGNATURE	DATE

I have explained the nature of this study to the subject and believe it to be understood.

_____	_____	_____
INVESTIGATOR (print)	SIGNATURE	DATE

Appendix C Medical Questionnaire

Name_____ Date of Birth (mm/dd/yy)_____
Address_____
City_____ Postal Code_____

Medical history

I. Do you have (have you ever had) any of the following conditions? (circle those which are appropriate)

a. heart disease
b. asthma
c. allergies
d. diabetes
e. high blood pressure
f. epilepsy
g. surgery
h. fractures
i. Orthopedic problems
back
hip
knee
ankle

II. Do you ever complain about the following during or after exercise? (circle those which are appropriate)

a. inability to keep up with other girls
b. chest pain
c. fainting
d. dizziness
e. irregular heart beat
f. wheezing
g. cough
h. other_____

III. Have you ever been hospitalized? If so, please list dates and reasons.

IV. Do you use any medications?

Type_____

Frequency of use_____

V. Has a physician ever suggested that you should be restricted from physical activity?

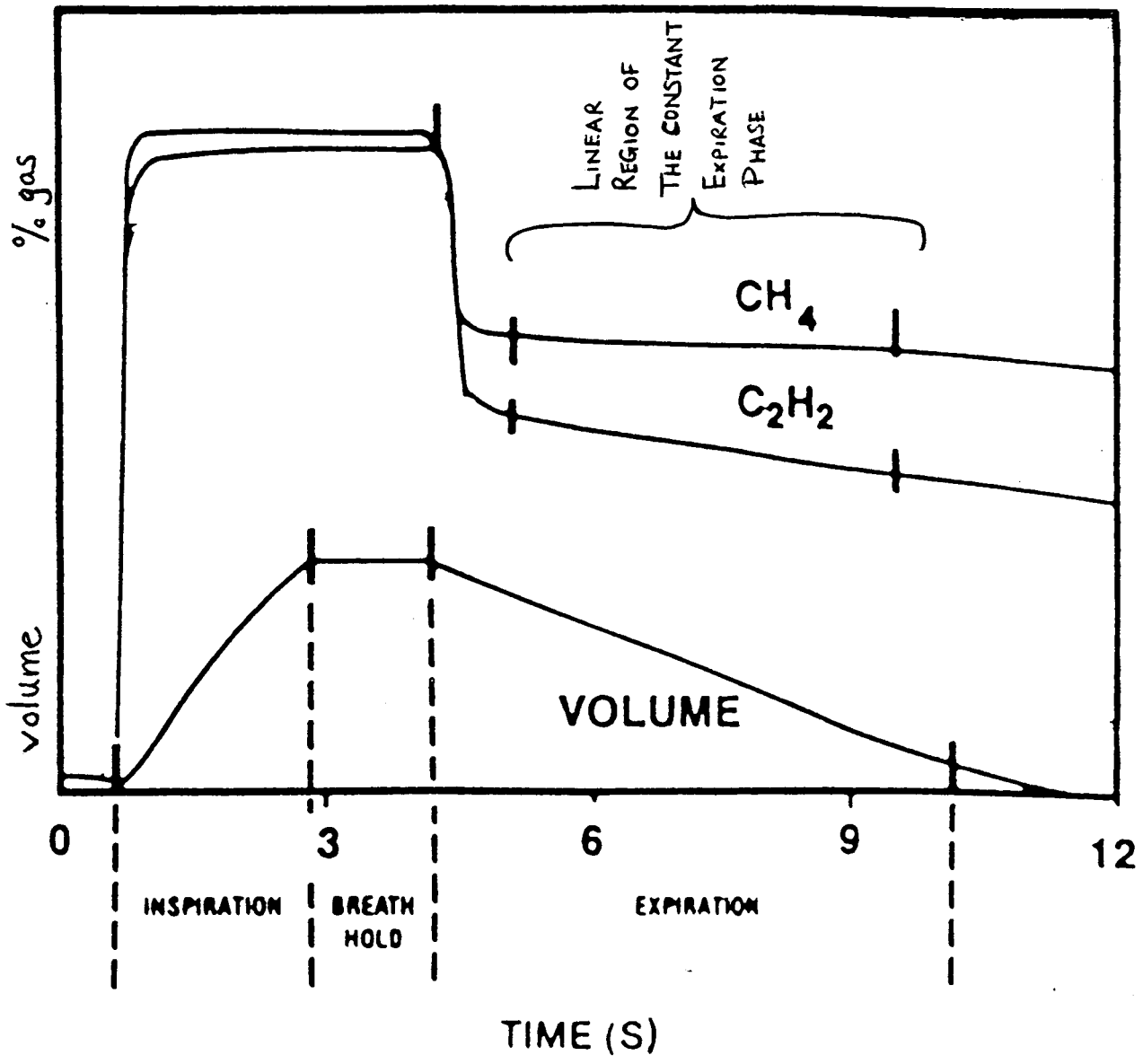
VI. VI Do you know of any medical reasons that would prevent you from participating in physical activity?

