

**WATER & ELECTROLYTE LOSSES AND REPLENISHMENT IN
CHILDREN DURING PROLONGED EXERCISE IN THE HEAT:
PHYSIOLOGICAL AND PERCEPTUAL CONSIDERATIONS**

by

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ABSTRACT

Recommendations regarding fluid replenishment for children who exercise are scarce and based on adult data. The objectives of this thesis were to evaluate and identify factors that would help optimize fluid replenishment for children who exercise in a warm environment.

Total sweat electrolyte losses were compared among maturational groups (males and females) exercising in the heat (chapters 2 and 3). Total sweat Na^+ and Cl^- losses, even when corrected for body weight, were greater in young men and women compared to their prepubescent and pubescent counterparts. Children and adults lost similar amounts of K^+ , lactate, and ammonia per kg body weight. Within the same maturational group, there were no gender differences in any of these electrolyte losses.

Chapter 4 summarizes the effect of hypohydration (induced by exercise in the heat) on thirst, drink preferences, and the subsequent voluntary rehydration. Thirst and drink preferences were assessed while children dehydrated up to 0.8% of their initial body weight in four separate trials (one flavored drink in each). Thirst intensity increased during dehydration. Grape was the preferred drink during the entire dehydration phase, but its desirability did not increase as much as it did with the orange, apple and water drinks. This is possibly due to a ceiling effect. During recovery, most subjects rehydrated with all drinks, exceeding baseline levels by 0.40% (apple) to 0.76% (grape). The

magnitude of overhydration, however, was greater with grape and orange than with water and apple.

Chapter 5 summarizes the effect of ingesting various electrolyte drinks on thermoregulation and performance of children who were kept euhydrated while exercising in the heat. Four grape-flavored drinks were tested. One drink was water and the other three had 6% carbohydrate (4% sucrose, 2% fructose) with different $[\text{Na}^+]$ (0, 8.8, 18.5 $\text{mmol}\cdot\text{l}^{-1}$). Rectal temperature, heart rate and performance were similar among trials. A negative Na^+ deficit occurred with all drinks but in a greater extent with Na^+ -free drinks. Na^+ intake did not modify plasma $[\text{Na}^+]$ or osmolality. Drink composition had no effect on thirst and stomach fullness sensations, nor did it affect voluntary intake during recovery.

The main conclusions of this thesis were: 1. Adults lost more Na^+ and Cl^- from sweat than children. 2. Children's thirst intensity and drink preferences increased in response to minimal dehydration levels. 3. Grape was the most desirable drink. 4. Children spontaneously overhydrated following hypohydration induced by exercise. 5. Compared to water, CHO-electrolyte drinks had no effect on thermoregulation, performance, and perceptual responses of children who were kept euhydrated while exercising in the heat.

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CHAPTER 1

INTRODUCTION

1.1. Background and Rationale

During exercise, hypohydration may occur as a result of both excessive water losses from sweating and inadequate fluid intake. To avoid the adverse effects of body water deficiency on thermoregulation, performance, and heat-induced illness, drinking specific volumes of fluids has been recommended for those who exercise for a long time in a hot environment (Gisolfi and Copping, 1974; Sawka and Pandolf, 1990; Senay, 1979).

Rehydration is aimed at replacing both the water and the electrolyte deficits and maintaining the body's euhydration status. To prevent hypohydration during prolonged exercise in the heat, the necessary volume of fluid intake depends to a great extent on sweat losses. Euhydration during exercise will also be dependent on the drive to drink, which involves the thirst sensation and other aspects of drinking behaviour. The optimal concentrations of electrolytes and carbohydrates in beverages to be ingested during exercise is a more controversial issue and depends on factors such as exercise duration, the amount of sweat electrolyte losses, drink palatability, and characteristics of the exercising individual such as heat acclimatization and age.

Water is a basic need of the human body. It provides an optimal solvent for the body solutes, in biochemical reactions, and its balance is essential for the maintenance of optimal functioning of the cardiovascular system. Euhydration refers to the normal body water content that, in an 80 kg man, may vary by about ± 165 ml per day ($\pm 0.23\%$ body weight) in thermoneutral conditions, and ± 380 ml (0.48% body weight) in the high ambient temperature conditions (Greenleaf, 1992). Clinical states above and below this range are called hyperhydration and hypohydration, respectively. Hypohydration can be further classified according to the percentage change in body weight: mild ($<4\%$), moderate ($5-8\%$), and severe ($8-10\%$) (Walmsley and Guerin, 1984). The process of losing water, from any hydrational state, is called dehydration, while rehydration refers to the process of gaining water from the hypohydrated state.

Research concerning water and electrolyte replacement during exercise has focused on adults. Thus, recommendations for fluid replacement for children have been based mostly on such studies (American Academy of Pediatrics, 1982; Squire, 1990). Children may require specific recommendations if, for example, the amount of their sweat electrolyte losses is different. This thesis is the first attempt to evaluate optimal fluid replacement for children during exercise in the heat. This is relevant not only to the athletic child but also to the non-athletic child who performs prolonged outdoor activities during the summer months. The information derived from this thesis may also have clinical implications since oral rehydration has been considered a more efficient therapy than intravenous fluid replenishment for treatment of acute and mild hypohydration in

children with gastroenteritis (Casteel and Fiedorek, 1990).

The following sections review some factors involved in determining optimal fluid replacement during exercise, and is based on studies performed with adults. These factors are then evaluated as to their applicability for the study of children.

1.1.1. Sweating and Sweat Electrolyte Composition

Much knowledge has been generated in the last few decades about the role of sweating in temperature regulation. The following is a brief outline of the main principles.

During exercise in the heat, the proportion of energy released as heat (up to 80%) may be such that, without heat dissipation, core temperature would increase 1°C every 5 min. There are several ways by which heat can be dissipated: conduction, convection, and radiation. At a mild environmental heat stress, body heat is lost through conduction, convection, radiation, and evaporation. However, when environmental temperature exceeds the skin temperature, evaporation is the main route for heat dissipation. Thus, for someone who exercises on hot days, heat loss occurs mostly through evaporation of water through the skin (sweating). At the same time, water and electrolyte losses from sweating can affect body fluid homeostasis.

Electrolyte losses through the skin are negligible unless a person sweats during exercise and/or exposure to a warm environment. The eccrine sweat glands, as opposed

to the apocrine glands, are those activated to excrete water as a way to achieve dissipation of heat. These glands also excrete electrolytes (Sato et al. 1989; Sato and Sato, 1987). This section focuses only on sweat from eccrine sweat glands.

Research on sweat electrolyte composition increased within the last 40 years, since the discovery that cystic fibrosis patients had high concentrations of Cl^- in their sweat (di Sant'Agnesse et al., 1953a, 1953b). Still, the understanding of eccrine sweat gland physiology and the "normal" sweat composition have yet to be firmly established. This is not surprising considering the different methods available to induce, collect, and analyse sweat. In addition, other individual-related factors such as age, gender, heat acclimatization, physical training and diseases may affect sweat electrolyte profile and sweating rate. Indeed, these factors explain some of the intra- and inter-individual variation in response to prolonged exercise in the heat. Their recognition has implications to understanding thermoregulation and assessing optimal fluid replacement.

Sweating is activated by central and peripheral (local) responses. The central response involves afferent impulses to the preoptic area of the hypothalamus. This afferent input for sweating is regulated by sensors which are activated when body temperatures increase. Core temperature elevation represents the major drive for sweating which can also be affected by changes in skin temperatures (Nadel et al. 1971a, 1971b). The peripheral response of the sweat gland is regulated predominantly by cholinergic stimuli, although it can also respond to adrenergic drive (Sato, 1977). Injection of cholinergic drugs, e.g. methacholine, is therefore a common method used to induce

sweating.

Sweat is formed in two steps: the secretion of "primary sweat" by the secretory coil, followed by partial absorption of NaCl and water in the reabsorptive duct (Sato, 1977). When sweat is released over the skin, it is hypotonic compared to plasma (Quinton and Tormey, 1976). The concentration of electrolytes in the primary sweat is believed to be similar to that in plasma (about 140, 110 and 4 mEq·l⁻¹ for Na⁺, Cl⁻ and K⁺, respectively) (Cage and Dobson, 1965). In the secretory coil, the primary sweat seems to be formed by active secretion of Na⁺ with passive diffusion of water through the permeable membrane. Na⁺ enters the cell coupled with Cl⁻ and then it is pumped out in exchange for K⁺ across the basolateral membrane.

In the reabsorptive duct, reabsorption of Na⁺ and Cl⁻ in exchange for K⁺ occurs through the Na⁺-K⁺ pump. The activated enzyme of this transport, Na⁺-K⁺ ATPase, has been identified by its inhibition with ouabain (Quinton and Tormey, 1976; Saga and Sato, 1988). Aldosterone acts in the sweat duct stimulating Na⁺ reabsorption similar to its role in the kidneys (Collins, 1966; Sato and Dobson, 1970). Water transport in the duct seems to follow osmotic forces. The final concentration of Na⁺ and Cl⁻ is therefore lower (20-60 mEq·l⁻¹) while that of K⁺ is higher (4-8 mEq·l⁻¹) than in plasma (Sato, 1989).

Other major solutes in sweat are lactate and ammonia (NH₃). Sweat lactate is produced in the secretory coil as a product of anaerobic glycolysis and its concentration ranges from 15 to 20 mEq·l⁻¹ (Sato, 1977). Concentration of NH₃ in sweat may range from 3.5 to 6.5 mEq·l⁻¹ which is 80 to 150 times greater than that of plasma (Sato et al.,

1989). This high $[\text{NH}_3]$ in sweat is probably due to the free nonionic diffusion of NH_3 from plasma. Movement of NH_3 across water solutions is in the direction of the more acidic compartment. Thus, the lower pH of the sweat duct as compared to plasma (1 to 2 units) favours NH_3 diffusion and its entrapment in the NH_4^+ form which is poorly diffusible (Sato et al., 1989).

Variations of sweat electrolyte concentrations have been explained as a function of sweating rate. It is believed that as sweating rate increases, reabsorption of Na^+ , for example, becomes limited. As a result, with increased sweating rate in the duct, there is an increase in $[\text{Na}^+]$ and $[\text{Cl}^-]$ (reabsorbed ions) and a decrease in $[\text{K}^+]$ (secreted ion). Such correlations, however, are not consistent and seem to depend on the methodology used. *In vitro* experiments with isolated sweat glands, unlike *in vivo* analysis, are more likely to reproduce the dependence of sweat composition on sweating rate (Bijman and Quinton, 1987; Schwartz and Thaysen, 1956). This interdependence of ion concentrations on sweating rate has yet to be proven.

Because sweating is activated by cholinergic agents, this is an alternative stimulus to heat and exercise for obtaining sweat samples. The content of a pharmacologically-induced (pilocarpine) sweat, however, does not entirely reproduce the thermal or exercise-induced sweat. Although sweat induced by pilocarpine iontophoresis is very useful for diagnosing cystic fibrosis (Gibson and Cooke, 1959), it was found to have a higher concentration of Na^+ compared to that induced by heat, irrespective of the sweating rate (Sato et al. 1970; Sato et al. 1990; Schwarz and Simpson, 1985).

Therefore, the type of stimulus should be considered when evaluating sweat electrolyte losses due to exercise in the heat.

Sweat may be collected in different ways: direct pipetting from skin under mineral oil (Brusilow, 1965; Quinton, 1982), use of capsules (Sens et al., 1985), gauze-pads or filter papers (Schwartz and Thaysen, 1956; Verde et al., 1982), impermeable bags (Boysen et al., 1984, Calvert et al., 1990), and total body washdown method (Vellar, 1968). Potential sources of error are evaporation of water, contamination, reabsorption by the skin, and leakage. The "anaerobic" method of Boysen and colleagues (1984) has the advantage of preventing these errors, in addition to allowing larger volume samples. This method consists of attaching a pocket-shaped plastic bag to the skin. Once sweat starts to drip, it accumulates between the plastic layers of the bag, thus avoiding reabsorption by the skin.

The composition of sweat may differ among various body sites. For example, ion concentrations ($[Na^+]$, $[K^+]$, $[Cl^-]$) are higher in the sweat from the hand as compared to the forearm (Verde et al., 1982). Thus, sweat harvest from a single site may not represent that of the whole body. One possibility is to collect sweat from several sites or to use a whole body washdown method (Vellar, 1968). In the latter, the body and clothing are washed with distilled water before and after exercise. The post-exercise sweat concentration is then calculated since the amount of distilled water used is known. This method however is not feasible, and it is susceptible to errors when the investigator is not familiarized with procedures (Lemon et al., 1986). Thus, for analysis of sweat

composition produced during exercise in the heat, the plastic bag is a practical, reliable (Calvert et al. 1990), and the least contaminated (Sato and Sato, 1990) method for collecting sweat. In addition, when applied to the back it does not interfere with the subjects's movements.

The heat acclimatization process involves earlier onset of sweating and increase in sweating rate (Gisolfi and Robinson, 1969; Kobayashi et al., 1980; Taylor, 1986; Wagner et al., 1972; Whyndham, 1967). Those changes were shown to be accompanied by an increase in the size of the sweat gland in monkeys (Sato et al., 1990). Sweat $[Na^+]$ and $[Cl^-]$ decrease with acclimatization, even for a given increase in sweating rate (Allan and Wilson, 1971; Kirby and Convertino, 1986; Ogawa et al., 1982). Acclimatization seems to increase the response of the sweat duct to aldosterone (Kirby and Convertino, 1986).

Physical aerobic training, not necessarily accompanied by acclimatization, can also increase sweating rate (Nadel et al., 1974). Buono and Sjolholm (1988) showed, by stimulating sweat with pilocarpine iontophoresis, that trained men and women had a greater sweating rate as compared to their untrained counterparts. As suggested by Sato and Sato (1983), training induces sweat gland hypertrophy as well as an increase in the cholinergic sensitivity and periglandular concentration of acetylcholine to the gland. The degree to which fitness affects sweat electrolyte concentration is unknown. Because physical training is usually accompanied by heat acclimatization, it is difficult to separate their effects. Thus, in designing a study on sweat electrolyte concentration or

thermoregulation, it is important to control for the influence of physical fitness and acclimatization.

So far, there is no confirmation that sweat electrolyte concentrations differ between genders. According to a report (Robinson and Robinson, 1954), sweat [Cl⁻] is lower in women than in men. Morimoto et al. (1967) did not find any gender-related differences in the sweat [Cl⁻], despite the higher sweating rate in men than in women.

1.1.2. Thirst and Drinking Behaviour during Exercise

Thirst is a sensation expressed as a compelling desire to drink. The mechanisms that induce thirst are sensitive to even slight body water deficit and increased plasma osmolality, thus serving as a defence against hypohydration. Earlier studies reported that thirst is not perceived until an individual has incurred a water deficit of about 2% of body weight (Rothstein et al., 1947). Others have reported that no body fluid decrease (Phillips et al., 1983), or a smaller deficit in body water (0.5-1.0%) is needed to stimulate drinking (Wolf, 1958). Several mechanisms are involved in the triggering of thirst and their relative importance is still under debate. Stimulation of thirst during exercise involves a reduction in blood volume, an increase in plasma osmolality (or plasma [Na⁺]), and cell dehydration. These are actually interrelated because hypovolemia induced by prolonged and moderate exercise causes increase in plasma osmolality which in turn shifts water out of the cells (Costill et al., 1976). This is because sweat is

hypotonic in comparison to plasma (Robinson and Robinson, 1954).

Central responses to osmotic and volume changes are triggered by the release of hormones. For example, an increase in plasma osmolality (above $290 \text{ mOsm}\cdot\text{l}^{-1}$) activates osmoreceptors, located in the supraoptic and paraventricular nuclei of the hypothalamus, to release arginine vasopressin from the posterior pituitary (Andersson, 1977; Yared and Ichikawa, 1989).

Independent of increased plasma osmolality, hypovolemia may induce thirst by directly stimulating the regulatory centre (Thrasher, 1982; Thompson et al., 1987), and by affecting both the kidney and the heart. Decreased central venous filling pressure and cardiac output, and the resultant decrease in renal perfusion pressure activate the renin-angiotensin II system (Gibbons et al., 1984). Angiotensin II has been considered a potent stimulator of thirst through several pathways such as indirectly increasing tubular reabsorption of Na^+ , and directly stimulating thirst centers and release of arginine vasopressin (Fitzsimons, 1976; Phillips et al., 1982; Simonnet et al., 1979). Stretch receptors in the right atrium may be involved in the control of drinking, because they are activated when hypovolemia accompanies a decrease in atrial pressure (Morimoto, 1990; Ramsay et al. 1975). These receptors are believed to activate the renin-angiotensin system, as indicated in experiments performed with dogs (Scheuer et al., 1989; Sobocinska, 1969).

In humans, it is harder to isolate osmotic- from volume-related mechanisms. Earlier studies used infusion of hypertonic solutions (Holmes et al., 1947) and blood

donation (Holmes et al., 1953) to examine human thirst in response to increased plasma osmolality or a decrease in blood volume. More recently, water immersion in men (Sagawa et al., 1992) was used to induce vascular volume shifts and release of antidiuretic hormone and renin-angiotensin. Indeed, it was found that water immersion suppressed increases in thirst perception and without an increase in plasma osmolality, even when men were hypohydrated.

Engell et al. (1987) studied the relative importance of plasma osmolality, plasma volume, and thirst sensation on volume intake using a multiple linear regression analysis. In this study, different hypohydration levels were induced in men by fluid restriction and exercise which was followed by *ad libitum* drinking. Increased plasma osmolality was the best predictor of volume intake ($r^2=0.58$). Low additional variances were found when plasma volume ($r^2=0.65$) and thirst sensation ($r^2=0.68$) were added to the equation. Thus, plasma osmolality may be a more important factor in determining the volume of fluid intake during and after hypohydration.

The volitional aspect of drinking during exercise is an intriguing phenomenon. It has been shown for many years and repeatedly confirmed that men do not fully rehydrate while working, walking, or exercising in the heat (Adolph and Dill, 1938; Greenleaf and Sargent, 1965; Greenleaf et al., 1983; Pitts et al., 1944; Rothstein et al., 1947). This phenomenon, initially named voluntary dehydration (Rothstein et al., 1947), is now being called involuntary dehydration because it occurs even when fluids are available *ad libitum*. As suggested by Nadel et al. (1993), the physiological mechanisms

of thirst can be attenuated because fluid moves to the intravascular compartment to compensate for the temporary plasma volume deficit (Nose et al. 1988b). This and other less understood factors such as inter-individual characteristics of drinking behaviour, fluid palatability, and gastro-intestinal symptoms may contribute to involuntary dehydration. The magnitude of the hypohydration also depends on a combination of different stress factors (individual factors, climatic heat, exercise, acclimatization) (Greenleaf and Sargent II, 1965).

Szlyk et al. (1989b) observed a great individual variability for involuntary dehydration. They classified humans as avid or reluctant drinkers of water offered *ad libitum*, according to their ability to remain within 2% of their initial body weight during prolonged exercise in the heat. Out of 33 men (23-33 years) who walked for 6 h in the heat (40°C), 13 were reluctant drinkers.

The magnitude of involuntary dehydration may increase with aging. Compared to young (21-29 yr) men, elderly (61-62) men showed a poorer ability to rehydrate after dehydration induced by resting 3 hours in the heat (45°C-25% relative humidity) (Miescher and Fortney, 1989). Ratings of thirst perception (analog scale) increased similarly in both groups despite the higher rectal temperature and hemoconcentration of the elderly men. Phillips et al. (1984) also found that young men (20-31 yr-old) drank twice as much water as their elderly (67-75 yr) counterparts after being dehydrated (about 2% body weight) by a 24 hour water deprivation. In this study, despite the greater increase in the plasma osmolality and vasopressin levels of the elderly, their thirst

perception ratings did not increase as much as in the younger men.

Drink temperature and flavor can also affect volume intake during exercise. After hypohydration, fluid intake was greater with cooler (15°C) than warmer (40°C) water temperature (Boulze et al., 1983). Hubbard et al. (1984) evaluated the voluntary intake of cool (15°C) or warm (40°C) fluids in men who were assigned one of 3 drinks (tap water, iodine-treated water, or a cherry-flavored drink) during a 6-hour walk in simulated desert conditions (40°C). Cooling and flavoring (cherry) increased the volume intake by 120% in men walking 6 hr in desert-like conditions. In another study similar study (Szlyk et al. 1989a), flavoring increased fluid consumption from 10 to 80% even in those men reluctant to drink. Although cooling and flavoring increased volume intake, subjects still presented involuntary dehydration.

Gastro-intestinal discomfort and symptoms related to decreased gastric emptying, such as stomach fullness, may further limit fluid intake during exercise. Also, gastric distention may be an important factor in the termination of drinking (Rolls et al., 1980). Exercise above 70% $\dot{V}O_2$ max delays the rate of gastric emptying (Brouns et al., 1987; Neuffer et al., 1989b). Running, as compared to cycling, induces more abdominal complaints.

Climatic heat and hypohydration decrease gastric emptying. Neuffer et al. (1989a) observed a decrease of about 35% in the rate of gastric emptying when subjects exercised (50% $\dot{V}O_2$ max) in the warm (49°C) as compared to thermoneutral (18°C) temperatures, and hypohydration further decreased gastric emptying. Large volume intakes (between

500-600 ml) of hypotonic drinks with carbohydrate contents below 10% tend to increase gastric emptying.

Thus, to avoid hypohydration during exercise in the heat, fluid intake beyond should be encouraged. This is facilitated by offering attractive flavored drinks which leave the stomach faster and stimulate further drinking, rather than just satisfy the thirst sensation. Drinking will be further encouraged by offering cooler (10-15°C) drinks.

1.1.3. Effect of Hydration State and Carbohydrate-Electrolyte Solutions during Exercise

The extent to which hypohydration impairs thermoregulation and physical performance depends on many interrelated factors such as its level, the duration and type of exercise, methods for inducing dehydration, and environmental conditions. Results on the effect of hypohydration on muscular strength and anaerobic performance (short-term exercise) are inconsistent. The procedure used to dehydrate subjects seems to affect results such that prolonged fluid restriction, as opposed to exercise/heat or diuretics, seems to decrease muscular strength at a greater extent (Nielsen et al., 1981; Sawka and Pandolf, 1990).

On the other hand, when exercise is prolonged and performed in a warm environment, hypohydration is consistently detrimental to thermoregulation and performance (Gisolfi and Copping, 1974; Pitts et al., 1944; Sawka et al., 1989, 1985).

Compared to the euhydrated state, core temperature is elevated by 0.1-0.2°C for each percentage of body weight deficit, depending on the environmental and exercise conditions. Beyond 2% dehydration, core temperature may be 0.4°C higher for each subsequent percentage point of dehydration (Gisolfi and Copping, 1974). Thus, as hypohydration and core temperature increase, exercise performance and heat tolerance decrease at a faster rate compared to the euhydrated state (Sawka and Pandolf, 1990, Sawka et al., 1992).

The main mechanism by which hypohydration increases hyperthermia and hampers performance during prolonged exercise in the heat remains unclear. During hypertonic hypohydration, sweating rate may not only decrease, but the core temperature for a given sweating rate is higher relative to when the body is kept euhydrated (Sawka et al., 1985). In addition, decreased plasma volume and increased plasma osmolality may increase the threshold temperature for sweating (Sawka et al., 1989).

Hyperosmolality and hypovolemia induced by dehydration increase core temperature and reduce heat dissipation through evaporation (sweating rate) and convection (skin blood flow). Plasma hyperosmolality may increase core temperature by affecting the hypothalamus (central) or the sweat glands (peripheral) (Senay, 1979). Studies with animals have demonstrated that the neurons of the thermoregulatory center in the anterior hypothalamus and preoptic region respond to changes in osmolality, such that infusion of a hypertonic solution increases core temperature (Greenleaf et al., 1976). Fortney et al. (1984) observed that hyperosmolality (even without hypovolemia) delayed

onset of sweating and cutaneous vasodilatation during exercise in the heat. One explanation is that the increase of the interstitial osmotic pressure inhibits water movement to the sweat gland (Greenleaf and Castle, 1971; Nielsen et al., 1971).

Isoosmotic hypohydration, induced by diuretics, reduced sweating rate for a given esophageal temperature (Fortney et al., 1981). It was then speculated that the decreased atrial filling pressure caused by hypovolemia alters the afferent neural information to the hypothalamic center that controls sweating rate. Another possible explanation is that hypohydration impairs thermoregulation during exercise by decreasing skin blood flow. This was recently (Montain and Coyle, 1992) investigated in men who cycled ($65\% \dot{V}O_2\text{max}$) and received one of the following treatments on separate occasions: oral fluid replacement, infusion of a volume expander, or no fluid replacement. Infusion of the volume expander and fluid replacement equally maintained blood volume at values above that of no fluid treatment. Forearm blood flow, however, increased only with the oral fluid replacement that most attenuated the rise in core temperature. This was despite similar sweating rates among treatments. Thus, it is possible that prevention of hypohydration by oral replacement improves thermoregulation during exercise by increasing skin blood flow which facilitates heat transfer from the core to the periphery.

Hypohydration also impairs aerobic performance through its effect on the cardiovascular system. Stroke volume is reduced as a result of the reduced blood volume (Nadel et al., 1980; Saltin et al., 1964). This is aggravated by climatic heat which dilates superficial veins of the skin, shifting part of the blood flow to the periphery (instead of

skeletal muscles) and further reducing venous pressure, venous return and cardiac output. Hamilton et al. (1991) showed that fluid replacement with volumes equal to water losses can prevent a decline in stroke volume.

While enough evidence has been gathered to support the benefits of keeping the body euhydrated during prolonged exercise in the heat, the need to replace electrolytes during prolonged exercise is more controversial. One argument against electrolyte ingestion during exercise is the possibility of increasing plasma osmolality and thus core temperature, as discussed above (Harrison, 1986). However, when the body is kept euhydrated by ingesting iso-osmotic drinks ($290 \text{ mOsm}\cdot\text{l}^{-1}$) with $[\text{Na}^+]$ up to $25 \text{ mEq}\cdot\text{l}^{-1}$, neither plasma $[\text{Na}^+]$ nor osmolality increased (Barr et al., 1991; Johnson et al., 1988).

On the other hand, hyponatremia, defined as a plasma $[\text{Na}^+]$ of less than $130 \text{ mEq}\cdot\text{l}^{-1}$, is the main concern when Na^+ is insufficiently replaced during prolonged exercise. Hyponatremia can be a manifestation of excessive Na^+ loss but can also occur secondary to excessive hypotonic fluid intake (Noakes et al. 1985). The accompanying decrease in plasma osmolality creates an osmotic gradient across the blood-brain barrier which causes water to move into the brain (Berry and Belsha, 1990). Apathy, nausea, vomiting, altered consciousness and even seizures are some of the neurological manifestations of hyponatremia (Gruskin et al., 1982). In fact, some of these symptoms were recently reported in an adolescent girl while walking for a long time in the desert (Geist and Barzilai, 1992), and in a 21-year-old man while exercising in a laboratory as a subject for an experiment on fluid replacement (Armstrong et al., 1993). In both

situations, hyponatremia was detected as a result of excessive water intake.

Inclusion of Na^+ in beverages has been further encouraged because it promotes carbohydrate (CHO) absorption from the intestine (Gisolfi et al., 1990; 1991; Leiper and Maughan, 1986). This is because glucose transport from the lumen through the enterocyte is coupled with Na^+ transport via a cotransporter located on the apical membrane (Crane, 1977). As a result, water absorption is increased since it moves passively towards the greater osmotic pressure. This optimization of water absorption achieved with CHO- Na^+ solutions has been a major rationale for the use of oral replacement, instead of intravenous therapy in situations of mild fluid losses.

It has been known for many years that CHO ingestion during prolonged physical activities improves performance in adults (Pitts et al., 1944). CHO provides an exogenous source of substrate and prevents a decrease in blood glucose (Coyle and Montain, 1992; Coyle et al., 1986; Davies et al., 1988; Murray, 1987).

1.1.4. Are Children Different from Adults in their Fluid Needs during Exercise ?

The proportion of water in the body decreases with age. At birth approximately 80% of body mass is comprised of water. This drops to about 61 and 63% in the first and third year of life, respectively. In male adults, body water ranges from 60 to 65% of body mass and is lower in females (50%) (Friis-Hansen, 1961). Thus, greater rates of decrease occur in the first year of life, and by 3 years the child's relative body water

approaches that of adults. The ratio of extracellular to intracellular water also decreases after birth and it becomes stable after 3 years of age. This is mainly due to a decrease in the proportion of extracellular water.

A unique aspect of fluid replacement in young children, compared to adults, is that they need more fluid daily as a percentage of body mass. Respective daily water turnover at one and six years is 13% and 11% of the total body mass, while in adults it is 6% (Hickman and Yasuda, 1986). This in part is due to the higher metabolic rate of children (per body mass). In addition, each 100 g of body mass increase will require 70 to 80 ml of net water gain (above normal maintenance requirements) for the production of new intracellular and extracellular water (Simmons and Ichikawa, 1989). The higher metabolic rate in children makes them more prone to developing metabolic acidosis when hypohydrated, because of the noncarbonic organic acid accumulation (Hickman and Yasuda, 1986).

When comparing children and adults with regard to fluid intake required to match their respective sweat losses during exercise, sweat volume in relation to their respective body mass (or total body water) is a basic factor to be considered. This is because hypohydration is evaluated as a percentage of body water loss in relation to the initial body weight.

It has been shown that the sweating rate of pre-pubescent boys is lower than that of men (Araki et al., 1979; Davies, 1981; Wagner et al., 1972) or adolescents (Falk et al., 1992). One study with females (Drinkwater et al., 1977) did not find any difference

in the sweating rate between girls and women under different environmental conditions. The main objective of these studies was to compare sweating rates in order to explain maturational differences in thermoregulation. Such an approach, combined with the fact that children have a larger surface area per unit of body mass, leads to the conclusion that, when exercising, children are less efficient in dissipating heat in very hot environments compared to adults (Bar-Or, 1989). Indeed, some studies have demonstrated that children are less efficient at dissipating and tolerating (performance time) heat as compared to adults in hot environments while exercising at the same relative intensity (Bar-Or et al., 1969; Drinkwater et al., 1977; Haymes et al., 1974; Wagner et al., 1972).

Maturational differences in sweating rate have not been applied to evaluate potential differences in dehydration levels. Based on data from a number of relevant studies, Table 1.1. shows the calculated hypohydration levels that would have been achieved during one hour had subjects avoided drinking, and assuming no sweating rate changes with time. Calculated values of hypohydration per kg body mass (as a percentage of initial body weight) were based on sweating rates and body masses reported in each study, while total body water was corrected for age and gender (Friis-Hansen, 1961). This Table includes only studies in which climatic and exercise conditions were similar for the children and the older group. In general, the magnitude of the potential degree of hypohydration was similar between children and adults. At lower climatic (Drinkwater et al., 1977) and metabolic (Araki et al., 1979) stresses, children may become slightly

more dehydrated than adults. Note that in women, percentage hypohydration per body water tends to be higher than that of men. This is because women's body water content does not increase after the age of 15 years and it is relatively lower than that of men (Friis-Hansen, 1961).

The second maturational aspect of fluid replacement during exercise concerns differences in electrolyte losses from sweating. This is discussed in more detail in chapter 2. (Sections 2.1. and 2.4). Briefly, for a given sweating rate, sweat $[Na^+]$ and $[Cl^-]$ tend to increase with age, and as a result sweat osmolality increases (Araki et al., 1979; Dill et al., 1967).

Children are not free from the involuntary dehydration induced by prolonged exercise in the heat. In a group of 11 boys (10-12 yrs) who cycled ($45\% \dot{V}O_{2max}$) intermittently for 3.5 hour in an environment of $39^{\circ}C$ and 45% relative humidity, the degree of involuntary hypohydration averaged 1% of initial body weight, or 0.3% per hour (Bar-Or et al., 1980). The rise in rectal temperature was fairly well correlated with the degree of hypohydration ($r=0.65$). More recently, Bar-Or et al. (1992) reported a greater degree of hypohydration in children with cystic fibrosis (1.57%) as compared to healthy (0.78%) children after 180-min of intermittent cycling ($45\% \dot{V}O_{2max}$) in the heat ($32^{\circ}C$, 45% relative humidity). In that study, however, there were no differences in thermoregulatory responses between groups as measured by increase in rectal temperature. In both studies, the fluid offered was chilled water ($15-17^{\circ}C$). It is unknown whether hypohydration would occur with flavored or CHO-electrolyte drinks. In addition,

the low osmolality of the water increases the urinary free water losses, enhancing hypohydration.

The reason for studying voluntary drinking in children with cystic fibrosis was that their larger NaCl losses through sweat would attenuate the increase in plasma osmolality caused by exercise-induced-hypohydration. As a result, this would lessen their ability to perceive thirst (Bar-Or et al., 1992). Although the rate of thirst perception was not measured during this study, the same rationale may be used when comparing children and adults. If indeed sweat $[Na^+]$, $[Cl^-]$, and osmolality of children are lower than those of adults, the increase in plasma osmolality of children during exercise-induced dehydration will be higher, assuming the same relative volume deficits and urinary electrolyte losses. Teleologically, the lower sweat $[Na^+]$ may be a compensatory mechanism for other maturational "disadvantages" of children.

As already mentioned, acuity of thirst perception seems to decrease with aging. Whether thirst sensation changes from childhood to adulthood is unclear. Studies in developing countries have included the "thirst symptom" to examine whether parents can evaluate the degree of dehydration of their children when they have diarrhea. In a survey (Herman et al., 1991) of 389 children (under the age of 5), only 8.5% of the parents perceived their child as "taking fluid eagerly". Thirst was also underestimated by parents of malnourished children with dehydration (Leal et al., 1990). However, rather than parental misinterpretation, the volitional aspect of thirst may be dependent on the child's cognitive and motor development which are not yet mature.

1.2. Outline and Objectives

The purpose of this thesis is to identify and evaluate physiological and perceptual responses that might contribute to an optimal fluid replacement for children during prolonged exercise in the heat. Some of these responses (discussed above) include: sweat volume and sweat electrolyte losses, fluid homeostasis and acid-base responses, thirst and taste sensations, and the effect of drinking different carbohydrate-electrolyte solutions. In the initial phases of the project, we estimated children's total sweat electrolyte losses (euhydrated state) and then evaluated how exercise-induced hypohydration affected their thirst sensation, flavor preferences and rehydration, using different flavored drinks. Based on these findings, drinks with different electrolytes, with and without carbohydrate, were formulated to evaluate their effects on physiological, biochemical, and perceptual responses of children exercising in the heat. The specific questions of each experiment within this thesis are defined in chapters 2-6.

The first study (chapter 2) compares sweat electrolyte composition and losses between children and adults. It is based on the rationale that the required amount of electrolyte replacement depends on its losses, and that both maturational and gender differences in sweat electrolyte losses have not been established. This study involved six distinct groups of prepubescents, pubescents and young adults (males and females), exercising at the same intensity (relative to their peak $\dot{V}O_2$) and under the same environmental conditions.

To further understand the maturational and gender differences in sweating and sweat composition, the second study (chapter 3) examines the concentration and losses of two other major organic solutes excreted in sweat: lactate and ammonia. Other objectives were to examine among groups, the changes with time in sweat lactate and ammonia, as well as the other ions, and relate them with sweat acidity. Subjects exercised under conditions that facilitate sweating, but prevented dehydration by forced drinking of water.

The third study (chapter 4) evaluates the effect of exercise-induced hypohydration on thirst sensation, drink preferences, and taste quality perceptions of four different flavored drinks. This was done to test whether children's perception of thirst is sensitive to small deficits in body fluid losses and whether the quality of drink intake affects such response. Information derived from these experiments could clarify factors that influence involuntary dehydration in children during exercise, such as the sensitivity of thirst perception or changes in preference pattern to a given drink. Twenty-four boys and girls were tested during four experimental sessions (one drink in each session) while gradually dehydrating by an intermittent exercise in a warm chamber. Because maintenance of hydration after exercise is also important for recovery, another objective was to examine the children's voluntary drinking following exercise that induced dehydration. Thus, the amount of the drink consumed was recorded, without the subject's knowledge, to assess the degree of voluntary rehydration and its relation to drink preferences.

The fourth study (chapter 5) examines the effect of beverages of various

electrolyte composition (with and without carbohydrate) on performance, thermoregulatory responses and electrolyte balance of children who exercise intermittently in the heat. To avoid the effect of hydration state, drinks were served at volumes that kept subjects euhydrated throughout the sessions. Based on the previous study (chapter 4), grape flavor was added to the drinks since it was the most desirable flavor (chapter 4). Each of twelve subjects was tested during four sessions (one drink in each session). These experiments provided the opportunity to study the influence of electrolyte (with and without carbohydrate) intake on total electrolyte losses (sweat plus urine), electrolyte balance, and metabolic responses.

The final study (chapter 6) is a further analysis of the study described in chapter 5. It evaluates the effectiveness of the various drink compositions on perceptual responses of euhydrated children exercising in the heat. These responses include thirst and gastric fullness sensations, ratings of thermal and overall comfort, and ratings of perceived exertion. In the third study (chapter 4), most children drank excessively to overshoot their initial body weight after exercise that induced mild-hypohydration. To examine whether such response occurs after an exercise protocol that prevents hypohydration and the effect of drink composition on voluntary drinking, volume intake during recovery was measured.

Chapter 7 provides conclusions and directions for future research, as based on this project.

TABLE 1.1. Potential Hypohydration per Hour Among Children and Adults (Males and Females), Under Various Climatic and Metabolic Stresses.

Values are calculated assuming no changes in sweating rate with time.

Study	Exercise	Climate	Age & Gender	HYPOHYDRATION	
				%body wt	%body H ₂ O*
Drinkwater et al., 1977	30% $\dot{V}O_2$ max (walking)	28°C-45%RH	♀ PP	0.50	0.87
			♀ YA	0.44	0.89
		35°C-65%RH	♀ PP	1.02	1.78
			♀ YA	0.79	1.58
		48°C-10%RH	♀ PP	1.53	2.66
			♀ YA	1.55	3.09
Araki et al., 1979	light	29°C-60%RH	boys	0.64	1.07
			men	0.41	0.68
	heavy	boys	1.14	1.90	
		men	2.41	4.61	
Wagner et al., 1972	5.6 km·h ⁻¹	49°C-17%RH not acclimatised	all ♂	0.83	1.38
			11-14yr	1.00	1.66
			15-16yr	0.89	1.48
		acclimatised	11-14yr	1.18	1.97
			15-16yr	1.78	2.97
			25-30yr	1.40	2.34
Davies, 1981	70% $\dot{V}O_2$ max	21°C-50%RH	♀ 14yr	0.82	1.37
			♂ 13yr	0.85	1.42
			♂ 36yr	1.28	2.14
Falk et al., 1992	50% $\dot{V}O_2$ max	42°C-20%RH	♂EP	0.99	1.64
			♂MP	0.99	1.65
			♂LP	1.08	1.81
Meyer et al., 1992	50% $\dot{V}O_2$ max	42°C-20%RH	♂ PP	0.81	1.34
			♂ P	0.75	1.25
			♂ YA	1.10	1.68
			♀ PP	0.65	1.13
			♀ P	0.73	1.30
			♀ YA	1.07	2.14

PP=prepubescent, P=pubescent, YA=young adults, EP=early-pubescent, MP=mid-pubescent, LP=late-pubescent RH= relative humidity. *based on Friis-Hansen (1961).

CHAPTER 2

SWEAT ELECTROLYTE LOSS DURING EXERCISE IN THE HEAT: EFFECTS OF GENDER AND MATURATION.

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2. 1. Introduction

During prolonged exercise in the heat, humans lose considerable amounts of both electrolytes and water from sweat. Most values for electrolyte loss in sweat were obtained from adults, mostly males. A study by Lobeck and Huebner (1962), that measured sweat electrolyte concentration in a wide age range of subjects, suggests that gender difference occurs only during adulthood. Men have higher $[\text{Na}^+]$ and lower $[\text{K}^+]$ in their sweat as compared to that of women. It has been shown, by pharmacologically induced sweating, that the concentration of Na^+ and Cl^- in healthy subjects increases with age; children having a sweat $[\text{Na}^+]$ of approximately $40 \text{ mEq} \cdot \text{l}^{-1}$ and adults, $60 \text{ mEq} \cdot \text{l}^{-1}$ (Anderson and Freeman, 1960; Kirk and Westwood, 1989). A study in desert walk (Dill et al., 1967) found that, while sweat $[\text{Cl}^-]$ of adults was around $30 \text{ mEq} \cdot \text{l}^{-1}$, boys' averaged $20 \text{ mEq} \cdot \text{l}^{-1}$ and girls $16 \text{ mEq} \cdot \text{l}^{-1}$.

Sweating rate is lower in boys as compared to men, even when corrected for body surface area (Araki et al., 1979, Wagner et al. 1972; Davies, 1979). However, no

difference in sweating rate was found between prepubescent girls and women (Drinkwater et al., 1977). Sweating rate of boys does not differ from that of girls (Lobeck and Huebner, 1962). Men and women also have similar sweating rate when both groups are matched for maximal aerobic power (Buono and Sjöholm, 1988).

Although some of the above studies have reported both sweat electrolyte concentration and sweating rate, there are no reports on the resultant differences in the total amount of electrolyte lost among maturational or gender groups. Such an approach may be valuable in recommending specific fluid-electrolyte intakes. Therefore, the objectives of this study were to: 1. compare sweat electrolyte concentration and sweating rate of prepubescent, pubescent and young adult males and females, under identical environmental conditions and relative exercise intensities. 2. compare the total amount of sweat electrolyte loss in these different groups.

2.2. Methods

Fifty-one Caucasian volunteers, 25 females and 26 males, participated in the study which had been approved by the McMaster University Ethics Committee. A written informed consent (Appendix 8.1.1) was obtained from the subject, or a parent if the subject was under 18 years old. Males and females were divided according to their maturational stage: prepubescents (PP, n= 8 ♀ and 10 ♂, Tanner I), pubescents (P, n= 9 ♀, Tanner II-IV, and 8 ♂, Tanner II-III) and young adults (YA, Tanner V, n= 8 ♀

and 8 ♂). Their physical and physiological characteristics are summarized in Table 2.1. The female groups were all different in age, height, weight and surface area. PP and P boys were similar in height, weight and surface area, however both groups were different from men in these variables. Within genders, subjects were similar in their adiposity (sum of 4 skinfolds) and peak $\dot{V}O_2$ per kg body weight. Men were significantly taller and heavier than the women and had a higher peak $\dot{V}O_2$ per kg. Boys had significantly higher peak $\dot{V}O_2$ per kg than did the girls. The P boys were lighter than the P girls. All were healthy, non-obese, unacclimatized and unacclimated to heat and not participating in any intensive physical activity program. Experiments were performed during late fall and winter in Canada.

The subjects came to the laboratory for two visits. On the first, they underwent a physical examination which included Tanner staging (pubic hair in males, breast development in females) for maturational stage (Tanner, 1968). Other measurements included anthropometry and peak $\dot{V}O_2$. Height was measured with a Harpenden wall mount Stadiometer 2109, weight with a Mott electro scale model LC2424 (20 g accuracy) and skinfolds with a Harpenden caliper. Peak $\dot{V}O_2$ was assessed by an open-circuit system (Beckman-Horizon Metabolic Measurement Cart) using a progressive (2-min stage), continuous, cycle ergometer test, lasting 6-10 minutes (Bar-Or, 1983). The test was terminated upon self-determined exhaustion or when the child could not maintain 50 rpm cadence in spite of encouragement by the investigator. Objective physiologic criteria for achieving max $\dot{V}O_2$ in our laboratory are: an increase in power load not

followed by an increase in O_2 uptake of more than $2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and a $\text{HR} > 195$ $\text{beats} \cdot \text{min}^{-1}$. Because some of the subjects did not fulfil these criteria, we are referring to this variable as peak $\dot{V}O_2$.

Approximately two weeks later, subjects came for the exercise-in-the-heat session. On the day of the experiment, subjects arrived at the laboratory after having refrained from exercise for 8 hours and caffeine or alcohol ingestion for 48 hours. To ensure a euhydrated state at the beginning of the session, subjects were instructed to drink regular amounts of fluids on the day of the test and, upon arrival to the laboratory, they were given 150-250 ml of water. Sweat collection bags were applied to both the right and left lower back. After the subjects had sat for 20 minutes, blood samples were drawn from the antecubital vein without stasis. During blood collection, subjects were sitting with and forearm flexed and maintained about 10 cm below the heart. A rectal thermistor (YSI 400 series) was inserted beyond the anal sphincter 8-10 cm in children and 12-15 cm in adults. A bipolar lead (Sport Tester PE3000 system) was used to monitor heart rate (HR). Subjects were dressed in athletic shorts and shoes and the girls wore also bikini tops. These clothes and the Sport Tester were weighed before entering the environmental room. Subjects emptied their bladder prior to the heat exposure. Temperature and relative humidity were maintained constant throughout the experiment ($40\text{-}42^\circ \text{C}$ and 18-20%, respectively). Air velocity was minimal ($< 0.2 \text{ m} \cdot \text{s}^{-1}$).

Upon entering the chamber, the subject's body weight, rectal temperature (T_{re}) and HR were determined. Exercise consisted of two 20-minute bouts separated by a

10-minute rest period. Subjects exercised on a cycle ergometer (Monark) at an intensity of 50% of their pre-determined peak $\dot{V}O_2$. The session lasted for 60 minutes or less if termination criteria were reached: $T_{re} > 39^\circ\text{C}$, $\text{HR} > 195 \text{ beat}\cdot\text{min}^{-1}$, or symptoms such as nausea, dizziness, chills and/or exhaustion. T_{re} and HR were monitored every 5 minutes. $\dot{V}O_2$ was determined in the middle of exercise bout 2. During the session, subjects were kept hydrated by drinking cool (8 to 12°C) water. Intake volume was determined by the investigator according to anticipated fluid losses based on their body size (Bar-Or et al., 1980). Hydration state was checked by weighing the subject in the resting period. Sweat was collected after each exercise bout. Blood was drawn from a vein in the dorsum of the hand, without stasis, in the last minute of bout 2 while subjects were still cycling and with the forearm maintained in a similar position as during blood sampling at rest. Following exercise bout 2, the subject was dried completely and body weight was obtained. Once the subject left the environmental room, they emptied their bladder and urine volume was recorded. The clothes and the Sport Tester were weighed again.

During the heat exposure visit, sweat was collected from a polythene bag modified from Boysen et al. (1984). The skin of the lower back was thoroughly cleansed with distilled water and dab dried with a sterile gauze. The bag, made of polythene film, was applied to the skin using Tegaderm tape (3M Co. Ltd.). To avoid contamination by the investigator's hands special care was taken while elaborating the bag and applying it to the skin. The accumulated sweat was collected with a disposable pipet through a

small opening. Two bags (on the right and left of the paravertebral line at the low back) were used to harvest enough sweat for analysis of electrolytes in duplicate. Each bag had a surface area of 100 cm² and 150 cm² for children and adults, respectively. This area covered about 1% of the total body surface area. The mean difference (\pm SD) in concentration of electrolytes between the right and the left bags from sweat collected at the same time was (n=25): 4.0 ± 2.2 , 3.5 ± 1.8 and 0.9 ± 0.7 mEq·l⁻¹ for Na⁺, Cl⁻ and K⁺, respectively. A weighted mean from the two bags was taken to reflect electrolyte loss throughout the session.

Concentrations of Na⁺ and K⁺ in sweat and plasma were analyzed using ion selective electrodes (Kodak Ektachem Analyzer 700XR). Concentration of Cl⁻ in sweat and plasma was measured by a Buchler-Cotlover chloride titrator. Osmolality was obtained by freezing point depression (micro-osmometer Model 3MO); hematocrit (Hct) and hemoglobin concentration (Hb) by a Coulter Counter analyser (Model S-plus STKR); and, plasma protein by the refractometry technique.

Total body sweating rate was calculated from differences in nude body weight obtained before and immediately after the experiment, corrected for fluid intake, respiratory water loss (Mitchell et al., 1972) and urine volume. Total sweat electrolyte losses were calculated by multiplying the volume of water from sweat by the concentration of the electrolyte. Water balance (net fluid gain or loss) was calculated by the change in nude body weight corrected for urine loss. Percentage change in plasma volume during exercise was calculated from changes in Hb and Hct (11).

Statistics. Results are presented as means \pm SD. Analysis of variances was used to determine differences between gender and maturational groups. A Tukey post-hoc procedure, modified for unequal sample sizes, was used to examine the significant differences among maturational groups. A two way analysis of variance was used to examine differences between rest and exercise plasma electrolyte values. Differences were considered significant at $p < 0.05$.

2. 3. Results

Four subjects did not complete the experimental session in the environmental room: two of them (1 ♂ P and 1 ♀ PP) were stopped by the investigator when their Tre reached 39° and the other two (1 ♂ PP and 1 ♀ YA) stopped for subjective reasons. These four subjects completed at least half of the second exercise bout then data was included in the analysis.

The increase in Tre from rest to the end of the session was similar among the 6 groups. Values (mean \pm SD) in °C were as following: 0.7 \pm 0.4 (♀ PP), 0.9 \pm 0.3 (♂ PP), 1.1 \pm 0.4 (♀ P), 0.7 \pm 0.4 (♂ P), 0.8 \pm 0.3 (♀ YA) and 0.8 \pm 0.2 (♂ YA). The % peak $\dot{V}O_2$ in the middle of bout 2 did not differ among groups. The mean and SD in % were: 49.8 \pm 1.6 (♀ PP), 48.7 \pm 3.8 (♀ P), 52.4 \pm 2.2 (♀ YA), 47.9 \pm 2.6 (♂ PP), 49.5 \pm 5.0 (♀ P) and 49.5 \pm 5.1 (♂ YA). Total water intake (mean \pm SD) in liters during the whole session in each group was: 0.38 \pm 0.25 (♀PP), 0.30 \pm 0.24 (♂PP), 0.41 \pm 0.29 (♀P),

0.42±0.26 (♂P), 0.35±0.11 (♀YA), and 0.56±0.19 (♂YA). Total urine output in liters in each of these groups was: 0.09±0.03 (♀PP), 0.04±0.02 (♂PP), 0.07±0.04(♀P), 0.07±0.03 (♂P), 0.05±0.03 (♀YA), and 0.12±0.04 (♂YA).

Sweat [Na⁺], [K⁺] and [Cl⁻] and sweating rate for each group during the whole session are illustrated in Figure 2.1. Despite the large variability, also found in other studies (Anderson and Freeman, 1960; Lobeck and Huebner, 1962, Verde et al., 1982), significant intergroup differences were found. Men had a higher sweat [Na⁺] than PP boys and a higher [Cl⁻] than all boys. Sweat [K⁺] was lower in YA (males and females) compared to that of children. However, the difference between YA males and P boys was not significant. Compared with YA, children (PP and P) had a lower sweating rate even when corrected for surface area. Gender differences, within the same maturational group, were found only in adults, with men having a higher sweat [Na⁺] and [Cl⁻] than the women.

Plasma osmolality at rest was similar in all groups (mean±SD= 289±5.1 mosm·kg⁻¹ H₂O) and, it did not change by the end of the exercise in any of the groups (mean±SD= 290±6.3 mOsm·kg⁻¹ H₂O). Sweat osmolality was lower than that of plasma (mean±SD = 117±39.5 mOsm·kg⁻¹ H₂O) and did not differ among groups.

The total amount of sweat electrolyte loss per kg body weight per hour showed a similar pattern in both genders: YA lost more Na⁺ and Cl⁻ compared to PP and P, whereas all groups had similar K⁺ loss (Table 2.2).

Compared with the values at rest, plasma [Na⁺] and [Cl⁻] did not change by the

end of the exercise in any of the groups (Table 2.3). Plasma $[K^+]$ increased in all groups and this increase was greater in both YA groups than in PP (14% vs 7% in the females and 18% vs 12% in males). All groups maintained close to their original body weight. The % changes (means \pm SD) in body weight (taking pre-chamber weight as 100%) were: 0.01 ± 0.21 (♀ PP), -0.09 ± 0.41 (♂ PP), 0.03 ± 0.32 (♀ P), 0.23 ± 0.24 (♂ P), -0.40 ± 0.36 (♀ YA), and -0.21 ± 0.43 (♂ YA). The changes in plasma volume from rest to the end of the exercise, and the respective changes in plasma protein, are shown in Table 2.4.

2.4. Discussion

The main finding of this study is that, during exercise (50% peak $\dot{V}O_2$) in dry heat, prepubescent and pubescent girls and boys tend to have lower sweat $[Na^+]$ and $[Cl^-]$ and higher $[K^+]$ compared to young adult women and men. The sweating rate of children (both PP and P) was lower than that of YA. As a result, the amount of Na^+ and Cl^- lost from sweat (absolute and per kg body weight) was lower in PP and P compared to YA, whereas no maturational difference was found in total K^+ loss. The only gender difference found in this study was the higher sweat $[Na^+]$ and $[Cl^-]$ of men compared to that of women.

The lower sweat $[K^+]$ and higher $[Na^+]$ and $[Cl^-]$ in the YA may be explained by the electrolyte transport mechanism in the sweat gland. The precursor sweat, derived from the interstitial fluid, has a similar electrolyte concentration to that of plasma (Sato,

1989). Plasma electrolyte concentration at rest and during exercise, in the present study, was similar in all groups (Table 2.3). Thus, the events that reflect maturational differences in the sweat electrolyte concentration probably occur in the reabsorptive duct, rather than the acinus of the gland. Sodium is reabsorbed in the duct in exchange for K^+ by the Na^+/K^+ pump (Quinton and Tomey, 1976).

At least two factors might explain the above differences in sweat electrolyte concentration. The first is related to differences in sensitivity of the duct to plasma aldosterone levels. The second is based on the hypothesis that sweat electrolyte concentration ($[Na^+]$ or $[Cl^-]$) is a function of sweating rate. During exercise in the heat, previous studies have shown an increase in plasma aldosterone levels (Costill et al., 1976; Falk et al. 1991a; Frye and Kamon, 1981). Similar to its effect in the kidneys, plasma aldosterone stimulates Na^+ reabsorption (and K^+ secretion) in the sweat gland duct (Collins, 1966; Sato, 1977). A recent study in our laboratory (Falk et al.; 1991a) showed that plasma aldosterone concentration at rest was similar in pre-, mid- and late-pubescent boys and it increased to the same extent after exercise in the heat in all three groups. Therefore, it appears that differences in the sweat $[Na^+]$ may not be related to plasma aldosterone levels. We cannot rule out, however, the possibility that the above differences in electrolyte concentration may reflect differences in the sensitivity of the receptors to aldosterone in the sweat gland as suggested by Kirby and Convertino (Kirby and Convertino, 1986), or in the number of receptors.

Although sweating rate does not seem to be affected by the menstrual cycle

(Francesconi et al., 1985), our finding that gender differences in sweat $[\text{Na}^+]$ and $[\text{Cl}^-]$ occurred only in adults (men higher than women) suggests that the sweat electrolyte transport may respond to other hormonal variations. Unfortunately, we did not obtain information on hormonal levels or the women's menstrual phase on the day of testing.

It has been suggested that sweat $[\text{Na}^+]$ (and $[\text{Cl}^-]$) increases with the increase of sweating rate (Sato et al., 1989). The explanation is that a higher sweating rate decreases the time available for Na^+ to be reabsorbed in the duct of the sweat gland. However when $[\text{Na}^+]$ was plotted against the sweating rate for all groups the correlation was low ($r = 0.15$). Similar findings were reported by Verde et al. (1982), in adults exercising in the heat, whose sweat $[\text{Na}^+]$ or $[\text{Cl}^-]$ did not correlate with the rate of sweating. Hjelm and co-workers (Hjelm et al., 1986), using pilocarpine iontophoresis, reported an inverse correlation between sweat $[\text{Na}^+]$ and the amount of sweat obtained from either healthy children or children with cystic fibrosis. Similar to our results, a study by Sato et al. (1990) showed a weak association between $[\text{Na}^+]$ collected from a sealed bag and the sweating rate of 6 healthy adults exercising in the heat. However, when measurements were taken from a single sweat gland a positive strong association was apparent. Therefore, such controversial results may be due to the method of stimulating and collecting sweat and the lack of association between $[\text{Na}^+]$ and sweating rate, as found in our study, may reflect the fact that these variables were not measured from a single sweat gland.

In our study, sweating rate was found to be higher in YA compared to both PP

and P (Figure 2.1). Previous studies support our findings that the sweating rate of boys is lower than that of adults (Araki et al., 1979; Wagner et al., 1972). In contrast, Drinkwater and colleagues (1977) found no significant difference in the sweating rate between 5 PP girls and 5 women who walked on a treadmill (30% $\dot{V}O_{2max}$) at three different heat exposures. However, during the warmest session (48°C, 10% RH), women tended to have a higher sweating rate compared to PP (9.6 vs 7.2 ml · m⁻² · min⁻¹). One might argue that the concentration of sweat electrolytes from the back does not reflect the concentration from the rest of the body. However, we have no reason to assume that such a discrepancy will be different among maturational or gender groups. In a recent study (Falk et al., 1991b), the regional differences in the sweat gland distribution were consistent among pre-pubescent, mid- pubescent and late pubescent boys.

Even though [Na⁺] and [Cl⁻] in sweat were lower than in plasma, [Na⁺] and [Cl⁻] in plasma were not altered at the end of the exercise (Table 2.3). An increase in concentration of these ions in plasma would be expected if no fluid was replaced. Since subjects were kept euhydrated with water, a plasma 'dilution' may have prevented an increase in plasma electrolyte concentration. There was an increase in plasma [K⁺] during exercise as has been documented in previous studies (Busse et al. 1989; Linton et al., 1984). The new observation in our study is the higher plasma [K⁺] increase in YA compared with children. Such a difference is probably due to a higher K⁺ shift from the contracting muscles of YA, since they were working with a greater mass and a higher absolute intensity.

In summary, our results suggest that sweat $[\text{Na}^+]$ and $[\text{Cl}^-]$ increase with maturation. Men have a higher sweat $[\text{Na}^+]$ and $[\text{Cl}^-]$ than both PP boys and women. Adults, males or females, have lower sweat $[\text{K}^+]$ than PP. When expressed in total loss per kg of body weight, adults lose more Na^+ and Cl^- from sweat than did PP and P of both genders. No maturational or gender differences seem to be found for total sweat K^+ loss. The above findings are related to Canadians of Caucasian origin. Whether they can be generalized to other ethnic or racial groups remains to be determined. The biological implication of these findings is not clear. They may suggest a protective mechanism in children against excessive salt loss, which accompanies their lower sweating rate.

TABLE 2.1. Physical and Physiological Characteristics of Subjects, by Maturation and Gender.

Maturation group	PP	P	YA	PP-P	PP-YA	P-YA
♀						
Tanner	I	II-IV	V			
N	8	9	8			
Age (yr)	9.1±1.4	11.7±0.7	21.4±0.7	**	**	**
Height (cm)	135.7±8	155.8±6	166.6±4	**	**	**
Weight (kg)	33.4±6.3	45.0±5.5	60.5±7.2	**	**	**
Surface area (m ²)	1.12±0.12	1.40±0.10	1.66±0.10	**	**	**
∑ 4 skinfolds (mm)	38.3±5.8	34.3±7.1	39.6±4.6	NS	NS	NS
Peak $\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	40.6±5.1	40.1±3.8	42.3±4.0	NS	NS	NS
♂						
Tanner	I	II-III	V			
N	10	8	8			
Age (yr)	9.1±1.3	11.0±1.2	23.4±2.0	**	**	**
Height (cm)	140.2±9	148.4±10	178.4±8	NS	**	**
Weight (kg)	33.9±8.1	38.3±7.0	76.3±7.3	NS	**	**
Surface area (m ²)	1.18±0.16	1.26±0.16	1.83±0.11	NS	**	**
∑ 4 skinfolds (mm)	30.6±4.7	28.4±5.7	37.8±6.5	NS	NS	NS
Peak $\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	46.3±1.9	49.2±1.2	48.6±1.5	NS	NS	NS

PP = prepubescent; P = pubescent; YA = young adults. *p < 0.01; NS = no difference. Values are mean ± SD.

TABLE 2.2. Total Electrolyte Loss in Sweat, by Gender and Maturation.
 Values are in $\text{mEq} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. (Mean \pm SD).

	Na ⁺	Cl ⁻	K ⁺
♀			
PP	0.13 \pm 0.05	0.08 \pm 0.04	0.07 \pm 0.04
P	0.22 \pm 0.09	0.13 \pm 0.08	0.09 \pm 0.03
YA	0.32 \pm 0.10	0.25 \pm 0.10	0.07 \pm 0.02
♂			
PP	0.23 \pm 0.14	0.15 \pm 0.12	0.06 \pm 0.03
P	0.29 \pm 0.11	0.19 \pm 0.12	0.07 \pm 0.03
YA	0.47 \pm 0.23	0.40 \pm 0.22	0.06 \pm 0.04
♀			
PP-P	*	NS	NS
P-YA	**	**	NS
P-YA	*	**	NS
♂			
PP-P	NS	NS	NS
PP-YA	**	**	NS
P-YA	*	*	NS

PP= prepubescents; P= pubescents; YA= young adults.

* $p < 0.05$; ** $p < 0.01$; NS= no significant difference.

There was no gender differences, within the same maturational group, in any of the electrolytes.

TABLE 2.3. Plasma Electrolyte Concentration in $\text{mEq}\cdot\text{l}^{-1}$ during Rest and Exercise.
(Mean \pm SD).

	[Na ⁺]		[Cl ⁻]		[K ⁺]	
	rest	exercise	rest	exercise	rest	exercise
♀						
PP	137 \pm 2.3	138 \pm 0.9	104 \pm 1.9	105 \pm 1.3	4.0 \pm 0.2	4.3 \pm 0.1*
P	139 \pm 2.7	139 \pm 2.7	105 \pm 1.6	106 \pm 3.0	3.9 \pm 0.2	4.5 \pm 0.3*
YA	137 \pm 1.9	137 \pm 1.9	104 \pm 2.3	106 \pm 2.0	4.1 \pm 0.3	4.7 \pm 0.3*
♂						
PP	140 \pm 1.7	140 \pm 2.2	105 \pm 1.2	105 \pm 1.6	3.9 \pm 0.2	4.4 \pm 0.1*
P	141 \pm 1.9	141 \pm 2.6	105 \pm 1.7	106 \pm 1.4	4.0 \pm 0.3	4.4 \pm 0.2*
YA	137 \pm 2.9	138 \pm 2.4	102 \pm 2.9	105 \pm 3.0	3.8 \pm 0.2	4.5 \pm 0.3*

* significant difference from the respective rest value ($p < 0.05$).

TABLE 2.4. Hemoglobin (Hb), Hematocrit (Htc), Plasma Protein and Changes in Plasma Volume (% Δ PV) and Protein from Rest to the End of Exercise. (Mean \pm SD).

Groups	Hb (g \cdot 100ml $^{-1}$)		Htc (%)		Protein (g \cdot l $^{-1}$)		% Δ PV	% Δ protein
	rest	exercise	rest	exercise	rest	exercise		
PP ♀	13.1 \pm 0.3	13.2 \pm 0.5	38.9 \pm 2.0	38.8 \pm 2.7	72.6 \pm 4.0	75.0 \pm 4.8	-0.6 \pm 4.3	3.2 \pm 3.0
♂	12.5 \pm 0.6	12.7 \pm 0.4	36.8 \pm 1.9	37.8 \pm 1.2	69.8 \pm 2.2	71.5 \pm 3.2	-3.7 \pm 2.9	2.4 \pm 3.1
P ♀	12.6 \pm 0.3	13.0 \pm 0.3	38.9 \pm 2.1	39.9 \pm 1.7	71.0 \pm 4.0	73.9 \pm 4.4	-5.3 \pm 3.7*	3.9 \pm 5.0
♂	12.3 \pm 0.6	12.6 \pm 0.5	36.4 \pm 1.9	37.2 \pm 1.6	73.3 \pm 1.9	74.8 \pm 1.9	-2.6 \pm 2.6	2.0 \pm 3.4
YA ♀	12.1 \pm 1.7	12.8 \pm 1.8	36.9 \pm 4.3	38.7 \pm 4.4	74.6 \pm 4.6	81.4 \pm 3.4	-8.8 \pm 2.1#	8.3 \pm 3.7
♂	15.4 \pm 0.7	16.2 \pm 0.7	44.9 \pm 2.3	47.2 \pm 1.9	73.0 \pm 3.2	80.2 \pm 3.5	-8.6 \pm 5.5#	9.8 \pm 3.0¶

PP = prepubescents; P = pubescents; YA = young adults

* = Different from the respective PP group ($p < 0.05$).

= Different from the respective PP and P groups ($p < 0.05$).

¶ = Different from the respective PP and P groups ($p < 0.01$).

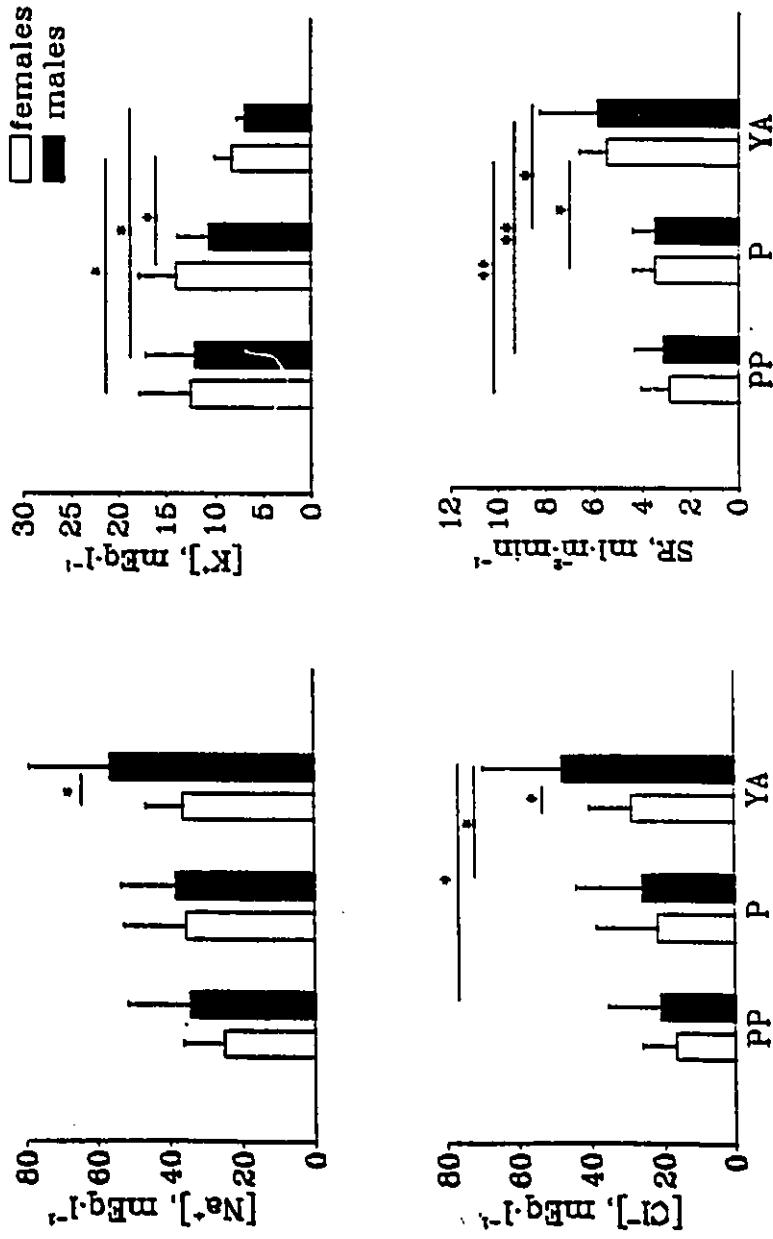


Figure 2.1. Sweat Electrolyte Concentrations and Sweating Rate (SR) from the Entire Session. Values are Means and Errors Bars are SD. *p<0.05, **p<0.01.

2.5. Addendum

In addition to the information in the above published study, total urine electrolyte losses were calculated, as shown in Table 2.5.

TABLE 2.5. Total Electrolyte Loss in Urine ($\text{mEq} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) by Maturation and Gender.

GROUP	Na ⁺	Cl ⁻	K ⁺
♀			
PP	0.20±0.10	0.34±0.22	0.23±0.18
P	0.08±0.07	0.12±0.07	0.15±0.05
YA	0.04±0.01	0.04±0.03	0.08±0.06
♂			
PP	0.13±0.13	0.15±0.10	0.15±0.08
P	0.22±0.22	0.23±0.10	0.14±0.08
YA	0.16±0.16	0.16±0.10	0.12±0.07
♀			
PP-P	NS	*	NS
PP-YA	*	*	NS
P-YA	*	*	NS
♂			
PP-P	NS	NS	NS
PP-YA	NS	NS	NS
P-YA	NS	NS	NS
♀-♂			
PP	NS	NS	NS
P	NS	NS	NS
YA	*	*	NS

PP= prepubescents; P= pubescents; YA= young adults. * $p < 0.05$; NS= no difference. Mean±SD.

Overall, women lost less Na^+ Cl^- and K^+ than girls and less Na^+ and Cl^- than men. The extent to which women's hormonal variation may have affected urine volume or electrolyte concentrations is unknown. It is unlikely that the women's lower urinary electrolyte losses, compared with the girls, are a compensation for their greater sweat electrolyte content, because such a compensatory response was not observed in the males.

The combined electrolyte loss from sweat and urine is shown in Table below.

TABLE 2.6. Combined Electrolyte Loss from Sweat and Urine ($\text{mEq} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) by Maturation and Gender.

GROUP	Na^+	Cl^-	K^+
♀			
PP	0.33 ± 0.13	0.42 ± 0.24	0.31 ± 0.20
P	0.29 ± 0.15	0.24 ± 0.08	0.24 ± 0.07
YA	0.36 ± 0.10	0.30 ± 0.10	0.16 ± 0.07
♂			
PP	0.37 ± 0.21	0.31 ± 0.15	0.21 ± 0.09
P	$0.51 \pm 0.21^*$	$0.43 \pm 0.19^*$	0.21 ± 0.08
YA	0.62 ± 0.32	0.55 ± 0.14	0.19 ± 0.09

PP= prepubescents; P= pubescents; YA= young adults. *different from ♀P. Mean \pm SD.

Total Na^+ and Cl^- losses tended to increase with maturation only in the males. This trend has yet to be confirmed because the study failed to control for the subjects' diet prior to the experiments. The amount of electrolyte intake acutely affects urinary losses since the kidney is a major regulator of body electrolyte stores.

CHAPTER 3

SWEAT LACTATE AND AMMONIA DURING EXERCISE IN THE HEAT: COMPARISONS AMONG GIRLS, BOYS, WOMEN, AND MEN.

3.1. Introduction

During prolonged exercise in the heat, two major solutes excreted in sweat are lactate (Lac^-) and ammonia (NH_3) whose respective concentrations are 10-15 and 80-150 times greater than those in plasma (Sato et al., 1989). The Lac^- in sweat is mainly derived from anaerobic glycolysis within the sweat gland (Sato, 1977; Sato and Dobson, 1973; Gordon et al., 1971). The high sweat $[\text{NH}_3]$ may originate from the sweat gland itself or from diffusion from plasma (Brusilow and Gordes, 1968; Sato et al. 1989; Czarnowski et al., 1992).

The large reported variability in sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$ may be explained by their dependence on factors such as the sweating rate (SR), sweat acidity, and the transport of other ions in the duct of the sweat gland. These factors may in turn determine the concentration of a person's sweat Lac^- and NH_3 . For example, SR was shown to inversely correlates with sweat $[\text{Lac}^-]$ (Falk et al., 1991; Lamont, 1987). Lamont (1987) found very high sweat $[\text{Lac}^-]$ ($43\text{-}100 \text{ mmol}\cdot\text{l}^{-1}$) in women. She suggested that this reflects women's lower SR. More recent and controlled studies found no gender differences in

SR in either adults (Bouno and Sjöholm, 1988) or children (Meyer et al., 1992). Thus, based on SR alone, it is unlikely that sweat $[\text{Lac}^-]$ would be different between genders. Because SR has been shown to increase with maturation, we hypothesised that sweat $[\text{Lac}^-]$ is higher in children than in adults. Indeed, sweat $[\text{Lac}^-]$ has already been shown to be higher in pre-pubescent than in late-pubescent boys at the first 20 min of moderate exercise ($50\% \dot{V}O_{2,\text{max}}$) (Falk et al. 1991). It is unknown whether such a difference persists through adulthood.

Much less is known about inter-individual differences in sweat $[\text{NH}_3]$ during exercise. NH_3 transport, between water compartments, depends on the pH gradient, moving towards the lower pH. It has been shown that sweat pH is lower (5 to 7) than blood pH (Sato et al., 1989). Whether sweat pH differs between children and adults during exercise has not been demonstrated. One might predict that sweat pH would be lower in children than adults for two reasons: the expected higher sweat $[\text{Lac}^-]$ in children and the increase of pH with SR (Bijman and Quinton, 1987; Kaiser et al., 1974). One possible way to prevent very low levels of sweat pH is to increase its NH_3 content. We therefore hypothesized that a lower sweat pH of children would be accompanied by a higher sweat $[\text{NH}_3]$.

The main purpose of this study was to compare sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$ and their respective losses among four groups (girls, boys, women, and men) who exercise intermittently (1 hour) at the same relative intensity ($50\% \dot{V}O_2$) and environmental conditions. Other specific objectives were to examine, among groups, the changes with

time in sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$ and other ions, and relate them to sweat $[\text{H}^+]$.

3.2. Methods

Data for this study were obtained from the previous experiment, thus a more detailed description of Methods can be found in Section 2.3.

Subjects. Forty-nine subjects (15 girls, 8 women, 18 boys and 8 men) were included in this study. Their physical characteristics are shown in Table 3.1. Of a total of 33 children, 17 were prepubescents and 16 (8 girls and 8 boys) at the start or in the middle of puberty. All subjects were healthy according to a medical interview and a physical examination. Experiments took place during the late fall and winter in Ontario, Canada which suggests that subjects were not acclimatized to the heat. This study was approved by the McMaster University Ethics Committee.

Procedures and Protocol. About two weeks prior to the experimental session, subjects came to the laboratory for anthropometric and peak $\dot{V}\text{O}_2$ determinations. Weight and height were measured and body surface area calculated (DuBois and DuBois, 1916). Adiposity was calculated from the sum of 4 skinfolds (triceps, biceps, subscapular, and suprailiac) measured in triplicate with a Harpenden caliper. Peak $\dot{V}\text{O}_2$ was assessed by a continuous progressive (2-min stages) protocol on a cycle ergometer at a constant cadence of 50 rpm, in a thermoneutral environment. The test was terminated upon subject's volitional exhaustion or when the target cadence could not be maintained. $\dot{V}\text{O}_2$

was measured by an open-circuit spirometry with respiratory gases analysed by an automated system (Beckman-Horizon Metabolic Cart).

On the day of the in-chamber experiment, subjects were advised to come to the laboratory having abstained from drinking alcohol for 48 h, caffeine for 8 h, and from exercising in the previous 2 hours. Subjects were encouraged to drink plenty of water on this day and, upon arrival to the laboratory, they were given 150-250 ml of water to reduce the likelihood of hypohydration. Before entering the chamber (40-42°C and 18-20% relative humidity), subjects voided, and dressed in shorts and sports shoes, plus bikini tops for females.

In the chamber, subjects cycled (Monark) for two 20-min bouts at 50% of their predetermined peak $\dot{V}O_2$, with a 10 min rest before bout 1 and between bouts. $\dot{V}O_2$ was measured in the middle of bout 2 (Beckman-Horizon Metabolic Cart). Heart rate (HR) (Sport Tester PE3000 system) and rectal temperatures (YSI 400 series) were measured continuously. Subjects were kept euhydrated by drinking cool (8-12°C water) throughout the session. Hydration state was checked by weighting subjects before, after bout 1, and at the end of the session (Mott electro scale LC2424, ± 20 g accuracy)

Sampling and analysis of sweat and blood. To guarantee enough volume, sweat was collected in two plastic bags (one in each side) attached to the lower back as previously described (chapter 2.2). Once collected, the sample was stored in microtubes and frozen (-4°C) until biochemical analysis. A separate sample was frozen at -70°C for determination of pH and [NH₃].

Sweat [Lac⁻] was determined using the Yellow Springs Instrument analyzer (model 23L), [NH₃] by spectrophotometry (colorimetric assay Sigma 70-UV), and osmolality by freezing point depression (Model 3M0). Sweat [Na⁺] and [K⁺] were measured using ion selective electrodes (Kodak Ektachem Analyser 700XR) and the [Cl⁻] with a Buchler-Cotlover chloridometer. Sweat pH was determined using Radiometer pH-(g2040c) and reference (K4040) electrodes coupled to a radiometer PHu62 pH meter.

Blood samples were drawn at rest after the subjects had rested for 15 min (seated) in the thermoneutral room, and during the last min of bout 2 while they were still cycling. To facilitate blood collection without stasis, subjects kept their hands in warm water (40–45°C) for 15 min prior to the collection. Using a 21-gauge butterfly, blood was collected (6 ml) from a vein of the dorsum of hand, without stasis, into a blood gas syringe and into two vacutainers (lithium heparin and EDTA). The blood collected anaerobically into a syringe (lithium heparinized), was freed from air bubbles, sealed and kept on ice until determination (within 1 h) of blood gases. HCO₃⁻ and pH (AVL 995/ Blood Gas Analyser with H⁺, O₂ and CO₂ electrodes). Blood [Lac⁻] was analysed immediately (model 23L, Yellow Springs Instruments) with the sample from one of the vacutainers (lithium heparin). Blood was then centrifuged and the plasma kept on ice for later analysis. Plasma electrolyte concentrations were measured using ion selective electrodes (Kodak Ektachem Analyser 700XR), protein by refractometry, and osmolality by freezing point depression (model 3M0). The blood sample collected into EDTA vacutainers was used for hematocrit and hemoglobin determinations (Coulter-Counter,

Model S-plus STKR).

Calculations. Sweating rate per surface area (m^2) and time (min), was calculated as a percentage of body mass, corrected for the water intake, urine output, increase in clothing weight, and respiratory water losses (Mitchell et al., 1972). Sweat lactate and NH_3 losses were calculated by multiplying the volume of total body sweat by its respective concentration. Hydration level was calculated by the change in body weight and expressed as % initial body weight, corrected for urine output and change in clothes weight. Percentage changes in plasma volume ($\% \Delta \text{PV}$) and red cell volume ($\% \Delta \text{RBC}$) were calculated from changes in hemoglobin and hematocrit (Harrison, 1985). Exercise blood $[\text{Lac}^-]$ was corrected for $\% \Delta \text{PV}$. Sweat pH was converted to the equivalent free hydrogen ion ($[\text{H}^+]$). Plasma and sweat strong ion difference (SID) were calculated as: $[\text{Na}^+] + [\text{K}^+] - [\text{Cl}^-] - [\text{Lac}^-]$ and $[\text{Na}^+] + [\text{K}^+] + [\text{NH}_3] - [\text{Cl}^-] - [\text{Lac}^-]$, respectively.

Statistics. Within genders, prepubescents and pubescents were found to be similar with respect to all sweat and blood parameters. All children were therefore pooled into two groups: boys and girls. A one way analysis of variance (ANOVA) was used to compare children vs adults and females vs males. A two way ANOVA was used to examine changes with time (e.g. bout 1 vs bout 2). Statistical significance was considered when $p < 0.05$. Values are expressed as mean \pm SEM.

3.3. Results

Physiological responses. The mean increase in rectal temperature (°C) from rest to the end of bout 2 did not differ among groups: 0.90 ± 0.08 (girls), 0.83 ± 0.08 (boys), 0.75 ± 0.11 (women), and 0.81 ± 0.07 (men). Heart rate (bpm) at the end of bout 2 was similar among groups: 165 ± 4 (girls), 162 ± 7 (boys), 162 ± 6 (women), 159 ± 8 (men). By the end of bout 2, most subjects maintained their hydration levels within 1% of initial body weight (range = -0.6 to 0.8%). The mean \pm SEM % change in body weight per group was: 0.11 ± 0.09 (girls), 0.01 ± 0.11 (boys), -0.09 ± 0.3 (women), -0.19 ± 0.02 (men).

Sweat composition. Children had 25% higher sweat [Lac⁻] than their respective counterparts after bout 1 ($p < 0.05$) (Figure 3.1). In each group, there was a drop in sweat [Lac⁻] from bout 1 to bout 2, and all groups ended the session with similar values (overall mean \pm SEM = 13.7 ± 0.4 mmol·l⁻¹). Within maturational group there was no gender difference in sweat [Lac⁻]. Mean sweat [NH₃] after the first bout was 66% higher in children than adults. Although sweat [NH₃] did not change significantly with time in any of the groups, opposite trends among groups resulted in men having similar value to boys but higher than women (Figure 3.1, bottom). The total sweat losses of Lac⁻ were higher in adults than in children, but similar among groups when corrected for kg body mass (Table 3.2). A similar trend was found for total NH₃. Men had a higher sweat [Na⁺] and [Cl⁻] compared to the other groups. Mens' sweat [Cl⁻] increased from bout 1

to bout 2 (Figure 3.2). Girls and boys had higher $[K^+]$ than their adult counterparts in the first bout. Overall, sweat $[SID]$ was similar among groups with one exception: at bout 2 it was higher in boys than men. Sweat $[H^+]$ was lower in men compared to boys during bouts 1 and 2, and compared to women during bout 2 (Figure 3.2). The lower sweat $[H^+]$ of women compared with girls was close to significance ($p=0.07$ in bout 1, and $p=0.06$ in bout 2). Sweat pH during both bouts was lower in girls and boys compared to their adult counterparts. Respective means \pm SEM were: 5.4 ± 0.2 (girls), 5.0 ± 0.1 (boys), 6.2 ± 0.5 (women), and 6.2 ± 0.4 (men) for bout 1, and 5.4 ± 0.2 (girls), 6.5 ± 0.5 (boys), 5.2 ± 0.2 (women), and 6.9 ± 0.4 (men) for bout 2.

While sweat osmolality decreased in boys and girls ($p<0.05$), it did not change in men and women (Figure 3.3, top). SR was consistently lower in girls and boys compared to their respective adult group (Figure 3.3, bottom).

Blood results. Blood pH at rest was similar among groups while at the end of exercise it was higher in girls than women (Table 3.3). However, the magnitude of the changes in blood pH, $[HCO_3^-]$ and PCO_2 was similar among the four groups (Table 3.3). Overall there was an increase in blood pH, and a decrease in $[HCO_3^-]$ and PCO_2 . When changes within groups were analyzed, significance ($p<0.05$) was found in all groups for blood PCO_2 , but not for the pH and $[HCO_3^-]$ (Table 3.3). While blood $[Lac^-]$ increased in men, it decreased in boys and it did not change in the female groups.

Plasma osmolality and RBC volume did not change from rest to the end of the exercise session. There was a decrease in PV and an increase in plasma proteins in all

groups, changes being significantly greater in adults ($p < 0.05$) (Table 3.4). There was no change in plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ from rest to the end of the exercise session in any of the groups, while plasma $[\text{K}^+]$ increased in an average of 12% in boys to 18% in men. In all groups, plasma $[\text{SID}]$ did not change from rest to the end of bout 2, with values (mean \pm SEM) of 37.7 ± 0.48 and 37.6 ± 0.5 $\text{mmol}\cdot\text{l}^{-1}$, respectively.

3.4. Discussion

The main purpose of this study was to compare sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$, and their respective losses, between children and adults of both genders during one hour of intermittent exercise (50% peak $\dot{V}\text{O}_2$) in the heat. Girls and boys had higher sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$ compared to their adult counterparts within 20 min of exercise. Thereafter, this difference persisted in females for $[\text{NH}_3]$ only. During this later stage of the session, men had higher sweat NH_3 than women. This was the only gender difference found within age groups. The total sweat Lac^- and NH_3 losses, corrected per kg body mass, were similar among groups.

The overall sweat $[\text{Lac}^-]$ of the boys (16.6 $\text{mmol}\cdot\text{l}^{-1}$) and men (15.1 $\text{mmol}\cdot\text{l}^{-1}$) in the present study agrees with those already reported in boys (Falk et al., 1991) and men (Fellmann et al. 1983; Czarnowski and Gorski, 1991) performing similar exercise protocols. The mean sweat $[\text{Lac}^-]$ of women (14.3 $\text{mmol}\cdot\text{l}^{-1}$) in the present study, however, was much lower than that of sedentary (100 $\text{mmol}\cdot\text{l}^{-1}$) or fit (43 $\text{mmol}\cdot\text{l}^{-1}$)

women (Lamont, 1987). The method of collecting sweat (plastic bag vs whole body washdown) and the exercise intensity (50% vs 70% $\dot{V}O_2$) may have accounted for such a difference. Although sweat from the bags represents only one site of the body, it is a reliable (Calvert et al., 1990) method, and involves the least contamination (Sato et al., 1990). Unless the investigator is very familiar with the procedures, the whole-body washdown is prone to errors, and whenever used its reliability should be reported (Lemon et al. 1986).

Sweat $[Lac^-]$, at the start of exercise, has previously been found higher in prepubertal compared to adolescent boys (Falk et al., 1991). Our findings are that, at this early stage of sweating, boys have a higher sweat $[Lac^-]$ than men and that corresponding differences exist in females.

Although children's sweat $[Lac^-]$ after bout 1 was higher than that of adults, this cannot be explained by their lower SR. We found a negative but very weak correlation between SR and sweat $[Lac^-]$ ($r=-0.27$, for all subjects). Such a relationship is not consistent since some have reported an inverse correlation (Falk et al., 1991; Fellmann et al, 1985; Lamont, 1983), while studies using single sweat glands have shown a significant positive (Bijman and Quinton, 1987), or no correlation (Kaiser et al., 1974).

It is unclear why children have higher sweat $[Lac^-]$ at the beginning of exercise. One possibility is that the energy required to start sweating is higher than to maintain sweating. The higher sweat $[Lac^-]$ of children at the start of exercise may be due to their higher sweating threshold (Araki et al., 1979; Wagner et al., 1972). Timing for sweat

collection was the same for all subjects and set according to exercise duration, which may not necessarily match for sweating duration. This time lag could be responsible for the higher sweat $[\text{Lac}^-]$ at the start of the exercise.

It is hard to affirm whether the sweat glands in children respond with a greater glycolytic rate at the start of exercise. Sweat $[\text{Lac}^-]$ may not be an accurate index of glycolytic rate of the sweat gland since some Lac^- , in conjunction with other ions or in exchange with H^+ , is reabsorbed in the sweat duct (Bijman and Quinton, 1984; 1987)

A consistent finding of the present study is that sweat $[\text{Lac}^-]$ dropped with exercise duration, which is in agreement with other studies where subjects cycled at constant work rates (Falk et al., 1991; Fellmann et al., 1983). The drop in sweat $[\text{Lac}^-]$ could be because, besides glycolysis, the sweat gland uses oxygen (oxidative phosphorylation) as a substrate for sweating (Sato, 1977; Fellmann et al., 1989). At the start of exercise, the vasoconstrictive effect of epinephrine release and reduced oxygen supply to the skin may cause the sweat glands to rely more on anaerobic glycolysis and produce more Lac^- . As exercise continues, especially in a warm environment, more blood is shunted to the skin allowing the sweat glands to work more aerobically.

Sweat $[\text{NH}_3]$ has rarely been studied during exercise. In the present study, the men's average sweat $[\text{NH}_3]$ ($3.2 \text{ mmol}\cdot\text{l}^{-1}$) was somewhat higher than that found when men cycled at 40% ($2.1 \text{ mmol}\cdot\text{l}^{-1}$), but much lower than that obtained at a higher exercise intensity ($7.1 \text{ mmol}\cdot\text{l}^{-1}$ at $80\% \dot{V}\text{O}_{2\text{max}}$) (Czarnowski and Gorski, 1991). This further indicate that sweat $[\text{NH}_3]$ increases with exercise intensity.

To our knowledge, this is the first report on sweat $[\text{NH}_3]$ of females and children who exercise in the heat. As expected, children had a higher sweat $[\text{NH}_3]$ in the first bout, and this can reflect a mechanism for avoiding further decrease in sweat pH. NH_3 diffusion is facilitated by increased pH gradient between compartments. NH_3 , as a base, diffuses from the extracellular space (higher pH) to the sweat gland duct (lower pH) where, once protonated to NH_4^+ , it becomes poorly diffusible (Brusilow and Gordes, 1968; Sato et al. 1989). Indeed, the children in the present study had a lower sweat pH than adults (5.2 vs 6.1 for bout 1; 5.3 vs 6.7 for bout 2) and, at least at the end of bout 2, they tended to have a higher blood pH (Table 3.3). To support this hypothesis, we found significant and positive correlations between sweat NH_3 and $[\text{H}^+]$ for all subjects combined ($r=0.56$). This is in agreement with other studies (Morimoto and Johnson, 1967; Brusilow and Gordes, 1968).

Although some sweat NH_3 may originate from plasma, it is unlikely that the higher sweat $[\text{NH}_3]$ of children in this study was a reflection of their greater plasma $[\text{NH}_3]$. At rest, plasma $[\text{NH}_3]$ in children is similar to that of adults (Colombo et al., 1981) and, in response to exercise (treadmill $\dot{V}\text{O}_{2\text{max}}$ test), the magnitude of its increase may even be higher in adults than in children (Nazar et al., 1992).

The mechanism involved in sweat acidification is not clear. It was suggested that the duct acidifies the precursor sweat by reabsorbing HCO_3^- and/or secreting H^+ in exchange of Na^+ (Quinton, 1982; 1983). In the present study, overall sweat $[\text{Na}^+]$ was inversely correlated with sweat $[\text{H}^+]$ ($r= -0.45$, $p<0.05$), but r values were greater in

children than adults (-0.43 vs -0.29). This indicates that lower sweat pH of children may be associated with their greater Na^+ reabsorption.

The average sweat [SID] ($7 \text{ mmol}\cdot\text{l}^{-1}$) in the present study was much lower than that of plasma ($37.6 \text{ mmol}\cdot\text{l}^{-1}$). Our values are close to those already reported in men as 'residual ions' (Bijman and Quinton, 1987), even though our calculations included ammonia. We did not find a strong inverse correlation between sweat $[\text{H}^+]$ and sweat [SID] ($r=-0.1$). This is probably due to a lower and small range of sweat [SID]. Besides [SID], the $[\text{H}^+]$ (or acid-base status) of a solution depends on two other variables: the PCO_2 and weak anions (mostly proteins). In fact, when [SID] is around zero, very small changes in PCO_2 may cause large change in pH (and $[\text{H}^+]$) (Stewart, 1983). On the other hand, proteins do not affect sweat $[\text{H}^+]$ because of their low concentrations in sweat ($2 \text{ mg}\cdot\text{dl}^{-1}$ vs $7 \text{ g}\cdot\text{dl}^{-1}$ in blood) (Sato and Sato, 1990).

Our finding that blood pH did not change over time in men is in agreement with a study that used a similar exercise/heat protocol (Sawka et al., 1980). Although in children an increase in blood pH achieved significance, the pH changes among groups were similar. This is somewhat divergent from high intensity exercise in which a fall in blood pH is considerably greater in adults than in children (Zanconatto et al., 1993). The overall decrease in blood pH in the present study may be due to increased ventilation (as shown by the PCO_2 decrease) and increased skin blood flow (perfusion) both promoted by increased body temperature (Senay and Christensen, 1967).

In conclusion, we have shown that children present a higher sweat $[\text{Lac}^-]$ and $[\text{NH}_3]$ compared to adults during the first 20 min of exercise in the heat. Within an hour of exercise, the overall sweat losses of Lac^- and NH_3 corrected per kg body mass, were similar among groups. These results are representative of an exercise plus heat stress which was too low to induce fatigue or blood acidosis. The extent to which such a pattern will persist during exercise-induced acidosis has yet to be determined.

TABLE 3.1. Physical Characteristics of Subjects. Mean \pm SEM.

VARIABLE	GIRLS (n = 15)	BOYS (n = 18)	WOMEN (n = 8)	MEN (n = 8)
Age (yr)	10.5 \pm 0.47*	9.9 \pm 0.35*	21.5 \pm 0.25	23.4 \pm 0.71
Height (cm)	148.6 \pm 3.1*	143.9 \pm 2.5*	166.4 \pm 1.4	178.4 \pm 2.9
Weight (kg)	40.2 \pm 2.1*	35.8 \pm 1.8*	60.5 \pm 2.5	76.3 \pm 2.6
Surface Area (m ²)	1.29 \pm 0.08*	1.22 \pm 0.04*	1.67 \pm 0.04	1.83 \pm 0.04
Σ 4 Skinfold (mm)	36.8 \pm 2.9	29.6 \pm 2.7	39.4 \pm 4.6	37.8 \pm 6.5
Peak $\dot{V}O_2$ (ml \cdot kg ⁻¹ \cdot min ⁻¹)	40.4 \pm 1.3	47.6 \pm 1.4 [#]	42.3 \pm 1.5	46.6 \pm 3.8

*different from respective adult group; [#] > girls, p < 0.05.

TABLE 3.2 . Total Sweat Lactate and Ammonia Losses by Groups.
 Values are also corrected per body mass (kg) and time (h).
 Mean \pm SEM.

GROUPS	LACTATE mmol	LACTATE mmol \cdot kg ⁻¹ \cdot h ⁻¹	AMMONIA mmol	AMMONIA mmol \cdot kg ⁻¹ \cdot h ⁻¹
GIRLS	5.45 \pm 0.94*	0.13 \pm 0.02	1.11 \pm 0.17	0.026 \pm 0.003
BOYS	4.83 \pm 0.54*	0.15 \pm 0.02	1.33 \pm 0.14*	0.022 \pm 0.004
WOMEN	9.22 \pm 1.36	0.15 \pm 0.02	1.35 \pm 0.17	0.037 \pm 0.002
MEN	11.4 \pm 2.30	0.14 \pm 0.03	2.35 \pm 0.46	0.032 \pm 0.006

*Different from the respective adult group, $p < 0.05$.

TABLE 3.3. Blood pH, [HCO₃], PCO₂ and [Lac] at Rest and at the End of Exercise Session. Mean ± SEM.

GROUPS	pH		[HCO ₃], mmol·l ⁻¹		PCO ₂ , mm Hg		[Lac], mmol·l ⁻¹	
	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE
GIRLS	7.33 ± 0.02	7.41 ± 0.01*	27.0 ± 0.6	23.7 ± 0.4*	51.6 ± 1.9	38.3 ± 1.0*	0.7 ± 0.1	1.1 ± 0.2
BOYS	7.31 ± 0.02	7.40 ± 0.01*	25.3 ± 0.6	24.8 ± 0.3	51.6 ± 1.9	41.1 ± 0.7*	1.0 ± 0.1	0.8 ± 0.1*
WOMEN	7.32 ± 0.03	7.35 ± 0.02	25.5 ± 1.0	23.8 ± 1.0	49.0 ± 3.3	41.5 ± 3.2*	1.1 ± 0.2	1.4 ± 0.2
MEN	7.30 ± 0.02	7.36 ± 0.02	26.0 ± 0.4	24.3 ± 0.5*	58.3 ± 2.2	43.9 ± 1.6*	1.0 ± 0.1	1.8 ± 0.3**

*Different from respective rest value, †different from women, ‡different from boys (p < 0.05).

TABLE 3.4. Percentage Changes in Plasma Volume (PV), Red Blood Cell (RBC), Plasma Protein, and Osmolality from Rest to the End of Exercise. Mean \pm SEM.

GROUP	% Δ PV*	% Δ RBC	% Δ PROTEIN*	% Δ OSMOLALITY
GIRLS	-3.6 \pm 1.4*	0.08 \pm 0.5	4.62 \pm 0.9*	0.09 \pm 0.6
BOYS	-2.3 \pm 0.4*	0.32 \pm 0.4	2.15 \pm 1.0*	0.62 \pm 0.8
WOMEN	-8.8 \pm 0.7	-1.25 \pm 0.9	9.22 \pm 1.4	0.95 \pm 0.6
MEN	-8.6 \pm 2.0	-0.09 \pm 0.4	9.97 \pm 1.19	0.94 \pm 0.6

*significant changes in each group; *different from the respective adult group ($p < 0.05$).

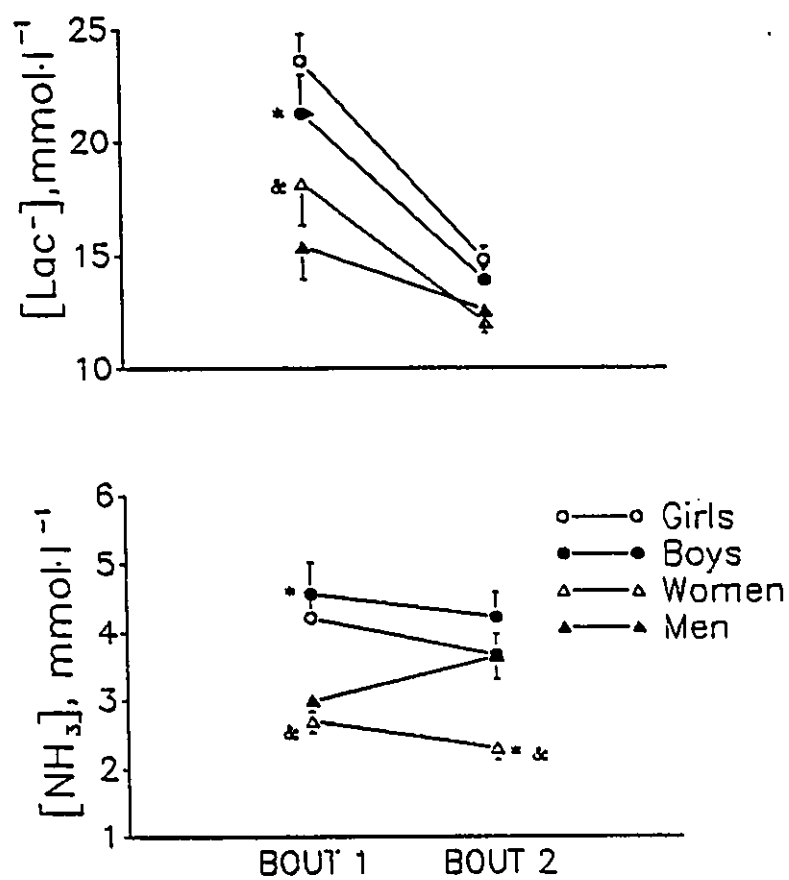


FIGURE 3.1. Sweat Lactate ([Lac⁻]) and Ammonia ([NH₃]) Concentrations.

*different from men, &different from girls (within bouts) ($p < 0.05$).

From bout 1 to bout 2, [Lac⁻] decreased in all groups ($p < 0.05$). Mean \pm SEM.

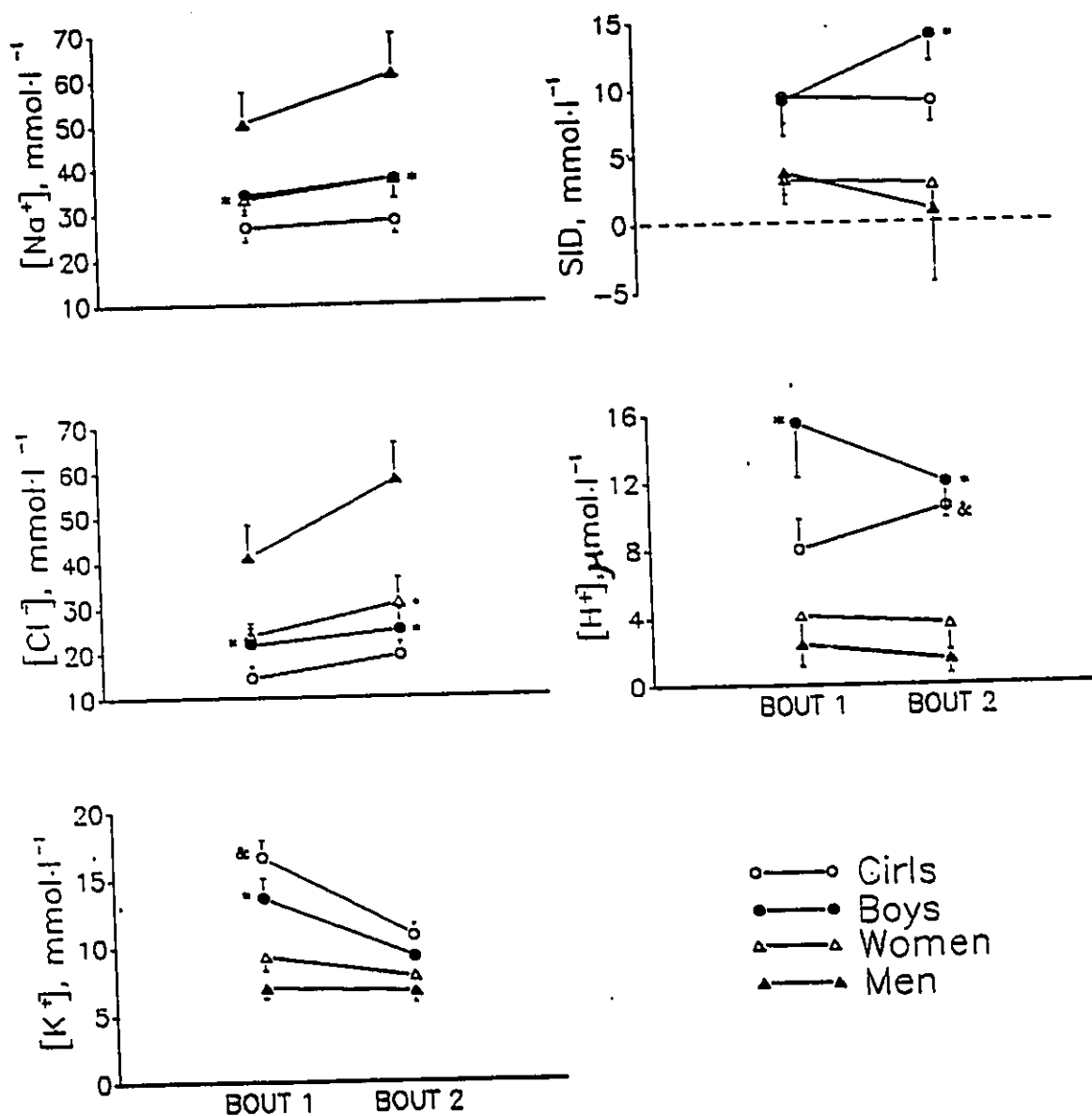


FIGURE 3.2. Sweat [Na⁺], [Cl⁻], [K⁺], Strong Ion Difference ([SID] and [H⁺].

*different from men, **different from women (within bouts) ($p < 0.05$). From bout 1 to bout 2, [Cl⁻] increased in men and [K⁺] decreased in the children ($p < 0.05$). Mean \pm SEM.

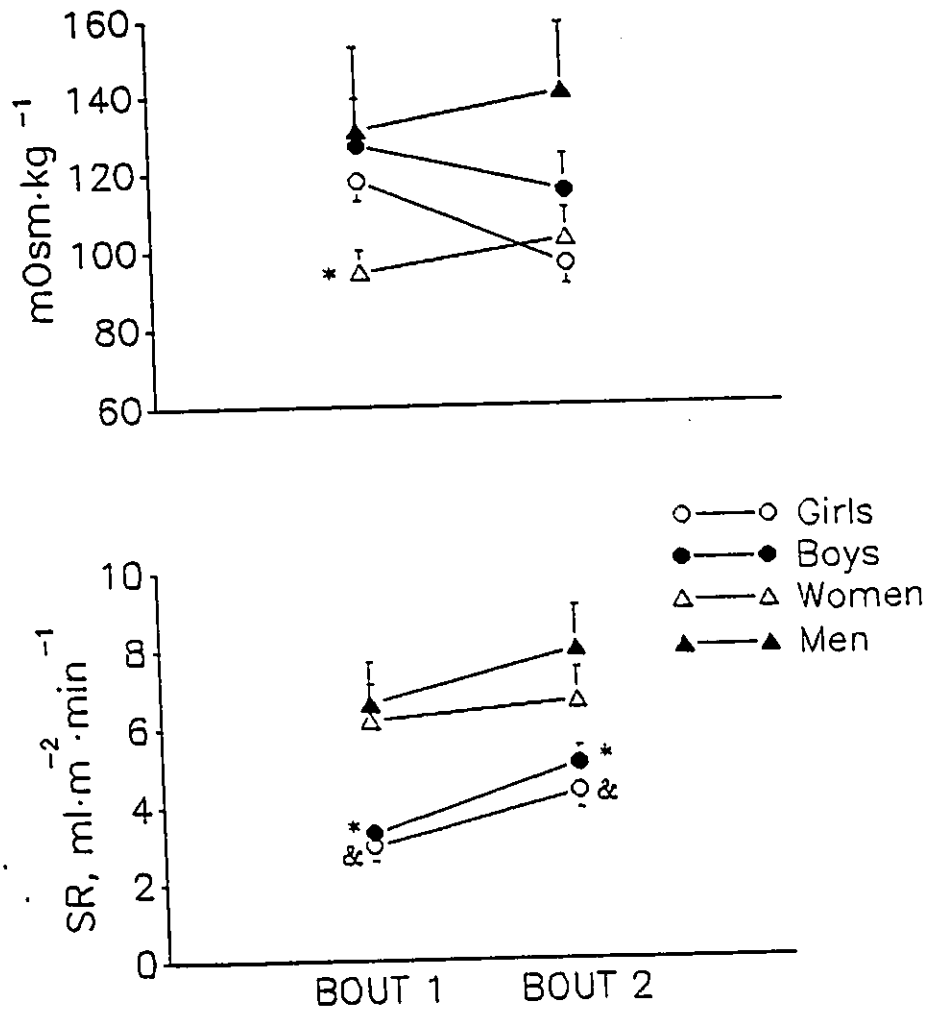


FIGURE 3.3. Sweat Osmolality and Sweating Rate (SR).

*different from men, &different from women (within bouts) ($p < 0.05$).
 From bout 1 to bout 2, osmolality decreased in the girls and boys.
 ($p < 0.05$). Mean \pm SEM.

CHAPTER 4

MILD HYPOHYDRATION DURING EXERCISE IN CHILDREN: EFFECT ON THIRST, DRINK PREFERENCES, AND REHYDRATION

(Accepted for publication in *Int. J. Sports Nutr.*)

4.1. Introduction

The benefits of adequate fluid ingestion during prolonged physical activities in the heat have long been recognized. The maintenance of a well-hydrated state can prevent an excessive increase in core temperature (Gaebelein and Senay, 1980; Morimoto, 1990), and a decrease of the working capacity (Sawka, 1992; Sawka and Pandolf, 1990). However, even when water is offered *ad libitum*, children (Bar-Or et al., 1980; 1992), like adults (Greenleaf and Sargent, 1965), dehydrate progressively. Whether such dehydration is due to an "impairment" in thirst perception facilitated by ingestion of water, or because of its specific taste is unknown. The extent to which exercise and exercise-induced dehydration affect thirst perception and drink preferences has not been reported for children. Extrapolation of adult-related data may be a wrong approach because the effect of hypohydration on thirst and drink preferences may change with age (Desor and Beauchamp, 1986; Desor et al., 1975).

Drink desirability is an important factor which influences its volume intake

(Boulze et. al, 1983; Hubbard et al., 1990; Szlyk et al. 1989a). A beverage can be perfectly balanced in its calorie and electrolyte requirements but it may have little benefit if the child does not like it. Thus, successful hydration during prolonged exercise may be more likely to be achieved with a drink that tastes good to the child.

It is important to fully rehydrate after exercise-induced dehydration for better recovery of heart rate and core temperature (Costill and Sparks, 1973; Gisolfi and Duchman, 1992). After exercise-induced dehydration of about 2.5%, adults spontaneously replenish most of their fluid losses within 1 hour (Nose et al., 1988a). However, full rehydration is not achieved even after 3 hours; and drinking behavior seems to be related to the composition of the drinks. Even when fixed volumes of fluids are given to dehydrated men, rehydration is faster with Na⁺ containing drinks as compared to water (Costill and Sparks, 1973; Nielsen et al., 1986). How children spontaneously rehydrate following hypohydration and to what extent the replenished volume is related to the drink preference are unexplored issues. Therefore, the main purposes of this study were to determine: 1) changes in the magnitude of thirst perception and taste-related variables of four different drinks during progressive dehydration, elicited by exercise in a hot climate, 2) children's drinking pattern and thirst perception, when given four different drinks during recovery from the hypohydrated state. A secondary purpose of this study was to examine whether children responded consistently (and logically) to a questionnaire regarding drink characteristics, which was evaluated in a preliminary session.

4.2. Methods

Subjects

Twenty-nine 9- to 13-year-old children (17 ♀ and 12 ♂) volunteered to participate in this study which had been approved by the McMaster Faculty of Health Sciences Ethics Committee. Five of them (3 ♀ and 2 ♂) dropped out after the initial visit for personal reasons (lack of time or interest). Thus, 24 subjects (14 ♀ and 10 ♂) completed all five visits. Boys and girls were similar in their physical characteristics. Their weight (mean±SD) was 38.4 ± 7.1 kg, height was 146.5 ± 7.7 cm, and peak aerobic power (peak $\dot{V}O_2$) was 42.0 ± 6.0 ml.kg⁻¹.min⁻¹ (♂ = 43.0 ± 5.16 , and ♀ = 41.6 ± 7.1). Adiposity, as assessed by the sum of four skinfolds, was 30.4 ± 11.7 mm (range 17 to 56 mm), suggesting that subjects were non-obese. They were healthy and physically active but not competitive athletes. Testing took place from mid-December to mid-April; thus, children were assumed to be unacclimatized to the heat.

Each child came to the laboratory for one preliminary and four experimental sessions, one week apart.

Preliminary Session

Prior to this session, the investigator informed a parent by phone that the child would be answering questions while tasting 4 drinks with distinct flavors: orange, grape, apple and water (Table 4.1). This was the only time that the nature of the flavors was disclosed. The commercial names of the drinks were not revealed during the recruitment,

nor at any time throughout the study. When the child came to the laboratory, the procedures of all sessions were explained to both the child and a parent, and then a written consent (Appendix 8.1.2) was obtained from the parent. Children were tested after school hours and they were asked to refrain from eating and drinking at least one hour prior to the beginning of the session. Health status was assessed by a medical history. Adiposity level was estimated by the sum of four skinfolds: triceps, biceps, subscapular and suprailiac, using a Harpenden caliper.

In addition to the assessment of subjects's physical characteristics and $\dot{V}O_2$, we evaluated the consistency in answering the sensory questionnaire at rest and in thermoneutral room. This was also intended to familiarize the subjects with the sensory questionnaire to be used in the experimental sessions.

Tasting Protocol. The child was first instructed, through a video tape, on how to fill out the sensory questionnaire. The tape explained, in detail, how to use analog line scales and the category box scales. Additional explanations were given by the investigator, whenever needed.

In order to examine whether children's perceptual responses were affected by the color of the drinks, each drink was served in two ways. First, they were presented in covered opaque cups, so that the child was unaware of their color. Subsequently, they were served in transparent cups. Both types of cups were odourless and tasteless. For both situations, the sequencing of the four drinks followed a Latin-Square order.

In this session, each child was tested alone, sitting comfortably in a thermoneutral

room. Drinks were served at the same temperature (about 8°C) and volume (25 ml), using a separate cup and questionnaire for each trial. Between samples, there was a 90-s interval and the child had a sip of water to clear the palate. Children did not see any commercial containers of the drinks during the study.

Sensory Questionnaire at rest. (Appendix 8.2.1) The perception of thirst and drink taste qualities such as intensity, sweetness, saltiness and sourness were assessed by visual analog scales. These scales were separate horizontal lines with anchor points at the ends and in the middle. The end points were labelled for example, as "not thirsty" to "very thirsty". The child was asked to mark responses to: "how thirsty you are"; "how strong in taste the drink is", "how sweet the drink is", "how salty the drink is", and "how sour the drink is".

Appropriateness of taste intensity, sweetness and sourness were also measured by 3-point "just right" box scales with the following categories: "too strong" "just right" and "not strong enough". The written instruction was to check the box which says how the child felt about the drink's level of "taste", "sourness" and "sweetness".

Preference of the drinks was measured on a 9-point horizontal box scale ranging from "super bad" (1 point) to "super good" (9 points). The questions included: "how much do you like the taste?" and "how much do you like the drink overall?". When drinks were served in transparent cups, a similar 9-point horizontal box scale was included with the question: "how much do you like the color?".

After the evaluation of the questionnaire, peak $\dot{V}O_2$ was assessed by a

progressive, continuous cycling test (The McMaster protocol- Bar-Or, 1980, pp 320-321) in a thermoneutral laboratory, using open circuit spirometry (SensorMedics Horizon metabolic chart). Peak $\dot{V}O_2$ was determined and used to standardize the exercise intensity in the experimental sessions.

Experimental Sessions

Procedures in these four sessions were identical, but in each of them the child was given a different drink (one of the 4 drinks tested in the preliminary session), following a Latin-Square order. All sessions were held in the afternoon. To ensure that children came to the laboratory euhydrated, they received oral and written instructions to drink regular amounts of fluids on the day of the test, and not to exercise during the preceding 8 hours. These were confirmed through a questionnaire once the child reported to the laboratory. In addition, the child was given a glass of water (150 ml) upon arrival at the laboratory.

Before entering the chamber, the subjects emptied their bladder. For safety reasons, core temperature was monitored throughout the in-chamber period, using a rectal thermistor (YSI 400 series) inserted 8 cm beyond the anal sphincter. We also monitored heart rate (HR) using a Sport Tester PE3000 system. Exercise would terminate if at least one of the following criteria were reached: rectal temperature $> 39^\circ\text{C}$, dehydration level $> 4.5\%$ of the initial body weight, nausea, dizziness, chills or exhaustion. Subjects were dressed in shorts and sport shoes and the girls also wore a bikini top.

Chamber conditions were 35°C, 18-20% relative humidity. The in-chamber protocol started with rest (15 min) followed by three 15-min cycling bouts (50% predetermined peak $\dot{V}O_2$), with 15-minute rest periods in between. Once the child entered the chamber, baseline values, including taste and thirst perceptions, were assessed. To calculate the degree of dehydration after each bout, the child was weighed on an Electro Mott scale model LC2424 (± 20 g accuracy). After that, thirst perception was assessed (analog scale) and then a sample (25 ml) of the tested drink was served at 8-10°C in a transparent cup, accompanied by the sensory questionnaire, similar to that used in the preliminary session (Appendix 8.2.2).

The child sat comfortably for the remainder of the rest period. To induce dehydration, no additional fluid was given during the dehydration stage, except for the tested drink (25 ml for each "sensory questionnaire"). Total time of the dehydration stage was 90 min.

The 30-min rehydration stage started after the third exercise bout. Children remained in the chamber and were allowed to drink as desired. The respective chilled drink was continuously provided and fluid volume was monitored (graded glasses) without the child's knowledge. The importance of drinking was not emphasized nor was the child reminded to drink. Body weight was determined at 15 and 30 min of the rehydration period. Throughout this phase, subjects read or did their school homework. Just before leaving the chamber, the child's thirst perception was evaluated. Then, she/he voided and the total urine output was measured. This volume was taken into account in

the calculation of the degree of rehydration.

To assess the degree of gradual hypohydration and the subsequent rehydration, changes of body weight were calculated from the baseline value. Difference in clothing weight was considered negligible (6 g). Sweat volume was calculated from the changes in body weight, corrected for the fluid intake, and urine volume.

Statistical Analysis

Data for each sensory variable were analyzed using a two-way ANOVA (subject and beverage). The effect of dehydration and rehydration on the sensory and physiological variables was analyzed by a two-way ANOVA with repeated measures (time). When the analysis produced a significant F ratio, a Duncan multiple range post-hoc procedure was used. This analysis was conducted using SPSS-X version 3.1. Correlations were determined by the Pearson product-moment method. To evaluate the effect of the type of drink, and the way of serving (clear vs opaque cup), a General Linear Model analysis (Minitab 7.1) was used. Differences were considered significant at $p < 0.05$. Results are presented as mean \pm SEM.

4.3. Results

Boys and girls presented similar physiologic and behaviour results; they were therefore pooled into a common group.

Evaluation of Sensory Questionnaire in the Preliminary Session. When children were resting in a thermoneutral room, results were similar whether or not the subjects saw the color of the drinks. This was despite of the fact that the color of the grape drink was preferable to that of water and apple drink (Figure 4.1). For a given sensory assessment, a similar pattern of responses was observed between the two types of questions: the 3-point category scales (left-sided graphs) and the analog scales (right-sided graphs) (Figure 4.2). The intensity of thirst perception remained constant during the resting session (score about 40), without differences among drinks.

Taste and Thirst Perception during Progressive Dehydration. All children tolerated the exercise in the heat well and completed each session. The increase in rectal temperature from the start of the session through the end of the dehydration session was $0.51 \pm 0.03^{\circ}\text{C}$ (mean \pm SEM), with no differences among drinks (0.48 ± 0.06 for apple, 0.50 ± 0.07 for grape, 0.51 ± 0.07 for orange and 0.52 ± 0.08 for water). Likewise, mean increase in heart rate was 56 ± 3 bpm (59 ± 4.7 for apple, 51 ± 4.9 for grape, 54 ± 4.2 for orange and 58 ± 4.3 for water).

The degree of hypohydration after each exercise bout (Figure 4.3, top) was similar among the four drinks and within each subject. By the end of the last bout, the average weight loss was 260 ± 12.5 g (263 ± 12.6 for orange, 261 ± 11.5 for water, 260 ± 13.0 for apple and 250 ± 12.3 for grape). This was equivalent to hypohydration of $0.70\% \pm 0.03$ of initial body weight. As the subjects gradually dehydrated, there was a corresponding increase in their thirst perception (Figure 4.3, bottom). The degree of

thirst increased significantly after each exercise bout, without any difference among the drinks at any given period of the dehydration phase.

As seen in Figure 4.4, while the preference ratings of orange, apple and water drinks gradually increased during dehydration, it remained more constant, but higher for grape drink. At the end of the session, orange was the drink that yielded the highest increase in preference ratings from the baseline values (21%). This increase was significantly higher than those for water (15%) and apple (9%). The relationship between a child's degree of liking a drink at rest (euhydrated) and at peak dehydration is shown in Figure 4.5. The Figure suggests a very good to fair agreement for apple, grape, and water, but not for orange. The latter was actually the drink for which desirability increased the most between rest (euhydration) and peak dehydration. A similar pattern was found in the other preference question ("How much do you like this drink overall?").

No significant changes with time occurred in perception of taste intensity of saltiness, sweetness, or sourness during the exercise-induced dehydration (Figure 4.6). Again, a similar response pattern was observed between measures of perceived intensity and the relative-to-ideal (just-right) scales. This is in agreement with the observations obtained during rest in a thermoneutral environment.

Drinking Pattern during Voluntary Rehydration. By the end of 30-min voluntary rehydration, volume intake in 68 out of the 96 sessions (71%) was enough not only to compensate for the fluid losses, but to overshoot by at least 0.1 kg of the initial body weight. Although overhydration occurred at a high frequency with all drinks

(apple=71%, grape=79%, orange=71% water=63%), average increase in body weight was higher with grape and orange than for water and apple ($p < 0.05$) (Figure 4.3, top). In 17 sessions (apple=5, grape=2, orange=4, water=6), subjects drank enough just to recover from their water loss. Two subjects consistently did not drink (or drank $< 100\text{ml}$) during any of the voluntary rehydration sessions. In other 3 sessions (grape, orange and water), 2 subjects drank insufficiently ($< 100\text{ ml}$) to match their body fluid losses. Thirst perception decreased to a similar degree with all drinks. Although the volume intake of grape and orange was greater than that of water and apple during the rehydration phase, there were no inter-session differences in the sweat or urine output (Table 4.2).

4.4. Discussion

This was the first published study in children to evaluate the effect of gradual dehydration, induced by exercise in the heat, on the intensity of thirst perception, the degree of liking of four drinks, and the subsequent voluntary rehydration. Our main findings were that children became more thirsty as they dehydrated, they preferred the grape drink, hypohydration was usually accompanied by an increase in the degree of drink liking (especially for an orange-flavored drink), and most children voluntarily overhydrated with all drinks during the recovery.

Evaluation of the Sensory Questionnaire. Consistency in response to the various questions (Figures 4.1, 4.2 and 4.6) suggests that 9- to 13-year-old girls and boys can discriminate consistently among taste intensities, sweetness, and sourness of drinks. Similar score patterns were observed for a given drink on both relative-to-ideal (just-right) and intensity (analog) scales. In addition, subjects rated consistently the sensory properties of the drinks throughout the exercise session while systematically reporting changes in the degree to which the drinks were liked. This is in agreement with the observation that children can reliably report exercise intensity (using the Borg scale) during exercise (Bar-Or and Ward, 1989). Steiner (1979) and Ganchrow et al. (1983) have demonstrated that children respond in characteristic and consistent ways to tastants varying in hedonic quality. Still, the distinct ingredients and flavors of the drinks in the current study could have "masked" or altered the perceived intensity of sweetness, sourness or saltiness (Kroeze, 1979). Further studies should clarify this question by serving children drinks that differ only in their sodium and/or carbohydrate content.

Overall, there was preference for a grape-flavored drink. The interpretation of this finding however cannot be based on flavor alone, because the four drinks differed also in their electrolytes and carbohydrate contents, and in osmolality (Table 4.1). One may argue that the preference for the grape drink was due to its higher carbohydrate content since children seem to prefer sweet tastes (Birch, 1987; Desor and Beauchamp, 1986). However, the apple and grape drinks tested in our study not only had similar carbohydrate content, but they were perceived as equally sweet (Figs. 3 and 6).

Effect of Dehydration on Thirst and Drink Desirability. Independent of the tested drink, thirst intensity increased as body weight decreased. Already after the first bout, when body weight loss was ~ 100 g, subjects showed an increase in the intensity of thirst perception (Figure 4.3). Such an increase in thirst intensity despite of an only minimal decrease in body weight in children is not commonly observed in adults working in the heat (Hubbard et al., 1990) even though, during daily activities, there seems to be an increase in thirst perception in men before plasma volume starts to decline. This was shown by Phillips et al. (1983) who assessed thirst perception concomitantly with blood samples in healthy men during normal working hours. Body fluid variables such as plasma volume, plasma osmolality and electrolytes remained unchanged while thirst intensity increased. In this case, as in our study, thirst may have been an anticipatory warning for further fluid losses. However, full fluid replenishment is probably postponed until recovery.

While perception of taste quality remained unchanged for all drinks during dehydration, the preferences for the orange, apple and water drinks increased. The lack of increase in the liking of the grape drink might be due to a ceiling effect.

Voluntary Rehydration. Our study showed that most children not only fully recovered from dehydration, but overshot their initial body weight (up to 1%) with all drinks. In contrast, young and old men restored less than 50% of their body fluid losses within 30 min of voluntary rehydration following a dehydration of 1.5-2.5% induced by resting (Miescher and Fortney, 1989) or exercise (Nose et al., 1988) in a hot environment.

Whether such a difference is due to the higher degree of dehydration in the adults or to age itself is still to be determined. Why children overhydrated following exercise that induced hypohydration is unclear. The fact that hypohydration was mild and forced, instead of voluntary, may have affected the drinking behavior of these children. However, we are presently observing (unpublished data) that overhydration during recovery may occur irrespective of whether the children are maintained euhydrated or drink *ad libitum* during exercise.

Although grape and orange in this study were the preferred drinks during dehydration, individual variability in volume intake was observed. Slzyk et al. (1989a, 1989b) noticed a great individual variability in volume intake among men who exercised for 6 hours in the heat. They classified subjects as either reluctant or avid drinkers of water, as measured by their ability to stay within 2% of their initial body weight. Out of 33 men exercising in the heat, 13 were reluctant drinkers (1989b). In the other study (1989a), 5 out of 11 men were reluctant drinkers but they increased the volume intake when a flavor was added to water. Our study suggests that children also follow this "classification" pattern, although lack of full rehydration was seen in only 4 (17%) of them.

In the present study, the volume of grape and orange drinks consumed during rehydration was significantly higher than water and apple, suggesting an agreement with the subjects' preference ratings. When no fluid was consumed, liking rates tended to be below 5 ("maybe I like maybe I don't"). However, overall correlation of the degree of

liking at peak dehydration with total volume intake was not strong ($r = 0.35$), with some variation among drinks (r values for apple = 0.57, grape = 0.33, orange = 0.26, and water = 0.35).

In conclusion, mild hypohydration in children who exercise in the heat induced an increase in thirst and in the degree of desirability of drinks. During voluntary rehydration, most children drank considerably to overshoot their initial body weight with all drinks. In line with the subjects' drink preferences, they drank larger volumes of the orange and grape drinks, as compared to apple and water. Drinking encouragement should be directed to those children who refrain from drinking during and following prolonged exercise. In addition to their applicability for healthy, active children, these results could also be useful in formulating oral fluid replenishment in common clinical conditions that lead to dehydration in children, such as gastroenteritis.

TABLE 4.1. Composition and Osmolality of the Four Drinks.

FLAVOR	CARBOHYDRATE g·dl ⁻¹	SODIUM mmol·l ⁻¹	POTASSIUM mmol·l ⁻¹	OSMOLALITY mOsm·l ⁻¹
GRAPE	13.9	< 5	6.7	728
APPLE	11.2	< 5	28	726
ORANGE	6.5	20	3.0	280
WATER	0.0	0.01	0.02	15

CHO= Carbohydrate

TABLE 4.2. Fluid Intake and Sweat Volume during the Rehydration Phase, and Urine Volume for the Entire Session. Values are in ml. Mean \pm SEM.

DRINKS	INTAKE	SWEAT	URINE
GRAPE	587 \pm 56.5*	67.3 \pm 7.6	45.0 \pm 9.1
APPLE	452 \pm 49.1	59.9 \pm 7.7	37.1 \pm 9.7
ORANGE	533 \pm 68.4*	70.1 \pm 10.3	50.8 \pm 12.1
WATER	453 \pm 59.5	72.0 \pm 6.1	49.2 \pm 12.3

* Significantly higher than apple and water ($p < 0.05$)

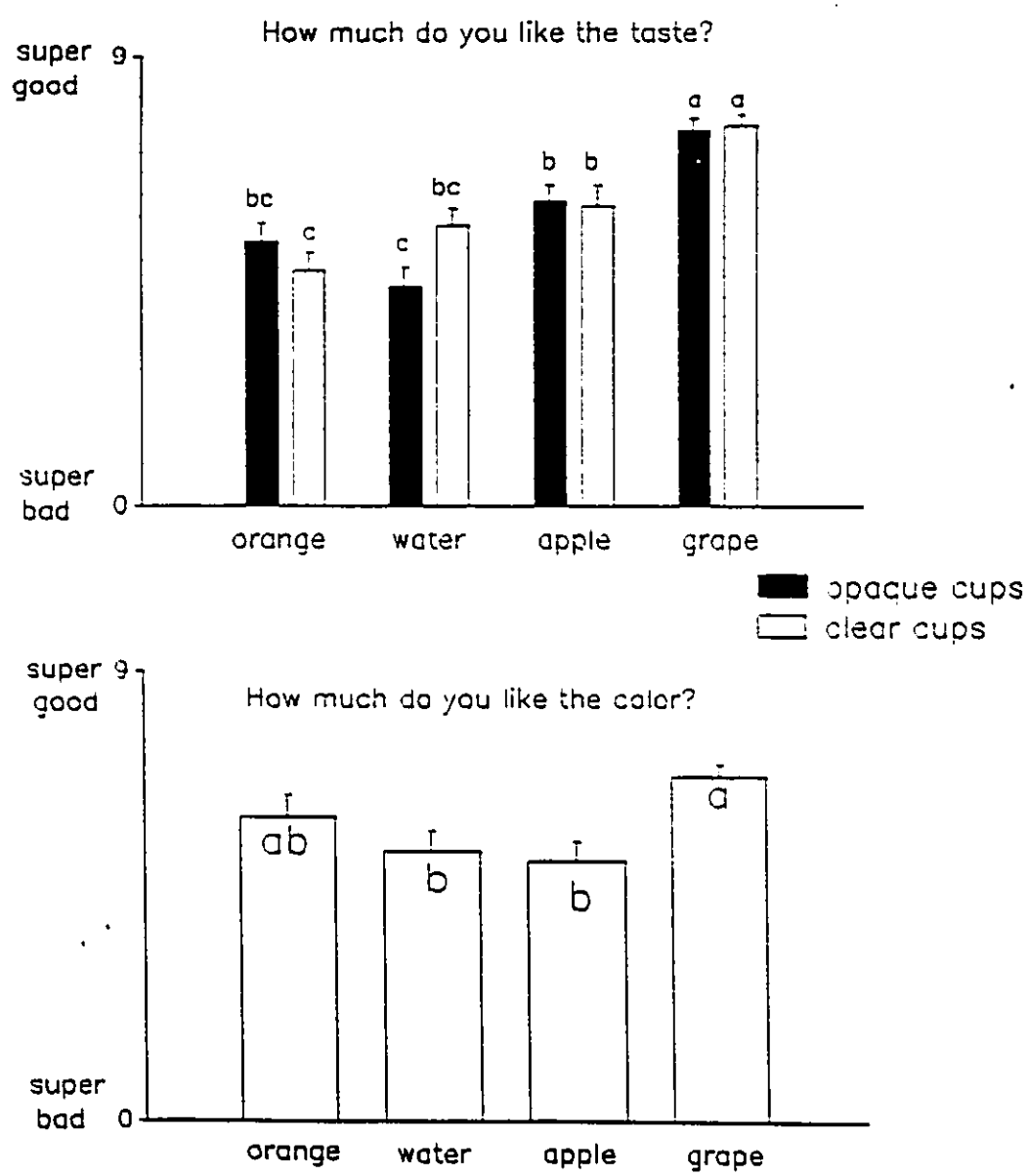


FIGURE 4.1. Drink and Color Preferences at Rest.

Values not sharing a common letter are different from each other ($p < 0.05$). (Mean \pm SEM).

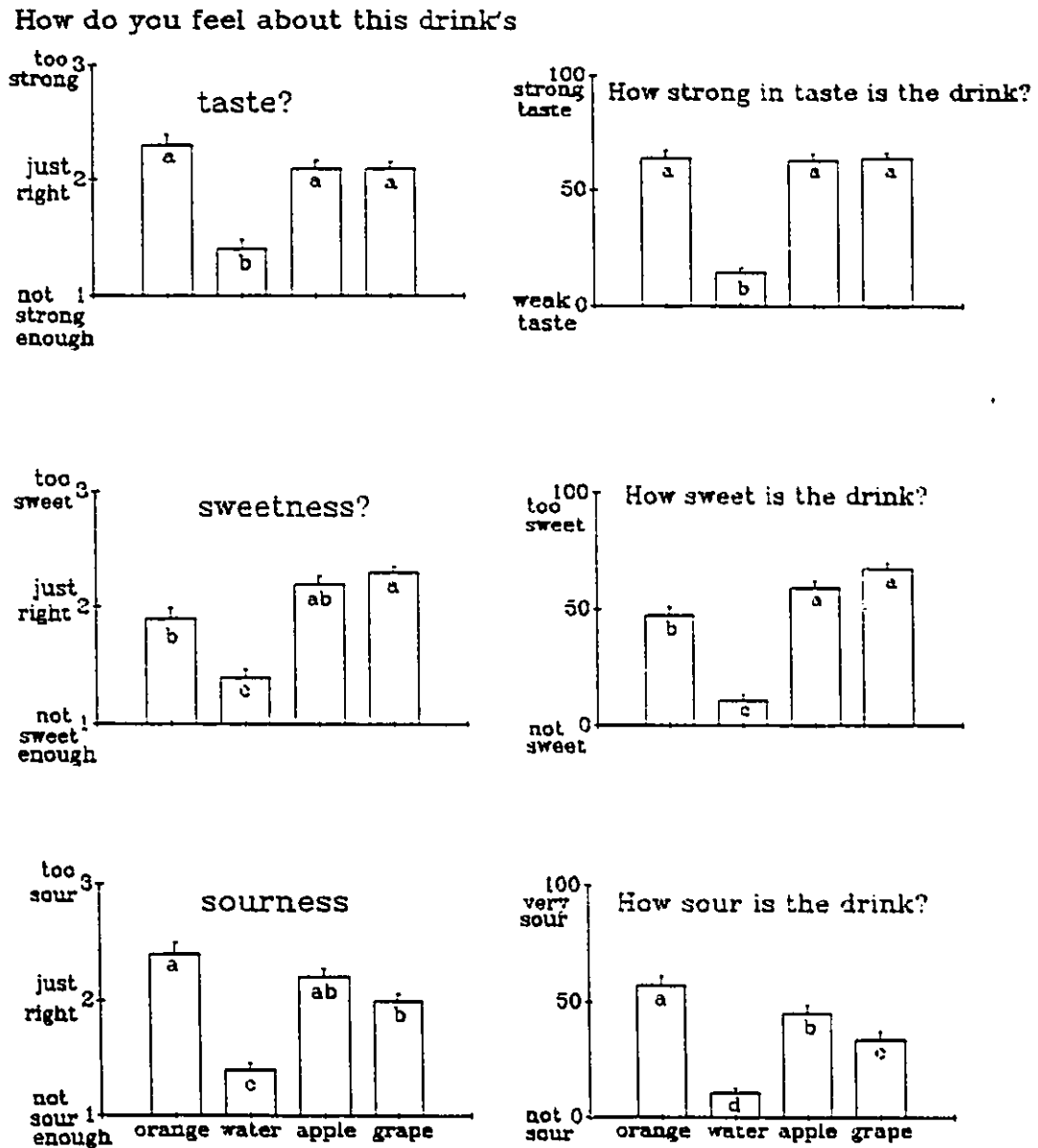


FIGURE 4.2. Taste Ratings During the Rest Session.

Values not sharing a common letter are different from each other ($p < 0.05$). Graphs on the left refer to the 3-point box scales questions, while those on the right, for visual analog scales.

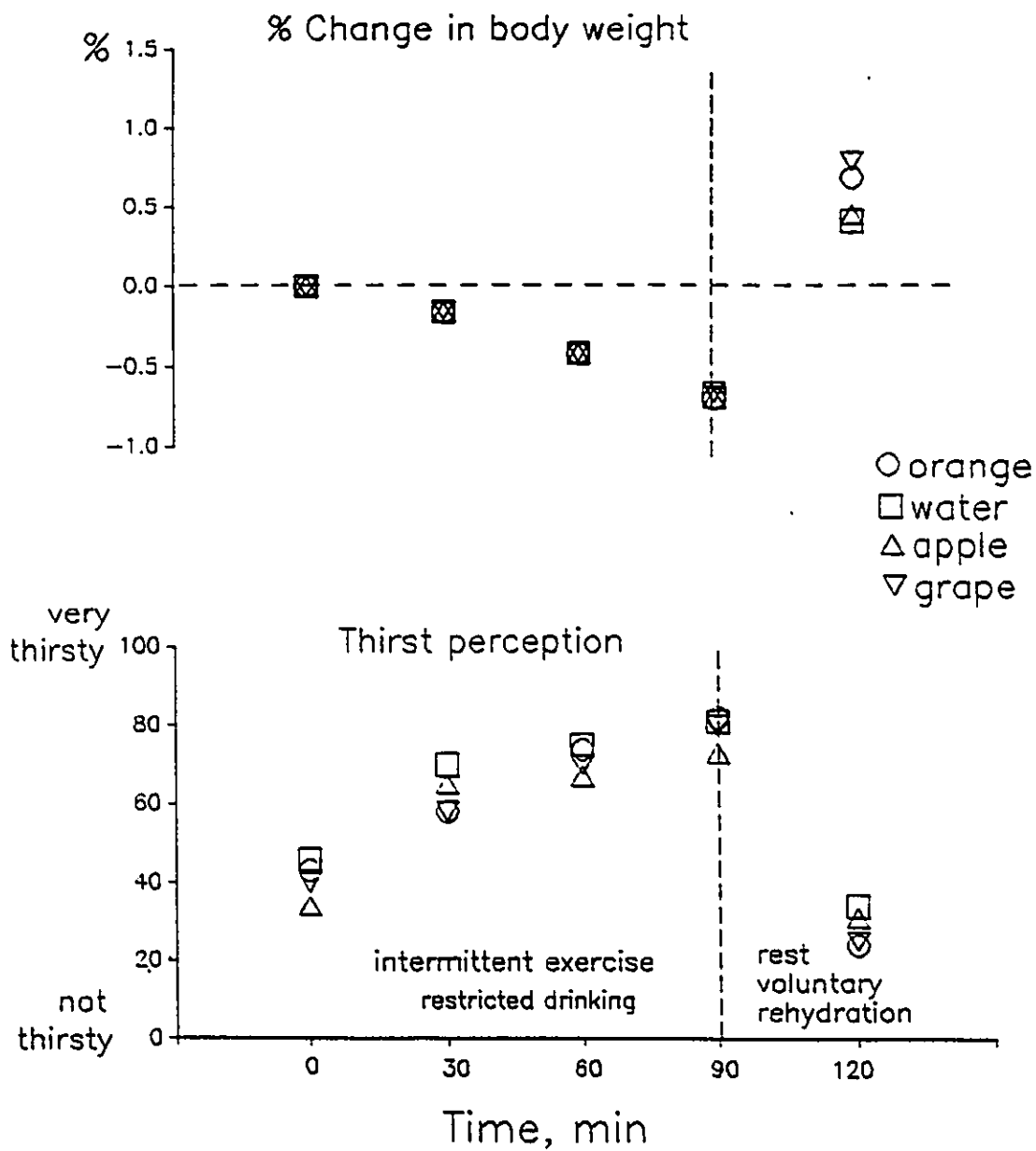


FIGURE 4.3. Changes in Body Weight and Thirst Perception During Dehydration and Rehydration for Each Drink. Means.

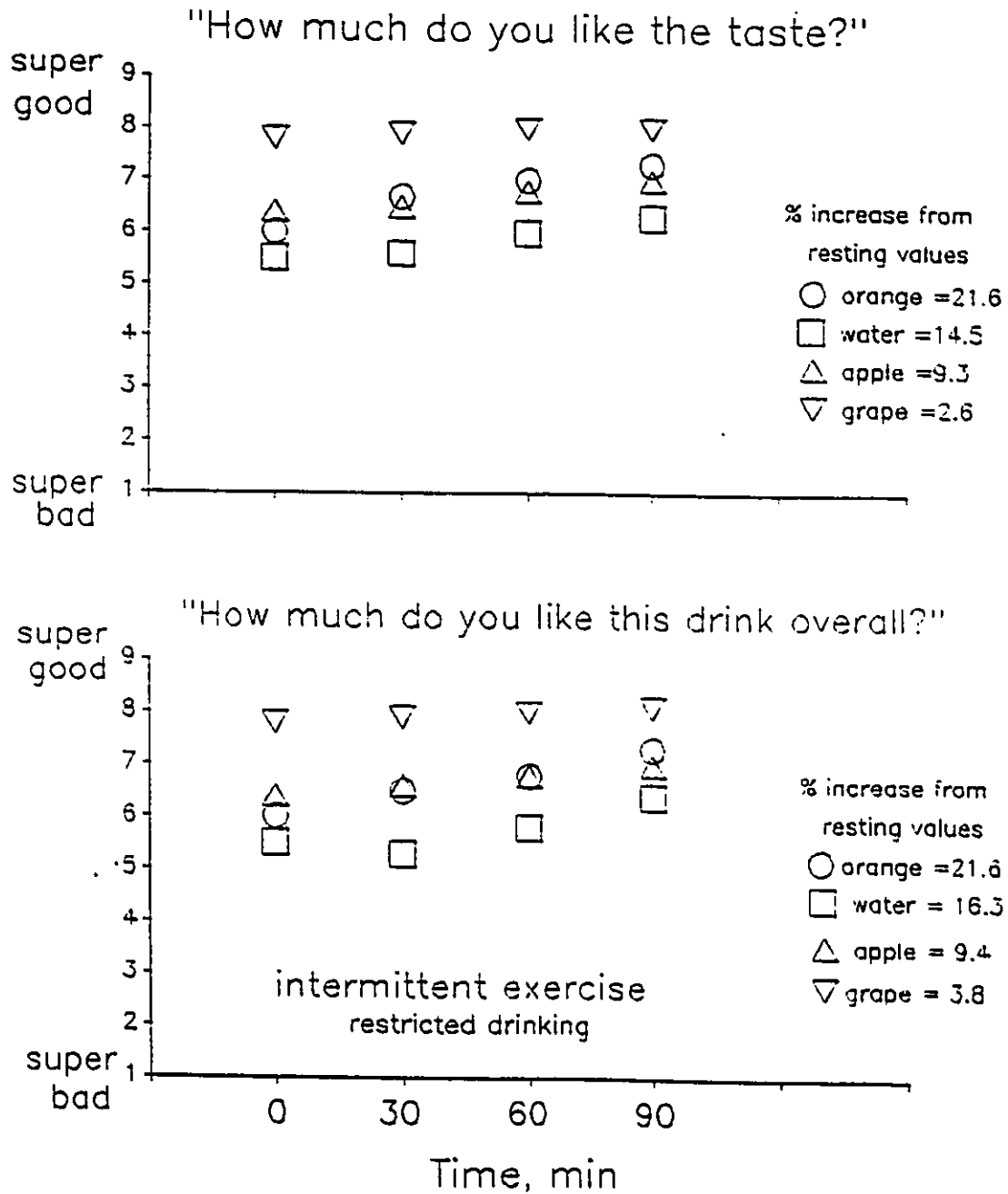
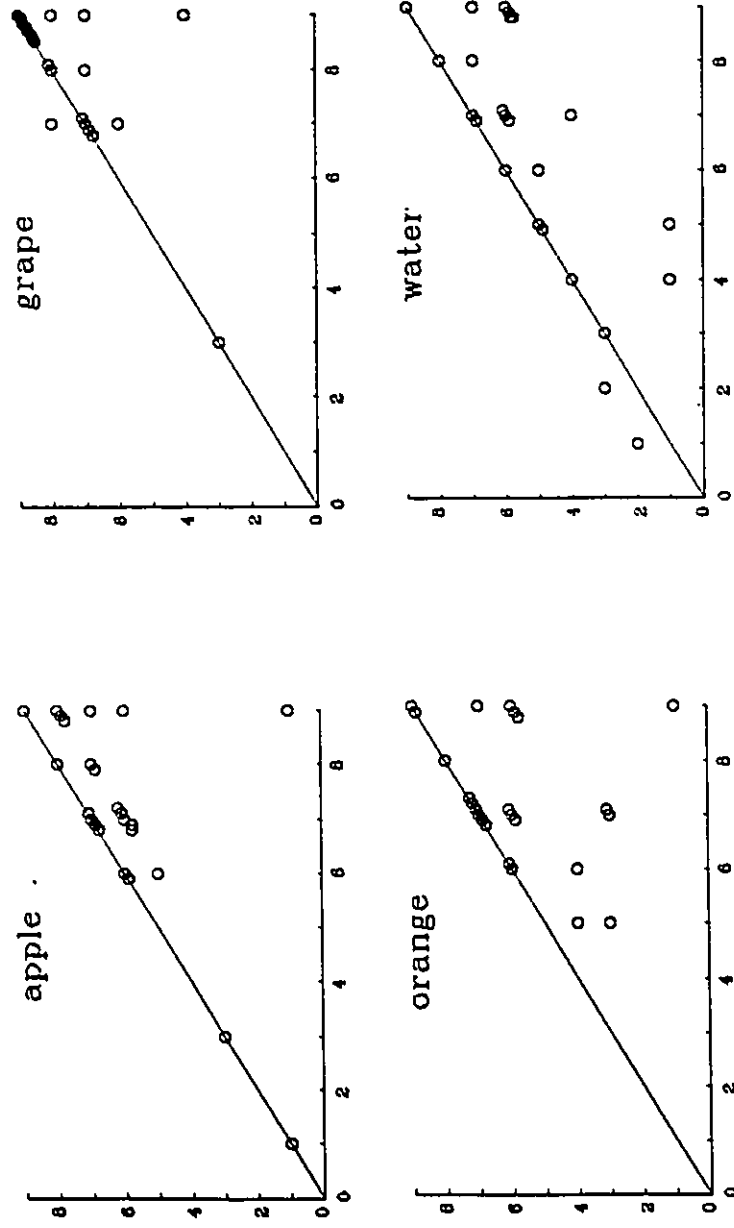


FIGURE 4.4. Taste Preferences during the Dehydration Session among the Different Drinks. Means.

How much do you like the taste?

preference at rest (euhydrated)



preference at peak dehydration

FIGURE 4.5. Individual Values Relating the Preference Scores Given at the Start of the Exercise Session and at Peak Dehydration.

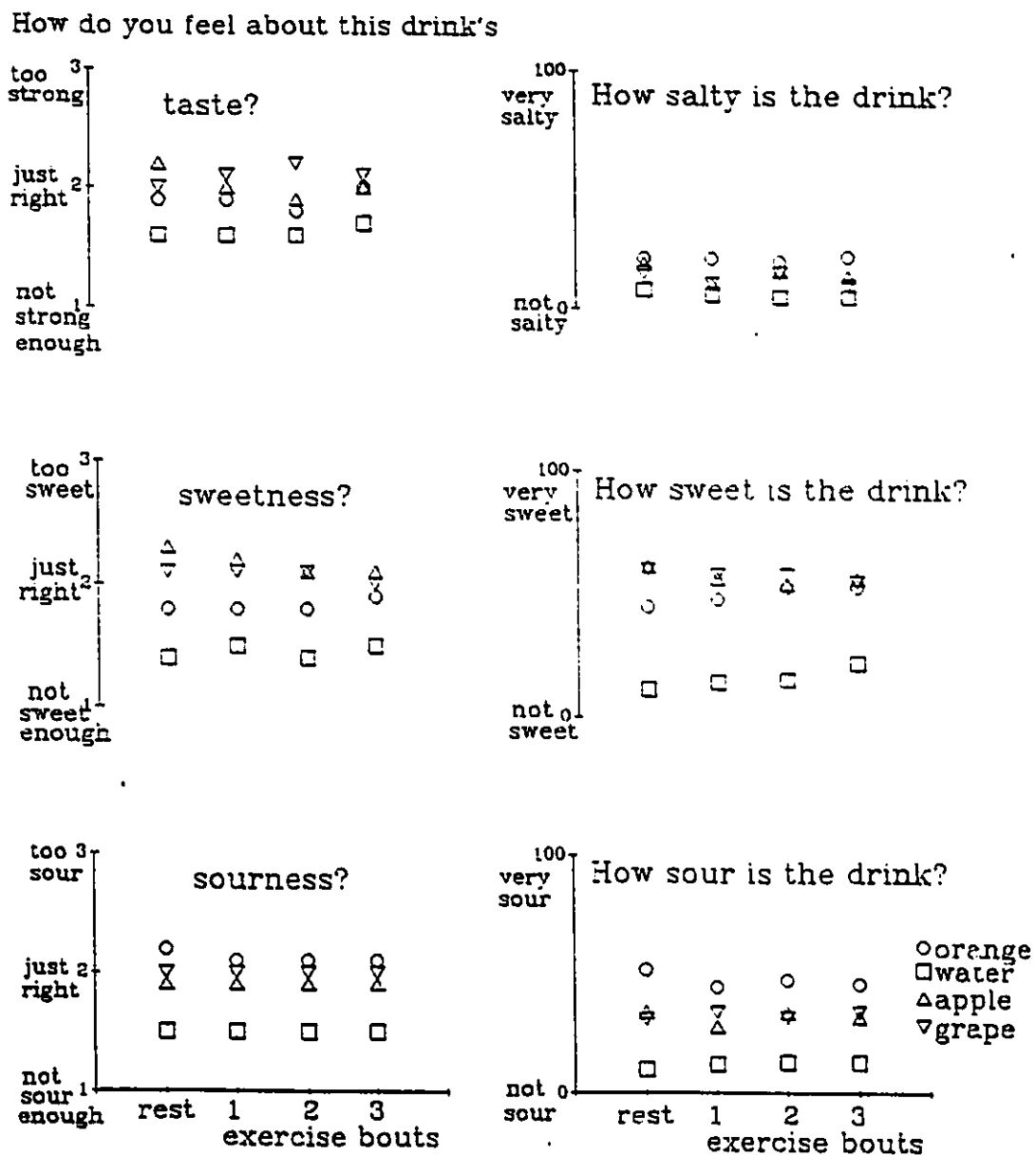


FIGURE 4.6. Taste Ratings during the Dehydration Session. Means.

4.5. Addendum to Chapter 4

4.5.1. Drink Selection After the $\dot{V}O_2$ max Test

In addition to measurements summarized in this chapter, we monitored which drinks children selected after a $\dot{V}O_2$ max test and whether such a preference agrees with the ratings given at rest and after the exercise-induced dehydration. Once subjects completed the $\dot{V}O_2$ max test, they were asked to select one of the four flavored drinks (grape, apple, orange and water) which were presented on a counter in clear plastic cups and in equal volumes.

Forty-eight percent of the subjects selected the grape drink; 28% water, 12% apple, and 12% orange. The higher preference for grape was statistically significant ($\chi^2=8.8$, $p<0.05$). To examine whether such preferences were consistent with the scores given at rest or after the exercise-induced dehydration, mean values were calculated according to the drink chosen after $\dot{V}O_2$ max. Results are summarized in Table 4.3. When grape was the chosen drink after the $\dot{V}O_2$ max, its mean scores were consistently higher than for the other drinks, as also found at rest and dehydration. A similar pattern was observed for orange and apple, but not for water. Even though water was the second most chosen drink after the $\dot{V}O_2$ max test, its scores in the other two conditions were low, especially for the first question at the hypohydrated state.

TABLE 4.3. Mean Preference Scores (\pm SEM) during Euhydrated States (Thermonutral and Heat), and at End of the Exercise-Induced Hypohydration (Heat). Subjects are divided according to their chosen drink following the $\dot{V}O_{2\max}$ test.

CHOSEN DRINK AFTER $\dot{V}O_{2\max}$	HOW MUCH DO YOU LIKE THE TASTE?			HOW MUCH DO YOU LIKE THE DRINK OVERALL?			
	GRAPE	APPLE	ORANGE	GRAPE	APPLE	ORANGE	WATER
GRAPE (n=11)							
euhydrated(22°C)	<u>8.2±0.3</u>	6.5±0.6	4.6±0.6	<u>8.4±0.2</u>	6.1±0.6	4.8±0.6	4.6±0.6
euhydrated(35°C)	<u>7.9±0.3</u>	6.6±0.3	5.8±0.4	<u>8.0±0.3</u>	6.3±0.4	5.9±0.5	4.4±0.5
hypohydrated(35°C)	<u>8.4±0.3</u>	7.0±0.4	7.1±0.5	<u>8.5±0.2</u>	7.0±0.5	7.4±0.4	5.5±0.7
APPLE (n=3)							
euhydrated(22°C)	8.7±0.3	8.0±0.8	5.0±1.2	8.0±0.1	7.0±0.9	5.7±0.7	4.3±1.7
euhydrated(35°C)	8.0±0.4	<u>7.6±0.5</u>	5.3±1.1	6.0±1.2	<u>7.3±0.2</u>	4.0±0.8	6.0±0.8
hypohydrated(35°C)	7.3±0.3	<u>8.0±0.5</u>	6.3±0.5	8.0±0.5	<u>7.0±0.9</u>	6.3±0.5	7.7±0.5
ORANGE (n=3)							
euhydrated(22°C)	4.0±2.1	5.0±1.4	<u>6.5±0.4</u>	4.0±2.1	5.5±1.8	<u>6.0±0.1</u>	6.0±0.1
euhydrated(35°C)	5.5±1.7	7.0±0.1	<u>7.7±1.0</u>	5.5±1.7	6.5±0.3	<u>7.5±1.0</u>	5.5±0.3
hypohydrated(35°C)	7.0±0.1	5.5±1.8	<u>8.0±0.7</u>	8.0±0.7	7.0±0.1	<u>5.5±1.8</u>	5.5±0.4
WATER (n=7)							
euhydrated(22°C)	6.7±0.4	6.3±0.7	4.0±0.8	6.5±0.5	6.2±0.8	4.2±0.7	<u>5.5±0.7</u>
euhydrated(35°C)	7.3±0.3	6.0±0.9	5.5±0.7	7.6±0.4	5.8±0.9	5.5±0.6	<u>6.0±0.6</u>
hypohydrated(35°C)	8.2±0.4	7.8±0.4	7.2±0.4	8.0±0.4	7.0±0.4	8.0±0.4	<u>7.2±1.0</u>
ALL SUBJECTS (n=24)							
euhydrated(22°C)	6.9±0.3	6.4±0.5	5.0±0.4	6.7±0.4	6.2±0.5	5.2±0.4	5.1±0.4
euhydrated(35°C)	7.2±0.3	6.8±0.4	6.1±0.4	6.8±0.3	6.4±0.4	5.7±0.4	5.5±0.4
hypohydrated(35°C)	7.8±0.3	7.1±0.3	7.2±0.2	8.1±0.3	7.0±0.2	6.8±0.3	6.5±0.4

Note: Underlined values are the scores corresponding to the drink chosen following the $\dot{V}O_{2\max}$ test.

4.5.2. Reliability of the Procedure for the Assessment of Taste Preference

The main questions of this analysis were: 1. Is the written questionnaire a reliable method to measure taste perception? 2. Are there any factors affecting the reproducibility of the answers, e.g. type of drink, its color, or the way the drink is served?

To test for the reliability of the taste questionnaire during the preliminary sessions (rest), the second drink of each order was repeated in the fifth position without the knowledge of the subject. This was done for both opaque and clear cup sequences. Two questions were analysed: "How much do you like the taste?" and, "How much do you like the drink overall?". The type of cup was not a significant factor affecting the reproducibility (mean values) of the answers for the first ($F_{1,24}=1.14$ $p=0.29$) or the second ($F_{1,23}=1.69$, $p=0.21$) question. Also, the type of drink was not a factor affecting the test re-test means in either of the questions ($F_{3,24}=1.41$ $p=0.26$ and $F_{3,23}=1.16$ $p=0.35$ for first and second question, respectively). The Table below shows the test re-test r values for all drinks combined and for each drink separately, according to the manner by which they were served. For all drinks, when served in clear cups, the reliability of the two questions was reasonably good (0.81 and 0.84). However, when the child could not see the color of the drink, the respective reliability for all drinks combined was only 0.54 and 0.56. When drinks were examined separately, r values were inconsistent, ranging from -0.11 to 0.90. Again, test-retest reliability was higher when the child could see the color. Orange was the only drink for which reliability remained high also when the cups were opaque.

Table 4.4. Test-Retest Reliability of the Taste Perception Questionnaire.

(2nd vs 5th position)

Drink	Type of cup	How much do you like the <u>taste</u> ?	How much do you like this drink <u>overall</u> ?
		r	r
ALL	both	0.68	0.70
	clear	0.84	0.81
	opaque	0.54	0.56
GRAPE	both	0.23	0.64
	clear	-0.45	0.23
	opaque	0.56	0.58
APPLE	both	0.64	0.64
	clear	0.90	0.90
	opaque	-0.11	-0.13
ORANGE	both	0.83	0.64
	clear	0.86	0.84
	opaque	0.86	0.76
WATER	both	0.49	0.49
	clear	0.77	0.50
	opaque	0.40	0.55

Although the high preference for grape was consistent among all three situations (euhydration-rest session, after $\dot{V}O_{2max}$, and dehydration sessions), the reliability of the questionnaire is still uncertain. It is hard to tell why r values were high for orange and low for grape. One possibility might be that the children who were chosen to repeat the

orange drink happened to rate more reliably than those who repeated the grape drink. A study design in which **the same child** repeats tasting of different drinks may clarify the effect of the taste quality on the reliability of the answers. Also, a larger sample size (n) would be necessary, since in our study sample sizes ranged from 6 to 8 subjects per drink. The higher r values in clear as compared to opaque cups suggest that visual (or color) memory plays a role in the reliability of the answers. Interestingly, when the question "How much do you like this color?" was analyzed for reliability, r values were lowest for orange (grape=0.74, apple=0.90, orange=0.58 and water=0.66). Thus, despite the agreement between the answers, further research is warranted to tell whether this questionnaire and procedures are reliable.

CHAPTER 5

**EFFECT OF DRINK COMPOSITION ON THERMOREGULATION AND
PERFORMANCE OF CHILDREN EXERCISING IN THE HEAT**

5.1. Introduction

Avoiding dehydration during prolonged exercise in the heat is important for temperature regulation and for performance. Ideally, the volume of water intake during exercise should match its overall lost from sweat, urine, and respiration. In addition to water, replenishment of Na⁺ combined with CHO has been recommended for adults who exercise for a long time in the heat (Gisolfi and Duchman, 1992).

Although there is no evidence that electrolyte replenishment during exercise improves performance or thermoregulation, one concern about excluding Na⁺ from drinks is that sweat Na⁺ losses combined with excessive water ingestion may lead to hypotonic hyponatremia during prolonged exercise (Barr and Costill, 1989; Noakes et al., 1985). Furthermore, addition of Na⁺ to a CHO-solution may facilitate restoration of plasma volume since net water absorption increases at the intestine (Gisolfi et al., 1991; 1992; Lifshitz et al., 1985).

One argument against Na⁺ ingestion during exercise is the possibility that increasing plasma Na⁺ and osmolality may increase core temperature by impairing sweating rate (Harrison, 1986). However, if during exercise euhydration is maintained

with iso-osmotic drinks containing up to 25 mEq·l⁻¹ of Na⁺, neither plasma Na⁺ nor osmolality increased (Barr et al., 1991; Johnson et al., 1988).

Sports drinks, therefore, usually contain Na⁺ (20 mEq·l⁻¹) and CHO (~6%). These drinks were originally designed for adults, but due to their promotion, they are also consumed by children. Whether these sport drinks are better than water and whether they affect plasma [Na⁺], thermoregulation or performance of children exercising in the heat has not been evaluated. The markedly lower amount of Na⁺ (and Cl⁻) lost through sweat by children compared to adults (chapter 2), suggests that children need to replace less, if any, Na⁺. Ingesting ~20 mEq·l⁻¹ Na⁺ drinks to maintain euhydration during exercise is unlikely to increase plasma [Na⁺] or impair thermoregulation since the average sweat [Na⁺] of children is still above 20 mEq·l⁻¹ (Figure 2.1). Thus, these drinks should not overload the extracellular Na⁺ content.

The purpose of this study was to compare the effects of ingesting four drinks with different electrolyte and CHO compositions on children exercising in the heat. Specific questions addressed were: 1. does CHO-electrolyte intake affect performance and thermoregulation of children exercising in the heat? 2. does the amount of ingested electrolyte affect its total losses (sweat and urine), and its overall balance? During prolonged activities in the heat, children may dehydrate even when water is available *ad libitum* (Bar-Or et al., 1980; 1992). To eliminate the confounding effect of hypohydration, volume intake of each drink was the same and calculated to keep subjects well and equally hydrated.

5.2. Methods

Subjects. Twelve 9- to 12-yr-old volunteers (6♀ and 6♂) completed the study. There were no gender differences in weight, height, surface area, adiposity and peak oxygen consumption ($\dot{V}O_2$). The respective mean \pm SD for the total group were: 11 \pm 0.3 yrs, 44.5 \pm 3.8 kg, 149 \pm 2.9 cm, 1.36 \pm 0.06 m², 21 \pm 2.4 % fat and 40.7 \pm 2.1 ml·kg⁻¹·min⁻¹. Subjects were in good health according to an interview and a written questionnaire completed by a parent. Ten subjects were habitually involved in recreational sports, and two were competitive swimmers. Experiments took place in Ontario, from February to July of 1992. Because of unusually low mean temperature during that summer (\sim 15°C), we assumed that subjects were not acclimatized to the heat.

After a screening session, subjects came to four trials (one week apart) that differed in the composition of the drinks ingested. Four drinks were assigned, one for each trial, in a Latin-Square order and in a double-blind design.

During the screening session, the procedures of the study were explained to the child and after obtaining his/her verbal assent, a parent signed a consent form (Appendix 8.1.3). The study was approved by the McMaster University Ethics Committee. We measured subjects' height (Harpeden wall-mount stadiometer model 2109), weight (Mott electro scale model LC2424-20 g accuracy), and adiposity (bioelectrical impedance-Valhalla Scientific). Peak $\dot{V}O_2$ was obtained using a progressive, 2-min-per-stage protocol (constant cycling cadence of 50 rpm) on a cycle ergometer (Fleisch-Ergostat Universal,

Metabo). This test lasted 6 to 10 min, ending with the subjects' volitional exhaustion or when they could not maintain the cadence, despite encouragement by the investigator. $\dot{V}O_2$ was measured by an automated open-circuit system (Beckman-Horizon Metabolic Measurement Cart).

Experimental protocol. All trials were held after school (about 5 pm), except for one child who was always tested in the morning. Subjects were given the following written and verbal instructions for the day of testing: 1) avoid exercising during the 8 hours before the session; 2) eat and drink regularly, but avoid salty and sweet snacks; 3) do not eat for three hours before the test, "because a snack will be provided at the laboratory". On the day of testing, compliance to these regulations was ascertained. Forty-five min prior to chamber entry, 2 slices of toast (white Wonder bread - no cholesterol) with 20 g of strawberry jam (E.D. Smith - no sugar added) were given at the laboratory. This was accompanied by 100 ml of the assigned drink. After that, shoes and shorts (plus bikini top, for girls) were weighed (Acculab scale, 1200, 0.24 g accuracy).

To collect sweat, polythene bags were attached to the lower back (Meyer et al., 1992a; Falk et al., 1991). Two bags were used: one to collect the sweat from the entire in-chamber period, and a contralateral bag to collect sequential samples from each exercise bout. Subjects voided and a urine sample was kept for later analysis. To monitor rectal temperature (T_{re}), a thermistor (YSI 400 series) was introduced 10 cm beyond the anal sphincter. A heart rate (HR) monitor (sport Tester PE 3000) was attached to the chest.

In-chamber protocol. Each trial consisted of intermittent exercise in an environmental room (34° -35°C, 42-45% relative humidity). Protocol and timing of measurements are shown in Figure 5.1. Following a 15-min rest, subjects performed one 20-min (bout 1) and two 15-min (bouts 2 and 3) cycling tasks at 50% of their pre-determined peak $\dot{V}O_2$. Each bout was followed by a 10-min rest. $\dot{V}O_2$ and respiratory exchange ratio (RER) were measured in the middle of bout 2 for five min.

Physical performance was evaluated in a fourth bout by asking the subjects to pedal for as long as they could at 90% of their peak power (See Addendum to this chapter). To ensure that children were highly and equally motivated for bout 4, we standardized the instructions by using a "point" system. Prior to bout 4 of each trial, the subject was told that every 30 s of cycling was worth 5 points and, at the end of the study, each child would get prizes according to the total number of points accumulated. The same investigator gave the standardized verbal encouragement. Subjects were considered exhausted when they spontaneously stopped pedaling, or when pedaling rate dropped to 40 rpm. Performance time was recorded, and total mechanical work output calculated.

HR, T_{re} , and skin temperatures (Mikron infrared thermometer 80 series) at the subscapula ($T_{subscapula}$) and forearm ($T_{forearm}$) were measured periodically during exercise and at rest. Thigh temperature (T_{thigh}) was measured only at rest. In bout 4, HR and the number of pedal revolutions were recorded every min, and T_{re} , every 2 min. Right after each exercise bout, subjects were weighed (Mott electro scale model LC2424). To keep

them well- and equally- hydrated throughout the trial, drinks were given in identical volumes and frequencies for each child: $1.8 \text{ ml}\cdot\text{kg}^{-1}$ every 13-15 min (once every rest and exercise period). These amounts, estimated to fully replenish sweat and urine losses, were based on a pilot study (See Addendum to this chapter) in which we examined body water losses of six 9- to 12-yr-old children performing a similar exercise protocol as in the present study.

Once subjects left the chamber, their clothes were weighed. They voided, urine volume was again measured, and a sample kept for later analysis.

Drinks. The composition and osmolality of the drinks are shown in Table 5.1. Three drinks had 6% carbohydrate content (sucrose 4% , and fructose 2%), but different $[\text{Na}^+]$: 0 ("CHO-0"), 8.8 ("CHO-8.8"), 18.5 ("CHO-18.5") $\text{mEq}\cdot\text{l}^{-1}$. One drink had neither carbohydrate nor electrolytes ("WATER"). K^+ was added, in equal amounts ($3 \text{ mEq}\cdot\text{l}^{-1}$), only to the drinks with Na^+ . Aspartame (0.035%) was added only to the WATER to match the sweet taste of the CHO drinks. All drinks in this study had the same grape flavor and color, based on a previous study experiment of children's preferences during and after exercise (Meyer et al., 1992b). Drinks were served at 8° to 10°C .

Blood, sweat and urine analysis. Venous blood (4 ml) was collected in lithium heparinised syringes before bout 1 (10th minute in-chamber) and after bout 4 (2nd min). Whole blood was immediately analyzed for lactate (YSI 1500 sport L-Lactate analyzer). Hemoglobin was measured using hemoximeter (OSM 3), and hematocrit was determined by the microhematocrit centrifuge technique. Two ml of whole blood was placed into

tubes to be centrifuged. Plasma was separated and kept at 4°C for later analyses. Whole blood (200 μ l) was deproteinized with 6% perchloric acid (400 μ l) and the clear supernatant was separated and kept frozen for determination of glucose concentration, in duplicate, by enzymatic fluorometric technique (Sigma Chemical). Plasma glycerol concentration was measured using this same technique (Boehringer Mannheim Bioquimica).

Plasma and urine $[\text{Na}^+]$, $[\text{Cl}^-]$ and $[\text{K}^+]$ were measured using ion-selective electrodes (Electrolyte Analyzer AVL 983S). Sweat $[\text{Na}^+]$ and $[\text{K}^+]$ were also analyzed using ion selective electrodes but with the Kodak Ektachem Analyser(700XR). Sweat $[\text{Cl}^-]$ was measured by a Buchler-Cotlover chloride titrator. Sweat lactate (YSI 1500 Sport L-Lactate analyzer) and sweat pH (Radiometer pH-G2040c) were also determined. Plasma, urine and sweat osmolality were measured by freezing point depression (micro-osmometer Model 3MO), and plasma protein by refractometry (Atago Model).

Calculations. Body surface area was calculated from body weight and height (DuBois and DuBois, 1916). Total sweat volume was calculated from changes in body weight, corrected for drink intake, urine output, respiratory water losses (Mitchell et al., 1972), and the increase in the weight of the clothes. The level of hydration was given as a % change of the initial body weight, corrected for urine volume and the increase in weight of the clothes (urine output was assumed to be constant throughout the trial). Total sweat and urine electrolyte losses were calculated by multiplying their concentration by the respective volumes. Total electrolyte balance was estimated as the difference between the

intake and the losses through urine and sweat. Percentage changes in plasma volume ($\% \Delta PV$) and red blood cells ($\% \Delta RBC$) were calculated from changes in hemoglobin and hematocrit (Harrison, 1985).

Statistics. A two-way ANOVA with repeated measures was used to examine the effect of the drink trial, and changes over time. Since one girl missed one trial (WATER), a general linear model (Minitab 8.2) was used to test the effect of the drink on variables measured once during a trial (e.g. performance time). A Tukey post-hoc test was employed when F-ratio was significant ($p < 0.05$). Results are presented as mean \pm SEM.

5.3. Results

A one-way analysis of variance (ANOVA) showed no gender differences in the physical characteristics, thermoregulatory responses, or sweat electrolyte losses. In a previous study, there were no gender differences in thermoregulation or sweat electrolyte composition among children (Meyer et al., 1992a). We therefore pooled the data for boys and girls in this study.

The % peak $\dot{V}O_2$ and the RER obtained in the middle of bout 2 were similar among trials ($50.6 \pm 0.74\%$, and 0.94 ± 0.01 , respectively), suggesting that subjects exercised at the same work intensity among trials. Subjects were well- and equally-hydrated throughout and among drink trials. After the last bout, the mean change in body wt of all trials was $-0.07 \pm 0.03\%$.

Physiological responses. Figure 5.2 shows changes with time in HR, T_{re} , $T_{subscapula}$ and T_{thigh} . HR and T_{re} changes were similar in all trials. T_{re} increased during the first 60 min and then gradually levelled off. Mean increase in T_{re} by the end of the bout 3 was $0.36 \pm 0.05^\circ\text{C}$. A steep rise of $\sim 0.1^\circ\text{C}$ was observed in each 2 min of the last bout (90% peak power), resulting in an increase of $0.75 \pm 0.07^\circ\text{C}$ (range 0.5 - 1.0°C) in those subjects who cycled for more than 10 min (2 boys in all drinks trials). The changes of $T_{forearm}$ followed the same pattern as those of $T_{subscapula}$ shown in Figure 5.2. T_{thigh} , measured at rest, was similar among trials and increased after bout 1.

Performance time (min) in bout 4 for each trial was 6.18 ± 1.19 (CHO-0), 5.11 ± 1.03 (CHO-8.8), 5.13 ± 1.13 (CHO-18.5) and 5.24 ± 1.30 (WATER). The corresponding work in kJ was 35.2 ± 5.2 (CHO-0), 31.7 ± 6.2 (CHO-8.8), 29.6 ± 6.2 (CHO-18.5), 33.4 ± 7.7 (WATER). There were no differences among drink trials in total time or work performed.

Biochemical responses. Plasma $[\text{Na}^+]$ before the trials ranged from 142 to 145 $\text{mEq}\cdot\text{l}^{-1}$. There was no inter-trial difference, nor was there any change observed at the end of the trial. The same occurred for $[\text{Cl}^-]$ (range 107-109 $\text{mEq}\cdot\text{l}^{-1}$). Plasma $[\text{K}^+]$ increased $3.8 \pm 1.42\%$ and similarly among trials. There was a decrease in the % ΔPV , an increase in plasma protein, and no changes in either the % ΔRBC or plasma osmolality (Table 5.2). All these responses were consistent among trials.

Plasma glycerol increased from 2.3 fold (CHO-8.8) to 4.25 fold (WATER) by the end of the trial, being higher with the WATER than in the other trials ($p=0.017$) (Figure

5.3). Blood [glucose] did not change significantly from rest ($5.19 \pm 1.13 \text{ mmol}\cdot\text{l}^{-1}$) to end of the trials ($4.85 \pm 0.14 \text{ mmol}\cdot\text{l}^{-1}$), nor was there any effect of drink trial. A similar increase in plasma [lactate⁻] of $1.6 \pm 0.18 \text{ mmol}\cdot\text{l}^{-1}$ was found among drink trials.

Total electrolyte losses and balance. The volume of urine after each trial, and urine electrolyte concentrations and osmolality before and after each trial are shown in Table 5.3. Except for CHO-18.5, there was a significant decrease in urine [Na⁺] compared to the pre-trial value. Urine [Cl⁻] and osmolality dropped with ion-free drinks.

At a given time point, mean sweat electrolyte concentration, pH, and sweating rate did not differ among drink trials. Data from the four trials of each subject at a given time were averaged, and a two-way ANOVA with repeated measures was used to examine the changes with time. Results are summarized in Figure 5.4. While sweat [Cl⁻] increased, [K⁺] and [lactate⁻] decreased over time. [Na⁺] tended to increase more gradually than [Cl⁻] during the trial. Sweating rate increased with time, especially from bout 1 to 2. Sweat osmolality, from the entire in-chamber time, did not differ among drink trials ($86 \pm 2.8 \text{ mOsm}\cdot\text{l}^{-1}$).

The various intake regimens did not affect the total electrolyte losses from urine or sweat (Table 5.4). A negative balance was found for Na⁺, Cl⁻ and K⁺, regardless of the amount of ion ingested. In the case of Na⁺, the deficit was higher with the ion-free drinks than in the others.

5.4. Discussion

This study showed that, compared to water, addition of electrolytes and CHO to a drink had no effect on regulation of body temperatures or on aerobic performance in children who were kept euhydrated and exercised intermittently in the heat (< 2 h). This is in agreement with studies performed in adults under similar (Francis, 1979) or different exercise conditions than our study (Levine et al., 1991; Johnson et al., 1988, Wells et al., 1985).

In a previous study (Meyer et al. 1993b, chapter 4), we found an increase of 0.53°C in T_{re} after 24 children slightly dehydrated (0.7% of initial body weight) by cycling three 15-min bouts (50% $\dot{V}O_2$ peak, at 35°C and 20% RH). In the present study, T_{re} did not increase as much (0.36°C) during the first three cycling bouts (50% peak $\dot{V}O_2$) (Figure 5.2), even though subjects cycled longer and at higher heat stress (35% RH). This may be because subjects were kept euhydrated. On the other hand, our study showed an effect of exercise intensity on the increase in core temperature as evidenced by the extent to which T_{re} increased during the cycling at 90% peak $\dot{V}O_2$. Most of the children in our study, even though euhydrated, presented a decrease in PV immediately following bout 4. In adults, both exercise and heat may lead to transient shift of fluid out of the vascular space despite the absence of dehydration (Harrison, 1985). The decrease of less than 6% in PV found in our study is still somewhat lower than that for adults (Barr et al., 1991; Davies et al., 1988; Wells et al., 1985). Indeed,

as already demonstrated in chapter 2 adults present a greater decrease in PV than children when they perform the same exercise protocol under the same environmental conditions.

In the present study, as well as in studies with adults (Barr et al., 1991; Johnson et al., 1988), Na^+ intake did not affect plasma $[\text{Na}^+]$. This can be explained by the relatively low Na^+ intake compared to total water and Na^+ body pool. The highest Na^+ intake (about 11.2 mEq) represented less than 1% of the total exchangeable Na^+ of the extracellular space (Yoshioka et al., 1990); and therefore, the negligible change that might have occurred in plasma $[\text{Na}^+]$ would not have been detected. It is also possible that plasma $[\text{Na}^+]$ was regulated by the kidney since, in our study, a greater decrease in urine $[\text{Na}^+]$ (and $[\text{Cl}^-]$) was observed when none (or low amounts) of these electrolytes was ingested. Resultant urine electrolyte losses were similar among drinks, however, because urine volume was somewhat lower with ion-free drinks.

Sweating rate increased from bout 1 to bout 2. Sweat $[\text{Na}^+]$ did not change over time, while $[\text{Cl}^-]$ rose from bout 1 to the end the trial (Figure 5.4). Conversely, Costill et al. (1976) showed that when men gradually dehydrated by exercising in the heat, there was a decrease in sweating rate as well as sweat $[\text{Na}^+]$ and $[\text{Cl}^-]$. The hypertonicity which results from hypotonic sweat loss is believed to impair sweating rate (Fortney et al. 1984; Sawka, 1992; Senay, 1973). In this case, euhydration probably prevented plasma osmolality from increasing and sweating rate from falling. A simple explanation is that sweating is modified according to the physiologic needs. The sweat gland may

prevent further body water losses by increasing NaCl reabsorption in its duct, since water follows the movement of these ions (Sato et al., 1989). More studies are required to clarify the effect of hydration state on the time course of changes in sweat composition.

There was no difference in the combined electrolyte losses from urine and sweat among trials. While Na⁺ and Cl⁻ losses were equally divided between urine and sweat, most of K⁺ losses (> 62%) came from urine. A negative electrolyte balance was observed with all drinks. Na⁺ deficit was about 100% greater with ion-free drinks than with the electrolyte drinks, but the magnitude of this difference is probably of little or no biological importance. The 0.16 mEq·kg⁻¹·h⁻¹ deficit difference in Na⁺ between the CHO-18.8 and WATER represents less than 1% of the total exchangeable Na⁺ in the extracellular space (about 42 mEq·kg⁻¹) (Yoshioka et al., 1990).

In the present study CHO alone did not reduce aerobic performance when subjects cycled at 90% peak $\dot{V}O_2$ after the 95 min of intermittent exercise in the heat. This difference from reports on adults (Coggan and Coyle, 1989; Coyle et al. 1983; Davis et al., 1988; Murray et al., 1987) could be due to a shorter protocol and a lower exercise intensity of our study. During prolonged and strenuous exercise (> 2 h), CHO ingestion has been recommended to maintain blood glucose concentration and CHO oxidation. This would spare muscle and liver glycogen stores which may be of limited supply toward the end of prolonged exercise.

Plasma glucose concentrations after each trial were similar, and at levels safely above hypoglycemia (>3.3mmol·l⁻¹). To prevent further decrease in blood glucose

during the WATER trial, regulatory mechanisms in glucose turnover rate may have occurred. Greater utilization of fat might be an alternative pathway to prevent hypoglycemia. Indeed, in the present study, plasma glycerol increased significantly in all trials, but such an increase was higher with WATER than with all CHO-drinks (317 vs 215%) (Figure 5.3). RER values (measured in the middle of bout 2) were similar among trials, which may suggest that a higher lipolysis during the WATER trial did not take place until the last exercise bout in which performance was determined.

Studies performed with adults also found a greater increase in plasma glycerol or free fatty acids when no CHO was ingested as compared to CHO drinks (Levine et al., 1991; Wells et al., 1985; Wright et al., 1991). A greater increase in plasma insulin concentration may have occurred in the early stages of exercise when CHO was ingested ($0.42 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$), causing a reduction in the rate of lipolysis. Wright et al. (1991) found that when well-trained cyclists ingested CHO (330 g) prior to and during (175 g) a cycling bout at 70% peak $\dot{V}O_2$ until exhaustion, plasma glycerol and free fatty acids did not increase as much as when no CHO was ingested. In Wrights' study, a higher plasma insulin level was observed during the 1½ h of cycling when subjects had CHO prior to and during exercise. Explanations derived from adult studies may not be suitable for children. Martinez and Haymes (1992) stated that, compared to women, "prepubescent girls rely more on fat utilization and less on CHO metabolism during exercise of moderate to heavy intensity". This was despite their finding that the magnitude of plasma glycerol increase did not differ between the girls and the women after 30-min run at 70%

peak $\dot{V}O_2$. Studies have been inconsistent in determining children's relative use of fat during exercise as compared to adults (Eriksson et al., 1971; Macek et al., 1976). Thus, further research is needed to clarify this aspect during prolonged exercise and to determine whether CHO ingestion affects fat utilization through insulin responses.

In conclusion, when children are kept well hydrated during prolonged, intermittent exercise in the heat, ingestion of CHO-electrolyte solutions and water are associated with similar thermoregulatory and performance responses. Effectiveness of CHO-electrolytes solutions is still to be determined when drinking is ad libitum and euhydration may not be maintained.

TABLE 5.1. Electrolyte and Carbohydrate (CHO) Composition, and Osmolality of the Four Drinks.

DRINKS*	[Na ⁺] mEq·l ⁻¹	[Cl ⁻] mEq·l ⁻¹	[K ⁺] mEq·l ⁻¹	CHO %	OSMOLALITY mOsm·l ⁻¹
CHO-0	0	0	0	6	263
CHO-8.8	8.8	7.1	3	6	281
CHO-18.5	18.5	15.5	3	6	292
WATER	0	0	0	0	26

* Numbers following "CHO-" indicate the [Na⁺]

TABLE 5.2. Plasma Protein ($\text{g}\cdot\text{l}^{-1}$) and Osmolality ($\text{mOsm}\cdot\text{l}^{-1}$), and the % Changes of Plasma Volume ($\%\Delta\text{PV}$) and Red Blood Volume ($\%\Delta\text{RBV}$) by Drinks. Mean \pm SEM.

DRINK	Protein		Osmolality		$\%\Delta\text{PV}$	$\%\Delta\text{RBC}$
	pre	post	pre	post		
CHO-O	76.8 ± 1.9	$80.8\pm 2.0^*$	289 ± 2	287 ± 2	$-6.3\pm 1.5^*$	1.22 ± 1.7
CHO-8.8	79.0 ± 2.5	$82.7\pm 1.5^*$	288 ± 2	292 ± 2	$-6.4\pm 1.6^*$	-0.33 ± 1.2
CHO-18.5	77.6 ± 1.7	$81.1\pm 1.8^*$	292 ± 4	289 ± 2	$-7.6\pm 1.5^*$	-2.80 ± 1.6
WATER	77.1 ± 2.1	$81.9\pm 2.1^*$	289 ± 2	286 ± 2	$-6.3\pm 2.0^*$	0.38 ± 1.3

Drink codes are the same as in TABLE 5.1.

*different from pre-exercise value ($p < 0.05$), * $p = 0.06$.

TABLE 5.3. Volume (ml) of Urine Collected after the Trial, Urine Electrolyte Concentration (mEq·l⁻¹) and Osmolality (mOsm·l⁻¹) before and after the Trials. Mean ± SEM.

DRINK	VOLUME URINE	[Na ⁺]		[Cl ⁻]		[K ⁺]		Osmolality	
		pre	post	pre	post	pre	post	pre	post
CHO-O	129 ± 31.1	133 ± 19.3	79 ± 13.7*	173 ± 18.4	118 ± 25.2 ^a	156 ± 48.7	154 ± 69.2	850 ± 78	655 ± 118 ^b
CHO-8.8	77 ± 12.7	120 ± 26.4	94 ± 21.9*	140 ± 24.1	132 ± 23.3	106 ± 25.3	132 ± 26.9	853 ± 108	898 ± 107
CHO-18.5	87 ± 13.0	146 ± 22.6	129 ± 15.7	176 ± 25.3	173 ± 19.1	198 ± 69.8	214 ± 76.6	904 ± 85	864 ± 77
WATER	124 ± 22.3	170 ± 15.4	113 ± 20.0*	193 ± 20.4	145 ± 27.8*	105 ± 23.4	99 ± 21.4	900 ± 87	704 ± 109*

Drink codes are the same as in TABLE 5.1.

*Significantly different from pre-exercise value for the same drink (p < 0.05).

^aChange from the pre-exercise value with this drink is significantly different from those with electrolytes (P < 0.05).

^bChange from the pre-exercise value with this drink is significantly different from the CHO-18.5 drink (P < 0.05).

TABLE 5.4. Electrolyte Balance by Drinks.

Values are expressed in $\text{mEq}\cdot\text{kg}^{-1}\cdot\text{body weight}\cdot\text{h}^{-1}$. Mean \pm SEM.

ION	DRINK	INTAKE	LOSSES		BALANCE
			URINE	SWEAT	
Na ⁺	CHO-0	0	0.14 \pm 0.05	0.19 \pm 0.03	-0.33 \pm 0.07*
	CHO-8.8	0.07 \pm 0.004	0.09 \pm 0.02	0.16 \pm 0.02	-0.18 \pm 0.03
	CHO-18.5	0.14 \pm 0.008	0.14 \pm 0.04	0.18 \pm 0.03	-0.18 \pm 0.05
	WATER	0	0.16 \pm 0.04	0.18 \pm 0.02	-0.34 \pm 0.04*
Cl ⁻	CHO-0	0	0.17 \pm 0.03	0.12 \pm 0.07	-0.29 \pm 0.05
	CHO-8.8	0.05 \pm 0.004	0.12 \pm 0.02	0.11 \pm 0.06	-0.18 \pm 0.03
	CHO-18.5	0.11 \pm 0.008	0.19 \pm 0.03	0.12 \pm 0.07	-0.20 \pm 0.05
	WATER	0	0.14 \pm 0.02	0.12 \pm 0.05	-0.26 \pm 0.04
K ⁺	CHO-0	0	0.18 \pm 0.08	0.06 \pm 0.01	-0.24 \pm 0.08
	CHO-8.8	0.023 \pm 0.0015	0.10 \pm 0.01	0.06 \pm 0.02	-0.14 \pm 0.01
	CHO-18.5	0.023 \pm 0.0015	0.19 \pm 0.06	0.06 \pm 0.02	-0.22 \pm 0.06
	WATER	0	0.11 \pm 0.02	0.06 \pm 0.02	-0.17 \pm 0.02

Drink codes are the same as in TABLE 5.1.

*Significantly different from the drinks with electrolytes ($p < 0.05$).

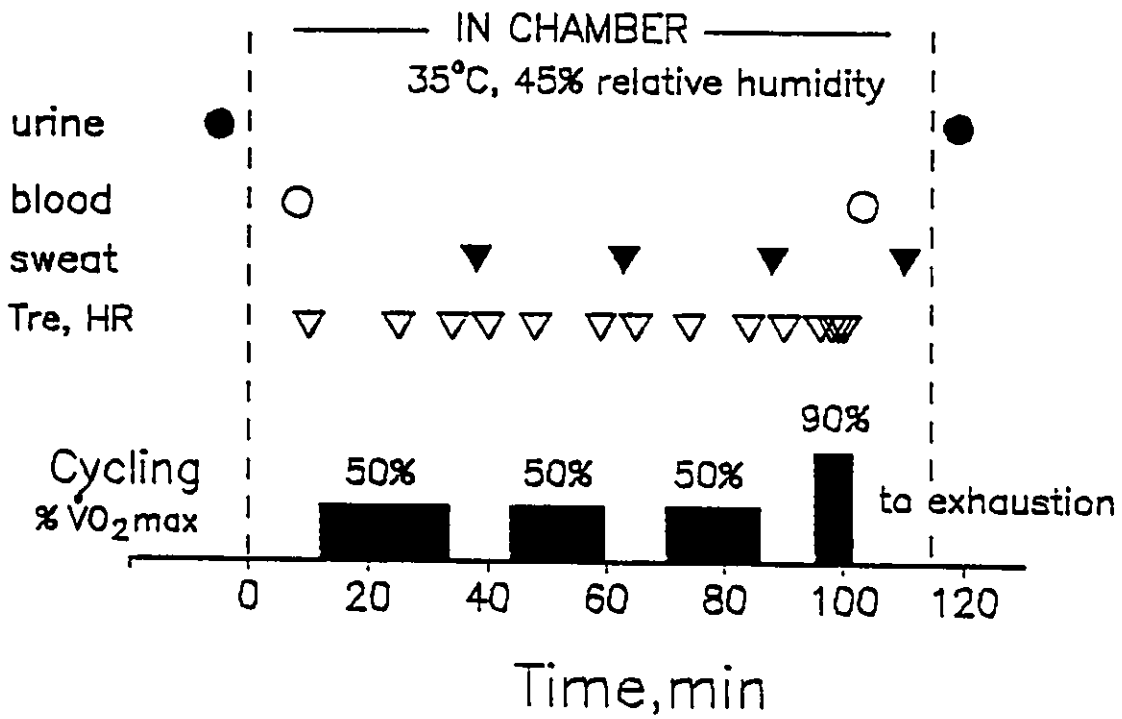


FIGURE 5.1. Schematic Diagram of the Intermittent Cycling Protocol, Timing of HR and T_{re} Measurements, and Collection of Sweat, Urine and Blood.

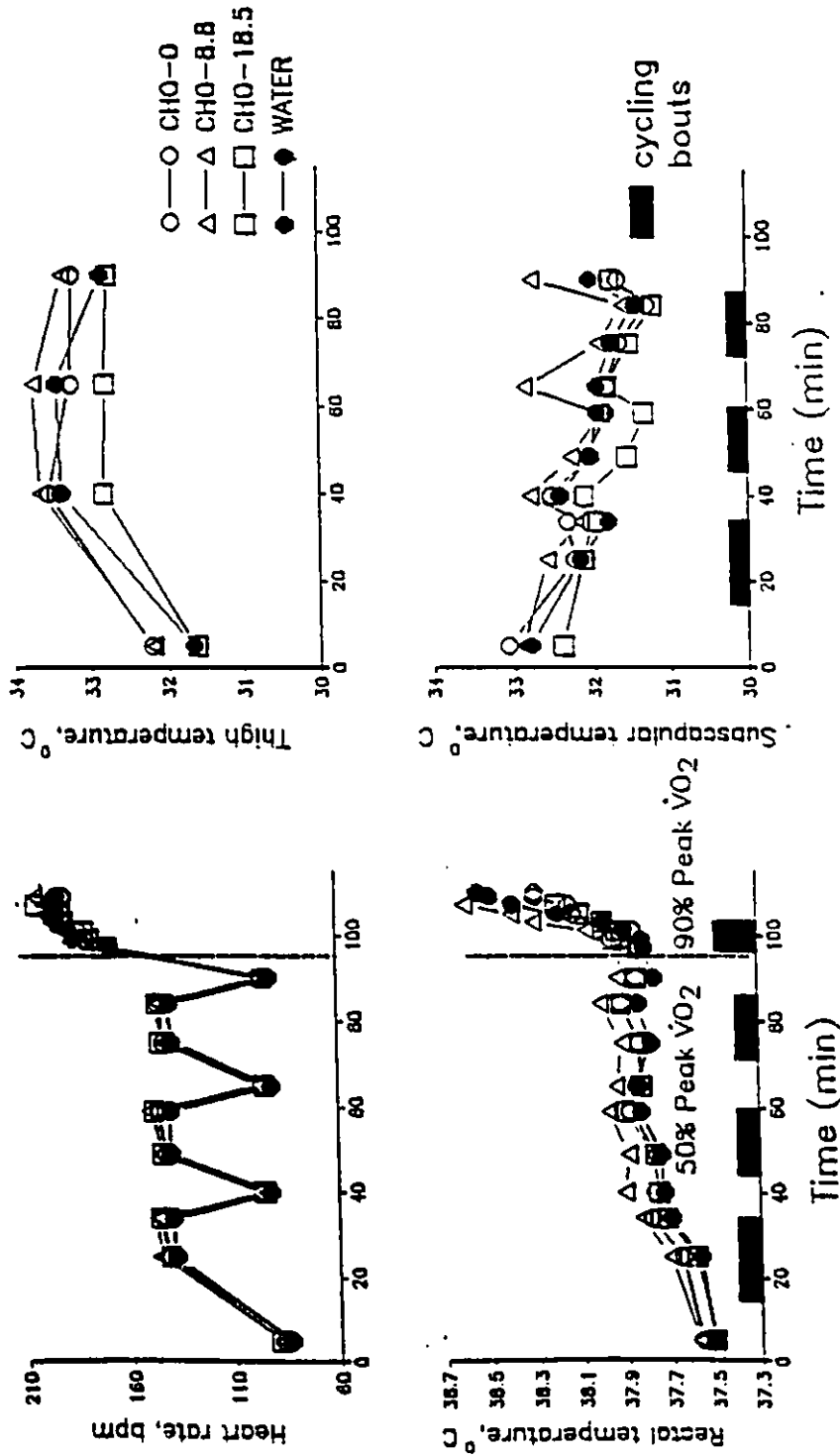


FIGURE 5.2. Heart Rate, Rectal and Skin (Subscapular and Thigh) Temperatures During the Trials.

Values are means. Drink codes are the same as in Table 5.1.

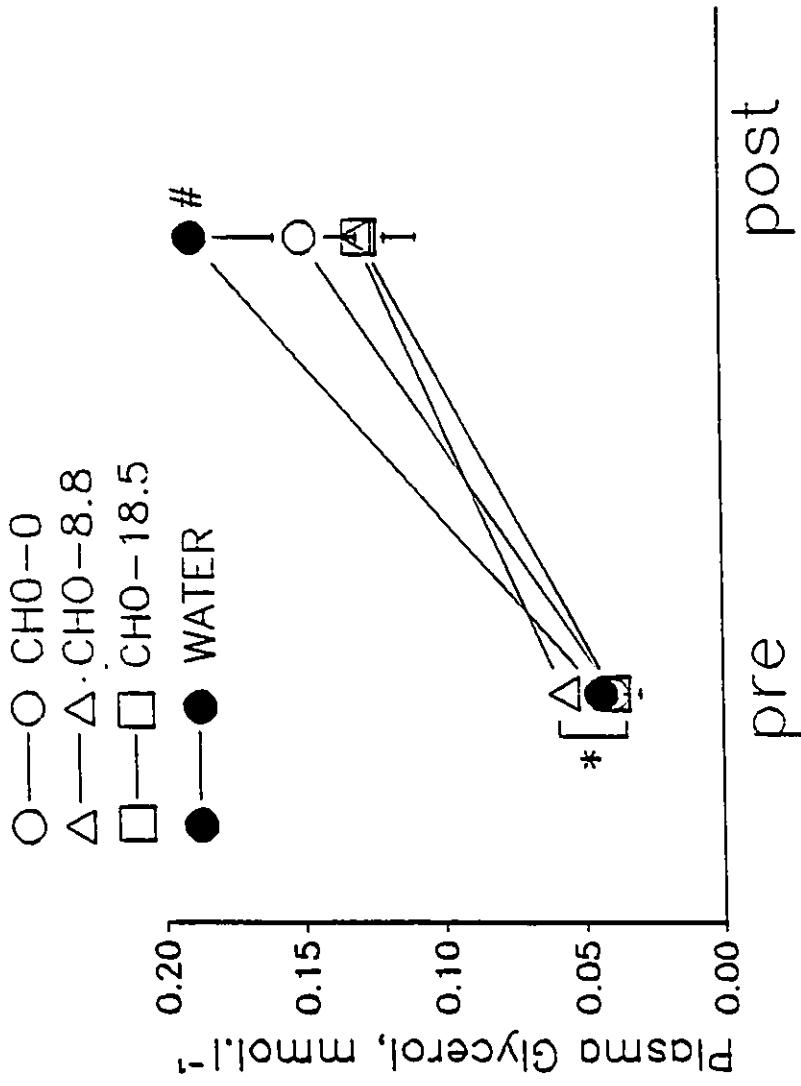


FIGURE 5.3. Plasma Glycerol Concentrations Before and After the Trials. Post-exercise values were corrected for % Δ PV. *different from post-exercise, #increase from pre-exercise value with this drink was greater from those with CHO ($p < 0.05$). Drink codes are the same as in Table 5.1. Mean \pm SEM.

Sweat electrolyte concentration

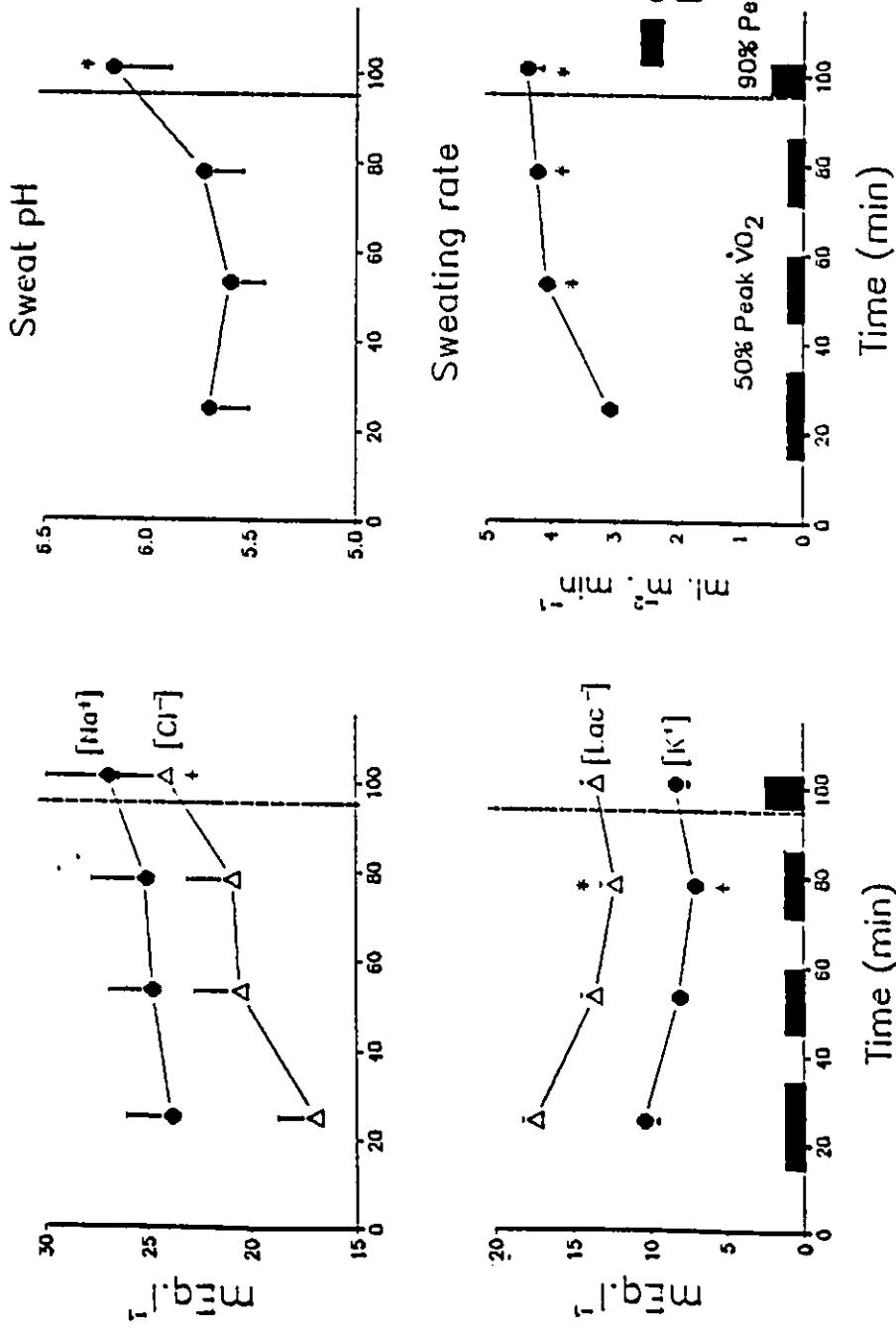


FIGURE 5.4. Time Course of Sweat Electrolyte Concentrations, pH, and Sweating Rate During the Whole Trial.
 Results are pooled means from the 4 drink trials and vertical lines are SEM.
 *different from bout 1, p < 0.05.

5.5. Addendum

Pilot Study

The purpose of this pilot study was to standardize an "all-out" exercise intensity which a child could sustain for 5-10 min after having cycled intermittently for 50 min at 50% $\dot{V}O_{2\max}$ in a warm climate (30°C and 50% RH). Seven healthy children 10-12 years old (4 boys and 3 girls) participated in this study. Each subject came to the laboratory for four visits. During the first visit, anthropometric measurements and assessment of $\dot{V}O_{2\max}$ on a cycle ergometer were taken. In the following three visits, the child cycled and rested in the heat, as shown in Table 5.5.

TABLE 5.5. Protocol for the Pilot Study.

Activity	rest	bout 1 50% $\dot{V}O_{2\max}$	rest	bout 2 50% $\dot{V}O_{2\max}$	rest	bout 50% $\dot{V}O_{2\max}$	rest	bout 4 85,90, or 95% $\dot{V}O_{2\max}$
Duration, min	10	20	10	15	10	15	10	?

Intensity of the last exercise bout was set at 85%, 90% and 95% of $\dot{V}O_{2\max}$. The sequence of these intensities was randomized. In each of the chamber visits, before starting the last bout, the child was instructed to maintain the set pace (50 rpm) for as long as possible. The encouragement given by the investigator was standardized during each of these sessions. The investigator was blinded to the work rate at which the bike was set by having someone else setting the bike weights and subjects were unaware of the purpose of the study. During the entire in-chamber period the child was kept nearly euhydrated by drinking 50 ml of cool water every 15 min.

The results of the endurance bout are summarized in Table 5.6. Not all subjects generated the planned power in all three sessions (deviating from the prescribed pedaling cadence). The Table therefore includes the planned $\dot{V}O_2\text{max}$ and the adjusted $\dot{V}O_2\text{max}$. Despite the inter-individual variability, it seems that most subjects could cycle for almost twice as long at 85% (or below) $\dot{V}O_2\text{max}$ than at 90 or 95%. Based on these results, we used 90% of $\dot{V}O_2\text{max}$, as the prescribed intensity for the endurance task of the study in Chapter 5.

TABLE 5.6. Total Work and Power Performed per Subject at each Exercise Intensity.

Subject & gender	Order	% $\dot{V}O_2\text{max}$		Bike wts number	Duration min	Revs number	Work kp m	Power	
		planned	adjusted					kp m min ⁻¹	W
1 ♂	1	85	85	24	15.0	750	10800	72	118
1	3	90	94	26	6.0	337	5249	875	143
1	2	95	90	29	7.0	225	3915	783	128
2 ♂	2	85	90	19	4.5	252	2867	659	108
2	1	90	87	22	2.4	118	1551	644	105
2	3	95	93	24	3.0	148	2124	708	116
3 ♂	3	85	85	17	15.4	753	7676	497	81
3	2	90	80	18	8.2	366	3953	485	79
3	1	95	94	19	7.1	357	4070	572	93
4 ♀	1	85	83	17	6.5	320	3259	501	82
4	3	90	90	18	4.6	248	2678	564	92
4	2	95	94	19	4.5	225	2559	569	93
5 ♂	2	85	75	15	15.0	751	6759	451	74
5	1	90	85	16	5.1	254	2443	477	78
5	3	95	95	17	5.1	250	2550	498	81
6 ♀	1	85	82	23	8.2	403	5561	681	111
6	2	90	88	24	5.2	258	3708	717	117
6	3	95	89	25	5.0	240	3600	720	118
7 ♀	1	85	82	20	2.1	97	1164	569	93
7	2	90	85	21	2.0	100	1260	630	102
7	3	95	83	22	2.8	127	1670	607	99

CHAPTER 6

CHILDREN'S PERCEPTUAL RESPONSES TO INGESTING DIFFERENT DRINK COMPOSITIONS DURING AND FOLLOWING EXERCISE IN THE HEAT

6.1. Introduction

When plain water was available *ad libitum*, children gradually dehydrated while exercising intermittently for 3 hours in the heat (Bar-Or et al., 1980, 1992). The reasons for this insufficient drinking remain unclear. One possibility is that the composition of the fluid ingested during exercise affects thirst sensation, gastric symptoms, and other perceptual responses that may interfere with children's voluntary drinking. In addition, children may wait for the recovery period to fully replace their body water losses.

One trigger for the increasing intensity of thirst sensation is an increase in plasma osmolality. Following dehydration, adults drank more and restored their fluid losses more effectively when plasma osmolality was maintained at higher levels, by ingesting Na⁺ with water (0.45 g per 100 ml), as compared to plain water (Nose et al., 1988a). It is possible that Na⁺ intake during and after exercise, as opposed to plain water, increased the perception of thirst by maintaining the plasma osmolality at an elevated level.

The amount and composition of fluid ingested after prolonged exercise may optimize the rate of rehydration and thus recovery of heart rate and core temperature

(Costill and Sparks, 1973; González Alonso et al., 1992; Nielsen et al., 1986; Gisolfi et al., 1992). Most children in the current project spontaneously overcompensated their body water losses during recovery from exercise-induced mild hypohydration (chapter 4, Figure 4.3). Whether such elevated volume intake occurs when hypohydration is avoided, and whether the drink composition affects the intake, are unexplored issues.

The objectives of this study were: 1. to compare the effect of various CHO-electrolyte solutions on drinking-related and other perceptual responses of euhydrated children during and following intermittent exercise in the heat; and 2. to quantify the voluntary drinking of the various solutions during recovery.

6.2. Methods

This is the same experiment as in chapter 5. A more detailed description of experimental protocol and physiological measurements is found in Section 5.2.

Subjects. A total of 12 children (6 boys, 6 girls) participated in this study which was approved by the McMaster University Ethics Committee. Boys and girls did not differ in age, weight, height, surface area (sa), adiposity, and peak oxygen uptake ($\dot{V}O_2$) (Table 6.1). Children were in good health conditions to participate in this study according to their medical history.

Procedures and Protocol. After explaining the procedures to both the child and a parent and obtaining their verbal consent, a parent signed a consent form (Appendix 8.1.3).

About one week prior to the experimental trials, subjects came for a screening visit in which anthropometric and peak $\dot{V}O_2$ measurements were assessed and a sensory questionnaire was applied.

A sensory questionnaire (Appendix 8.2.3) was applied (before peak $\dot{V}O_2$ test) to evaluate whether the composition of the drinks affected the subjects' drink preferences (9-point-category scale), and their ability to distinguish taste intensities of saltiness and sweetness (analog scales). For this test, a sample of 30 ml of each drink was given, in randomized order, while the child was sitting comfortably in a thermoneutral room.

Each subject then attended four in-chamber sessions, about one week apart, which differed as to the composition of the drinks they ingested. Three of the drinks were 6% carbohydrate (CHO) with different $[Na^+]$: 0 (CHO-0), 8.8 (CHO-8.8), 18.5 (CHO-18.5). One drink had neither CHO nor Na^+ (WATER) (Table 5.1). All drinks had the same grape flavor and color and were assigned in a double-blind design and a Latin-square order.

On the day of each trial, subjects were instructed to eat and drink regularly. Once subjects arrived at the lab, they drank 100 ml of the assigned drink and euhydration was further assured through a questionnaire. Each trial consisted of intermittent exercise in a warm chamber (34-35°C, 42-45% relative humidity). Subjects cycled (Fleish-Ergostat Universel, Metabo) one 20-min (bout 1) and two 15-min (bouts 2 and 3) bouts at 50% of the pre-determined peak $\dot{V}O_2$ (at 50 rpm). In a fourth bout, subjects cycled at 90% peak $\dot{V}O_2$ (still at 50 rpm) for as long as they could sustained the prescribed pace. Before

exercise bout 1 and between bouts, there was a 10-min rest, thus total time in-chamber was at least 105 min. Throughout that period, subjects were kept euhydrated by drinking a set quantity of the assigned drink ($1.8 \text{ ml}\cdot\text{kg}^{-1}$ every 13-15 min).

Rating of perceived exertion (RPE) was evaluated by the Borg 6-20 category scale (Borg, 1972) at the midpoint of bouts 1, 2, and 3 and at the end of bout 1 and 3. In bout 4, RPE was obtained every 2 min. Rating of how warm or cold the subjects felt and their overall comfort was done by asking them periodically to point, on respective charts, at a number corresponding to "How hot or cold do you feel now?" or "How comfortable do you feel now?". These 9- and 5- point category scales ranged from +4 ("very hot") to -4 ("very cold"), and 1 ("comfortable") to 4 ("very uncomfortable"), respectively.

Immediately after each exercise bout, subjects were weighed (Mott electro scale model LC2424), and their sensations of thirst (analog scale) and stomach fullness (category scale) were rated. The subject was instructed to mark an "X" on a horizontal line (146 mm) which showed "How thirsty are you?". This scale had anchor points at the ends and in the middle and only the end points were labelled from the left to the right as "not thirsty" to "very thirsty". Sensation of stomach fullness was assessed through a 1 to 5 category scale ranging from "not full at all" to "extremely full", in response to the question "How full does your stomach feel now?". Any other spontaneous complaints of pain or aches were registered.

Heart rate (HR), Sport-Tester PE3000 system, and rectal temperature (T_{re}), YSI 400 series, were measured periodically throughout the in-chamber period. In bout 4, RPE

and T_{re} were recorded every 2 min, while HR and the number of pedal revolutions were recorded every min. Subjects voided before and after the in-chamber session and after recovery, and urine volume was weighed (Acculab scale, 1200, 0.24 g accuracy).

Recovery. After leaving the chamber, subjects sat comfortably for 30 min in a thermoneutral room (22°C, 60%RH), and were allowed to drink *ad libitum*. The assigned drink was constantly provided and intake was measured, without the subject's knowledge. Perception of thirst intensity and stomach fullness was assessed once, at the end of recovery, and T_{re} and HR in its midway and at the end.

Venous blood was collected before bout 1, and within 2 min after bout 4. Whole blood was analyzed for lactate (YSI 1500 sport L-Lactate analyzer), hemoglobin (hemoximeter-OSM 3), and hematocrit (microhematocrit centrifuge). Blood glucose concentration was determined by enzymatic fluorometric technique (Sigma Chemical Company). Plasma was analysed for $[Na^+]$, $[Cl^-]$ and $[K^+]$ using ion-selective electrodes (Electrolyte Analyzer AVL 983S) and osmolality by freezing point depression (micro-osmometer Model 3MO).

Calculations. Hydration status was calculated as the change in body weight relative to initial body weight, corrected for increase in clothing weight and for urine volume (urine output was assumed to be constant throughout the session). Sweat volume was calculated from changes in body weight, corrected for drink intake, urine output, respiratory water losses (Mitchell et al., 1972), and the increase in the weight of the clothes. Percentage change in plasma volume ($\% \Delta PV$) was calculated from changes in hemoglobin and

hematocrit (Dill and Costill, 1974).

Statistics. Analyses of variance for repeated (time) and nonrepeated measures were used to determine the effect of the drink trial. A t-test was employed when the F-ratio was significant ($p < 0.05$). Results are presented as mean \pm SEM.

6.3. Results

Because there were no gender differences in the subjects' physical, physiological and perceptual characteristics, results for boys and girls were pooled.

Subjects maintained euhydration during the in-chamber period (Figure 6.1, top). Despite identical volumes of intake among drink trials, the WATER trial induced a drop of $0.10 \pm 0.02\%$ of body weight (about 50 ml) from bout 3 to bout 4. This was probably due to larger - but not statistically significant - water losses from combined urine and sweat during this trial. Urine and sweat losses (mean \pm SEM) during CHO-0, CHO-8.8, and CHO-18.5 and WATER trials were: 129 ± 31 , 77 ± 13 , 87 ± 13 and 124 ± 22 ml for urine; and 330 ± 29 , 335 ± 32 , 354 ± 26 , and 375 ± 37 ml for sweat.

On average, subjects felt their stomach "somewhat full" (rating=2) during all trials (Figure 6.1., middle). One subject complained of stomach ache during the CHO-18.5 trial, despite reporting her stomach being just "somewhat full". The perceived intensity of thirst was similar among the drink trials and subjects did not become more thirsty during the in-chamber period (Figure 6.1., bottom). There was a considerable fall

in the intensity of thirst by the end of the voluntary drinking ($p < 0.05$), irrespective of the nature of the drink.

Ratings of perceived exertion were similar among trials (Figure 6.2., top). There was a rise in RPE during bout 1 (from 12.0 to 13.5), and it was even more remarkable during each 2 min of bout 4 (90% peak power). Comfort ratings were similar among sessions and, on average, subjects felt "comfortable" (Figure 6.2., middle). At any given time, they felt equally warm among drink trials (Figure 6.2., bottom). Overall ratings of perceived heat increased from baseline value to the end of each exercise bout: 0.99 ± 0.14 (bout 1), 0.91 ± 0.17 (bout 2), and 0.89 ± 0.17 (bout 3), with no differences among drink trials. None of these perceptual responses were correlated with T_{re} or HR responses within any of the drink trials.

Changes in T_{re} from pre-exercise values (37.6°C) were similar among drink trials during the intermittent exercise (in-chamber) and recovery period (Figure 6.3). Mean increase in T_{re} by the end of bout 4 was 0.42°C , dropping to pre-exercise values within 15 min of recovery and even further (37.4°C) by 30 min of recovery. HR did not differ among drink trials, and it remained quite constant during the first 3 exercise bouts (144 ± 3 bpm). Performance time of the last bout averaged 5.4 ± 0.56 min (range 1.2 to 15.5 min), with no differences among drink trials.

There were no changes in plasma $[\text{Na}^+]$ or $[\text{Cl}^-]$ in any of the drink trials. On average, there was an increase in plasma $[\text{K}^+]$ ($3.8 \pm 1.42\%$), with no difference among drink trials. Plasma osmolality did not change in any of the drink trials. Respective pre

and post values (mean \pm SEM, in mOsm \cdot l $^{-1}$) in each drink trial were: 289 \pm and 287 \pm 2 (CHO-0), 288 \pm and 292 \pm 2 (CHO-8.8), 292 \pm 4 and 289 \pm 2 (CHO-18.5), and 289 \pm 2 and 286 \pm 2 (WATER). There was a similar increase of 1.6 mEq \cdot l $^{-1}$ in blood lactate in all trials. Blood glucose concentration did not change significantly from rest (5.19 \pm 1.13 mmol \cdot l $^{-1}$) to the end of the trials (4.85 \pm 0.14 mmol \cdot l $^{-1}$), nor was there any effect of drink trials. Similar changes in % Δ PV occurred among drink trials: -6.3 \pm 1.5% (CHO-0), -6.4 \pm 1.6 (CHO-8.8), -7.6 \pm 1.5 (CHO-18.5), and -6.3 \pm 2.0 (WATER).

Voluntary drinking during recovery. Total volume intake during the 30-min recovery was similar among drinks (201 \pm 27.1 ml), although during the first 10 min subjects drank more CHO-8.8 than CHO-18.5 (Figure 6.4).

During the screening visit, subjects preferred the taste of WATER as compared to CHO-18.5 ($p < 0.05$) even though they could not discriminate the drinks by their [Na $^{+}$] or CHO content since the saltiness and sweetness intensity did not differ among drinks (Figure 6.5).

6.4. Discussion

The aim of this study was to examine the effect of drinks with various Na $^{+}$ (up to 18.5 mEq \cdot l $^{-1}$) and CHO (none vs 6%) contents on perceptual responses of children during intermittent exercise in the heat, and to quantify voluntary drinking during recovery. While subjects were exercising, drinks had no effect on thirst and stomach

fullness sensations, nor on perceived exertion, thermal and comfort ratings. Voluntary drinking during recovery was similar among drinks.

Independent of the drink trial, subjects did not become significantly more thirsty during the in-chamber period. This response could be expected since, in this study, Na⁺ ingestion was not accompanied by an increase in plasma osmolality (or [Na⁺]) which is a mechanism involved in triggering thirst (Engell et al., 1987; Rolls et al., 1980). Decreased body water volume is another way by which thirst is activated (Greenleaf, 1982; Engells et al., 1987). Note that during the WATER trial, as body hydration levels tended to drop (in-chamber), thirst sensation tended to increase (Figure 6.1). The analog scale used to assess thirst intensity seemed to be a fairly sensitive method to evaluate changes in thirst sensation as children gradually dehydrated or rehydrated (Meyer et al., accepted).

The finding that sensation of stomach fullness was similar among drinks might imply that drink composition did not affect the rate of gastric emptying. Based on studies with adults, solutions containing up to 8% of CHO have similar rates of gastric emptying (Coyle and Montain, 1992). However, increased perception of stomach fullness does not necessarily indicate a slower gastric emptying. In a recent study with adults (Zachwieja et al., 1992), gastro-intestinal comfort sensations were similar between drinks that caused a 13.3% difference in the rate of gastric emptying.

Ratings of perceived exertion were similar among drink trials. This is in agreement with studies performed in adults in that RPE did not differ between water and

a CHO-electrolyte solution while they were running (Wells et al., 1985); or between water and an electrolyte solution while cycling (Barr et al., 1991). However, increase in RPE was significantly greater when no fluid was replaced (Barr et al., 1991). In the present study, the somewhat low increase in RPE during the first three bouts (50% $\dot{V}O_{2max}$) is probably because children maintained euhydration, despite being in the warm environment. During this period, RPE levelled off at about 13 ("somewhat hard") which is a similar rating to that given by children while cycling at a similar intensity in a thermoneutral environment (Bar-Or and Reed, 1986), but about 2 points lower to that given by boys while cycling at 55% $\dot{V}O_{2max}$ in a heat of 43°C and 20% RH (Bar-Or and Inbar, 1977). In adults who cycled for one hour at various intensities (48, 60 and 68% of $\dot{V}O_{2max}$), RPE was not affected by increasing environmental temperature (24°, 44°, and 54° C) (Noble et al., 1973). In the present study RPE rose sharply only when exercise intensity increased to 90% peak $\dot{V}O_{2max}$.

Maintenance of euhydration is probably another reason why subjects in the present study did not feel very hot (Figure 6.2) and did not feel uncomfortable. In adults, hypohydration speeds the rate of core temperature elevation (Sawka, 1992), in addition to reducing the core temperature at which exhaustion from heat strain occurs (Sawka et al., 1992).

During the voluntary drinking, the total volume intake was similar among drink trials (201 ml, 4.5 ml·kg⁻¹). This volume is at least 2.5 times smaller (500 ml, 12 ml·kg⁻¹) than that consumed after children dehydrated mildly (0.7%) from intermittent exercise

in the heat (chapter 4). By the end of 30-min, volume intake was such that an 0.4% increase of initial body weight (pre-exercise) occurred, as opposed to a 0.6% increase when children dehydrated (chapter 4). Thirst triggering and increased drink preferences may explain why children drank relatively more following exercise that induced hypohydration.

In the present study, thirst intensity at the end of the last bout and volume intake during recovery were mildly correlated ($r=0.51$). There was, however, some variability among drink trials, in that thirst intensity and volume intake were strongly correlated for the Na^+ -free drinks ($r=0.70$ and 0.86 with WATER and CHO-0, respectively), but not for CHO-8.8 and CHO-18.8 ($r=0.39$ and 0.01 , respectively). This indicates that, during rehydration, volume intake may also depend on increase of salt appetite as recently reviewed by Nadel et al. (1993). It was previously found (chapter 4) that during exercise-induced mild hypohydration, children's degree of drink liking increased the most for the drink with the highest $[\text{Na}^+]$ ($18.5 \text{ mEq}\cdot\text{l}^{-1}$). Since we did not measure rate of salt appetite and sequential plasma $[\text{Na}^+]$ in the present study, it is hard to confirm the role of salt palatability on voluntary fluid intake during recovery in children.

Voluntary intake during recovery did not seem to be related to the subjects' taste preferences which were evaluated in the screening visit. Subjects preferred WATER as compared to CHO-18.5, even though they could not distinguish among sweetness and saltiness of the drinks. Thus, it is possible that a greater increase in the desirability of CHO-18.5 occurred during the in-chamber period, as it did in the previous study (chapter

4), resulting in similar drink intakes.

In conclusion, when children cycled intermittently in the heat for approximately two hours, intake of either water or CHO-electrolyte drinks (CHO=6% and $[\text{Na}^+]$ up to $18.5 \text{ mEq}\cdot\text{l}^{-1}$) did not affect intensity of thirst or stomach fullness sensations, nor any other perceptual variables. Drink composition had no effect on the voluntary drinking following the in-chamber session.

TABLE 6.1 Physical Characteristics of the Subjects (mean \pm SD).

Variable	Boys (n=6)	Girls (n=6)
Age (years)	11.0 \pm 1.1	11.0 \pm 1.3
Weight (kg)	40.5 \pm 4.14	48.4 \pm 18.0
Height (cm)	145.4 \pm 5.14	153.7 \pm 18.0
SA (m ²)	1.28 \pm 0.06	1.44 \pm 0.28
SA/weight (cm ² ·kg ⁻¹)	317 \pm 20	317 \pm 61
Fat (%)	19.1 \pm 5.10	22.4 \pm 9.8
Peak $\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)	43.8 \pm 5.43	37.8 \pm 8.24

SA = surface area

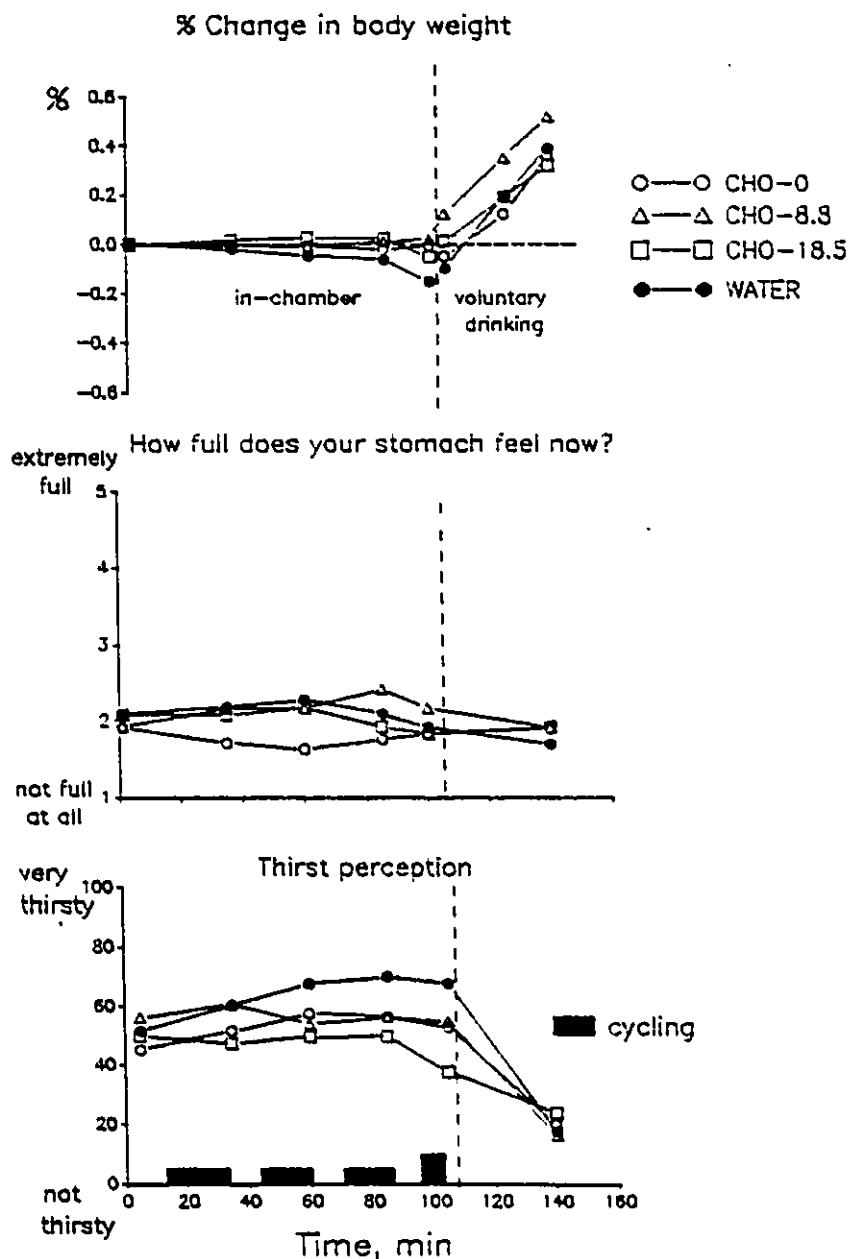


FIGURE 6.1. State of Body Hydration and Perceived Intensity of Stomach Fullness and of Thirst.

Vertical line denotes end of in-chamber period. Values are means. Drink codes starting with CHO- indicates presence of 6% carbohydrate followed by the drink [Na⁺]. WATER is the drink with neither CHO or [Na⁺].

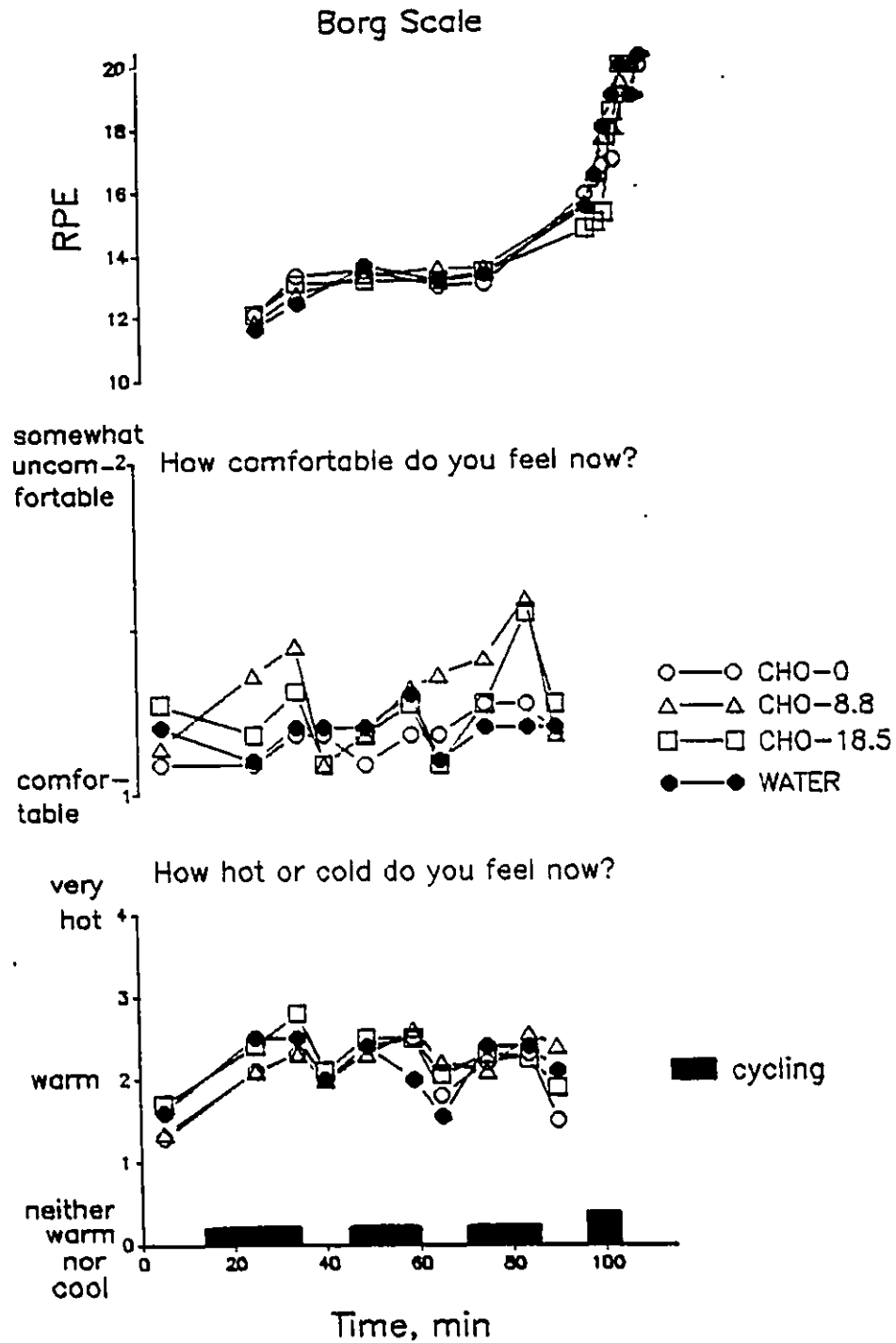


FIGURE 6.2. Subjective Ratings of Perceived Exertion (RPE), General Comfort, and Perception of Heat and Cold.

Values are means. Drink codes are the same as in Figure 6.1.

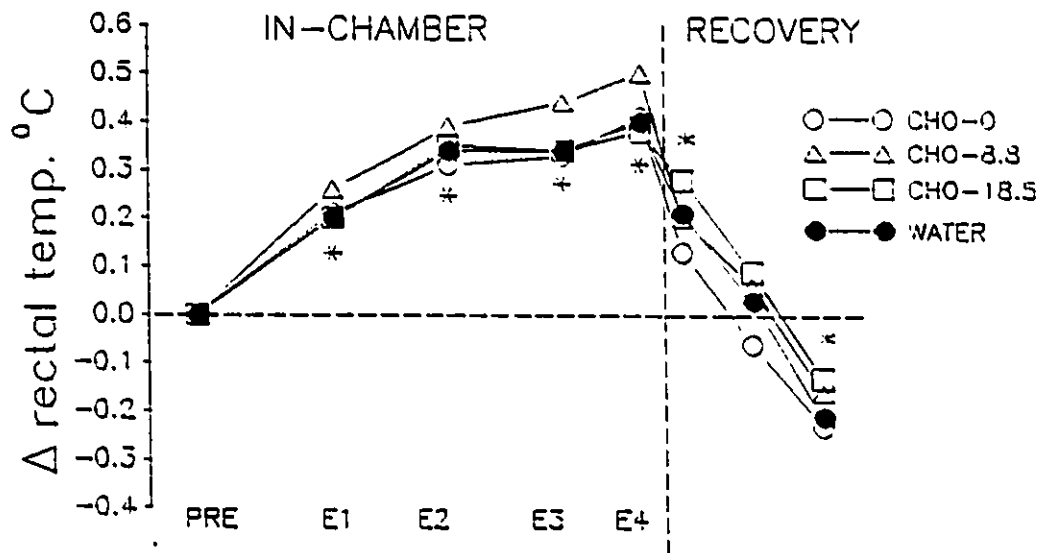


FIGURE 6.3. Changes in T_{re} from Pre-Exercise (PRE) at the End of Each Exercise Bout (E), and during Recovery (R).

E4= T_{re} when subjects stopped pedaling minus PRE. There was a ten min interval between E4 and R0. Values are Means.

Voluntary drinking during recovery

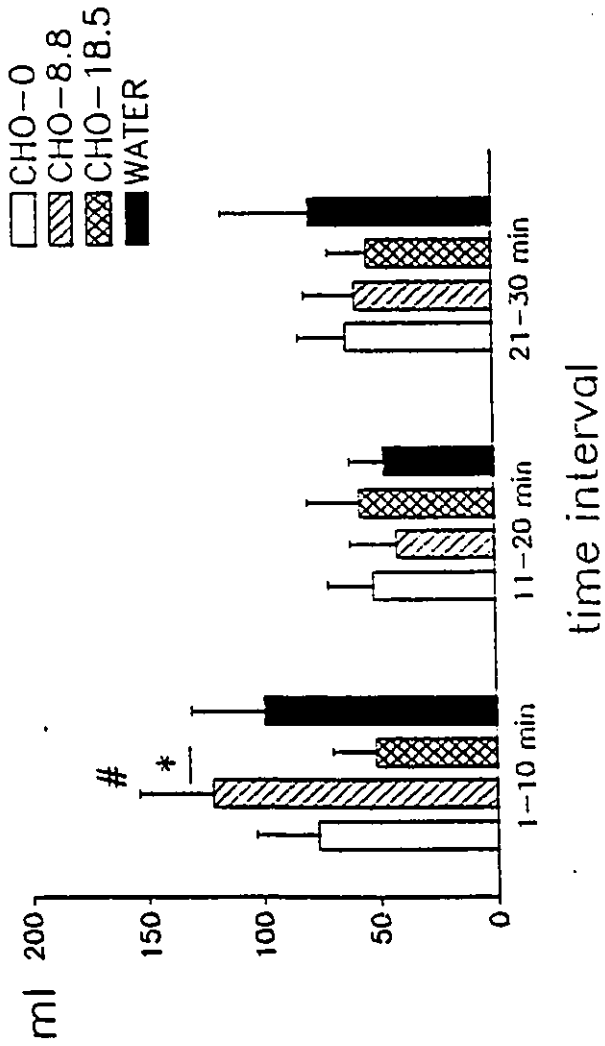


FIGURE 6.4. Volume Intake during Voluntary Drinking.
 * > CHO-18.5, at this interval, # > other intervals with this drink ($p < 0.05$).
 Mean \pm SEM. Drink codes are the same as in Figure 6.1.

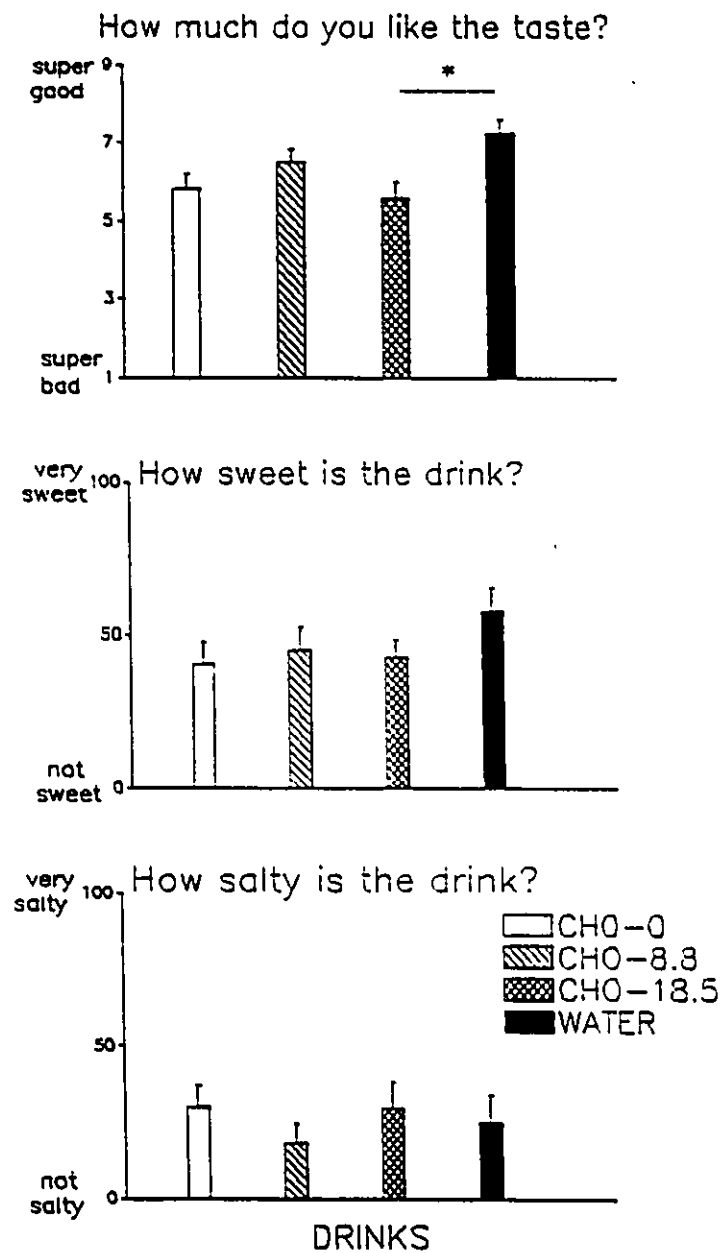


FIGURE 6.5. Drink Preferences and Taste Qualities Measured in the Screening Session.

* $p < 0.05$. Mean \pm SEM. Drink codes are the same as in Figure 6.1.

CHAPTER 7

CONCLUSIONS, AND DIRECTIONS FOR FUTURE RESEARCH

These studies explored physiological and perceptual mechanisms that would help establishing guidelines for oral fluid replenishment for children who exercise in the heat. First, children's sweat and urine electrolyte losses were compared to those of adults, to determine whether one might expect differences in specific fluid-electrolyte requirements during exercise. Second, to identify factors that could increase children's drink acceptability, the changes in thirst and taste preferences for different drinks were examined at rest and while children gradually dehydrated during exercise in a warm environment. Finally, the effects of ingesting various CHO-electrolyte solutions on thermoregulation and performance were examined in children who were kept euhydrated during intermittent exercise in the heat. The following conclusions can be drawn:

1. When children (pre-pubescent or pubescent) and adults, both males and females, underwent similar heat/exercise stresses:

- Girls and boys had a lower sweating rate than their adult counterparts.
- The total Na^+ loss from sweat ($\text{mEq}\cdot\text{kg body wt}^{-1}$) was twofold greater in the adults than in children. Differences were even greater for the sweat Cl^- loss.
- The total sweat losses of K^+ , lactate and ammonia ($\text{mEq}\cdot\text{kg}\cdot\text{body wt}^{-1}$) were similar

among maturational and gender groups.

- Overall, women lost less Na^+ , Cl^- and K^+ from urine than girls and less Na^+ and Cl^- than men.

- Combined sweat and urine losses tended to increase with maturation in the males.

2. When children gradually dehydrated in four separate trials (one flavored drink in each) by exercising in the heat with a limited fluid supply:

- By the end of 90-min of intermittent exercise, they dehydrated up to 0.7% and the mean increase in rectal temperature was 0.5°C. without differences among drinks.

- There was an increase in the intensity of thirst sensation, independent of the type of drink. The degree of thirst started increasing as early as at the end of the first exercise bout, when body fluid loss was still minimal (0.03%).

- The grape-flavored drink remained the drink of choice, in agreement with the preferences ratings evaluated in the non- exercise state.

- While the preference ratings of orange, apple and water drinks gradually increased, it remained constant for the grape drink, suggesting a ceiling effect. Orange drink yielded the highest increase in preference ratings from the baseline values.

- Independent of the drink, there was no change during dehydration in the perception of taste intensity, saltiness, sweetness, or sourness.

3. When children could drink *ad libitum* during recovery from exercise-induced hypohydration:

- Most children drank enough, not only to recover, but also to overshoot by ~0.1% their initial body weight. This occurred with all drinks but at a greater magnitude with those most desirable at peak dehydration: grape and orange .
- Thirst perception gradually decreased, at a similar rate, in all drinks.

4. When children exercised intermittently in the heat and were kept euhydrated by drinking in four separate occasions WATER or one of three CHO solution with different $[\text{Na}^+]$: 0, 8.8 and 18.5 mEq·l⁻¹:

- By the end of 95 min of intermittent exercise, mean increase in rectal temperature was 0.36°C, without differences among drinks.
- Drink composition did not affect aerobic performance (constant-rate exercise to exhaustion).
- Urine $[\text{Na}^+]$ decreased with all drinks, but at a lower magnitude with Na^+ -free drinks.
- Drink composition did not affect total sweat or urinary electrolyte losses.
- A negative Na^+ balance occurred with all drinks, but with a greater magnitude with ion-free drinks. Such Na^+ deficit differences were, however, of minor biological importance since they represented less than 1% of the total exchangeable Na^+ in the extracellular space.
- Na^+ intake did not affect plasma $[\text{Na}^+]$.

- On average, plasma glucose tended to decrease, but post-exercise values ($\sim 4.8 \text{ mmol}\cdot\text{l}^{-1}$) did not differ among drinks .
- Plasma glycerol increased in all drink trials. The extent of such an increase was greater with WATER than in all CHO-drinks (317 vs 215%), suggesting that a greater lipolysis during the WATER trial.
- The intensity of thirst sensation did not change, independent of drink composition.
- Drink composition did not affect stomach fullness sensations, perceived exertion, or perceived thermal and comfort ratings.

5. When children could drink *ad libitum* during a 30-min recovery from exercise that did not induce hypohydration:

- Average volume intake was 2.5 times smaller than that consumed after children mildly (0.7%) dehydrated from intermittent exercise in the heat.
- Voluntary fluid intake was not affected by the drink composition, nor was it related to the drink preferences.

Based on these findings, guidelines regarding fluid replacement for children who exercise in the heat can become more specific. Drinking should be encouraged during prolonged exercise. The amount calculated ($1.8 \text{ ml}\cdot\text{kg}^{-1}$ every 15 min) to keep children euhydrated is adequate when exercise is moderate (45-50% $\dot{V}O_{2\text{max}}$) and performed in a dry heat. A higher intake should be considered if the child is heat acclimatised and/or

performing higher exercise intensities, or exposed to greater climatic heat stresses. Euhydration will more likely be maintained with grape flavored drinks, compared to orange, apple and water. Addition of CHO (4% fructose, 2% sucrose) and Na⁺ (up to 18.5 mEq·l⁻¹) does not affect thermoregulatory responses or performance of children who are kept euhydrated during intermittent exercise (50% $\dot{V}O_2$ max) that lasts less than two hours.

Sports drinks which generally contain CHO and electrolytes are now very popular. These drinks are designed for adults, however consumption is probably promoted among children by attractive advertisements such as "game over for water boys". Although sports drinks do not seem to do any harm to children, it is still undetermined whether they are better than water. Under the conditions of the present studies (chapters 5 and 6), CHO-electrolyte drinks did not show any physiological or perceptual benefit compared to water. More important than drink composition, the experiments that maintained children euhydrated (chapter 5) tended to attenuate the increase in rectal temperature, compared to those when children dehydrated (chapter 4).

To more specifically set guidelines on fluid replacement for children, the following questions should be considered. The effect of drink composition and flavoring on the voluntary drinking of children exercising in the heat has not yet determined. When plain water is available *ad libitum*, children dehydrate about 1% within three hours of intermittent exercise (Bar-Or et al., 1980, 1992). Preliminary results from an on-going project at the Children's Exercise and Nutrition Centre suggests that children

do not dehydrate when grape-flavored drinks (either water or CHO-electrolyte drinks, as in chapter 5) are available for them *ad libitum*.

It will be of interest to evaluate the need for Na⁺ and CHO intake when children exercises for a longer time (>2 hours) in the heat. In the chapter 5 study, total Na⁺ deficit was higher with Na⁺-free drinks, but such a negative balance had no biological significance. It is unknown whether more prolonged activities in warm environments, combined with excessive water intake, will reduce plasma [Na⁺] to levels of clinical concerns. Based on this same study, CHO ingestion did not improve performance after 95-min of intermittent exercise in the heat. Results also suggested a compensatory response by increasing fat utilisation, especially when no CHO was ingested. Whether children would benefit from CHO ingestion when exercising for a longer time, and whether they use relatively more fat compared to adults is undetermined.

Another unexplored topic is the regulation during exercise of fluid availability at the gastro-intestinal level in children. Even if CHO ingestion fails to show any improvement in performance, it is possible that when CHO is combined with Na⁺, intestinal water absorption will be optimized, as shown in adults. This possibility has yet to be confirmed. It is also unknown whether the same drink- and exercise-related factors that affect gastric emptying rate in adults are true for children. Considering the ethical issues of doing research with children, some studies might be postponed until less invasive methods of measuring intestinal absorption and gastric emptying are available.

Another consideration in formulating a sport drink for children is the appropriate selection of "inert ingredients" such as sweeteners, flavorings and dyes. Although rare, adverse effects may occur from these ingredients. For example, aspartame has been reported to cause allergic reactions (Kumar et al., 1993). Discriminate labelling of these ingredients should therefore be mandatory.

The finding that most children spontaneously overhydrated, especially following exercise that induced dehydration, suggests that guidelines on fluid composition should be extended to the recovery period, especially for those who tend to overhydrate.

The above studies were performed in North America, with healthy Caucasians and heat unacclimatised children. Conclusions are therefore restricted to such subjects. Fluid replenishment guidelines for children who live in tropical places, or those with other conditions such as cystic fibrosis, or diabetics have yet to be determined.

CHAPTER 8

APPENDICES

8.1. Consent Forms

8.1.1. Studies 1 and 2 (Chapters 2 and 3)

SWEAT ELECTROLYTES: RELATIONSHIP TO GENDER AND MATURATION

I, _____, consent to allow my son/daughter _____ to participate in a study designed to collect his/her sweat in a warm environment of a climatic chamber. Dr. Oded Bar-Or (521-2100 ext 7615), the principal investigator, or Flavia Meyer, the co-investigator, has explained that my child will be invited to the laboratory for 2 visits.

FIRST VISIT: Physical examination by a physician, height, weight and skinfolds thickness. An exercise test on a cycle ergometer to determine maximal aerobic power (breathing through a mouthpiece) and muscle power will be performed.

SECOND VISIT: My son/daughter will be asked to perform two 20-minute moderate exercise bouts on a cycle ergometer, with 5-10 minute rest in between. This will be done in a climatically controlled room where the temperature will be warm (41-43° C), and the humidity low (15-20% relative humidity). Body temperatures, heart rate and body weight will be measured periodically.

A sample of 8-10 ml (volume of 2 teaspoons) of venous blood and a urine sample will be collected at the beginning and at the end of this session. In the climatically controlled room, the heart rate will be monitored through two electrodes attached to the child's chest. Skin temperature will be determined by applying a thermometer on 3-4 sites, and rectal temperature will be monitored by a flexible plastic thermometer.

No known harmful effects occur during or following any of the above observations. Following the session in the climatically controlled room, my son or my daughter may feel tired and warm for 30-60 minutes. I understand that there are no direct benefits to my child to take part of this study. I further understand that my child can withdraw at any time from participation in this study, even after I have signed this form, without in any way affecting the care given to him. Any information which is collected will be kept confidential, and will not identify my child in any way. This is also true if results are published.

NAME(print)

SIGNATURE

DATE

WITNESS(print)

SIGNATURE

DATE

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

INVESTIGATOR(print)

SIGNATURE

DATE

8.1.2. Study 3 (Chapter 4).

CHILDREN'S TASTE PREFERENCES OF DIFFERENT DRINKS DURING REST AND EXERCISE.

I, _____, consent to allow my son/daughter _____ to participate in a study designed to test his/her taste preferences during rest and exercise in a warm environment. Dr. O. Bar-Or (521-2100 X 7615), the principal investigator, or F. Meyer, the co-investigator, has explained that my child will be invited to the laboratory for 5 visits.

VISIT I: The visit will include:

- a) Physical examination by a physician, assessment of height, weight and skinfolds thickness.
- b) At rest, my son/daughter will drink a sample of four different common beverages. Following each sample he/she will answer a questionnaire related to his/her taste preferences.
- c) Aerobic fitness test: An exercise test on a stationary bicycle to determine maximal aerobic power (breathing through a mouthpiece). During this test, heart rate will be monitored (two small electrodes will be attached to the chest to monitor heart rate).

VISITS II, III, IV and V: During these sessions my son/daughter will perform several 15-min cycling tasks with 15-min resting between for a total of 60-90 minutes, in a warm room (30-35 degrees C, 50-60% relative humidity). Drinking will be restricted during this period in a way that he/she will gradually lose up to 2-2.5% of the initial body weight (a similar water loss to that reached during an afternoon activities in a play ground). After that, the taste preference and drinking pattern of my child will be observed while he/she is resting comfortably for additional 30 min. A flexible disposable rectal thermometer will be used to measure his/her body temperature throughout each visit. The interval between visits will be approximately one week.

I understand that no harmful effects occur during and following any of the above observations. Following the session in the warm room, my son/daughter may feel tired, thirsty and warm for 30-60 minutes. I understand that there are no direct benefits to my child from taking part of this study, even after I have signed this form, without in any way affecting the care given to him. Any information which is collected will be kept confidential, and will not identify my child in any way. This is also true if results are published.

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

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

_____ INVESTIGATOR(print)	_____ SIGNATURE	_____ DATE
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8.1.3. Studies 4 and 5 (Chapters 5 and 6).

CHILDREN'S RESPONSES TO DIFFERENT BEVERAGES WHILE EXERCISING INFORMATION SHEET

Drinking fluids in sufficient amounts and proper content is important for the well-being and performance of children who exercise in a hot climate. A team of investigators at the Children's Exercise and Nutrition Centre (McMaster University) is about to study the effects of various beverages, similar to those available commercially, on children, who rest and exercise in a warm environment. The study will include four visits to the laboratory at Chedoke Hospital.

VISIT I: This visit will include: Physical examination by a physician, assessment of height, weight and % body fat. An exercise test on a cycle ergometer (8-12 min) to determine maximal aerobic power (breathing through a mouthpiece) will be performed.

VISITS II, III, IV and V: During these sessions the child will perform three cycling tasks, with a 15-min rest period in between, in a warm room (30-32 degrees C, 50-60% relative humidity).

These conditions reflect a typical hot day in Southern Ontario. The first 3 tasks will be at a moderate intensity lasting 20 min each, the 4th will be more intense, lasting as long the child can sustain the effort (about 5-10 min). The child will be given one of the four beverages periodically during each session, in order to prevent body fluid losses. Body temperatures, heart rate and body weight will be measured periodically. A sample of 5 ml (volume of one teaspoon) of venous blood and a urine sample will be collected at the beginning and at the end of this session. Sweat will be collected from a small plastic bag attached to the lower back. The heart rate will be monitored through two electrodes placed on the chest. Skin temperature will be determined by applying a thermometer to 3 skin sites, and rectal temperature will be monitored by a flexible plastic thermometer. The interval between visits will be about one week.

I, _____, consent to allow my son/daughter _____, to participate in a study designed to test his/her physiologic responses while exercising in a warm environment, and drinking various beverages. Dr. O. Bar-Or (521-2100 X7615), the principal investigator, or F. Meyer(521-2100 X 7259), the co-investigator, has explained that my child will be invited to the laboratory for 4 visits, as outlined in the information sheet overleaf. I understand that no known harmful effects occur during or following the above observations. However, the child may feel tired and hot for 1-2 hours after the session. A slight bruising from the site of the blood taking may occur. I understand that there are no direct benefits to my child from 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 which is collected will be kept confidential, and will not identify my child. 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 child's parent and believe he/she understood it.

INVESTIGATOR(print)

SIGNATURE

DATE

8.2. Taste Questionnaires

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8.2.1. Taste Questionnaire at Rest

BV182
Drink Order _____
ID Number _____

NAME _____ DATE _____

PRODUCT CODE _____

Before drinking, please place an X on the line to show how thirsty you are.

Not _____ Very
Thirsty _____ Thirsty

Now, taste the beverage by taking 2 or 3 sips.

Please answer these questions. (Take additional sips as needed.)

How much do you like the COLOR?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

How much do you like the TASTE?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

How much do you like this drink OVERALL?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

CONTINUE GRADING YOUR SAMPLE ON PAGE 2

BV 182 - PAGE 2

Please check the box which says how you feel about this drink.

<u>TASTE</u>	<u>SOURNESS</u>	<u>SWEETNESS</u>
<input type="checkbox"/> Too Strong	<input type="checkbox"/> Too Sour	<input type="checkbox"/> Too Sweet
<input type="checkbox"/> Just Right	<input type="checkbox"/> Just Right	<input type="checkbox"/> Just Right
<input type="checkbox"/> Not Strong Enough	<input type="checkbox"/> Not Sour Enough	<input type="checkbox"/> Not Sweet Enough

This drink is for (check one box only)

- Me Only
 Me and My Parents
 My Parents Only

How likely would you be to ask your parent to buy this drink for you?

- Definitely Would Ask For It
 Probably Would Ask For It
 Don't Know
 Probably Would Not Ask For It
 Definitely Would Not Ask For It

CONTINUE GRADING ON PAGE 3 . . .

BV 182 - Page 3

Place an X on the line to show how strong in taste the drink is:

Weak Taste _____ Strong Taste

Place an X on the line to show how sweet the drink is:

Not Sweet _____ Very Sweet

Place an X on the line to show how salty the drink is:

Not Salty _____ Very Salty

Place an X on the line to show how sour the drink is:

Not Sour _____ Very Sour

8.2.2. Taste Questionnaire during Exercise

BV 188
Drink Order _____
ID Number _____

NAME _____ DATE _____

PRODUCT CODE _____

Before drinking, please place an X on the line to show how thirsty you are.

Not Thirsty _____ Very Thirsty

Now, taste the beverage by taking 2 or 3 sips.

Please answer these questions. (Take additional sips as needed.)

How much do you like the TASTE?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

How much do you like this drink OVERALL?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

CONTINUE GRADING YOUR SAMPLE ON PAGE 2

BV 189 - PAGE 2

Please check the box which says how you feel about this drink.

<u>TASTE</u>	<u>SOURNESS</u>	<u>SWEETNESS</u>
<input type="checkbox"/> Too Strong	<input type="checkbox"/> Too Sour	<input type="checkbox"/> Too Sweet
<input type="checkbox"/> Just Right	<input type="checkbox"/> Just Right	<input type="checkbox"/> Just Right
<input type="checkbox"/> Not Strong Enough	<input type="checkbox"/> Not Sour Enough	<input type="checkbox"/> Not Sweet Enough

Place an X on the line to show how strong in taste the drink is:

Weak Taste _____ Strong Taste

Place an X on the line to show how sweet the drink is:

Not Sweet _____ Very Sweet

Place an X on the line to show how salty the drink is:

Not Salty _____ Very Salty

Place an X on the line to show how sour the drink is:

Not Sour _____ Very Sour

Check the box which says how your stomach feels.

- No Stomach Upset
- Mild Stomach Upset
- Severe Stomach Upset

8.2.3. Taste Questionnaire at Rest for Study in Chapter 5

BV 272
Drink Order _____
ID Number _____

NAME _____ DATE _____

PRODUCT CODE _____

Before drinking, please place an X on the line to show how thirsty you are.

Not Thirsty _____ Very Thirsty

Now, taste the beverage by taking 2 or 3 sips.

Please answer these questions. (Take additional sips as needed.)

How much do you like the TASTE?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

How much do you like this drink OVERALL?

Super Good	Really Good	Good	OK Nothing Special	Maybe I Like it Maybe I Don't	Not Too Bad	Bad	Really Bad	Super Bad Yuk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Place an X on the line to show how sweet the drink is:

Not Sweet _____ Very Sweet

Place an X on the line to show how salty the drink is:

Not Salty _____ Very Salty

Place an X on the line to show how sour the drink is:

Not Sour _____ Very Sour

CHAPTER 9

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