Appendix D In-Chamber Form

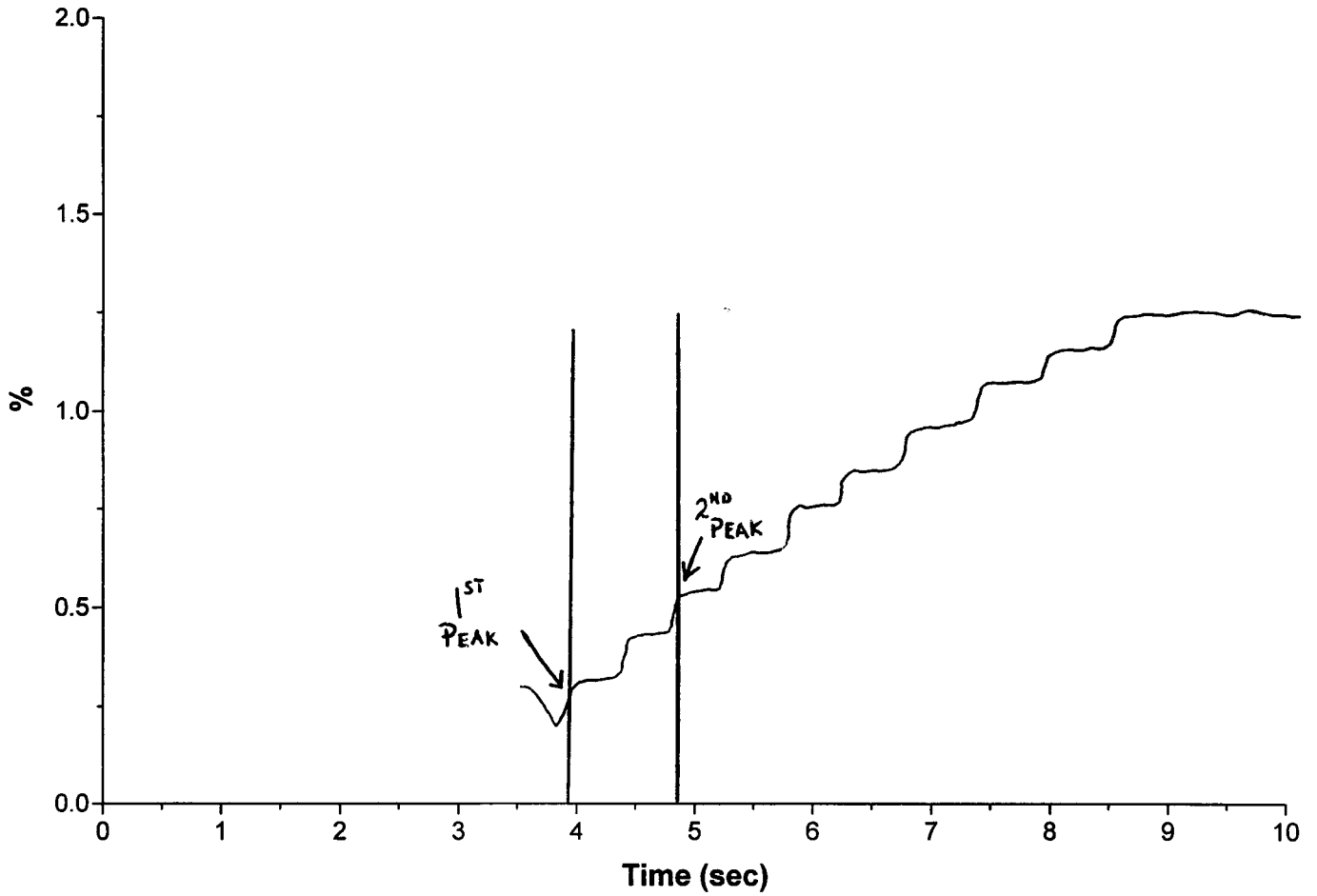
	PRE-EXERCISE 15-MIN		BOUT 1 20-MIN			REST 1 10-MIN	
Chamber Time (min)	0	14	15	25	34	35	44
Body Wt. (kg)							
Temp (ºC) Forearm							
Sub-scap							
Thigh							
Forearm Blood Flow $ml \bullet 100ml^{-1} \bullet min^{-1}$						Min 36-37	Min 38-39
Heart Rate $beats \bullet min^{-1}$							
Cardiac Output $l \bullet min^{-1}$							
VO_2 $ml \bullet kg^{-1} \bullet min^{-1}$							
Blood Pressure mmHg							
Borg Scale							
Thermal Sensation							
Comfort Scale							

	BOUT 2 20-MIN			REST 2 10-MIN	
Chamber Time (min)	45	55	64	65	75
Body Wt. (kg)					
Temp (°C) Forearm					
Sub-scap					
Thigh					
Forearm Blood Flow $ml \bullet 100ml^{-1} \bullet min^{-1}$				Min 66-67 / Min 68-69	
Heart Rate $beats \bullet min^{-1}$					
Cardiac Output $l \bullet min^{-1}$					
VO_2 $ml \bullet kg^{-1} \bullet min^{-1}$					
Blood Pressure MmHg					
Borg Scale					
Thermal Sensation					
Comfort Scale					

Appendix E Editing: Cardiac Output



Appendix F Editing: Forearm Blood Flow



Mode: Strain Gauge
range : 0.50%

Inflow rate: