EFFECTS OF HYPOGLYCEMIA ON HUMAN THERMOREGULATION

by

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ABSTRACT

The effect of hypoglycemia on autonomic thermoregulatory responses and thermal perception was examined in ten male subjects who participated in the study on two different occasions. On one occasion they were rendered euglycemic (blood glucose 5.0 mM) and on the other hypoglycemic (blood glucose 2.8 mM), using the hyperinsulinemic glucose clamp technique. In both cases they were immersed in a 28 °C water and exercised on an underwater cycle ergometer for twenty minutes at 50% of their maximum work rate (exercise phase). Thereafter they remained immersed for an additional 99 minutes (cooling phase). During the exercise phase, skin temperature (Tsk) and the change in esophageal temperature relative to resting values (ΔTes) were similar for the euglycemic and hypoglycemic conditions. Oxygen uptake ($\dot{V}O_2$) was higher during exercise in the euglycemic compared with the hypoglycemic condition ($p \leq 0.004$). Skin flux from the skin ($\dot{Q}$) and and skin blood perfusion (SkBP) values were also higher in the euglycemic condition compared to the hypoglycemic condition during the last 5-6 minutes of exercise ($p \leq 0.04$). Passive vasodilation and sweating were initiated at similar ΔTes values and the gain of their response was unaffected by hypoglycemia. In the cooling phase, the Tsk, $\dot{Q}$ and SkBP were similar in the two conditions. ΔTes was greater ($p \leq 0.005$) during the hypoglycemic than in the euglycemic condition. Hypoglycemia caused a greater decrease in Tes ($p \leq 0.001$) by shifting the ΔTes threshold for onset of shivering from -0.09 ± 0.07 °C in the euglycemic condition to -0.65 ± 0.12 °C in the hypoglycemic condition ($p \leq 0.001$). Hypoglycemia did not affect subjective thermal perception during the exercise-induced mild hyperthermia, but decreased the sensitivity of thermal perception during mild hypothermia ($p \leq 0.02$). The present results indicate that hypoglycemia (2.8 mM) does not affect either the thermal perception or the autonomic thermoregulatory responses activated during exercise-induced hyperthermia. In contrast, it decreases the sensitivity of thermal perception and the ΔTes threshold for shivering during mild hypothermia. Lack of a similar effect of

hypoglycemia upon the thermoregulatory defence responses activated during mild hyperthermia versus those activated during mild hypothermia indicates separate control for these different thermoregulatory responses. In conclusion, hypoglycemia does not interfere with thermal balance during exercise-induced mild hyperthermia, but may predispose subjects to hypothermia when exposed to a relatively high heat loss environment, while are not exercising.

DEDICATION

To my parents, Christo and Pipitsa,

whose unconditional love and moral support illuminates my way to "Ithaka".

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PREFACE

The present thesis examines the effects of hypoglycemia on thermoregulatory autonomic responses and thermal perception. The underlying rationale, sources of inspiration and the aims of the thesis are presented in Chapter *One* (Introduction). On a general level, the aim is to reveal some aspects of the multi-faceted interaction between the thermoregulatory and glucoregulatory systems as the interaction appears at the whole-body level of physiological function. Therefore, a review of thermoregulation and glucoregulation is provided in Chapter *two* (Literature Review), with particular focus on studies examining the interaction between these two homeostatic systems.

Results of the experimental study designed to address specific issues are reported in Chapters *three*, *four* and *five* :

Chapter *three*: Effects of hypoglycemia on thermoregulation during exercise-induced mild hyperthermia;

Chapter *four*: Effects of hypoglycemia on thermoregulation during immersion hypothermia;

Chapter *five*: Thermal perception during hypoglycemia.

The findings of these studies are summarized in Chapter *Six*.

CHAPTER ONE: INTRODUCTION

STATEMENT OF PROBLEM-RATIONALE

Studies in mammalian thermoregulation have revealed a great deal of information regarding the location and response characteristics of thermosensors, as well as the nature of the physiological mechanisms by which body heat loss and production can be varied to maintain body temperature constant. The relationships between central and peripheral thermal disturbances imposed on the thermoreceptors and the thermoregulatory responses activated to achieve thermoregulation have also been documented.

However, the mechanisms which exist within the central nervous system (CNS), linking the afferents from the thermal receptors with the efferents to the effector organs, are not clear.

It has been suggested that thermoregulatory integration takes place simultaneously at many different levels within the CNS (Satinoff, 1978). Nevertheless, the main site of integration seems to be the preoptic area anterior hypothalamus (PO/AH). Different suggestions regarding the mechanism(s) underlying thermoregulatory integration have been proposed (Hardy, 1961; Hammel *et al.*, 1963; Bligh, 1984; Boulant and Dean, 1986). However, the specific "neural foci" within the hypothalamus, or the central structures which participate in the integration of thermal information within the CNS, remain a "black box" with regard to our appreciation of body temperature regulation.

Non-thermal factors and thermoregulation

One of the main problems in understanding thermoregulation resides in the great number of non-thermal factors which can influence the sensor-to-effector pathways by acting synaptically on them (Bligh, 1984). However, it seems that central thermosensitive structures can be affected by a variety of endogenous non-thermal factors, in addition to synaptic inputs from afferent pathways (Boulant and Dean, 1986).

A very common observation in physiological studies is that different homeostatic systems use the same physiological effector mechanisms in their operation. A typical example of such an overlap is the use of the peripheral vasomotor tone for the regulation of both body temperature and arterial blood pressure. It is therefore likely that physiological overlaps take place not only at the effector level, but also at different levels of a homeostatic reflex loop. Namely, neurons of the PO/AH, in addition to temperature regulation, could also play a role in the regulation of other variables, such as body water, metabolite and hormonal levels and sexual behaviour.

In fact, it has been shown that PO/AH neurons are sensitive to changes in temperature, osmotic pressure, glucose concentration, and the concentration of circulating reproductive steroids, testosterone and estrogen (Baker and Doris, 1982; Silva and Boulant, 1984). Boulant (1980) suggested that PO/AH neurons are characterized by functional specificity, that is to say that, the temperature sensitive neurons of this area are responsible for much of the regulation of body temperature, whereas the temperature insensitive neurons participate in the control of other regulatory systems.

Interestingly, the work by Silva and Boulant (1984) demonstrated existence of a interelationship between thermoregulation and other regulatory systems, which contradicts the notion of functional specificity among PO/AH neurons. Using a tissue slice technique, they investigated the effect of low glucose and/or hyperosmotic media on the firing rate of temperature sensitive and temperature insensitive neurons in the PO/AH. These experimental perfusions affected one-third of the temperature insensitive neurons and nearly half of the thermosensitive neurons.

In addition to ruling out functional specificity of PO/AH neurons, the important finding of the latter studies is that it is the population of thermosensitive neurons rather than temperature insensitive neurons, which contains the majority of the osmosensitive, glucosensitive and steroid sensitive neurons. This indicates that the basis for the interaction between different regulatory systems could be neuronal. Particularly for

thermoregulation, this is evidence that central thermosensitive structures may be affected by a variety of endogenous non-thermal factors in addition to synaptic inputs from afferent pathways. Furthermore, it may suggest that temperature can affect the regulation of other homeostatic systems.

Thermoregulatory research has, to date, focused primarily on the manner in which thermal afferent information is integrated to initiate appropriate responses for maintainance of thermal balance. In many circumstances, integration of thermal afferent information alone, can not explain the effector responses. It is now becoming evident that non-thermal factors play an important role in modulating thermal regulation. It is proposed that this may be achieved in two ways: a) endogenous non-thermal factors, such as glucose and reproductive steroid levels, osmotic pressure, etc, can act directly on the sensor-to-effector pathways, in essence influencing the conveyance and integration of the neural-coded information; b) other homeostatic mechanisms may modulate thermal regulation by introducing inhibitory and/or excitatory input synapses converging on the sensor-to-effector thermoregulatory pathways.

Thus, further investigation of the interactions between the different homeostatic systems will reveal more information about the nature of each system *per se*, as well as about its role in whole-body homeostasis. With a specific focus on the thermoregulatory system, qualification and quantification of the effect of each one of the non-thermal factors on each different thermoregulatory response will help our understanding of the thermoregulatory integrative processes.

Gellhorn's hypothesis

With respect to the interaction between different homeostatic systems, it is well accepted to date, that lowering blood glucose concentration, a non-thermal variable, to levels below normal causes a decrease in body temperature, even during exposure to a normothermic environment. It has been hypothesized (Gellhorn, 1938) that in the face of

the diminished blood glucose concentration, this particular physiological response probably functions to decrease the metabolic demands of the body for glucose, thus preventing any harmful consequence of glucose deprivation on the brain tissue.

Although the observations relating the decrease in body temperature to lowering blood glucose concentration have commonly been considered as a reafirmation of Gellhorn's hypothesis (Freinkel *et al.*, 1972; Gale *et al.*, 1981), further examination of this hypothesis by examining the effect of hypoglycemia on all the different thermoregulatory responses, is uninvestigated.

It is well known that glucose is the predominant metabolic fuel of the CNS and that an acute reduction of circulating glucose triggers a complex physiological response aimed at restoring the blood glucose concentration (Cryer and Gerich, 1983). This restoration is produced by both an increase in glucose supply to the circulation (Rizza et al., 1978) and by elimination of glucose use in the peripheral tissues (Porte *et al.*, 1966; Fineberg and Merimee 1974). The stimulation of the appropriate responses is mediated by circulatory factors, whereas the central glucosensitive structures also seem to play a role, especially with respect to the acute physiological responses (Frohman, 1980).

Decreased body temperature could therefore be considered an additional mechanism capable of decreasing the glucose uptake from the circulation, since the former decreases the overall metabolic rate and therefore tissue glucose requirement. Consequently the occurence of hypothermia during hypoglycemia may be explained in accordance with the original Gellhorn's hypothesis (1938).

In a recent study, Buchanan *et al.*, (1991) investigated whether hypothermia is critical for survival during prolonged insulin-induced hypoglycemia in rats. The authors clamped plasma glucose concentration at a hypoglycemic level during an 8 hour period by continuous infusion of insulin in two groups of animals. The group exposed to normal room temperature (22 to 24 °C) became hypothermic during hypoglycemia (mean nadir core temperature, 31 °C). In contrast, hypothermia was prevented in the other group of

animals during hypoglycemia by warming the air temperature in their cages. None of the animals in the normothermic group survived the test for more than 7 hours, whereas all animals in the hypothermic group survived the 8 hour period and returned to normal after euglycemia was restored at the end of the insulin infusion. These findings support the suggestion that hypothermia is necessary for survival during prolonged insulin-induced hypoglycemia in rats, and seem to be consistent with Gellhorn's hypothesis (1938). Nevertheless, the relevance of these findings to larger mammalian species remains to be investigated.

Although the authors (Buchanan *et al.*, 1991) did not examine the thermoregulatory responses of the hypothermic group, other studies have shown that hypoglycemia decreases body temperature both by increasing heat loss (sweating, vasodilation) and suppressing shivering thermogenesis during exposure to a thermoneutral and/or a cold environment (Gale *et al.*, 1981; Haight and Keatinge, 1973). The effect of hypoglycemia on the thermoregulatory responses when the body is exposed to heat or during mild body hyperthermia has not been investigated.

PURPOSE OF THE PRESENT STUDY

In light of the study by Silva and Boulant (1984), suggesting that a low glucose tissue perfusion excites a majority of warm-sensitive neurons of the PO/AH (tissue slice preparation) whereas it inhibits the cold-sensitive neurons, it may be suggested that the preoptic neurons are directly involved in the hypothermic response usually observed during hypoglycemia. Namely, considering that warm-sensitive neurons facilitate heat loss whereas cold-sensitive neurons facilitate heat production responses (Hammel, 1965; Boulant, 1980), the effects of a low glucose tissue perfusion on these thermosensitive neurons as shown by Silva and Boulant (1984), can explain the thermoregulatory responses usually observed during hypoglycemia concominant with cold exposure (Gale *et al.*, 1981; Haight and Keatinge, 1973). Furthermore, the study by Silva and Boulant

strongly supports the notion of an interaction between thermoregulation and glucoregulation which constitute two different homeostatic systems. However, the extent of the influence of one system upon the other, in an *in vivo* preparations, has not been previously attempted. The whole aspect of the interaction between these two homeostatic mechanisms at the systemic level of physiological function can be revealed both, by examining and quantifying the effect of low or high blood glucose concentrations on the individual thermoregulatory responses and conversely by examining the effect of low or high body temperatures on the glucoregulatory responses. The individual thermoregulatory responses may be characterized and quantified according to their *threshold*, the *gain*, and the *maximum intensity or magnitude* of the response.

The present study was designed to investigate one aspect of this interaction by quantifying the effect of a low blood glucose concentration on the different thermoregulatory responses as each is activated during exposure to an appropriate thermally challenging condition (hyperthermia and hypothermia). Quantification focussed on the effect of hypoglycemia on the core temperature thresholds, gain and maximum intensity of the responses.

Furthemore, the present study aimed to re-evaluate Gellhorn's (1938) teleological hypothesis outlined earlier. Gelhorn's hypothesis addressed the issue of body temperature decrease during hypoglycemia as it has been observed during exposure to both thermoneutral and cold environments. It did not specifically address this topic in relation to heat exposure or hyperthermia. The present thesis extrapolating from the initial hypothesis re-evaluates Gellhorn's proposal for the case of hyperthermia. It is suggested that if Gellhorn's hypothesis applies during hyperthemia, then low blood glucose concentration should decrease the core temperature threshold values and, in general, enhance the thermoregulatory responses which are activated during hyperthermia (sweating, peripheral vasodilation), and should thus prevent an elevation in core temperature.

Finally, this study examined the effects of low blood glucose level on subjective thermal perception. Assuming that subjective thermal perception is directly related to thermoregulatory behavior this would allow a comparison between the effects of an endogenous non-thermal factor such as hypoglycemia on autonomic and behavioral thermoregulation.

To address the above questions, 10 male volunteers were immersed in a 28 °C water bath as previously described (Mekjavić *et al.*, 1991) on two different occasions. On one occasion the subjects were rendered hypoglycemic and on the other euglycemic. During the first phase of the experimental protocol (exercise phase) subjects' core temperature was increased by exercise on an underwater cyclergometer. The exercise was 20 minutes in duration at an intensity equivalent to 50% of each subject's maximal work rate. The effects of hypoglycemia on the autonomic thermoregulatory responses activated during the exercise-induced increase in core temperature were examined during this phase. Following the exercise session, subjects remained seated and immersed for an additional 99 minute period or until their core temperature decreased by 2 °C from its resting value (cooling phase), thus rendering the subjects mildly hypothermic. The effects of hypoglycemia on autonomic thermoregulatory responses activated during mild hypothermia were examined during the cooling phase. Results from physiological variables recorded during the exercise and cooling phases are presented in Chapters three and four, respectively. The effects of hypoglycemia on thermal perception were examined in parallel with the autonomic thermoregulatory responses during exercise and the cooling phases. Thermal perception data are presented in Chapter five.

Specifically, the present study investigated the manner in which hypoglycemia affects: a) sweating and skin passive vasodilation during exposure to exercise-induced mild heat stress, b) shivering thermogenesis and skin vasoconstriction during immersion hypothermia, c) subjective thermal perception of hot and cold

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CHAPTER TWO: LITERATURE REVIEW

CENTRAL REGULATION OF BODY TEMPERATURE

The existence of a "heat center" in the brain has been proposed since 1887 by Isaak Ott who was the first to localize this center by showing that a puncture at the "anterior inner end of the optic thalami" caused an increase in rectal temperature of the rabbit, due to increased heat production. Barbour (1912) demonstrated that direct thermal stimulation of the brainstem had an effect on rectal temperature in rabbits. These observations served as the basis for the hypothesis that there are thermosensitive cells in the brain stem which participate in temperature regulation. Subsequent studies have served to localize the thermally sensitive cells in the anterior hypothalamus (Magoun *et al.*, 1938; Hemingway *et al.*, 1940). Since then, much effort has been made to relate the function of the hypothalamic areas to the thermosensory inputs from various peripheral and central locations and the activity of the thermoregulatory effectors. The information obtained, provided detailed insights regarding the location and the properties of the thermoreceptors and the thermo-effector organs, as well as the neurochemical features of the hypothalamic cells (Zotterman, 1953; Bligh, 1973; Hensel, 1981; Boulant, 1981; Boulant and Dean, 1986; Simon *et al.*, 1986).

It is now well established that thermal input to the thermoregulatory integrating centers is provided by peripheral and central warm and cold receptors. Peripheral thermal receptors are located in the skin (Zotterman, 1953), abdominal cavity (Rawson and Quick, 1972) and skeletal muscle (Jessen *et al.*, 1983). It has also been established that thermosensitive neurons within the CNS are located in the hypothalamus (Hammel *et al.*, 1960; Boulant, 1981), spinal cord (Simon, 1974) and medulla (Lipton, 1973). The following short review will focus on some of the integrative processes of thermal inputs which occur at various levels of the CNS, with emphasis on hypothalamic integration.

Integration and central processing of thermal inputs

Tracing neurally coded thermal information from the thermoreceptors to various sites of the thermoregulatory neuraxis is probably a simplistic way to understand the complex integrative thermoregulatory processes of the CNS. Nevertheless, it is probably the only way which is presently afforded, considering technological and technical limitations. Using this method, a great deal has been learned about the neural activity at the sites where thermal information is synaptically conveyed within the CNS.

Spinal cord

There is a substantial degree of neurological signal processing at the spinal level. Processing occurs in the dorsal horn neurons (DHN), which receive thermoreceptor afferents from the skin. Dissimilarity observed between the activity of the skin thermoreceptors and that of the DHN neurons suggest the existence of spinal integration at this level. This dissimilarity is profound with respect to the scrotal thermal inputs (Neya and Pierau, 1980; Pierau *et al.*, 1984).

Trigeminal nucleus

Secondary neurons at this level receive synaptic input from primary thermoreceptors ascending from the facial areas. The integration of facial sensory information in the trigeminal nucleus is minimal compared with that of scrotal temperature in the lumbar spinal cord (Poulos, 1975). In addition, thermal signal processing in the trigeminal nucleus seems unaffected by other neural sites. In other words, it appears that the thermal information from the face is transmited to the thalamus almost uninterrupted (Gordon and James, 1986).

Thalamus

There is a high degree of processing of thermal information at the thalamic level, especially from the scrotal area. Thermoresponsive and non-thermoresponsive thalamic neurons exhibit a characteristic bursting activity. It has been shown that some units respond to decreasing scrotal temperature by altering their discharging response from a relatively rapid rate of tonic activity to bursting activity (Jahns, 1975). The warm sensitive units of the thalamus have been called "switching neurons", because of their on-off behavior. This "switching" response of thalamic neurons to scrotal heating can be abolished by blocking activity of the cerebral cortex or lesioning the nucleus raphe magnus (NRM). The "switching" response of thalamic neurons has been attributed to the existence of a positive feedback cortex-thalamus-cortex loop (Hellon and Taylor, 1982), which suggests that higher cortical centers influence thalamic thermoresponsiveness.

Midbrain

The NRM of the midbrain seems to be a key processing site for thermal information from peripheral and central stimuli. Heating and cooling the midbrain elicits responses from many warm sensitive neurons in NRM, but few or no cold sensitive neurons. Most of the NRM neurons, which are sensitive to midbrain temperature alterations, also respond to temperature changes in skin of the abdomen. It has also been shown that there is a high degree of spinal cord input to NRM, whereas recent studies suggest that the ascending thermal input from the skin passes first through the NRM on its way to thalamus and hypothalamus (Hellon, 1983).

Hypothalamus

Thermo-receptive characteristics

Early studies involving single neuron unit activity have shown that local warming increases the firing rate of the warm sensitive preoptic area anterior hypothalamic (PO/AH) neurons and decreases the firing rate of the cold sensitive neurons. Conversely, local cooling decreases the firing rate of the warm sensitive neurons and increases the firing rate of the cold sensitive neurons (see Boulant and Demieville, 1974; Boulant, 1980).

It has also been observed in animal studies that a variety of thermoregulatory responses may be elicited by altering the temperature of the PO/AH (Boulant, 1980; Boulant and Dean, 1986). Thus, PO/AH warming elicits heat loss responses, whereas PO/AH cooling elicits heat production responses. Slight changes of the PO/AH temperature above and below normal values causes changes in skin blood flow and thermoregulatory behaviour.

However, there are indications that factors other than the local temperature also influence neural activity of the PO/AH thermosensitive neurons. Namely, anesthesia affects the response of the cold sensitive neurons. It has been shown that 15-20% fewer cold sensitive neurons in the PO/AH region responded to local cooling in anesthetized animal preparations compared with the non-anesthetized ones (see Boulant and Dean, 1986). Similarly, in studies using tissue slice techniques it has been shown that the majority of the thermosensitive PO/AH cells are sensitive also to low glucose and hyperosmotic media or to testosterone and estradiol media (Boulant and Dean, 1986). These studies indicate a neuronal basis for the interaction between different regulatory systems, and more specifically, they show that temperature can affect the regulation of other homeostatic systems and vice versa.

Integrative characteristics

Thermoregulatory afferent and efferent pathways to and from the hypothalamus are complex and numerous (see Gordon and Heath, 1986). It seems that the hypothalamic region provides a major integrative site for thermoregulatory processes. Many studies have demonstrated that stimulation at almost any site of the CNS elicits integrative responses of hypothalamic neurons (see Satinoff, 1978).

More specifically, it has been shown (Boulant and Hardy, 1974) that the major part of the PO/AH that responded positively to local warming (warm receptors) also responded positively to skin and spinal cord warming. This is an indication that a convergence of thermal inputs occurs within the CNS (Bligh, 1984). However, PO/AH neurons are ten times more sensitive to local than to skin stimulation (Reaves, 1976). Nevertheless, thermal stimulation of the skin facilitates the hypothalamic thermoresponsive neurons, especially in the PO/AH area.

In more recent studies with unanaesthetized animal preparations, has been shown that the percentage of PO/AH neurons which responded to a change of skin temperature is 75% (see Gordon, 1981; Gordon and Heath, 1981; Reaves, 1976). On the other hand, studies on hypothalamic thermoresponsiveness using anaesthetized animals have shown that 34% of the neurons in the PO/AH responded to skin thermal stimulus (see Boulant and Hardy, 1974). This decrease in hypothalamic thermoresponsiveness to skin stimulation in anesthetized preparations compared with unanesthetized ones is another indication of the effects of anesthesia on thermoregulation.

A better understanding of the thermoregulatory integrative processes has been achieved by correlating the neural activity of the thermoresponsive CNS neurons at different levels of the thermoregulatory neuraxis with temperature sensation (Kenshalo, 1990). In addition, differences in the response characteristics between the hypothalamic thermosensitive neurons have also been used to explain the differences in the nature of the various thermoregulatory responses. For instance, there are thermoresponsive neurons

in the PO/AH which, for the same thermal stimulus, respond more rapidly to skin thermal stimulation than others (Gordon and White, 1985). Each group of these neurons is likely to be responsible for driving different thermal responses. Gordon (1981) suggested that the rapid responding thermosensitive PO/AH cells should drive quick behavioural responses, whereas the more slow responding neurons should drive slow motor outputs, such as vasomotor tone and metabolic processes. This suggestion seems to agree with the neuronal model suggested by Boulant (1981), according to which, each one of the three different PO/AH neuron groups (in terms of thermosensitivity) elicits different thermal responses.

Neuronal models in thermoregulation

Despite our detailed knowledge of the individual components (central and peripheral receptors, effector organs) and neural pathways involved in the thermoregulatory processes, the nature of their "linking" within the central nervous system (CNS) resulting in the maintenance of a constant body temperature is not known. Existing experimental data is not sufficient to answer this question.

In an effort to expand present knowledge, many investigators using research findings inspired by systems engineering control methods, have created a variety of mathematical and neuronal based analog models of mammalian thermoregulatory control system (Hammel, 1965; Stolwijk and Hardy, 1966; Nadel *et al.*, 1970; Wissler, 1985). The basic concept incorporated in many models is that of a "set point", in which the core temperature is compared with a set value or "set point". Any difference between the actual temperature and the set-temperature is termed error, and the effector responses are proportional to the magnitude of this error.

Whereas most thermal models incorporate a single integrator with multiple inputs and outputs, Satinoff (1978) added a new dimension to the problem by introducing the idea of a multicontrol system. According to this concept there exists not a single

thermostat, but as many as there are thermoregulatory responses, represented at several different levels of the neuraxis and operating simultaneously and in a parallel fashion with each other. Although these thermostats are capable of independent action, they normally act in concert, because they are arranged in parallel and hierarchically (Satinoff, 1983).

Following a different approach, Bligh (1990) suggested the representation of the central nervous interface between the afferent pathways from the thermosensors and the efferent pathways to the thermoregulatory effectors, by two simple sensor-to-effector pathways: one from the warm sensors to heat loss effectors and the other from the cold sensors to the heat production effectors. In other words, this model proposes that all inputs from peripheral and central warm receptors converge to a sensor-to-heat-loss-effector pathway and similarly, all inputs from cold receptors converge to a cold sensor-to-heat production effector pathway. The model also proposes that there is a reciprocal cross inhibition between the two pathways. This model demonstrates that cross inhibition of thermal afferent information establishes thresholds for the effector responses, based on the special characteristics of the cold and warm sensors, and thus eliminates the need for a comparator or "set-point" in a thermoregulatory model.

Considering that convergence of all thermal inputs from the warm and cold peripheral and central receptors will create two multi-synaptic pathways, then any non-thermal factor which affects the firing rate of thermoresponsive neurons or the synaptic transmission of the neurally coded thermal information should be expected to affect the thermoregulatory responses for a given thermal stimulus. Anesthesia as such is a non-thermal factor, and it has been shown for example that N_2O affects synaptic transmission within the CNS (Davis *et al.*, 1957; Hasley, 1974). Recent studies have also demonstrated that N_2O modifies heat production in humans during cold exposure by affecting both the threshold and the slope of the shivering response (Mekjavic *et al.*, 1992; Passias *et al.*, 1992).

Similarly, Silva and Boulant (1984) have shown that the firing rate of thermosensitive neurons in rat preoptic tissue slices is affected by perfusions with low-glucose and/or hyperosmotic solution. It seems possible that these effects could represent in the *in vivo* preparation a deviation of the normal thermoregulatory responses to a given thermal stimulus. Further investigation of the effects of different blood glucose concentration and/or plasma osmolarity on the various thermoregulatory responses observed in humans, should reveal the extent of the influence of such endogenous non-thermal factors on thermoregulation.

Extensive examination of the effect of non-thermal factors upon thermoregulatory responses will also offer insight on the integrative thermoregulatory processes and perhaps about the manner in which different physiological systems integrate to maintain the internal environment ("milieu interior").

HYPOTHALAMIC REGULATION OF BLOOD GLUCOSE

The role of the central nervous system (CNS), and more specifically the hypothalamus, in the regulation of energy metabolism has been recognized since 1849, when Claude Bernard observed that puncture of the floor of the fourth ventricle in dogs resulted in hyperglycemia and glycosuria.

The above observations were followed by studies of Cannon *et al.*, (1924), who suggested central regulation of plasma glucose, implicates the sympathetic nervous system and adrenal glands. In these studies, hypoglycemia was induced by insulin injection in cats, after the authors had denervated the heart of the experimental animals. It was observed that, following the insulin injection and after blood glucose concentration had dropped below a critical concentration value (70 to 80 mg per 100 cc), the rate of the denervated heart increased. This was considered to be an indication of sympathetic activation caused by hypoglycemia. However, since the heart was denervated, the increase in heart rate was attributed to increased epinephrine secretion by the adrenal

glands. This was deemed particularly so since, removal of one of the adrenal glands and denervation of the other resulted in a failure of the rate of the denervated heart to increase during insulin induced hypoglycemia. These results suggested that epinephrine secretion increases during hypoglycemia due to central neural excitation of the adrenal glands. Furthermore, it was observed that increase of the rate of the denervated heart (an indication of epinephrine secretion) was accompanied by a decrease in the rate of decline of blood glucose level. This indicated that epinephrine plays a role in the restoration of the plasma glucose concentration during hypoglycemia, by mobilizing glucose from the liver and it was suggested that this is probably one of the reasons that epinephrine secretion increases during hypoglycemia. However, it had been shown earlier by Griffith (1924) that glycogenolysis can also occur by direct stimulation of the hepatic nerve.

Later studies by Feldman *et al.*, (1940) and Gellhorn *et al.*, (1941) contributed further to the formation of the idea that blood glucose is controlled by the autonomic nervous system. They investigated the role of the two components of the autonomic nervous system, the sympatho-adrenal axis and vago-insulin axis, on blood glucose regulation. It was shown that activation of the sympatho-adrenal component favours increase of the blood glucose concentration, resulting in hyperglycemia. On the other hand, activation of the vago-insulin component seemed to increase insulin release, increasing glucose removal from the blood and resulting in hypoglycemia. A constant blood glucose level, therefore, demands a balanced function of the two components. Both studies investigated the effects of two different types of "stress stimuli" on blood glucose levels, as well as the mechanisms underlying the variations in blood glucose cooncentration. Feldman *et al.*, (1940) examined the effect of anoxia, induced by breathing air containing 7% oxygen, on blood glucose in rats and rabbits. It was shown that the animals became hyperglycemic as a result of the exposure to anoxia. The authors hypothesized that this was due to a relatively hyper stimulation of the sympatho-adrenal component of the autonomic nervous system, compared with the vago-insulin

component, induced by anoxia. In order to test this hypothesis, the animals were adrenalectomized and it was observed that adrenalectomized animals developed hypoglycemia. This again was attributed by the authors to a relatively hyper insulin secretion, due to the fact that the vago-insulin system was the only one of the two components of the autonomic nervous system remaining functional after the adrenalectomy. The latter suggestion was examined by performing a vagotomy on the animals, in addition to adrenalectomy. It was shown, that vagotomy, which severs the vago-insulin system, abolished the hypoglycemia during exposure to anoxia, confirming the hypothesis.

Similar results were observed by Gellhorn *et al.*, (1941), who investigated the effects of emotional excitement on the sympatho-adrenal and the vago-insulin or parasympathetic-insulin system in rats. The authors used a similar methodological approach as the one used by Feldman *et al.*, (1940). Namely, they used normal, adreno-demedullated and adreno-demedullated-vagotomized rats. It was shown that fear and struggle, in the same way as anoxia, produce hyperglycemia in normal animals, hypoglycemia in adreno-demedullated animals and no change or small increase in the blood glucose concentration in adreno-demedullated-vagotomized animals. The authors interpreted their results in accordance with the interpretation of Feldman *et al.*, (1940), assigning an important role for blood glucose regulation to the autonomic nervous system.

Subsequent investigations focused on the role of higher centers in the CNS on blood glucose regulation (Niijima, 1977). In these studies, a glucose solution was injected into the carotid artery of the rabbit, and efferent discharge were recorded from fine filaments dissected from splanchnic nerve branch innervating the adrenal glands and from vagal nerve branch innervating the pancreas. It was shown that the efferent discharge in the adrenal nerve was decreased, probably leading to a decreased epinephrine release, whereas the firing rate in the pancreatic branch of the vagus nerve was increased, most

likely causing an increased insulin output. It was suggested that glucose sensitive areas in the brain affect the activity of the two nerve branches, thus probably affecting the release of insulin and epinephrine.

It has also been demonstrated (Niijima, 1975) that a gradual decrease in arterial blood glucose concentration, after intravenous insulin injection, caused a gradual increase in the firing rate in the adrenal nerve, which was accompanied by a gradual decrease in the efferent discharge rate in the pancreatic branch of the vagus nerve. These results indicated the reciprocal roles of the sympathetico-adrenal and vago-insulin components of the autonomic nervous system regarding blood glucose regulation, as well as the relationship between blood glucose concentration and the rate of stimulation of these two autonomic components. It was further proposed that the activation of the two nerve branches was mediated by the CNS. Other studies (see Frohman, 1980) located the CNS region involved in carbohydrate metabolism and blood glucose regulation, and discovered some of the mechanisms underlying these regulations.

The hypothalamus has been shown to be one of the major CNS centers involved in the regulation and in fact, to be involved in both short-term and long-term metabolic regulatory mechanisms (Frohman, 1980). For the purpose of the present literature review, short-term mechanisms will be described in more detail. Short-term regulation of carbohydrate metabolism and blood glucose regulation has been studied mostly by electrical or by chemical stimulation of the hypothalamus.

There are two major efferent hypothalamic connections involved in neurometabolic regulation: one via the pituitary and the other via the autonomic nervous system. The hypothalamic-pituitary neuroendocrine axis regulates the secretion of hormones which modulate energy metabolism (growth hormone, adrenocorticotropic hormone and adrenal glucocorticoids, thyroid-stimulating hormone and thyroid hormones, gonadotropins and gonadal steroid). However, it should be mentioned that the effects of those hormones are slow in onset and relatively prolonged. Thus, the major

hypothalamic connection with respect to the acute control of energy metabolism is that with the autonomic nervous system.

Hypothalamic electrical stimulation causes liver glucose degradation and the secretion of insulin and glucagon

Insulin and glucagon are two very important hormones participating in the regulation of blood glucose. It has been shown that the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic nucleus (LH) affect blood glucose concentration both directly, by promoting liver glycogenolysis and indirectly, by neurally mediating the secretion of the two hormones mentioned above. The following review will focus on studies which have related VMH and LH with liver glucose degradation and the secretion of insulin and glucagon.

Direct hypothalamic effect on liver glycogenolysis

Shimazu *et al.*, (1966) electrically stimulated the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic nucleus (LH) during a 20-hour period in male rabbits. It was shown that blood glucose concentration increased, by up to 60% of the resting values, during VMH stimulation. In contrast, stimulation of the LH resulted in a decrease of about 20% in blood glucose concentration. In addition, the liver glycogen content at the end of the 20-hour stimulation period was significantly depleted when VMH was stimulated, whereas it was unaffected when LH was stimulated.

These observations suggest VMH stimulation causes degradation of liver glycogen, resulting in an increase of plasma glucose. Based on the findings by Ban (1964, 1975), according to which VMH is one of the nuclei of the sympathetic area of the hypothalamus and the LH of the parasympathetic area, the authors concluded that excitation of the sympathetic system promotes the increase of glycogenolysis in the liver.

Thus, it seems that hypothalamus neurally affects liver glycogenolysis through the sympathetic system.

Frohman and Bernardis (1971) studied the hypothalamic control of plasma glucose in rats further, by electrically stimulating the ventromedial region of the hypothalamus and reported an increase in the blood glucose concentration, whereas stimulation of the lateral hypothalamic area or of the cerebral cortex did not have any significant effect on the blood glucose concentration. In the same experiments, it was observed that the rate of glucose removal from the plasma was the same during VMH, ventrolateral hypothalamic area (VLH) or cortex stimulation, indicating that the hyperglycemic response during VMH stimulation occurs due to increased hepatic glucose output.

In order to ascertain whether the increased hepatic glucose output was directly stimulated by the hypothalamus without being mediated by epinephrine, the animals were adrenalectomized. It was shown that the initial glucose rise (first 3 minutes of stimulation) was not affected by adrenalectomy but the response was markedly impaired at 10 to 15 minutes. Similar results were observed in other studies using pancreateatomized and hypophysectomized animals thus excluding the contribution of other hormones (see Frohman, 1980).

These results suggested that the hypothalamus exerts a direct neural effect on liver glyconenolysis which is not mediated by any associated hormonal responses. The hypothalamic effect is at least responsible for the initial rise in blood glucose concentration during direct electrical hypothalamic stimulation.

Direct hypothalamic effect on Insulin release

Electrical stimulation of the hypothalamus also seems to affect insulin release. It has been observed that VMH stimulation exerts an inhibitory effect upon insulin release (Frohman and Bernardis, 1971; Frohman, 1980), whereas it remains to be elucidated

whether electrical stimulation at some other hypothalamic site can enhance insulin secretion.

Frohman and Bernardis (1971) suggested that in rats, the mechanisms underlying the suppressive effect of VMH stimulation upon insulin release are adrenally mediated. In contrast, it seems that in dogs, insulin is suppressed by direct neural sympathetic activation, since section of the splanchnic nerve (which carries sympathetic fibers) abolished this decrease and in addition caused an increase of insulin secretion rate (Miller, 1975). This indicates some species variability with respect to the effect of hypothalamus on insulin secretion.

In humans, direct neural suppression seems to be of greater importance in the decrease of insulin secretion than are catecholamines of adrenal origin (Frohman, 1980). In fact, it has been shown that in man surgical stress suppresses the insulin secretion response which follows an intravenous glucose injection. However, adrenalectomy does not seem to obviate this suppression of insulin secretion (Frohman, 1980). Therefore, considering that surgical stress activates the sympathetic nervous system and since adrenal catecholamines do not participate in this suppression of insulin secretion, it seems that the suppression is mediated neurally.

In general, it may be suggested that insulin suppression is mediated by the sympathetic system, whether this occurs by direct neural stimulation or through adrenal catecholamine secretion. This sympathetically mediated insulin inhibition involves the α-adrenergic receptor of the pancreatic β (insulin secreting) cells (Frohman, 1980). Thus, considering the suggestion by Ban (1975) that VMH constitutes the sympathetic center, the suppressive effects of VMH electrical stimulation on insulin secretion may be better understood. In addition, the effects of enhanced sympathetic activity, such as during psychological stress, hypothermia, anoxia etc., appear to be related to an inhibition of insulin secretion.

On the other hand, neural stimulation of insulin is mediated by the parasympathetic nervous system. It has been shown that direct vagal stimulation increases insulin release in dogs and this stimulation is more effective when glucose blood concentration is elevated (Bergman and Miller, 1973). Nevertheless, there is only weak evidence regarding the enhancement of insulin secretion as a response to hypothalamic electrical stimulation. It has been shown (Stephens and Morrisey, 1975) that electrical stimulation of the posterior hypothalamus causes an increase in blood insulin concentration, which subsequently leads to hypoglycemia. However, the observed insulin response in these studies was delayed (approximately one hour) and of a small magnitude. Similarly, Steffens *et al.*, (1972) observed an increase in both glucose and insulin levels in the blood during feeding, elicited by electrical stimulation of the lateral hypothalamus. Nevertheless, these results are difficult to interpret because of the concommitant food intake. Thus, an unequivocal increase in insulin secretion after stimulation of a specific hypothalamic site, which is characteristic of a neurally mediated response, has not yet been demonstrated.

Direct hypothalamic effect on glucagon secretion

Electrical stimulation of the VMH also elicits the release of glucagon (Frohman and Bernardis, 1971). Since in these studies the same response was observed even in adrenalectomized animals, it was suggested that glucagon response is unrelated to the adrenal. However, it seems that glucagon secretion is mediated via the sympathetic nerves. Esterhuizen and Howell (1970) observed that although stimulation of the vagal nerve, which contains parasympathetic fibers, did not affect the release of glucagon in cats, sympathetic stimulation resulted in a significant increase of glucagon levels in the blood. Morphological changes in the pancreatic α (glucagon secreting) cells were also observed after sympathetic stimulation, suggesting a direct effect of sympathetic fibers on these cells.

Similarly, Bloom *et al.*, (1973) observed that blood glucagon concentration increased during stimulation (within the physiological frequency range) of sympathetic innervation of the pancreas, and the increase was approximately proportional to the frequency of stimulation. As in the study by Frohman and Bernardis (1971), it was once again observed by Bloom *et al.*, (1973) that glucagon secretion elicited by activation of the splanchnic nerve, is independent of the adrenal glands, which in these studies had been removed from the experimental animals prior to the experiment. Furthermore, it was observed that the glucagon response was fast in onset and ended shortly after the termination of the stimulation, indicating the direct neural effect of the sympathetic innervation upon α cells. In contrast, epinephrine infusion in humans stimulates glucagon secretion (Gerich *et al.*, 1972). Luyckx *et al.*, (1975) suggested that the glucagon concentration increase in adrenalectomized rats is a compensatory mechanism for the absence of circulating epinephrine.

In summary, electrical stimulation of VMH elicits an increase in the concentration of glucose and glucagon in the blood, possibly by direct neural stimulation of glycogen degradation in the liver and of α-cells in the pancreas, respectively. These responses seem to be mediated by the sympathetic innervation of the liver and pancreas and both may occur indepedently of the adrenal glands. Conversely, insulin secretion is inhibited during VMH stimulation, a response which also seems to be mediated by the sympathetic innervation of islets of Langerhans and is independent of the adrenal glands. On the other hand, parasympathetic or vagal stimulation increases insulin release, but does not seem to affect glucagon secretion. Furthermore, electrical stimulation of LH decreases the blood glucose concentration, probably because insulin secretion increases during the stimulation. The effects of LH stimulation on the secretion of glucagon and insulin remains unclear.

Effects of chemical stimulation of CNS by 2-DG and epinephrine on plasma glucose

Compounds which can enhance or suppress glucose metabolism, such as insulin or glucose analogs, as well as neurotransmitters with their agonists and antagonists, have been used to clarify the neurochemical mechanisms of central control of metabolism. The fact that these substances affect CNS function as well as the regulation of metabolism and blood glucose concentration, indicates a relation between these regulations and the CNS. In the following short review, studies examining the effects of the glucose analog, 2-deoxyglucose (2-DG), and epinephrine will be discussed.

Frohman and Nagai (1976) used the compound 2-DG, which is a non-metabolizable analog of glucose. This compound competes with glucose for carrier-mediated transport across cell membranes and thus, decreases the CNS glucose metabolism. Cerebroventricular administration of 2-DG in dogs elicited responses similar to those observed during electrical stimulation of the ventromedial hypothalamus (Shimazu *et al.*, 1966; Frohman and Bernardis, 1971); namely, hyperglycemia, hyperglucagonemia and suppression of insulin secretion.

Since cerebroventricular administration of 2-DG affects primarily the CNS neurons, the study by Frohman and Nagai (1976) further justified the existence of a central control of the observed responses. However, the exact central neurochemical mediation of these responses is not clear although, the peripheral mediation is very similar to those observed during hypothalamic electrical stimulation, since administration of α-adrenergic blockades can block all these responses (Frohman, 1980).

Franklin and DiStefano (1962) showed that the CNS is responsible for at least half of the hyperglycemic response to epinephrine administration in cats since the hyperglycemic response to intravenous epinephrine administration was reduced by 50% in decapitated animals, in comparison with the response observed in intact animals. These results indicated that although the hyperglycemic response to epinephrine is partly due to

some peripheral effects of epinephrine, probably directly on the liver, the brain is necessary for the full hyperglycemic response to epinephrine.

Proceeding a step further, Franklin and DiStefano examined the same response following spinal transection of the animals. In these experiments the animals were not decapitated and thus, the brain and its circulation remained intact. Consequently, any hormonal secretion occurring within the brain could affect the response. However, the hyperglycemic response to epinephrine injection in these spinally transected, but not decapitated animals was again reduced by 50% of the control response in intact animals. Since the response in decapitated animals was the same with the response in the spinally transected animals, it was suggested that the response is neurally mediated. In other words it was proposed that there is a hyperglycemic centre in the brain, which is partly responsible for the hyperglycemic response to epinephrine (due to its stimulation by epinephrine).

Franklin and DiStefano (1962) also observed that microinjection of epinephrine into specific loci in the wall of the fourth ventricle elicits a hyperglycemic response. This was an indication that sites in the brain other than the hypothalamus play a role in short-term metabolic regulation.

Ezdinli *et al.*, (1968) confirmed the findings of Franklin and DiStefano (1962) in dogs. They observed similar results, namely, that cervical cord lesions substantially decreased the hyperglycemic response to epinephrine. The study by Ezdinli *et al.*, (1968), further supports the concept that the CNS plays a role in short-term glucose metabolic regulation.

Glucoreceptors in the hypothalamus and other areas

Most of the information regarding the regulation of glucose metabolism has been revealed from studies related to feeding behaviour. Similarly, the discovery and description of the characteristics of glucosensitive cells in the hypothalamic area, as well

as in other areas has been strongly promoted in the interest of understanding the regulatory mechanisms of food intake behaviour.

It is commonly accepted that the regulatory centers responsible for feeding behaviour are located in the lateral hypothalamic area (LH) and the ventromedial hypothalamus (VMH) (Oomura, 1980). Feeding is initiated by the LH, whereas VMH is responsible for satiation. These areas also contain cells that are sensitive to blood glucose fluctuations.

Anand *et al.*, (1961) recorded the discharge activity of neurons located in the above areas in monkeys and cats, before and after the induction of hyperglycemia and insulin-induced hypoglycemia. It was shown that the firing rate of the VMH neurons was increased in response to hyperglycemia but diminished in response to insulin-induced hypoglycemia, whereas in general, the firing response of LH neurons was decreased by hyperglycemia and slightly increased during hypoglycemia.

In a further study (Anand *et al.*, 1964), it was observed that the activity of VMH neurons showed a stronger correlation with the arteriovenous (a-v) glucose difference measured in the femoral vessels, than with blood glucose levels *per se*. In the same study it was observed that the activity of the LH neurons was inversely related to the activity of the VMH neurons. This was an indication that these neurons are likely affected either directly by their glucose utilization, or indirectly by glucose utilization in the periphery. In fact, Chhina *et al.*, (1971) showed that glucosensitive hypothalamic centers respond to glucose utilization rate in the brain, as this is indicated by the a-v glucose difference, independently of any peripheral information. However, this evidence does not rule out the possibility that blood glucose concentration *per se* provides a stimulus for those neurons.

In general, it seems that the activity of neurons in the VMH or satiety center increases when glucose utilization in the body is increased, whereas the neurons in the LH or feeding center exhibit the inverse relationship. Neurons tested in other

hypothalamic regions and in the cortex areas did not respond to changes in blood glucose and insulin concentration. The studies by Anand *et al.*, (1961 and 1964) support the existence of hypothalamic glucoreceptors, which seem to play a role in the feeding behaviour of animals and most likely in the physiological processes involved in the regulation of blood glucose concentration. Very similar results have been reported by Chhina *et al.*, (1971).

Oomura *et al.*, (1969) investigated the percentage of VMH and LH neurons that respond to blood glucose alterations. They suggested that approximately 40% of the VMH neurons which were tested, increased their firing rate following glucose administration, whereas the remaining 40% were unaffected. Conversely, 30% of the tested neurons in the LH area increased their firing rate after glucose administration, 25% decreased their firing rate, and the remaining 40% were unaffected. In the same study it was observed that the spontaneous firing rate of the neurons in the cortical and thalamic areas did not change after glucose administration. Once again, it was suggested that the glucose-sensitive elements in the CNS are located in the hypothalamic region.

Summarising the studies related to hypothalamic glucoreceptors and to the effects of glucose and insulin infusions on their response characteristics, Oomura (1976) pointed out the common observation that glucose tends to decrease the firing rate of glucose sensitive neurons in the LH, whereas it increases the firing rate of the neurons in the VMH, in a dose-response manner. It was also indicated that insulin alone tends to mildly inhibit VMH neuronal discharge, but enhances the excitatory effect of glucose when insulin and glucose are applied together. In the LH area, only the glucose-sensitive neurons respond to insulin and they can be strongly excited in a dose-response manner. Oomura also discussed the mechanisms behind the effects of insulin on the firing rate of these neurons. Based on the findings of Creese and Jenden (1968), who showed that insulin caused hyperpolarization of the muscle membrane, Oomura suggested that insulin probably causes hyperpolarization in neural membranes, which would explain the

inhibitory effects of insulin. Furthermore, it was suggested that increased glucose uptake by the neurons, which is facilitated by insulin infusion, could be the explanation of the excitatory effects of insulin when it is administered with glucose. The latter suggestion is in agreement with Anand *et al.*, (1964). However, Oomura questioned whether the effects of glucose in the VMH and LH neurons is caused by simple binding of glucose to the receptors or by metabolism of glucose by the neuron.

Glucoreceptors have been discovered in other areas outside the CNS. Niijima (1969) reported the existence of glucoreceptors in the liver of the guinea pig. In this study, the liver was excised, and the portal vein was perfused with solutions which differed from each other in their glucose content. The afferent discharge of the hepatic branch of the vagal nerve was recorded, and it was observed that the afferent firing rate was almost completely depressed by higher glucose concentrations, whereas a lesser degree of depression was caused by lower doses. This clearly suggested the existence of hepatic glucoreceptors. However, it could not be concluded from this study whether the neural information from the hepatic glucoreceptors is directed through the vagal nerve to the VMH and LH neurons to affect their discharge and consequently blood glucose regulation.

Schmitt (1973) showed that the firing rate of neurons in lateral hypothalamus was affected by injection of glucose solutions into the portal vein of the rat. Some neurons increased and some decreased their firing rates in response to these injections. Since similar injections into the general circulation via the jugular or a tail vein did not affect the firing rate of lateral hypothalamic neurons, it was suggested that there was a neural pathway connecting the hypothalamic neurons with glucoreceptors into the portal system or the liver. This suggestion was further supported by the fact that spinal transection at the level of T_5 and on a different occasion, severance of the splanchnic nerves, abolished any further hypothalamic neuron response to portal vein glucose injection.

Therefore, it seems that peripheral input from liver (Niijima, 1969; Schmitt, 1973) and intestinal (Sharma and Nasset, 1962) glucoreceptors, as well as gastric and esophageal mechanoreceptors (Niijima, 1967) somehow affects the activity response of those centers, which most likely play an integrative role in the regulation of blood glucose concentration.

In conclusion, the hypothalamic neuron population includes some neurons in VMH and LH, called chemoneurons, which receive visceral sensory input and are sensitive to glucose concentration in the blood. Blood glucose strongly stimulates the VMH neurons and mildly inhibits the LH neurons. Insulin, when infused alone, without glucose, mildly inhibits VMH neurons and strongly excites the LH neurons. When insulin and glucose are infused together, insulin enhances the excitatory effect of glucose on the VMH neurons, whereas glucose diminishes the excitatory effect of insulin on the LH neurons.

The studies mentioned in the foregoing discussion strongly indicate the participation of the CNS, and more specifically of the hypothalamus, on blood glucose regulation.

Some interesting similarities may be observed by comparing the central regulatory mechanisms of the thermoregulatory and glucoregulatory systems. The existence of hypothalamic neurons sensitive to local temperature, and neurons sensitive to local glucose variations, is one such similarity. In addition, it has been shown that there is a convergence of neurally coded information conveyed by the peripheral thermoreceptors and glucoreceptors to the central thermoregulatory and glucoregulatory areas, respectively, indicating a similarity in the integrative mechanisms between the two regulatory systems. Thus, all the observations imply the existence of two separate control mechanisms with similar functional features. Furthermore, the fact that the region containing thermosensitive neurons has been located in a different site in the hypothalamus (PO/AH) than the region with glucosensitive neurons (VMH, LH) supports

the notion of functional specificity for these neurons. However, the observations by Silva and Boulant (1984) that half of the thermosensitive neurons in the tissue slices from the PO/AH responded also to low glucose perfusion media, added a new dimension to the perception of the physiological regulatory mechanisms. It indicates a neural basis for an interaction between the two different physiological systems. The extent or the limits of this interaction remain to be demonstrated.

HYPOGLYCEMIA AND TEMPERATURE REGULATION

Hypoglycemia is a generic term which indicates that glucose has been removed from the blood at a faster rate than it has been replenished, resulting in a decrease of blood glucose concentration to abnormally low levels. Hypoglycemia can be caused by different factors, but mainly it is the result of various physiological disturbances (Conn and Seltzer, 1955). It can also be caused by alcohol intake while the hepatic glycogen stores are depleted (Freinkel *et al.*, 1963; Madison, 1968), as well as by prolonged and strenuous exercise (Levine *et al.*, 1924; Saltin and Hermansen, 1967).

For experimental purposes, hypoglycemia has been induced by insulin injections or by ethanol intake after an exhaustive exercise session. Neural glucodeprivation has been induced in humans and other animals by systemic or cerebral injections of 2-DG.

In a thermoneutral environment

Evidence for an effect of hypoglycemia on body temperature

The effects of insulin-induced hypoglycemia on core temperature in humans were described relatively early by Mayer-Gross and Berlinger (1942). In this study, insulin coma was used as a treatment of psychoses. Nine patients were injected with insulin (40 to 200 units) while exposed to a normal room temperature. It was observed that rectal temperature started to decline 2-3 hrs after the insulin injection and while the clinical signs of the coma appeared. Coma and the decline of core temperature were interrupted

by glucose injection, after which the patient regained consciousness and core temperature rose slowly, returning to its original level. It was speculated that the fall of core temperature was the result of a central disturbance of the hypothalamic thermoregulatory or autonomic centers by hypoglycemia. Similar observations and suggestions have been reported by Conn and Seltzer (1955).

Although hypothermia had been observed in patients in hypoglycemic coma, Kedes and Field (1964) were the first to emphasize hypothermia as one of the symptoms in clinical and asymptomatic hypoglycemia. They presented 5 case studies of 5 patients suffering from hypoglycemia caused by different reasons. In all cases, the patients suffered a small degree of hypothermia (35-35.5 °C core temperature). The consistency of occurrence of hypothermia in hypoglycemic patients compelled the authors to conclude: "...decreased body temperature could be a useful clue, especially in patients with absent or minimal symptoms or signs of hypoglycemia, when the diagnosis would be otherwise unsuspected". On the above experiments hypothermia was always reversed with elevation of blood glucose concentration after intravenous glucose injection. It was speculated by the authors that hypothermia during hypoglycemia was due either to increased heat dissipation, a result of skin vasodilation caused by the disfunctional hypothalamic heat regulating center, or to decreased heat production due to the absence of glucose.

In another case report, Jaffe and Paed (1966) presented the case of a hypoglycemic patient who was admitted to the hospital in a stuporous state while he was cold and sweating. His blood sugar level was 50 mg/100 ml and his oral temperature was 34.4 °C. Administration of glucose intravenously had a dramatic effect, reversing all of his symptoms and increasing his temperature to 36.5 °C within 20 minutes. The authors considered as an imbalance of the autonomic nervous system the fact that the patient was sweating (an indication of sympathetic discharge), whereas pulse rate and systolic blood

pressure (another indication of sympathetic discharge) were unaltered during hypoglycemia.

These studies describing the clinical symptoms of hypoglycemia, including hypothermia (Mayer-Gross and Berliner 1942, Conn and Seltzer 1955), as well as the above mentioned case reports on hypoglycemia (Kedes and Field, 1964; Jaffe and Paed, 1966) offered a good first insight regarding the effects of hypoglycemia on body temperature. However, these studies were not designed to investigate the effects of hypoglycemia on body temperature directly. Therefore their observations regarding the relation between the blood glucose concentration and thermoregulation were not obtained systematically, under controlled conditions. The first systematic assessment and clinical documentation regarding this relation was provided by Strauch *et al.*, (1969).

In this study, two groups of patients were investigated. One group consisted of 15 patients who arrived at the hospital hypoglycemic and comatose, and the other group comprised 20 patients who were transferred to the emergency room comatose or stuporous, with an etiology other than hypoglycemia. It was shown that in 53% of the hypoglycemic patients the rectal temperature was below 35.6 °C, whereas only 1 patient of the nonhypoglycemic group was hypothermic. The hypoglycemic patients were all treated with 25 gr of glucose which was injected intravenously with 50 ml of water and as a result, body temperature returned to normal within the next 2 hrs. Although no correlation could be demonstrated between levels of plasma glucose and body temperature in the hypoglycaemic, hypothermic group, it was suggested by the authors that hypothermia is not a non-specific manifestation of depressed CNS function but is related to the effects of hypoglycemia to the CNS function.

Since all the previous clinical information was obtained from patients who were already hypothermic when they were brought to the hospital, Molnar and Read (1974) tried to control the conditions better by using subjects who were initially normothermic and hypoglycemic. They induced hypoglycemia by injecting insulin in 36 subjects, who

were exposed to a room temperature of 28 to 30 °C, while temperatures (skin and core), sweating and blood glucose concentration were monitored. Seventeen of their subjects became slighly hypothermic. The range of their core temperature was 34.9 to 35.8 °C. The rest of the subjects, who had high initial temperature (above 36.8 °C), experienced a decrease in their core temperature during insulin-induced hypoglycemia, but their core temperatures did not decrease below 36 °C.

There is a possibility that the hypothermia which was observed in the above mentioned clinical studies was due to cold exposure of the patients prior to their delivery to the hospital. However, the observations by Jaffe and Paed (1966) and Molnar and Read (1974) which were obtained in the hospital and therefore under normal room temperature condition, are in agreement with those from the other studies. Therefore, based on all these observations, it could be suggested that a decrease in core temperature may be induced by hypoglycemia even in a thermoneutral environment.

In contrast to the common sugestion that hypoglycemia causes a decrease in body temperature, Ramos *et al.*, (1968) reported the manifestation of fever as a result of hypoglycemia in 14 out of 75 diabetic patients who had been transferred to the hospital suffering from hypoglycemia. However, the observed hyperthermia was not attributed to hypoglycemia directly, but rather to increased intracranial pressure or brain swelling, as implicated by the increased opening spinal fluid pressure (350 mm Hg), observed in one patient, and the indications of cerebral edema which were shown during postmortem examination in some of the other patients. In light of previous observations that hypothermia was always reversed with elevation of blood glucose concentration after intravenous glucose injection (Kedes and Field, 1964; Jaffe and Paed, 1966; Strauch *et al.*, 1969), the fact that intravenous administration of glucose in the study by Ramos *et al.*, (1968) failed to bring patients' increased core temperatures back to normal values, supports the notion that hyperthermia was not the result of hypoglycemia in this study.

Therefore, overall it seems more likely that hypoglycemia causes a decrease in core temperature than an increase. The question is, what are the mechanisms of such an effect ? It has been speculated that the decrease in core temperature is due to the effect of hypoglycemia upon the hypothalamic thermoregulatory centers (Mayer-Gross and Berlinger, 1942; Kedes and Field, 1964), or to a defective autonomic nervous system (Jaffe and Paed, 1966). Unfortunately, the mechanisms of hypoglycemia-induced hypothermia cannot be discerned from these studies due to their methodological limitations.

Mechanisms of hypoglycemia-induced hypothermia

Freinkel *et al.*, (1972) conducted an interesting study in which two major questions related to the mechanisms of hypoglycemia-induced hypothermia were addressed. The first was whether the effects of hypoglycemia arise either due to an extracellular fuel limitation, or to selective deprivation of glucose within the cells, and second, whether decrease in body temperature is caused either by action on the peripheral thermogenic mechanisms, or upon the thermoregulatory centers within the CNS.

To answer these questions, seven male subjects were exposed in 25 °C while 50 mg per kilogram of 2-deoxy-D-glucose (2-DG) were injected into their bloodstream. It was shown that rectal temperature decreased by 1.1 °C 2.5 hrs after the injection and thereafter started increasing without reaching preinjection values within the entire 6 hrs duration of the experimental period. Since 2-DG blocks intracellular glucose utilization, it was suggested by the authors that hypothermia occurred due to intracellular glucose deprivation, rather than to a limitation of extracellular glucose.

In order to distinguish between peripheral and central effects of 2-DG in relation to the thermoregulatory responses, saline solutions or 2-DG in saline were injected intravenously or in to the cerebral ventricles of mice. The mice were kept at a constant environmental temperature of 22 °C. Hypothermia developed in mice as a result of the 2-

DG injection, during both the intravenous and the intracerebral administration. However, the degree of hypothermia was fivefold greater following the intracerebral compared with intravenous administration. These results seem to imply an effect of glucopenia upon the central hypothalamic thermoregulatory centers, rather than upon peripheral mechanisms of thermogenesis. Whether glucopenia affects these centers directly or some other areas in the brain, which induced an effect on the thermoregulatory centers cannot be deduced from these observations.

Thompson *et al.*, (1980) also used 2-DG in human subjects to investigate, whether increased heat loss or decreased heat production was mostly responsible for the decrease in core temperature resulting from 2-DG-induced glucodeprivation. It was observed that tympanic temperature was decreased by 0.9 °C within the first 30-90 minutes after the begining of the intravenous injection of 2-DG (50 mg/kg), which lasted 20 minutes. However, heat production, as reflected by the oxygen uptake values, was increased by 30% in the group of subjects injected with 2-DG, as compared with the stable heat production values recorded in the control group, which was injected with saline solutions. Subjects did not shiver, nor were they restless or active during the experiment and thus, the increase in heat production could not be attributed to any of these factors. Most likely, the catecholamine concentration in the blood, which were monitored to be increased during the 2/3 of the experimental time period, could account for the increased thermogenesis. On the other hand, all the subjects sweated after the 2-DG administration. Although skin blood flow was not measured in this study, it was suggested by the authors that the 2-DG-induced glucopenia caused a decrease in core temperature by increasing heat loss through sweating and cutaneous vasodilation rather than by decreasing heat production, which in fact was raised.

In contrast, Molnar and Read (1974) sugggested that heat production decreased during insulin-induced hypoglycemia by 5 to 42%. However, heat production was not measured directly in their study, but it was calculated based on the difference between

ambient and skin temperature. The calculations were based on values obtained before hypoglycemia and during the post-hypoglycemic recovery period, when rewarming was occurring and thus the possibility of a decreased skin temperature due to vasoconstriction was high. Therefore, these calculations are questionable.

Unpublished observations from two subjects receiving intravenous insulin injections (0.15 U/kg) are also described by Thompson *et al.*, (1980). In one of the subjects, tympanic membrane temperature decreased by 0.7 °C within 60 minutes after the insulin administration, whereas a moderate sweating response was observed 40 and 50 minutes after the injection. Heat production increased by 18%, while a peak fivefold increase in plasma catecholamines was observed 40 minutes after insulin infusion. In the second subject, a decrease of core temperature by 1 °C by 120 minutes was related to a marked sweating response from 30 to 40 minutes and a moderate response from 50 to 90 minutes. Heat production also increased in this subject by 25%.

Thus, it seems that there is a consistency in the results obtained from insulin-induced hypoglycemia and 2-DG neural glycodeprivation studies, with respect to their effect of decreasing body temperature in subjects exposed to a thermoneutral environment. It may be suggested that although heat production increases during hypoglycemia or glucopenia, body temperature decreases, most likely due to increased sweating response.

Gale *et al.*, (1981) further clarified the effects of insulin-induced hypoglycemia on heat production and body temperature. They also investigated the effect of hypoglycemia on peripheral blood flow, a very important heat dissipation and conservation mechanism. It was shown that insulin-induced hypoglycemia decreased the core temperature of 11 subjects exposed in a normothermic environment (25 °C), by 0.51 °C. Core temperatures reached their minimum values 58-78 minutes after the initiation of the 40 minute insulin infusion period, whereas sweating developed after 30 minutes. Calf and hand blood flow increased at 33 and 43 minutes respectively, after insulin was given, and heat production,

as indicated by the oxygen uptake values, was increased by 23% after 30 minutes. It was suggested that core temperature decreased, despite the observed rise in heat production, due to increased peripheral vasodilation and sweating.

The increase in heat production observed by Thompson *et al.*, (1980) and Gale *et al.*, (1981) during 2-DG-induced neuroglucopenia and during insulin-induced hypoglycemia, respectively, has been attributed to catecholamine-stimulated thermogenesis. In fact, Macdonald *et al.*, (1982) showed that metoprolol (a β_1-selective antagonist) reduced the heat production response to insulin-induced hypoglycemia in humans exposed to a normothermic environment, whereas propranolol (a β_1 and β_2 antagonist) abolished this response. These results indicated that the increase of heat production during hypoglycemia results mainly through β-adrenoceptor-mediated mechanisms. Considering that catecholamines increase heat production by acting upon both β_1- and β_2-adrenoreceptors it may be suggested that the increased heat production observed during hypoglycemia is due to an increased plasma catecholamine concentration.

In summary, it has been suggested that insulin- induced hypoglycemia and 2-DG-induced neuroglycopenia in humans exposed to a thermoneutral environment leads to a decrease of core temperature by 0.5 to 1.0 °C. This is probably due to intracellular glucose deprivation of the neurons in the hypothalamic thermoregulatory centers or other brain areas which may indirectly influence the former. An increased heat loss mediated by sweat secretion and peripheral vasodilation are both mechanisms responsible for this decrease of body temperature. Heat production increases during the state of hypoglycemia or glucopenia, most likely due to the thermogenic effect of plasma catecholamines on β-adrenoreceptors. It seems that the increased heat loss is high enough to cause a decrease in core temperature.

In a cold environment

The association of hypoglycemia with a considerable lowering of body temperature during cold exposure has been recognized for many years (Cassidy *et al.*, 1925, 1926; Dworkin and Finney, 1927).

Cassidy *et al.*, (1925) and Dworkin and Finney (1927) showed that insulin-induced hypoglycemia renders animals "poikilothermic", since they lose their ability to shiver and thus, their ability to thermoregulate when exposed to cold. Interestingly, this state of poikilothermia was terminated by glucose injection, which caused shivering to start almost immediately (within 1 or 2 minutes after the glucose injection), body temperature to rise, and the animal returned to normal.

Cassidy *et al.*, (1926) investigated the effects of insulin injection on domestic fowl. The response of blood glucose concentration to insulin was observed to be in parallel with the rectal temperature. It was shown that the blood glucose concentration falls within 40 to 60 minutes after injection of insulin, and recovery commences between 3 and 6 hours after the administration of insulin. Body temperature fell 1 to 3 °C below normal as a result of hypoglycemia. Core temperature started decreasing after hypoglycemia had reached a certain level (between 140 mg and 100 mg per cent). Further decrease of blood glucose level is followed by a concurrent decrease of core temperature. Shivering never accompanied this fall in core temperature. However, the increase in blood glucose was followed by a rise in core temperature. These results seemed to indicate that hypoglycemia inhibits shivering. To further verify this hypothesis, hypoglycemic fowls were immersed in tap water (20 °C). The body temperature at the begining of the immersion was 40.8 °C. Initiation of shivering was observed almost 30 minutes later at a core temperature of 33 °C. It should be noted that in normal fowl shivering starts immediately after immersion in water of 16 °C temperature or after a fall of the core temperature by 0.3 to 0.8 °C. These additional observations confirmed the suggestion that hypoglycemia inhibits shivering in the fowl as it does in mammals.

Not many studies have been conducted in humans investigating the effects of hypoglycemia or neuroglucopenia on the thermoregulatory responses during cold exposure. In one of them (Haight and Keatinge, 1973), hypoglycemia was induced by ethanol ingestion in young male volunteers. Ethanol causes hypoglycemia by impairing hepatic gluconeogenesis, in circumstances of depleted liver glycogen reserves. Cases like this occur after exercise to exhaustion, or starvation.

Haight and Keatinge (1973), administered ethanol to the subjects orally (approximately 34 ml of ethanol diluted with 500 ml water) after an exercise session performed in a thermoneutral environment at 70% of their maximal oxygen consumption which lasted until exhaustion (approximately 2 hours). Hypoglycemia appeared when the subjects were given ethanol upon completion of the exercise. Subsequently the subjects were exposed in a cold room (14.5 °C) for 30 minutes, in an air flow of approximately 15 km/hr. Volunteers completed this experimental protocol twice. On one occasion they were exposed to cold in a hypoglycemic state, whereas in the other they were loaded with glucose either orally or intravenously (normoglycemic). It was shown that rectal temperature was maintained at a normal value during the normoglycemic condition. In contrast, rectal temperature decreased to 34.5 °C during hypoglycemia. The metabolic rate or shivering failed to increase in the hypoglycemic condition during cold exposure, but increased when subjects were loaded with glucose. This observation can probably account for the occurance of hypothermia during the hypoglycemic state.

Gale *et al.*, (1981) also investigated the effects of insulin-induced hypoglycemia on thermoregulatory responses in man during exposure in a room temperature of 18-19 °C in an air flow of 1.7 m/s. Five volunteers in a normoglycemic state were initially exposed to the above described environmental conditions until they had developed a sustained shivering for 10 minutes. This period was reached within 60-75 minutes from the begining of the exposure. Heat production had doubled during this period due to the shivering response, and rectal temperature was maintained constant. Insulin

hypoglycemia was then induced and 8.5 -15 minutes later, when plasma glucose was in the range of 2.5 -2.7 mM, shivering was completely suppressed and as a result, heat production decreased below initial levels by the end of the experiment. Rectal temperature decreased below 35 °C in all five subjects approximately 1 hour after the insulin infusion, while cold discomfort was greatly reduced. Calf and hand blood flow, which were low during the pre-insulin-infusion period, exhibited a transient increase with hypoglycemia. Visible sweating was observed in two of the subjects for a few minutes. Therefore, it seems that increased peripheral blood flow and sweating secretion might make a small contribution to the development of hypothermia in hypoglycaemic subjects during cold exposure. Nevertheless, the most important factor for the development of hypothermia was the suppression of shivering which results in a decreased heat production.

These observations are in agreement with those by Haight and Keatinge (1973), suggesting that hypoglycemia decreases core temperature during cold exposure due to suppression of shivering. This was even more dramatically demonstrated when glucose was injected intravenously in 2 of the subjects from the Gale *et al.*, (1981) study while they were hypothermic (35.2 °C) and hypoglycemic. Shivering response, as indicated by EMG activity recorded in the quadriceps muscle, was restored within 40 seconds after the glucose injection.

Gale *et al.*, (1981) in the same series of experiments, showed very elegantly that the suppression of shivering has a central rather than peripheral origin. They arterially occluded the leg of one of two hypothermic and hypoglycemic subjects who were injected with glucose. It was observed that shivering was restored in the arterially occluded limb at the same time as in the other limb after the injection of glucose. It seems that shivering in both legs was triggered by the central thermoregulatory regions after the injected glucose reached those areas through the blood circulation. There was no need for increased blood glucose levels to perfuse the muscles of the occluded leg in order to start

shivering, thus implying that glucose deprivation of the central thermoregulatory regions was the reason of shivering suppression during hypoglycemia.

In summary, it has been shown that ethanol-induced hypoglycemia in man, and insulin-induced hypoglycemia in man and other animals suppresses the shivering response during cold exposure, resulting in hypothermia. Shivering may be restored immediately with intravenous injection of glucose. It seems that the effect of hypoglycemia upon the central thermoregulatory areas rather than upon the skeletal muscles is responsible for the suppression of shivering. Peripheral vasodilation and sweating probably play a small role in the development of hypothermia during hypoglycemia and cold exposure.

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CHAPTER THREE: EFFECTS OF HYPOGLYCEMIA ON THERMOREGULATION DURING EXERCISE-INDUCED MILD HYPERTHERMIA

INTRODUCTION

Insulin-induced hypoglycemia causes skin vasodilation during exposure to thermoneutrality (French and Kilpatrick, 1955; Allwood *et al.*, 1959; Gale *et al.*, 1981), by releasing the sympathetic vasoconstrictor tone (Middleton and French 1974; Berne and Fagius, 1986) and stimulates sweating (Molnar and Read 1973, 1974; Macdonald *et al.*, 1982; Gale *et al.*, 1983), a response which is also sympathetically mediated (Berne and Fagius, 1986). The decrease in body temperature that usually accompanies hypoglycemia in thermoneutrality has been attributed to the increased heat loss resulting from the elevated skin blood flow and sweating (Kedes and Field, 1964; Gale *et al.*, 1981). It has been suggested that the reduction of body temperature during hypoglycemia may, by decreasing the metabolic requirements of the brain for glucose, protect it from consequences which are potentially fatal when lowering core temperature is prevented (Gellhorn, 1938; Buchanan *et al.*, 1991).

The effects of hypoglycemia on body temperature during exposure to environmental and/or exercise-induced heat stress have not been established. Extrapolating from Gellhorn's (1938) teleological suggestion and the observations during exposure to thermoneutrality, it might be hypothesized that an increase in core temperature would be prevented by hypoglycemia compared to euglycemia during externally and/or exercise-induced heat stress, in which case the mechanisms involved would need to be elucidated.

Core temperature (Tc) during exercise increases and stabilizes at a level proportional to the work intensity (Nielsen, 1966). In the event that, at a given work rate, vasodilation and sweating were enhanced by a non-thermal factor such as hypoglycemia, then this would alleviate an exercise-induced elevation in Tc normally observed during euglycemia. The present study examines the effect of hypoglycemia on the Tc and the Tc

threshold value for initiation of sweating and cutaneous passive vasodilation as well as the gain of each response during exposure to exercise-induced mild hyperthermia.

METHODS

Subjects

The experimental protocol used in the present study was approved by the Simon Fraser University Ethics Committee.

Ten healthy male volunteers participated in the present study after giving their informed written consent. Their participation was subject to physician's approval and they were familiarized with the experimental protocol and the possible risks involved prior to the experiments. None of the subjects was a smoker, was obese, or was taking medication. The majority of the subjects could be characterized as physically active and none had a history of hypertension or endocrine disorder.

Experimental protocol

Each subject, dressed in swimming shorts, was immersed to the chest in a water bath at 28 °C and exercised at 50 % of his maximal work rate (determined previously on a separate occasion) on an underwater cycle ergometer for 20 minutes, or until the rise in core temperature reached a plateau and sweating secretion was stimulated. Each subject followed the same experimental procedure on two different occasions. On one occasion the blood glucose level was maintained at 2.8 mM (hypoglycemia) and in the other at 5.0 mM (euglycemia), using the glucose clamp technique (DeFronzo *et al.*, 1979). The rate of insulin infusion for both conditions was 60 $mU.m^{-2}.min^{-1}$ and the rate of glucose infusion was adjusted accordingly in order for the plasma glucose concentration to be maintained at the desired level. The two trials were spaced at least one week apart, to avoid acclimation to the experimental protocol, and the order in which the trials were conducted was alternated among subjects. Subjects were unaware of the condition (euglycemia or

hypoglycemia) they were undertaking. Any effects of circadian rhythm were minimized by conducting each trial at the same time of the day for each subject. Each subject agreed to avoid strenuous exercise during the day preceding the experiment and to fast for 12 hours prior to testing. The subject was asked to maintain a similar diet as well as physical activity and sleeping schedules during the 2 day period preceding each of the two experimental trials, thus diminishing the potential effect of these factors on the recorded physiological responses.

An esophageal thermistor probe inserted to a length determined from sitting height (Mekjavić and Rempel, 1991) was used to measure core temperature. Heat flux and skin temperature were measured at six sites (arm, chest, abdomen, back, thigh, and calf). Sweat secretion was measured with the use of a specially designed ventilated capsule placed on the skin surface of the subject's forehead. A Laser Doppler probe was placed on the contralateral side of the forehead for skin blood perfusion (SkBP) measurements. The subject breathed through a mouthpiece during the experiment. ECG was monitored continiuously throughout each trial.

A catheter was inserted in the left forearm (antecubital vein) for insulin and glucose infusion. Blood samples were taken via another catheter inserted in the left hand (contralateral vein). The catheterized hand was placed in a heated chamber (70 °C). This results in sufficient arteriovenous shunting to arterialize the venous blood sample, thus eliminating the need for arterial catheterization in these studies (McGuire *et al.*, 1976). The basal blood glucose and catecholamine concentrations were determined from the first blood sample taken thirty minutes after the catheterization. This was followed by the implementation of the glucose clamp procedure. Plasma glucose reached the desired concentration within approximately 30 minutes of the initiation of the glucose clamp procedure. Resting values were recorded for 5 minutes (rest) while the subject was sitting on the underwater cycle ergometer immersed in the water to the chest. The core temperature values corresponding to the initiation of sweating and to the first SkBP

values exceeded resting levels were determined during the exercise and were characterized as the sweating initiation and the passive vasodilation thresholds, respectively. Following the exercise session, the subject remained in the water bath for an additional 99 minute period, or until the core temperature decreased by 2 °C from its resting value, as has been described previously (Mekjavć *et. al.*, 1991). Results from the cooling phase are presented in Chapter four.

Maximal exercise test

The $\dot{V}O_2$ max test was performed on a separate occasion prior to the initiation of the experiments on the underwater cycle ergometer while the subject was immersed to the chest in 28 °C water. The protocol included 2 minutes of rest while the subject was seated on the underwater ergometer, followed by 2 minutes of unloaded pedalling at 55 revolutions per min (RPM). Thereafter the intensity of the exercise was incremented by 0.5 kp at 2 minute intervals until exhaustion.

Instrumentation

The immersion tank (2.1 x 1.05 x 2.1 metres) contained approximately 4,000 liters of water, continuously agitated throughout the immersion by a PAC-FAB hydropump (El Monte C.A., USA) and maintained at 28 °C. Water temperature was recorded with a YSI 701 thermistor.

Heat flux ($\dot{Q}$,W.m $^{-2}$) and skin temperature (Tsk, °C). Heat flux from the skin surface was measured with heat flux transducers (Concept Engineering, Old Saybrook, CT). Thermistors embedded in the transducers' surface placed on the skin, allowed concurrent measurement of skin temperature. The transducers were attached to the skin with waterproof tape (Elastoplast, Lachine, Quebec).

Core temperature (Tc, °C). Esophageal temperature (Tes) was assessed using a YSI 702 (Yellow Springs Instruments, Ohio, USA) thermistor.

Ventilation ($\dot{V}_I$, L.min $^{-1}$). The volume of the inspired air was measured with an Alpha Technologies Ventilation Module (Model VMM110, California). The subject was breathing through a low-resistance two way valve. The expiratory side was connected by Collins corrugated tubing to a 9 liter fluted plexiglass mixing box.

Oxygen uptake ($\dot{V}O_2$, L.min.$^{-1}$). and carbon dioxide production ($\dot{V}CO_2$, L.min $^{-1}$). $\dot{V}O_2$ and $\dot{V}CO_2$ were determined from the analysis of mixed expired gas and inspired minute ventilation. A continuous 500 ml.min.$^{-1}$ sample of mixed expired gas was drawn from the mixing box and analysed for oxygen and carbon dioxide contents using an Applied Electrochemistry Oxygen Analyser (S-3A) and a Statham Godart Capnograph, respectively.

Sweat rate(Esw, g.m^{-2}.min^{-1}). Sweat rate was measured with the sweat capsule positioned on the skin surface of the forehead (surface area of capsule = 4.5 cm^2). The capsule was ventilated at a rate of 330 ml.min^{-1} with dry air supplied from a compressed air cylinder, which was maintained at room temperature. The air entering the capsule was dry and its temperature equal to room temperature. Temperature and relative humidity of the air exiting the capsule was measured with a temperature and relative humidity sensor (Smart Reader 2, ACR Systems Inc., Surrey, B.C., Canada), respectively. The value for sweat secreted was determinedy by calculating the difference between the water content of the air entering and leaving the capsule.

Skin blood perfusion (SkBP, non-dimentional (N.D.)). Skin blood perfusion was measured with a Laser Doppler perfusion monitor (Periflux PF3, Sweden), and is reported as the ratio of skin blood perfusion at any given time (SkBP(t)) relative to average skin blood perfusion values recorded during the 5 minutes of resting period preceding the exercise ($SkBP_{rest}$). Thus at rest, SkBP = SkBP (t) / $SkBP_{rest}$ = 1.

Heart rate (HR, min^{-1}). Heart rate was obtained with a Lifepak 8 Cardiac monitor (Physio-Control Systems, Redmond, WA) using a bipolar precordial lead. Contamination

of the recorded signal from water entry between the skin and the surface electrode was prevented by using waterproof tape.

Data acquisition. All physiological variables were recorded at one minute intervals. With the exception of heart rate and sweat rate, physiological variables were measured on-line with an HP 3497A data acquisition system controlled by an Apple Macintosh II computer (using LabVIEW, National Instruments, Austin Texas). For the measurement of sweating the temperature and humidity modules were controlled with an ATS, PC computer (Model SX-30).

Blood samples and analysis. Glucose content of the sampled blood was determined by a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma glucagon and insulin were measured by radioimmunoassay, whereas, norepinephrine and epinephrine by high performance liquid chromatography (HPLC) with electrochemical detection, as described by Meneilly *et al*., (1985).

Statistical analyses

The response of each recorded variable was compared between the two experimental conditions using a two-way analysis of variance (ANOVA) with repeated-measures design. Threshold and gain values for sweating and passive vasodilation were determined for each subject separately and they were chosen from Esw-ΔTes and SkBP-ΔTes plots, respectively. The threshold and gain values were compared between the two experimental conditions using paired t-test. The core temperature threshold for initiation of sweating was considered to be the core temperature value corresponding to the first sweating response higher than the values observed during the resting period which correspond to insensible water loss. Similarly, core temperature threshold for passive vasodilation was considered to be the value corresponding to the first SkBP response exceeding resting level (ratio > 1). Threshold values were chosen by an investigator who was naive to the conditions and subjects involved. The slope of the Esw-ΔTes and SkBP-

ΔTes regression lines as they were obtained with a linear regression analysis were considered to be the gains of the Esw and SkBP responses, respectively. The 5% level was chosen as the level of significance for all statistical analyses.

RESULTS

Results are presented as mean ± SE for the subject group. Subjects' physical characteristics are presented in Table 1.

Esophageal temperature

The group mean Tes value was throughout the pre-exercise resting period identical between the two conditions (36.57 ± 0.08 °C in the euglycemic and 36.43 ± 0.07 °C in the hypoglycemic condition). The change in Tes from resting values (ΔTes) was used for the analysis of the core temperature response. As may be seen in Fig. 1, ΔTes increased from 0.04 ± 0.02 at the last minute of the resting phase to an end-exercise value of 0.68 ± 0.12 °C during euglycemia and from -0.02 ± 0.01 to 0.66 ± 0.10 °C during hypoglycemia. ΔTes followed a similar pattern of increase in both experimental conditions and started reaching a plateau during the last 5 minutes of exercise. There was no difference in the ΔTes values between the two experimental conditions.

Skin temperature

The group mean Tsk was similar during the last minute of the resting phase between the two experimental conditions (30.26 ± 0.19 for the euglycemic and 30.37 ± 0.15 °C for the hypoglycemic conditions). After a small decrease of approximately 0.5 °C during the first 5 minutes of exercise, skin temperature increased reaching an end-exercise value of 30.82 ± 0.20 for the euglycemic and 30.51 ± 0.14 °C for the hypoglycemic conditions, respectively (Fig. 1). Although Tsk had a tendency to be higher

in the euglycemic compared with the hypoglycemic condition, especially during the last 5-7 minutes of the exercise, the difference was not statistically significant.

Oxygen consumption

Oxygen consumption increased from 0.48 ± 0.02 and 0.43 ± 0.02 $l.min^{-1}$ during rest to 2.5 ± 0.05 and 2.32 ± 0.07 $l.min^{-1}$ by the fourth minute of exercise (Fig. 2) in the euglycemic and hypoglycemic conditions, respectively. $\dot{V}O_2$ reached a plateau after the 3-4 first minutes of exercise in both experimental conditions. In contrast to ΔTes, $\dot{V}O_2$ was higher during the euglycemic condition ($p \leq 0.004$), throughout the exercise period.

Heart rate

HR, was similar in the two experimental conditions (Fig. 2). HR increased from 68 ± 2 and 69 ± 4 min^{-1} at the last minute of the resting phase to 134 ± 4 and 136 ± 4 min^{-1} at the fourth minute of exercise during the euglycemic and hypoglycemic conditions, respectively. HR reached a plateau after the first 4-5 minutes of exercise and was maintained at this level until the end of exercise under each experimental condition.

Ventilation

$\dot{V}_I$ increased from 13.65 ± 0.63 $L.min^{-1}$ at the last minute of the resting period to 66.18 ± 2.72 $L.min^{-1}$ by the end of exercise during the euglycemic condition and from 12.94 ± 0.51 to 68.46 ± 4.59 $L.min^{-1}$ during the hypoglycemic condition (Fig. 2). Similarly to HR, ventilation reached almost a plateau after the first 3-4 min of exercise. There was no difference in $\dot{V}_I$ between the two experimental conditions.

Respiratory exchange ratio

Respiratory exchange ratio (RER) did not vary between resting and exercise periods (Fig. 3). In the euglycemic condition, the RER value of 0.93 ± 0.04 at the end of

resting period was maintained with very small fluctuations during exercise reaching end-exercise values of 0.94 ± 0.03. Similarly, in the hypoglycemic condition the RER value of 0.93 ± 0.02 at the end of the resting period was maintained at 0.95 ± 0.02 up to the end of exercise. There was no difference in group mean RER value between the two experimental conditions.

Heat flux

Heat flux began to increase after 5 minutes of exercise. Thus, $\dot{Q}$ increased from 123.3 ± 5.6 and 125. 6 ± 8.8 $W.m^{-2}$ at the fifth minute of exercise to 165.2 ± 5.3 and 146.3 ± 8.0 $W.m^{-2}$ at the end of exercise for the euglycemic and hypoglycemic conditions, respectively (Fig. 4). Heat flux was higher in the euglycemic compared with the hypoglycemic condition during the last 5 minutes of exercise ($p \leq 0.04$).

Skin blood perfusion

Skin blood perfusion increased after the fifth minute of exercise (Fig. 4) as did the $\dot{Q}$ and Tsk values in both experimental conditions. SkBP was higher in the euglycemic than the hypoglycemic condition during the last 6 min of exercise ($p \leq 0.01$). As may be seen in Fig. 7, SkBP initially increased linearly with ΔTes in both experimental conditions. However, a further increase in core temperature was followed by an elevation in SkBP in the euglycemic, but not during the hypoglycemic condition. There was no difference in the respective ΔTes value which corresponded to the first SkBP response exceeding resting values (passive vasodilation threshold) between the two experimental conditions (-0.02 ± 0.07 for the euglycemic and -0.02 ± 0.05 °C for the hypoglycemic conditions; Table 2). The regression analysis which was performed for the linear portion of the SkBP-ΔTes relation indicated that there was no difference in the gain of the passive vasodilation response between the two experimental conditions (4.53 ± 1.23 $.^{o}C^{-1}$ in the euglycemic and 2.81 ± 2.57 $.^{o}C^{-1}$ in the hypoglycemic condition; Table 2).

Sweating

Average time for initiation of sweating was approximately 14 minutes after the start of exercise for both experimental conditions (Fig. 4). Sweating secretion followed a similar pattern of increase in both experimental conditions reaching an end-exercise value of 6.31 $\pm$ 0.68 and 5.80 $\pm$ 0.75 $g.m^{-2}.min^{-1}$ in the euglycemic and hypoglycemic condition, respectively. Although there was a tendency for a higher ΔTes value at which sweating commenced (core temperature threshold values for initiation of sweating) in the euglycemic (0.34 $\pm$ 0.1 ^{o}C) compared with the hypoglycemic (0.22 $\pm$ 0.1 ^{o}C) condition, the difference was not statistically significant ($p \leq 0.35$, power 0.14, Fig. 7, Table 3). Similar results were obtained by using the Wilcoxon Sign Rank non-parametric statistical test. There was no difference in the gain of the sweating response between the two expermental conditions (11.75 $\pm$ 1 $g.m^{-2}.min^{-1}.^{o}C^{-1}$ in the euglycemic and 11.07 $\pm$ 1.8 $g.m^{-2}.min^{-1}.^{o}C^{-1}$ in the hypoglycemic condition; Table 3).

Glucose and hormonal values

Plasma glucose concentration decreased from 5.2 $\pm$ 0.1 mM to 2.9 $\pm$ 0.1 mM thirty minutes after the initiation of insulin infusion during the hypoglycemic condition and was maintained at this level for the remainder of the experiment (Fig. 5). Plasma glucose was maintained at a normal level (approximately 5.0 mM) during the euglycemic condition (Fig. 8).

Plasma insulin concentration increased from 76 $\pm$ 5 and 81 $\pm$ 5 (basal values) to 806 $\pm$ 54 and 849 $\pm$ 76 $pmol.L^{-1}$ 30 minutes after the implementation of glucose clamping, during the euglycemic and hypoglycemic conditions, respectively (Fig. 5). Insulin concentration was maintained at a similar level for the remainder of the experiment. There was no difference in the plasma insulin concentration between the two experimental conditions.

The basal glucagon concentration was 116 ± 6 and 113 ± 7 $pg.ml^{-1}$ in the euglycemic and hypoglycemic condition, respectively. During hypoglycemia, glucagon concentration began to increase 30 minutes after the initiation of insulin infusion and reached a peak value of 224 ± 26 $pg.ml^{-1}$ towards the end of exercise. Glucagon concentration was maintained at basal levels throughout the experiment during the euglycemic condition (Fig. 6). Plasma glucagon concentration was higher ($p \leq 0.001$) during the hypoglycemic compared to the euglycemic condition after the first 30 minutes of the glucose clamping.

Plasma epinephrine increased during the hypoglycemic condition from 91 ± 14 $pg.ml^{-1}$ (basal value) to 627 ± 64 $pg.ml^{-1}$ sixty minutes after the initiation of insulin infusion and was maintained at this level for the remainder of the experiment. Conversely, epinephrine was maintained at an almost basal level (89 ± 16 $pg.ml^{-1}$) during the euglycemic condition throughout the experiment (fig. 6). Epinephrine concentration was higher ($p \leq 0.0001$) during the hypoglycemic compared to the euglycemic experimental condition after the first 30-40 minutes of the glucose clamping.

Plasma norepinephrine concentration increased from basal value of 276 ± 24 and 254 ± 23 $pg.ml^{-1}$ to a peak value of 674 ± 104 and 578 ± 48 $pg.ml^{-1}$ during the first 60 minutes of the glucose clamping for the euglycemic and hypoglycemic conditions, respectively (Fig. 6). There was no difference in plasma norepinephrine concentration between the two experimental conditions.

DISCUSSION

The present study demonstrates that hypoglycemia does not affect the core temperature threshold for initiation of sweating and passive skin vasodilation or the gain of those responses during exposure to exercise-induced mild hyperthermia. As a result hypoglycemia does not affect the exercise-induced increase in core temperature.

Oxygen uptake

$\dot{V}O_2$ was higher during exercise in the euglycemic compared to the hypoglycemic condition. This is surprising considering that the same absolute work rate was performed during both experimental conditions and judging by the similar heart rate and ventilation responses. Furthermore, the same metabolic substrates were used during both conditions as indicated by the similar RER values. The only variation between the two conditions which, could possibly account for the difference in $\dot{V}O_2$, is related to the glucose infusion rates. Namely, during exercise glucose infusion rate exhibited a transient linear increase in the euglycemic condition (from 10.81 $\pm$ 1.06 at last minute of rest, to 19.06 $\pm$ 2.02 $mg.kg^{-1}.min^{-1}$ at the end of exercise) and a transient linear decrease in the hypoglycemic condition (from 3.36 $\pm$ 1.09 at the last minute of rest to end-exercise values of 1.74 $\pm$ 0.89 $mg.kg^{-1}.min^{-1}$). In case that the larger amount of glucose infused during euglycemia was higher than the exercise energy demands, it could be suggested that the excessive amount of glucose was converted and stored as glycogen. This conversion requires ATP and could be partly accounted for the increased $\dot{V}O_2$ observed during the euglycemic compared to the hypoglycemic condition (Ravussin and Bogardus, 1982; Thiebaud *et al.*, 1983; DeFronzo *et al.*, 1984). However, for an exercise intensity of 2.1 $L.min^{-1}$ (50% of mean $\dot{V}O_2$ max for the particular subject group) and a RER value of 0.95, the glucose oxidation rate is approximately 2.4 $g.min^{-1}$ or 34 $mg.kg^{-1}.min^{-1}$. Since glucose infusion rate during exercise in the euglycemic condition was only 19

$mg.kg^{-1}.min^{-1}$, it is obvious that there is no excess glucose uptake by the muscle. Thus, energy costs of glycogen storage are highly unlikely, and consequently, the higher $\dot{V}O_2$ values observed during euglycemia can not be explained based on the present results.

However, the higher $\dot{V}O_2$ observed during euglycemia it is likely that resulted in elevated heat dissipation. This was reflected by the higher SkBP and consequently higher $\dot{Q}$ values especially during the last few minutes of exercise. It appears that dissipation of the extra heat during the euglycemic condition allowed core temperature to be maintained at values corresponding to the performed work rate and thus similar to those during hypoglycemia.

Skin blood perfusion

Skin blood flow in the non-acral body regions is controlled by sympathetic vasoconstrictor nerves which cause cutaneous vasoconstriction when activated and sympathetic vasodilator nerves which cause cutaneous vasodilation when activated (Edholm *et al.*, 1957; Fox and Edholm, 1963; Kellogg *et al.*, 1991a,b).

The 28 °C water bath temperature used in the present study provides a rather cool environment which can result in a decreased skin blood flow due to increased sympathetic vasoconstriction (Kellogg *et al.*, 1989). It is likely that the exercise-induced increase in core temperature observed during the first 15 minutes of exercise caused the release of this sympathetic vasoconstrictor tone (passive vasodilation), resulting in the observed increase of SkBP. The similarities in the pattern of the increase in core temperature as well as in the core temperature thresholds and gains for passive vasodilation (Table 2), between the two experimental conditions indicate that hypoglycemia did not affect the release of the sympathetic vasocontriction caused by the increase in core temperature.

A further increase in core temperature exerted an elevation in skin blood perfusion during the euglycemic but not during the hypoglycemic condition. It is not possible based

on the present results to distinguish the termination of passive vasodilation from the initiation of active vasodilation. Consequently, it is difficult to assess whether active vasodilation was ever initiated or was partly or fully stimulated in either of the conditions of the present study. In previous studies, the distinction between passive and active vasodilation has been achieved with the use of local iontophoresis of bretylium (Kellogg *et al.*, 1989, 1991b). It was shown in these studies that after the removal of any vasoconstrictive tone by skin temperature elevation to 38 °C, an exercise-induced active vasodilatory response increases SkBF by 6-7 fold. When skin vasoconstriction was activated by cold exposure (decreasing the Tsk to 30-32 °C), SkBF decreased by 40-50% below the values measured at Tsk of 34-35 °C (normothermia). Based on these findings, the 3-4 fold increase in SkBP observed in the present study may, to a certain degree, be attributed to active vasodilation. This appears more likely during the hypoglycemic condition, especially during the last 5-10 minutes of exercise. It is possible that low blood glucose concentration restricted active vasodilation during the euglycemic condition. This effect could be due to a hypoglycemia-induced central inhibition of the active vasodilatory response. On the other hand, it can not be excluded that the high epinephrine levels observed during hypoglycemia exerted skin vasoconstriction which masked an active vasodilatory response. Finally, it is also possible that the higher SkBP values observed at the end of exercise in the euglycemic compared to the hypoglycemic condition (Fig. 4, 7), simply reflected higher heat dissipation resulting from the amplified heat production during euglycemic exercise.

Sweating

The magnitude and gain of the sweating response and the core temperature threshold at which sweating was initiated, were not affected by hypoglycemia. Assuming that sweating during hypoglycemia in subjects exposed to thermoneutrality serves a thermoregulatory purpose, namely to decrease body temperature, then it appears that

hypoglycemia should potentiate sweating during exercise-induced mild hyperthermia. The present results indicate that this is not the case. One possible explanation for the absence of hypoglycemic potentiation of the exercise sweating response is that the thermal stimulus exerted by exercise-induced core temperature elevation was strong enough to mask any potentiation in the sweating response caused by hypoglycemia per se (Gale *et al.*, 1981; Macdonald *et al.*, 1982; Gale *et al.*, 1983). Alternatively, hypoglycemia does not affect thermoregulatory sweating, as indicated from the present results. Possibly, sweating secretion exerted by hypoglycemia during exposure to thermoneutrality is a sympathetic response, which is not meant to serve any thermoregulatory purpose although it indirecty causes a decrease in core temperature.

Thus, although the notion that the thermoregulatory system mediates the decrease in core temperature caused by hypoglycemia during exposure to thermoneutrality cannot be rejected based on the present results, it can be postulated that this notion does not apply in the case of exercise-induced mild hyperthermia.

Exercise effects on core temperature threshold for passive vasodilation and sweating

In both experimental condition, sweating commenced during the exercise and abated during the passive cooling phase (reported in chapter four), at similar ΔTes threshold values. This indicates that exercise did not affect the core temperature threshold at which the sudomotor system is activated and is in agreement with previous findings (Johnson and Park, 1981; Kellogg *et al.*, 1991b).

Analogous comparisons were performed for the passive vasodilation threshold. It was similarly observed that exercise did not exert any effect on passive vasodilation threshold. On the contrary, it has been shown that exercise shifts the threshold for active vasodilation to higher core temperature values (Kellogg *et al.*, 1991b). These findings, in conjunction with the present results, seem to support the concept of separate control for the two mechanisms of vasodilation (passive and active; Kellogg *et al.*, 1991b). However,

it should be pointed out that the above observations regarding the effects of exercise on sweating and passive vasodilation based on the present results should be considered with caution, since Tskin was lower by approximately 2 °C in the cooling (chapter four) compared to the exercise phase in the present study. It is possible that the difference in the thermal input from the skin affected the core temperature thresholds of the studied responses therefore masking the effect of exercise.

Previous studies have shown that initiation of dynamic exercise causes cutaneous vasoconstriction in subjects exposed to normothermia (Johnson and Park, 1981; Taylor *et al.*, 1988; Kellogg *et al.*, 1991a, 1991b). No such effect was observed in the present study during the initiation of exercise most likely because the vasoconstrictive effect had already been exerted by the peripheral cold stimulus induced by immersion masked any exercise-induced vasoconstriction.

Re-examining Gellhorn's hypothesis

Conclusive remarks regarding the interesting teleological hypothesis proposed by Gellhorn (1938), cannot be made on the basis of the present results. Buchanan *et al.*, (1991) compared the effects of hypoglycemia in two resting animal groups. Hypoglycemia-induced hypothermia was prevented in one group but not in the other. It was shown that long term exposure to insulin-induced hypoglycemia was fatal for the animal group in which hypothermia was prevented. On the contrary, the animals in which group that hypothermia was not prevented survived and recovered after hypoglycemia was reversed. Although these findings seem to support Gellhorn's hypothesis, the mechanisms responsible for this protective effect and whether this protective effect of hypothermia applies to humans remains to be determined. The inhibitory effect exerted by hypoglycemia on shivering thermogenesis in human subjects during cold exposure (Haight and Keatinge, 1973; Gale *et al.*, 1981; present study: Chapter four) seem also to support Gellhorn's hypothesis. Namely, it indicates that a thermoregulatory response

which is necessary for cold defence (shivering) is modulated (inhibited), allowing hypothermia to occur. On the other hand this is not the case regarding the thermoregulatory responses used for heat defence during exercise-induced mild hyperthermia. The responses are not modulated in order to prevent hyperthermia in any way different from that exhibited during euglycemia. Assuming that the physiological systems operate in a teleological manner, the above observations indicate an asymmetry in regulation and activation of different components (responses) of a particular physiological system (thermoregulation) towards pursuing the determined target. It is also likely that physiological systems do not follow a multiple-step approach towards pursuing their final aims but adopt rather a more simple one-step approach. For example, if the aim of hypoglycemia is to decrease body temperature (step 1) in order to lessen the requirements for glucose by the brain cells (step 2) so that the brain can survive during the period of low glucose availability (step 3), then hypoglycemia should potentiate heat loss responses during hyperthermia. This was not demonstrated in the present study. It appears that the "logic" for the particular example given could be teleological, but more simple. Namely, when blood glucose is low, any glucose demanding process is inhibited. Therefore, since shivering requires glucose, it is inhibited (step 1) via a CNS mediated response. It appears that during hypoglycemia a first priority of physiological mechanisms is to "shut off" all the glucose or energy requiring processes rather than to decrease body temperature. The fact that body temperature decreases may then be a consequence of achieving this priority rather than the main target of the physiological responses observed during hypoglycemia.

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Table 3-1. Subject's physical characteristics

Subjects	Age (yr)	Weight (kg)	Height (cm)	$\dot{V}O_2$ max ($L.min^{-1}$)	$\dot{V}O_2$ max ($ml.kg^{-1}.min^{-1}$)
1	24	62.5	179	3.6	57.6
2	28	68.0	181	4.2	61.0
3	30	79.5	173	3.9	49.0
4	27	68.0	170	4.4	64.7
5	22	66.0	175	4.2	62.9
6	29	81.0	181	4.0	49.4
7	27	66.0	168	4.1	61.4
8	28	71.0	180	4.9	69.0
9	29	70.0	170	4.1	58.6
10	36	70.0	175	4.1	58.6
Mean	28	70.2	175	4.1	59.2
$\pm$ SE	1.17	1.9	1.5	0.1	2.0

Table 3-2. The ΔTes threshold values for passive vasodilation and the gain of the response (SkBP/ΔTes) during exercise in the euglycemic and hypoglycemic conditions

	ΔTes threshold for SkBP (°C)		SkBP/ΔTes ($^{o}C^{-1}$)	
Subjects	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
1	-0.17	0.02	5.60	3.06
2	0.13	0.13	13.80	2.93
3	~	~	~	~
4	0.00	0.00	2.97	2.65
5	0.04	0.04	2.47	1.34
6	0.08	0.00	4.19	1.29
7	-0.52	-0.38	3.36	4.81
8	0.08	0.00	1.11	1.21
9	0.15	0.00	4.02	5.81
10	0.00	0.00	3.28	2.18
Mean ± SE	-0.02 ± 0.07	-0.02 ± 0.05	4.53 ± 1.23	2.81 ± 2.57

~ missing data

Table 3-3. The ΔTes threshold values for initiation of sweating and the gain of the response (Esw/ΔTes) during exercise in the euglycemic and hypoglycemic conditions

	ΔTes threshold for Esw (°C)		Esw/ΔTes ($g.m^{-2}.min^{-1}.°C^{-1}$)	
Subjects	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
1	-0.29	0.27	7.92	14.60
2	0.18	0.71	7.14	13.53
3	0.39	0.05	11.77	13.35
4	0.66	0.61	11.55	6.75
5	0.55	0.37	17.25	8.59
6	0.44	0.29	11.40	6.54
7	-0.12	-0.26	12.13	8.72
8	0.71	0.35	13.19	24.55
9	0.33	-0.10	15.40	6.75
10	0.51	-0.14	9.69	7.32
Mean ± SE	0.34 ± 0.1	0.22 ± 0.1	11.75 ± 1.00	11.07 ± 1.8

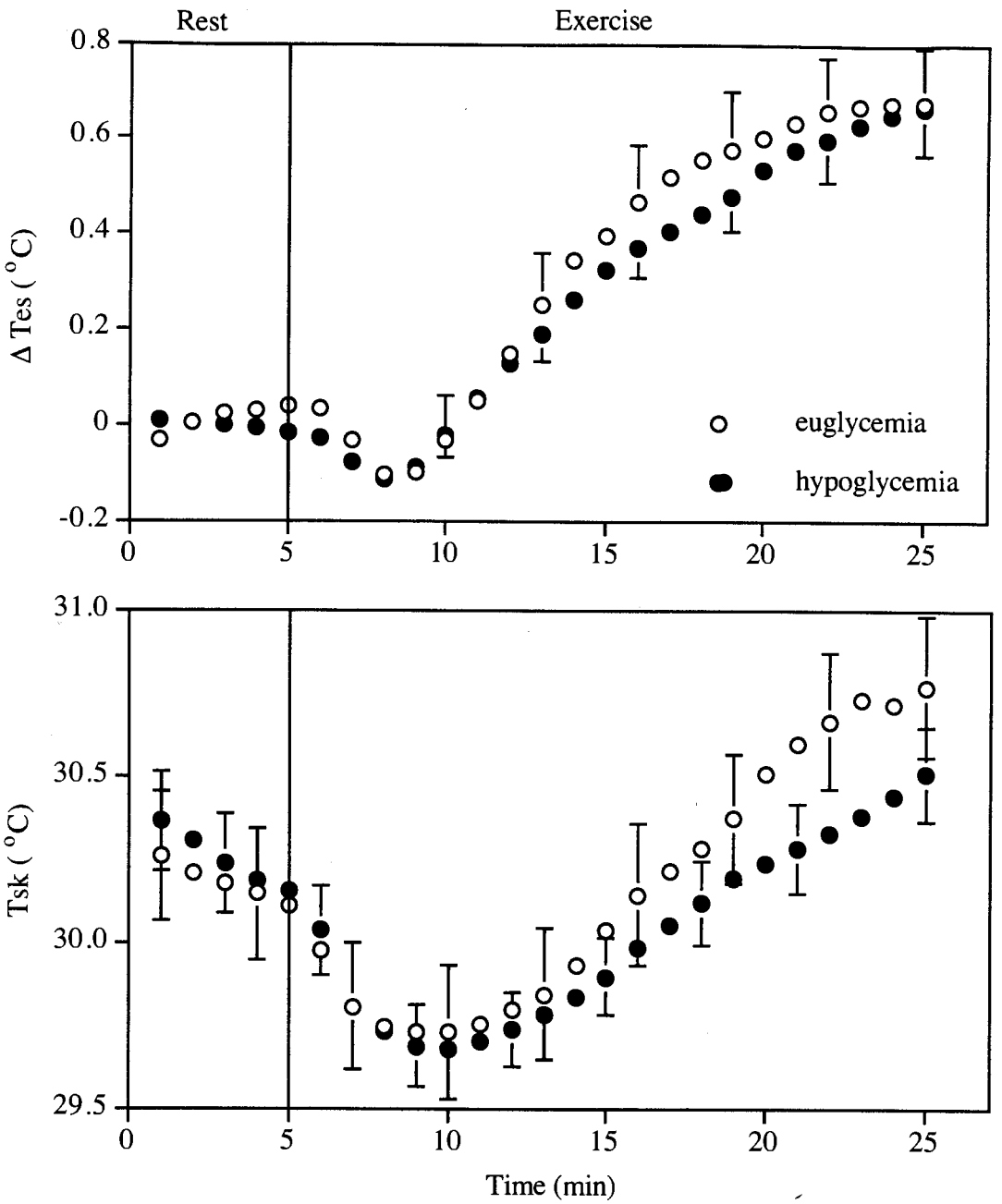

Fig. 3-1. Changes in esophageal temperature relative to resting values (ΔTes,*top*) and skin temperature (Tsk, *bottom*) responses (mean ± SE), during rest and exercise periods. Subjects were either euglycemic (open circles) or hypoglycemic (closed circles).

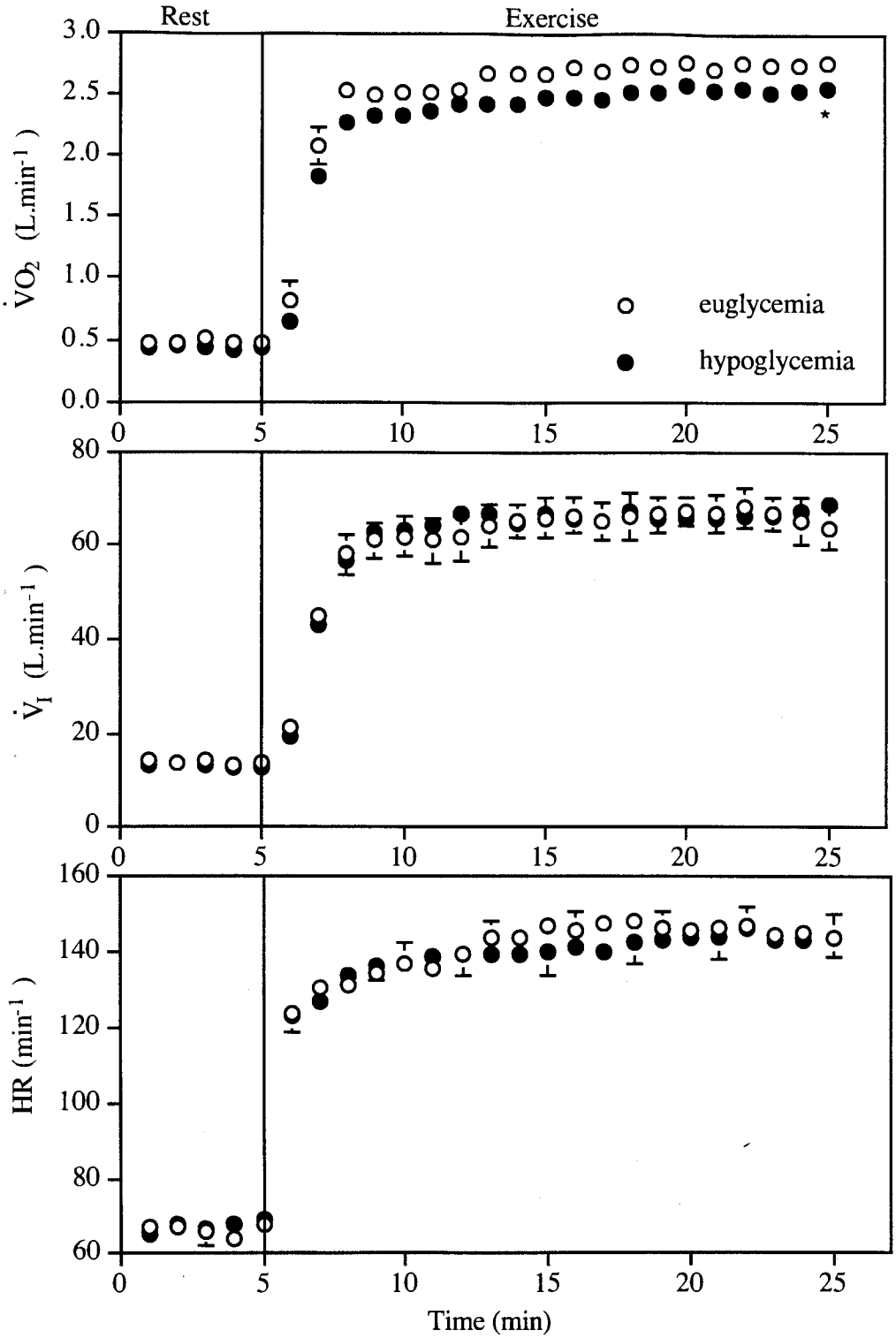

Fig. 3-2. Oxygen uptake ($\dot{V}O_2$, *top*), inspired ventilation ($\dot{V}_I$, *middle*) and heart rate (HR, *bottom*) responses (mean ± SE), during the rest and exercise periods in the euglycemic (open circles) and hypoglycemic (closed circles) conditions, $p \leq 0.004$.

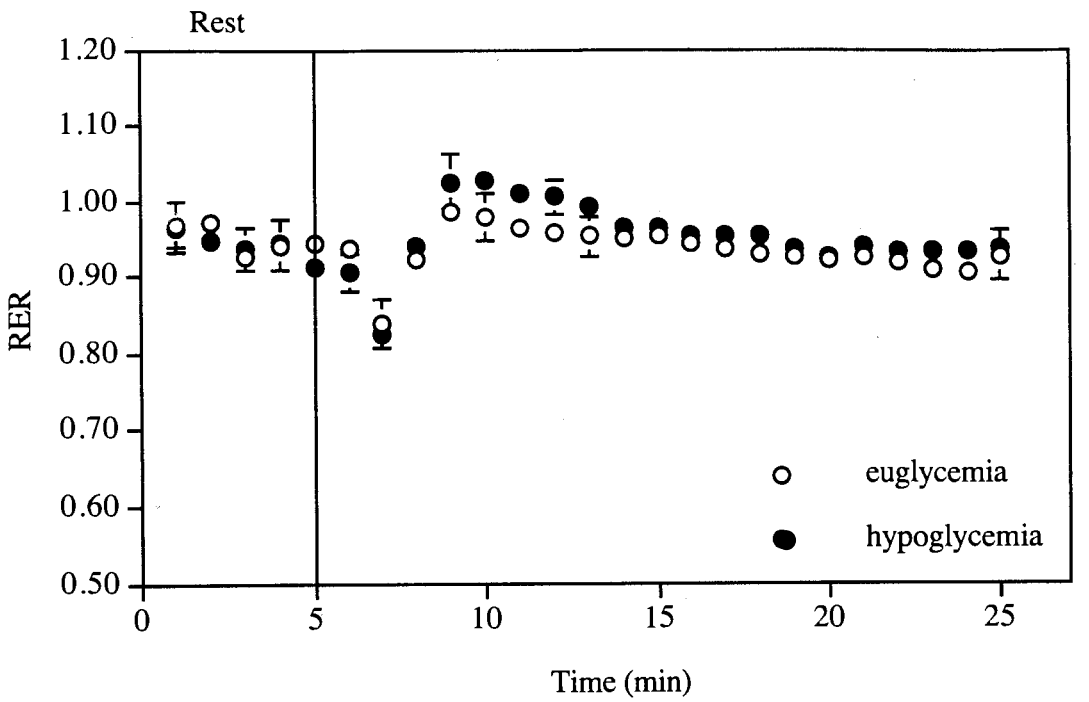

Fig. 3-3. Respiratory exchange ratio (RER) values (mean ± SE), during the rest and exercise periods in the euglycemic (open circles) and hypoglycemic (closed circles) conditions.

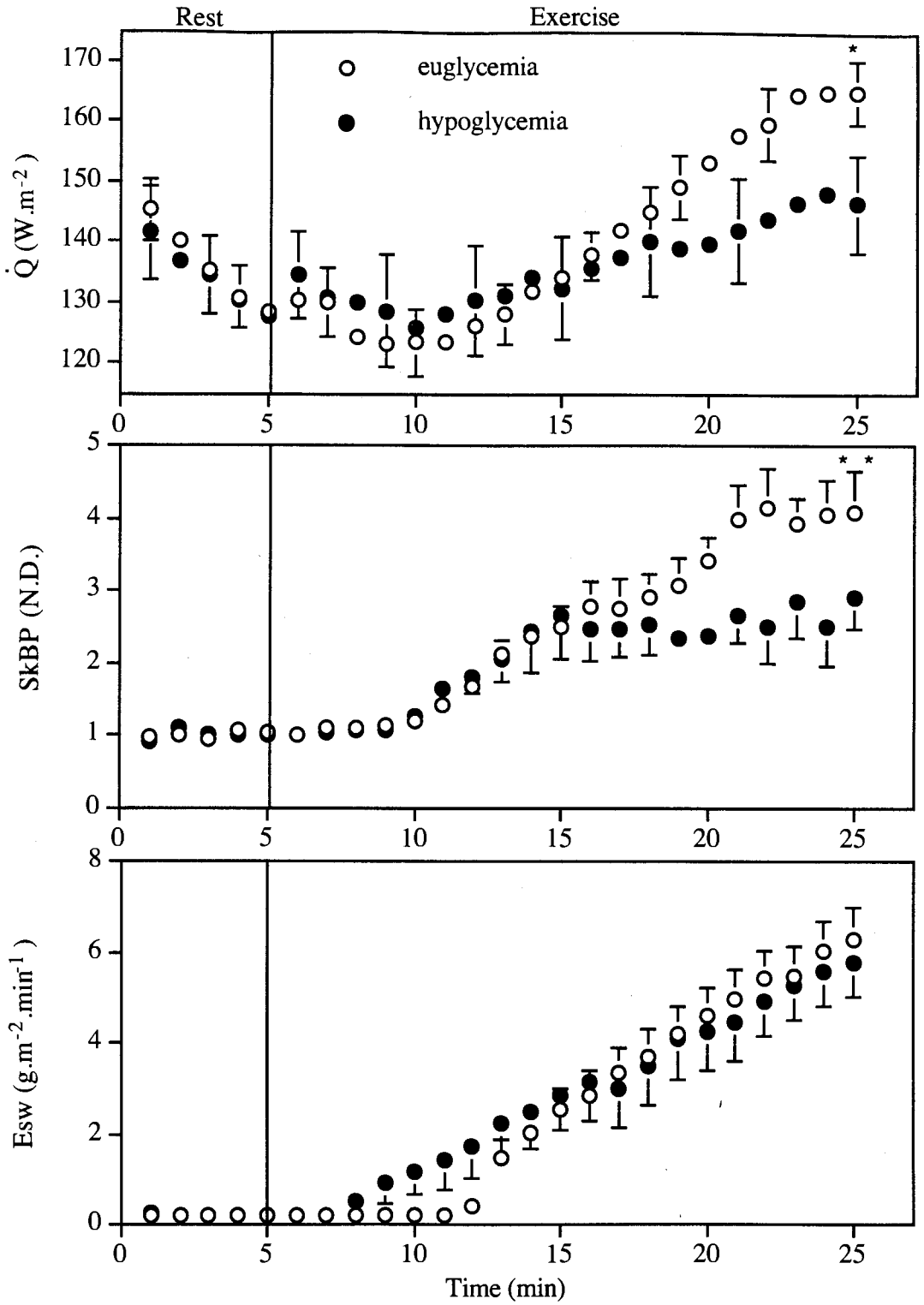

Fig. 3-4. Heat flux from the skin ($\dot{Q}$, *top*), skin blood perfusion (SkBP, *middle*) and sweat rate (Esw, *bottom*) responses (mean ± SE), during the rest and exercise periods in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. *$p \leq 0.04$ during the last 5 min of exercise, **$p \leq 0.01$ during the last 6 min of exercise.

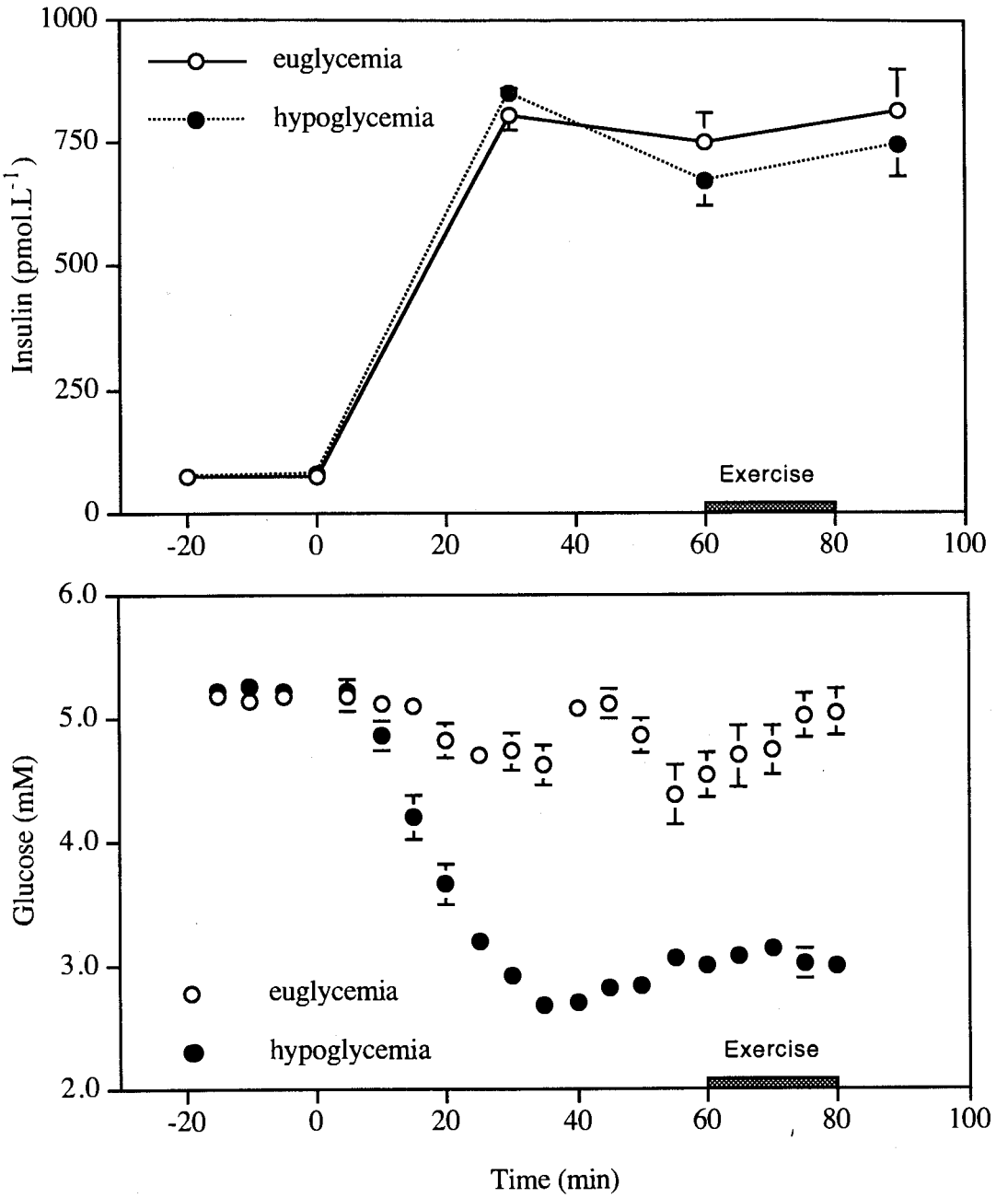

Fig. 3-5. Plasma insulin (*top*) and glucose (*bottom*) values (mean ± SE) during the rest and exercise periods in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. Glucose clamping was initiated at time zero.

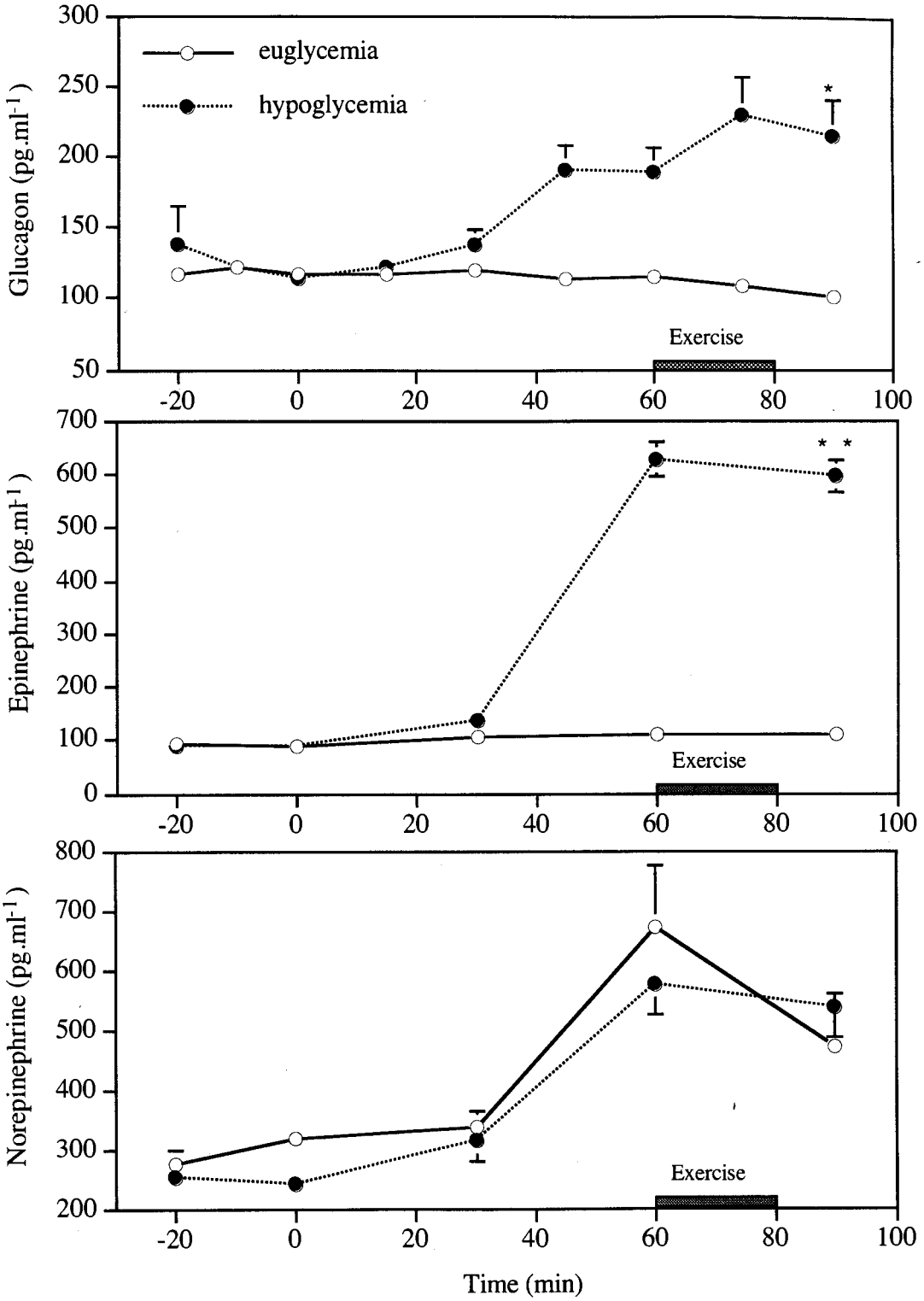

Fig. 3-6. Plasma glucagon (*top*), epinephrine (*middle*) and norepinephrine (*bottom*) values (mean ± SE) during the rest and exercise periods in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. Glucose clamping was initiated at time zero. *$p \leq 0.001$ from minute 40 onward, **$p \leq 0.0001$ from minute 60 onward.

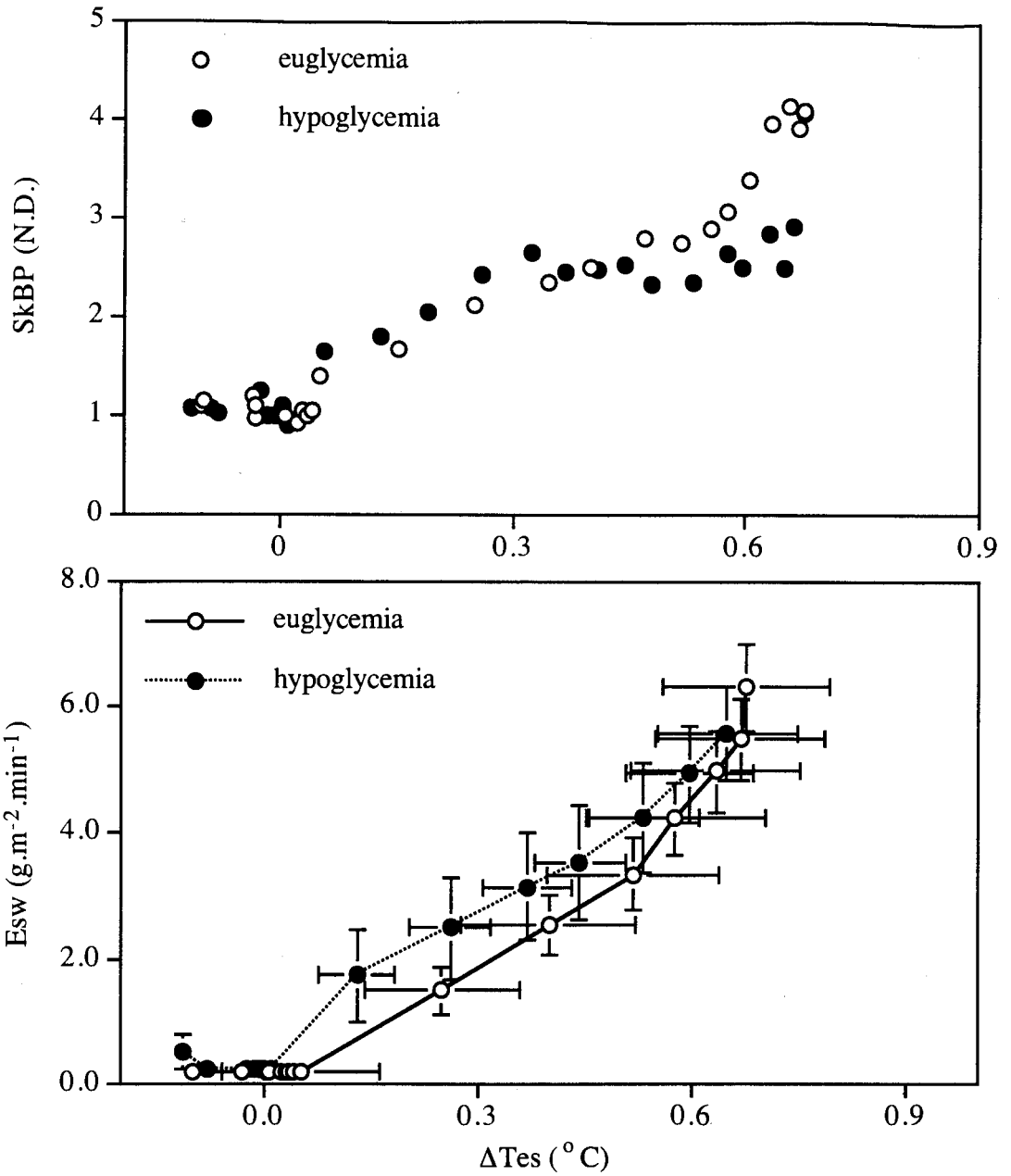

Fig. 3-7. Skin blood perfusion (SkBP, *top*) and sweat rate (Esw,*bottom*) as a function of the change in esophageal temperature relative to resting values (ΔTes) during the exercise period in the euglycemic (open circles) and hypoglycemic (closed circles) conditions.

CHAPTER FOUR: EFFECTS OF HYPOGLYCEMIA ON THERMOREGULATION DURING IMMERSION HYPOTHERMIA

INTRODUCTION

Hypoglycemia induced by infusion of insulin in humans decreases core temperature during exposure to a thermoneutral environment at rest by inducing peripheral vasodilation and sweating and thus an increase in heat loss (Allwood *et al.*, 1959; Middleton and French 1974; Gale *et al.*, 1981; Gale *et al.*, 1983; Berne and Fagius, 1986). Studies in animals (Cassidy *et al.*, 1925, 1926; Dworkin and Finney, 1927) and humans (Haight and Keatinge, 1973; Gale *et al.*, 1981) have shown that hypoglycemia decreases body temperature further during cold exposure by inhibiting shivering thermogenesis. However, the specific effects of hypoglycemia on the core temperature threshold and intensity of shivering thermogenesis have not been assessed.

Insulin-induced hypoglycemia also stimulates sweating during exposure at rest to a thermoneutral environment (Molnar and Read 1973 and 1974; Gale *et al.*, 1981; Macdonald *et al.*, 1982; Gale *et al.*, 1983; Berne and Fagius, 1986). Since this response coincides with an increased catecholamine secretion (Macdonald *et al.*, 1982), it might be supposed that it is a hypoglycemia-induced sympathetic effect and consequently is not meant to serve a thermoregulatory purpose. It was shown in chapter three, that hypoglycemia does not affect sweating or skin passive vasodilation when these responses are instigated mainly for thermoregulatory purposes, such as during exercise-induced mild hyperthermia. However, the effects of hypoglycemia on sweating and skin blood perfusion responses during mild hyperthermia free from factors, such as exercise, with possible confounding effects upon the studied responses, remain to be elucidated.

The present study was designed to quantify the effects of hypoglycemia on the threshold and intensity of shivering thermogenesis during immersion mild hypothermia and the sweating and skin blood perfusion responses during recovery from exercise-induced mild hyperthermia. In view of suggestions of an interrelationship between the thermoregulatory and glucoregulatory systems (Silva and Boulant, 1984) it was envisaged that quantification of the effects of hypoglycemia upon the different

thermoregulatory responses would offer further insight into the interactions between these homeostatic mechanisms, as they are expressed at the integrated whole-body level of physiological function.

METHODS

Subjects

Ten healthy male volunteers participated in the present study after giving their informed written consent. Their participation was subject to physician's approval and they were familiarized with the experimental protocol and the possible risks involved prior to the experiments. The study was approved by the Simon Fraser University ethics committee.

Experimental protocol

Each subject followed a similar experimental protocol as described previously by Mekjavić *et al.*, (1991), which allows the determination of shivering and sweating responses to changes in core temperature, while maintaining skin temperature constant. Namely, each subject, dressed in swimming shorts, was immersed to the chest in a water bath maintained at 28 °C and exercised at 50% of his maximal work rate (determined previously on a separate occasion) on an underwater cycle ergometer for 20 minutes, or until the rise in core temperature reached a plateau and sweating secretion was stimulated. Following the exercise session the subject remained in the water bath for an additional 99 minute period or until the core temperature decreased by 2 °C from its resting value. During this period the water was agitated by activating a propeller to increase convective heat loss and thus facilitate core cooling. Each subject followed the same experimental procedure on two different occasions. On one occasion the blood glucose concentration was maintained at 2.8 mM (hypoglycemia) and in the other at 5.0 mM (euglycemia), using the glucose clamp technique (DeFronzo *et al.*, 1979). The rate of insulin infusion

for both conditions was 60 $mU.m^{-2}.min^{-1}$ and the rate of glucose infusion was adjusted accordingly in order for the plasma glucose concentration to be maintained at the desired level. The two trials were spaced at least one week apart, to avoid acclimation to the experimental protocol, and the order in which the trials were conducted was alternated among subjects. Subjects were unaware of the condition (either euglycemia or hypoglycemia) they were undertaking. Any effects of circadian rhythm were minimized by conducting the trials at the same time of the day for each subject. Each subject was asked to avoid strenuous exercise during the day preceding the experiment and to fast for 12 hours prior to testing. The subject was also asked to maintain a similar diet as well as physical activity and sleeping schedules during the 2 day period preceding each of the two experimental trials, thus diminishing the potential effect of these factors on the recorded physiological responses.

Core temperature was measured with an esophageal thermistor probe inserted to a length determined from sitting height (Mekjavić and Rempel, 1991). Heat flux and skin temperature were measured at six sites (arm, chest, abdomen, back, thigh and calf). A ventilated capsule was placed on the skin surface of the subject's forehead for sweat secretion mesurements. Skin blood flow was measured on the contralateral side of the forehead by a Laser Doppler probe. The subject was breathing through a mouthpiece during the experiment. ECG was monitored continuously throughout each trial.

Insulin and glucose were infused through a catheter inserted in the left forearm (antecubital vein), and blood samples were taken via another catheter inserted in the left hand (contralateral vein). The catheterized hand was placed in a heated chamber (60-70 ^{o}C). This results in sufficient arteriovenous shunting to arterialize the venous blood sample, thus eliminating the need for arterial catheterization in these studies (McGuire *et al.*, 1976). The first blood sample was taken thirty minutes after the catheterization to determine the resting values of blood glucose and catecholamine concentration. This was followed by the implementation of the glucose clamp procedure. Plasma glucose reached

the desired level within approximately 30 minutes after the initiation of the glucose clamp procedure. Resting values were recorded for 5 minutes (rest) while the subject was sitting on the underwater cycle ergometer immersed in the water to the chest. The core temperature values corresponding to the cessation of sweating (sweating cessation threshold values) and the initiation of shivering (shivering threshold) were determined during the 99 minute period of immersion following the exercise. During the same period, the core temperature values at which the decreasing SkBP attained resting values was characterized as the core temperature threshold for passive vasodilation. Following the immersion, subjects were rewarmed and normal blood glucose level was reinstated.

Maximal exercise test

The $\dot{V}O_2$ max test was performed on a separate occasion prior to the initiation of the experiments on the underwater cycle ergometer while the subject was immersed to the chest in 28 °C water. The protocol started with 2 minutes of rest while the subject was seated on the underwater ergometer. This was followed by 2 minutes of unloaded pedalling at 55 revolutions per min (RPM). Thereafter, workrate was incremented by 0.5 kp at 2 min intervals.

Instrumentation

The immersion tank (2.1 x 1.05 x 2.1 metres) contained approximately 4000 liters of water, continuously agitated throughout the immersion by a PAC-FAB hydropump (El Monte C.A., USA) and maintained at 28 °C. Water temperature was recorded with a YSI 701 (Yellow Springs Instruments, Ohio, USA) thermistor.

Heat flux ($\dot{Q}$,W.m $^{-2}$) and skin temperature (Tsk, °C). Heat flux from the skin surface was measured with heat flux transducers (Concept Engineering, Old Saybrook, CT). Thermistors embedded in the transducers' surface placed on the skin, allowed concurrent measurement of skin temperature. The transducers were attached to the skin with waterproof tape (Elastoplast, Lachine, Quebec).

Core temperature (Tc, ^{o}C). Esophageal temperature (Tes) was assessed using a YSI 702 (Yellow Springs Instruments, Ohio, USA) thermistor.

Ventilation ($\dot{V}_I$, L.min $^{-1}$). The volume of the inspired air was measured with an Alpha technologies Ventilation Module (Model VMM110, California). The subject was breathing through a low-resistance two way valve. The expiratory side was connected by Collins corrugated tubing to a 9 liter fluted plexiglass mixing box.

Oxygen uptake $\dot{V}O_2$ (L.min^{-1}) and carbon dioxide production ($\dot{V}CO_2$, L.min $^{-1}$). $\dot{V}O_2$ and $\dot{V}CO_2$ were determined from the analyses of mixed expired gases and inspired minute ventilation. A continuous 500 ml.min.$^{-1}$ sample of mixed expired gas was drawn from the mixing box and analysed for oxygen and carbon dioxide contents using an Applied Electrochemistry Oxygen Analyser (S-3A) and a Statham Godart Capnograph, respectively.

Sweat rate (Esw, $g.m^{-2}.min^{-1}$). Sweat rate was measured with the sweat capsule positioned on the skin surface of the forehead (surface area = 4.5 cm^2). The capsule was ventilated at a rate of 330 ml.min^{-1} with compressed dry air supplied from a tank which was maintained at room temperature. The air entering the capsule was dry and its temperature equal to room temperature. The temperature and the relative humidity of the air exiting the capsule was measured with a temperature and relative humidity sensor (Smart Reader 2, ACR Systems Inc., Surrey, B.C., Canada), respectively. By calculating the difference between the water content of the air entering and leaving the capsule, the value for sweat secreted was determined.

Skin blood perfusion (SkBP, non-dimentional (N.D.)). Skin blood perfusion was measured with a Laser Doppler perfusion monitor (Periflux PF3, Sweden), and is reported as the ratio of skin blood perfusion at any given time (SkBP(t)) relative to average skin blood perfusion values recorded during the 5 minutes of resting period preceding the exercise ($SkBP_{rest}$). Thus at rest, SkBP = SkBP (t) / $SkBP_{rest}$ = 1.

Data acquisition. All physiological variables were recorded at one minute intervals. With the exception of heart rate and sweat rate, physiological variables were measured on-line with an HP (Hewlett Packard) 3497A data acquisition system controlled by an Apple Macintosh II computer (using Labview software, National Instruments, Austin Texas). For the measurement of sweating the temperature and humidity modules were controlled with an ATS (model SX-30) PC computer.

Blood samples and analysis. Glucose content of the sampled blood was determined by a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma glucagon and insulin were measured by radioimmunoassay, whereas norepinephrine and epinephrine by high performance liquid chromatography (HPLC) with electrochemical detection, as described by Meneilly *et al*., (1985).

Statistical analyses

The responses of all recorded variables were compared between the two experimental conditions using a two-way analysis of variance (ANOVA) with repeated-measures design. A regression analysis was conducted for the ΔTes response obtained during cooling, for each subject separately. The slopes obtained this way were used as an indication of the cooling rate of esophageal temperature. Comparison of the slopes between the two experimental conditions was conducted using a paired t-test. Threshold values for sweating, passive vasodilation and shivering were determined for each subject separately, and they were chosen from Esw-ΔTes, SkBP-ΔTes and $\dot{V}O_2$-ΔTes plots, respectively. The core temperature threshold value for shivering was considered to be the core temperature corresponding to the first oxygen uptake value which was higher than the median of the $\dot{V}O_2$ response recorded during the first 10 minutes of the cooling phase. The core temperature threshold value for cessation of sweating was considered to be the core temperature value corresponding to the last sweating response higher than the values observed during the resting period which correspond to insensible water loss. Similarly,

core temperature threshold for passive vasodilation was considered to be the value at which the SkBP ratio reached resting values (ratio =1). Threshold values were chosen by an investigator who was naive to the conditions and subjects involved. The slopes of the Esw-ΔTes and SkBP-ΔTes relations, obtained with a linear regression analysis were considered to be the gains of the Esw and SkBP responses, respectively. The threshold and gain values of all the responses were compared between the two experimental conditions using paired t-test. The 5% level was chosen as the level of significance for all analyses.

RESULTS

In general, subjects felt more hungry, tired and shivered visibly less during the hypoglycemic compared with the euglycemic condition. Some of the subjects also reported that they sweated during the hypoglycemic condition while they were waiting to enter the water tank to start the experiment. Subjects' personal descriptions regarding shivering and sweating were very consistent with the investigators' subjective observations regarding the time and intensity of appearance of the described responses. Results are reported as mean ± SE for the subject group.

Esophageal temperature

Since resting Tes was similar between the two experimental conditions (36.57 ± 0.08 and 36.43 ± 0.07 °C during the euglycemic and hypoglycemic conditions, respectively), core temperature was expressed as the relative change of esophageal temperature from the resting value (ΔTes, °C). During the post-exercise cooling phase core temperature decreased in an almost linear manner during both experimental conditions (Fig. 1) from 0.68 ± 0.12 to -0.6 ± 0.06 °C in the euglycemic condition and from 0.66 ± 0.1 to -1.2 ± 0.08 °C in the hypoglycemic condition. Tes was lower during the hypoglycemic compared with the euglycemic condition after the first 5 minutes of the

experiment ($p \leq 0.006$). The average cooling rate of Tes as determined by linear regression analysis, was greater ($p \leq 0.0002$) during the hypoglycemic (-1.2 ± 0.06 °C.hr^{-1}) compared to the euglycemic condition (-0.78 ± 0.06 °C.hr^{-1}).

Skin temperature

Tsk decreased from 31.0 ± 0.2 to 28.7 ± 0.3 °C in the euglycemic condition and from 30.6 ± 0.1 to 28.3 ± 0.1 °C in the hypoglycemic condition (Fig. 1). The greater portion of this decrease in skin temperature occurred during the first few minutes of the cooling phase and it was elicited by the agitation of the water by a propeller which was activated after the end of exercise. There was no difference in Tsk between the two experimental conditions.

Oxygen consumption

$\dot{V}O_2$ attained its approximate resting level after the end of exercise, and remained at this level for a further 10-20 minutes before it began to increase again (Fig. 2). The elevation in $\dot{V}O_2$, concominant with the onset of visible shivering, was observed early in the euglycemic condition. The increase in $\dot{V}O_2$ from minutes 25 to 70 was significantly higher during euglycemia ($p \leq 0.004$) than during hypoglycemia. During the remaining 25 minutes of the experiment, there was a tendency for higher $\dot{V}O_2$ in the euglycemic compared with the hypoglycemic condition, albeit the difference was not statistically significant. When $\dot{V}O_2$ was expressed in relation to ΔTes (Fig. 6), the ΔTes values corresponding to the initiation of the increase in $\dot{V}O_2$ (shivering threshold, Table 1) were lower ($p \leq 0.001$) in the euglycemic (-0.06 ± 0.08 °C) compared with the hypoglycemic condition (-0.65 ± 0.12 °C).

Heat flux

$\dot{Q}$ exhibited a transient increase following the activation of the propeller at the end of exercise, and stabilized at values similar to, or lower than, resting at approximately 25-30 minutes after the initiation of the cooling phase (Fig. 3). $\dot{Q}$ reached peak values of 266 ± 15 in the euglycemic and 216 ± 16 $W.m^{-2}$ in the hypoglycemic condition, respectively during the transient increase. End immersion values of 90 ± 4 and 91 ± 6 $W.m^{-2}$ during the euglycemic and hypoglycemic conditions, respectively were recorded. Although there was a tendency for heat flux to be higher during the euglycemic compared with the hypoglycemic condition, the difference was not statistically significant.

Skin blood perfusion

SkBP was similar in both experimental conditions (Fig. 3). It exhibited a 2-3 fold decrease during the first 40 minutes of the cooling phase, in both experimental conditions. During the remainder of the immersion SkBP was stabilized at values slightly below resting. The ΔTes values at which SkBP reached resting levels were not significantly different between the two experimental conditions (0.14 ± 0.09 and 0.01 ± 0.17 °C for the euglycemic and hypoglycemic conditions, respectively; Table 2). The regression analysis which was performed for the linear portion of the SkBP-ΔTes relation included the SkBP data points that were higher than one. It was observed that there was no difference in the gain of SkBP response between the two experimental conditons (3.84 ± 0.97 $.^{o}C^{-1}$ in the euglycemic and 3.22 ± 0.86 $.^{o}C^{-1}$ in the hypoglycemic condition; Table 2)

Sweating

Esw decreased slowly and abated in almost all the subjects during the first 30 minutes of the cooling phase in both experimental conditions. As can be seen in Fig. 3, there was no difference in the sweating response between the two experimental

conditions. Similarly, although the ΔTes corresponding to the cessation of sweating (threshold values for cessation of sweating, Fig. 6) was lower during the hypoglycemic compared to the euglycemic condition (0.15 ± 0.14 and 0.38 ± 0.07 °C, respectively), the difference was not statistically significant ($p \leq 0.13$, power 0.32, Fig. 6, Table 3). Similar results were obtained by using the Wilcoxon Sign Rank non-parametric statistical test. Comparison of the slopes obtained from the linear regression analysis of the Esw-ΔTes relation indicated that although the gain of the Esw response was higher in the euglycemic (12.54 ± 1.13 $g.m^{-2}.min^{-1}.°C^{-1}$) compared to the hypoglycemic (9.18 ± 1.61 $g.m^{-2}.min^{-1}.°C^{-1}$) condition, the difference was not statistically significant (Table 3).

Glucose and hormonal values

Insulin concentration was maintained relatively stable during both experimental conditions (Fig. 4). Insulin levels of 814 ± 83 and 745 ± 64 $pmol.L^{-1}$ at the beginning of the cooling phase reached end immerson values of 955 ± 70 and 879 ± 69 $pmol.L^{-1}$ for the euglycemic and the hypoglycemic conditions, respectively. There was no difference in plasma insulin levels between the two experimental conditions.

The initial plasma glucose concentration of 5.2 ± 0.2 and 3.0 ± 0.1 mM were maintained relatively stable during the experiment. The glucose values at the end of the immersion were 5.0 ± 0.1 and 2.7 ± 0.1 mM for the euglycemic and the hypoglycemic conditions, respectively (Fig. 4).

The initial glucagon values of 101 ± 4 and 215 ± 25 $pg.ml^{-1}$ were maintained relatively stable throughout the immersion in both conditions reaching end immersion values of 100 ± 4 and 190 ± 40 $pg.ml^{-1}$ during the euglycemic and the hypoglycemic condition, respectively (Fig. 5). Glucagon values were higher ($p \leq 0.05$) during the hypoglycemic compared to the euglycemic condition.

Plasma epinephrine remained consistently higher ($p \leq 0.007$) during hypoglycemia compared to the euglycemic condition throughout the experiment (Fig. 5).

Epinephrine values of 109 ± 25 and 597 ± 113 pg.ml^{-1} recorded at the beginning of the cooling phase were maintained during the experiment and reached end immersion values of 96 ± 25 and 617 ± 115 pg.ml^{-1} during the euglycemic and the hypoglycemic conditions, respectively.

Plasma norepinephrine increased during the cooling phase of the experiment from 473 ± 87 and 538 ± 49 pg.ml^{-1} at the beginning to end immersion values of 727 ± 123 and 989 ± 190 pg.ml^{-1} during the euglycemic and the hypoglycemic conditions, respectively (Fig. 5). There was no significant difference in plasma norepinephrine concentration between the two experimental conditions.

DISCUSSION

The present study demonstrates that during immersion in 28 °C of water, hypoglycemia induces a greater cooling of the core, which appears to be mediated by a reduction in heat production rather an enhancement of heat loss.

Heat production

The most interesting findings of the present study are that: 1) hypoglycemia (2.8 mM) reduces, but does not totally abolish, hypothermia-induced heat production, 2) this reduction is achieved by decreasing the core temperature threshold for hypothermia-induced heat production by approximately 0.6 °C and the amplitude of heat production by approximately 20%, compared with the respective values during euglycemia.

The observed difference in heat production between the two experimental conditions, as reflected by oxygen uptake values (Fig. 2), could be attributed either to a greater thermogenic effect exerted by the infusion of insulin and glucose during the euglycemic compared to the hypoglycemic conditions, and/or to an inhibition of shivering thermogenesis caused by hypoglycemia. It has been shown that combined infusion of insulin and glucose exerts a thermogenic effect in humans, reflected by a 5-

11% increase in resting oxygen consumption (Ravussin and Bogardus 1982; Ravussin *et al.*, 1983; Thiebaud *et al.*, 1983; DeFronzo *et al.*, 1984) and that approximately 50-70% of this increment in energy expenditure may be accounted for by the conversion of glucose to glycogen (Ravussin and Bogardus 1982; Thiebaud *et al.*, 1983; DeFronzo *et al.*, 1984). In the present study, $\dot{V}O_2$ was higher in the euglycemic than in the hypoglycemic condition from minute 25 to 70 of cooling (post exercise period). This difference in $\dot{V}O_2$ could be partly attributed to the greater thermogenic effect exerted by insulin and glucose infusion during the euglycemic than the hypoglycemic condition. Since insulin infusion rates were similar between the two experimental conditions any difference in the thermogenic response could be only linked to the greater glucose infusion rates, which were required during the euglycemic compared to the hypoglycemic condition in order to maintain higher blood glucose levels in the former condition. However, $\dot{V}O_2$ values were almost identical in the two conditions during the first 10-20 minutes of cooling, during which period the differences in glucose infusion rates between the two experimental conditions, if anything, were higher compared to the rest of the experiment. Namely, glucose infusion rate decreased in the euglycemic condition from 18.53 ± 1.92 to 13.71 ± 1.72 $mg.kg^{-1}.min^{-1}$ by minute 20 of the cooling phase and was increased from 2.32 ± 0.83 to 3.81 ± 1.01 $mg.kg^{-1}.min^{-1}$ during hypoglycemia. The values observed at minute 20 of the experiment were maintained at this level for the remainder of the experiment reaching end immersion value of 14.11 ± 1.16 and 4.50 ± 1.59 $mg.kg^{-1}.min^{-1}$ in the euglycemic and hypoglycemic conditions, respectively. This confirms that the observed $\dot{V}O_2$ difference between the two experimental conditions most likely reflects differences in shivering thermogenesis.

As for the mechanism of this inhibition, Gale *et al.*, (1981) demonstrated very elegantly that shivering, as indicated by electromyographic activity (EMG), was restored quickly in hypoglycemic subjects after intravenous glucose injection even in the limb

which was isolated from circulation by an arterial occlusion cuff. This demonstrated that shivering inhibition was due to central rather than peripheral effects of hypoglycemia.

The present study quantified this effect of hypoglycemia on shivering thermogenesis. The approximately 20% lower $\dot{V}O_2$ values observed during the hypoglycemic compared with the euglycemic condition (Fig. 2) could be attributed to shivering inhibition at both the central and/or the peripheral level. The 0.6 °C shift in the core temperature threshold for shivering (Fig. 6) suggests an effect on central thermoregulatory integration. This interpretation is in accordance with the observations by Silva and Boulant (1984) that low glucose perfusion media inhibits the cold-sensitive neurons of the PO/AH tissue slice preparation in the rat, considering that these cold-sensitive neurons facilitate heat production responses (Hammel, 1965; Boulant, 1980).

Whether there is a blood glucose threshold concentration below which shivering is totally suppressed cannot be deduced from the present results. However, it is clear that hypoglycemia at a blood glucose concentration of approximately 2.8 mM does not totally abolish shivering thermogenesis, but it rather shifts the core temperature threshold to lower values. It has been shown that hypoglycemia exerts a "dose-response" rather than an "all-or-none" type of effect on other physiological responses (Gale *et al.*, 1983). Whether this is the case for shivering thermogenesis remains to be elucidated. Two of the ten subjects in the present study did not exhibit any shivering response during the hypoglycemic condition although their core temperature reached 35 °C, at which point the experiment had to be terminated. The remaining volunteers shivered at core temperature values which were close to or clearly above 35°C. It could be suggested that most likely in these two subjects hypoglycemia (2.8 mM) shifted their shivering thresholds to values below 35 °C rather than it totally abolished shivering.

Heat conservation and heat loss mechanisms

Skin blood perfusion

Neural and hormonal factors are involved in the regulation of skin blood flow during hypoglycemia. Using microelectrode recordings of skin sympathetic nerve activity it has been shown that hypoglycemia releases the sympathetic vasoconstrictor tone of the skin blood vessels (Berne and Fagius, 1986; Fagius and Berne, 1989), thus causing skin vasodilation. However, the increased plasma epinephrine concentration caused by hypoglycemia can exert an α-adrenergic mediated vasoconstrictive effect on skin blood vessels (Rowell, 1986; Maggs and Macdonald, 1992).

The release of the sympathetic vasoconstrictor tone of the skin blood vessels is exerted during the early stages of hypoglycemia, thus explaining the skin flushing or skin vasodilation reported in previous studies (French and Kilpatrick, 1955; Gale *et al.*, 1981). However, prolonged hypoglycemia exerts a vasoconstrictor effect especially in the distal body areas, which is similar to that caused by epinephrine infusion, indicating that the release of epinephrine can account for the majority of the peripheral vascular responses observed during sustained hypoglycemia (Maggs and Macdonald, 1992).

In the present study, relative changes of skin blood perfusion from baseline were similar in both experimental conditions. Probably, the severity of the peripheral cold stimulus applied on the immersed part of the body in combination with central cold stimulus, exerted a strenous reflex cutaneous vasoconstrictive effect, thus masking any vasoconstrictor action of the increased plasma epinephrine concentration observed during hypoglycemia or overiding any sympathetically mediated cutaneous vasodilatory effect of hypoglycemia.

The unique feature of the study reported in chapter three and the present study, compared with previous reports (Gale *et al.*, 1981, 1983; Berne and Fagius, 1986) is that cutaneous vasomotor tone was decreased and increased, respectively, for thermoregulatory purposes. It has been shown in the former two studies that

hypoglycemia does not affect the sympathetically mediated regulation of skin blood perfusion when it is driven for thermoregulatory reasons, such as during exercise-induced mild hyperthermia and/or during recovery from it.

Heat flux

In the present study heat flux from the skin had a tendency to be higher during the euglycemic compared with the hypoglycemic condition, but this difference was not significant. Therefore, the higher core cooling rate which was observed during hypoglycemia cannot be attributed to increased convective heat loss.

Sweating

Previous studies have shown that hypoglycemia stimulates sweat secretion even during exposure to a thermoneutral environment and at a normal body temperature (Gale *et al.*, 1981; Macdonald *et al.*, 1982; Gale *et al.*, 1983). Using microelectrode recordings of sympathetic signals in the peroneal nerve, it has been confirmed that the sweating during insulin-induced hypoglycemia reflects an increase in sudomotor activity directed to the skin (Berne and Fagius, 1986). Similar results have been observed during tissue glycopenia induced by infusion of 2-deoxy-D-glucose (Fagius and Berne, 1989). Thus, it seems that the sweating during hypoglycemia is due to sympathetic activation. In the present study, six subjects reported the perception of sweating during the rest, pre-immersion period of the hypoglycemic trial. Values of sweating during these observations are not available as the sweating monitor was not applied prior to immersion. Subjects did not report any differences in sweating during the immersion period in the two conditions. It was shown previously (chapter three) that hypoglycemia does not affect the core temperature threshold value for initiation of sweating neither the gain of the sweating response during exercise-induced mild hyperthermia. The present results confirm the latter observations by showing that neither the core temperature

threshold for cessation of sweating nor the gain of the sweating response were affected by hypoglycemia during recovery from exercise-induced mild hyperthermia. In light of the similarities in the observations reported in chapter three and the present results it could be suggested that exercise per se did not constitute a confounding factor for the interpretation of the effects of hypoglycemia on the thermoregulatory responses during exercise-induced mild heat stress. The present findings in combination with those reported in chapter three seem to indicate that sympathetically-induced sweating should be distinquished from the thermoregulatory sweating during hypoglycemia. It seems that sweating develops during hypoglycemia even at thermoneutral body and environmental temperatures due to sympathetic activation. This is probably a stress-related rather than a thermoregulatory-related response, although the latter cannot be totally excluded. However, exercise-induced mild hyperthermia, and/or recovery from it, such as in the present study, requires and activates thermoregulatory sweating which does not seem to be affected by hypoglycemia.

Theoretical considerations

The neurophysiological evidence provided by Silvaand Boulant (1984), that 50% of the examined preoptic area (PO/AH) thermosensitive neurons do not exibit any variation in their firing rates in response to low glucose media, whereas the remaining 50% were affected, lends support to the present findings. Namely, that the responses which were either affected less (sweating) or were not affected by hypoglycemia (skin blood perfusion) in the present study, are facilitated mostly by the thermosensitive neurons in the PO/AH region which do not exhibit any variation in their firing rates in response to low glucose media. Conversely, heat production mechanisms, such as shivering, are facilitated by the thermosensitive neurons that respond to low glucose media and as a result appeared to be affected by hypoglycemia. The dissimilar effects of hypoglycemia on the three examined thermoregulatory responses ($\dot{V}O_2$, SkBP and Esw)

indicate that they are regulated by separate "integrators" or regions within the CNS as suggested by Satinoff (1978). A "single integrator" neural model would have predicted similar effect of hypoglycemia on all the effector responses.

In addition, the possibility that hypoglycemia affected the non-thermal neural inputs converging on the thermal sensor-to-effector pathways in the CNS (Bligh, 1990), cannot be excluded as an alternative explanation of the present observations. In this manner, hypoglycemia may have affected the non-thermal synaptic inputs on the pathways from the cold sensors to heat production effectors resulting in inhibition of shivering thermogenesis. In contrast, a limited or no effect of hypoglycemia on the non-thermal synaptic inputs on the pathways from the warm sensors to heat loss effectors resulted in a normal function of the heat loss mechanisms (sweating, skin blood perfusion) during hypoglycemia.

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Table 4-1. The ΔTes theshold values for shivering.

Subjects	ΔTes threshold for shivering (°C)	
	Euglycemia	Hypoglycemia
1	0.09	-0.80
2	-0.10	-0.35
3	-0.50	-1.10
4	0.12	-0.40
5	0.06	-0.40
6	-0.17	-1.08
7	-0.20	-0.77
8	0.20	-0.30
9	-0.18	†
10	-0.24	†
Mean ± SE	-0.09 ± 0.07	-0.65 ± 0.12

† Subjects did not shiver

Table 4-2. The ΔTes threshold values for passive vasodilation and the gain of the response (SkBP/ΔTes) during cooling in the euglycemic and hypoglycemic conditions

	ΔTes threshold for SkBP (°C)		SkBP/ΔTes ($^{o}C^{-1}$)	
Subjects	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
1	0.33	0.10	2.60	3.73
2	-0.18	0.00	10.29	8.54
3	~	~	~	~
4	~	~	~	~
5	0.42	0.60	4.26	2.35
6	0.14	-0.08	2.12	0.78
7	0.18	-0.50	2.35	2.03
8	0.43	0.21	2.12	4.05
9	0.00	-0.78	4.31	2.99
10	-0.20	0.50	2.69	1.27
Mean ± SE	0.14 ± 0.09	0.01 ± 0.17	3.84 ± 0.97	3.22 ± 0.86

~ missing data

Table 4-3. The ΔTes threshold values for cessation of sweating and the gain of the response (Esw/ΔTes) during cooling in the euglycemic and hypoglycemic conditions

	ΔTes threshold for Esw (°C)		Esw/ΔTes ($g.m^{-2}.min^{-1}.°C^{-1}$)	
Subjects	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
1	0.29	-0.17	13.23	12.03
2	0.43	0.83	7.24	8.25
3	0.27	-0.19	11.01	10.81
4	0.67	0.91	17.00	21.97
5	-0.10	0.28	8.42	8.57
6	0.30	0.12	12.36	6.83
7	0.44	0.06	15.88	3.73
8	0.65	0.30	10.13	6.39
9	0.33	-0.30	17.94	5.47
10	0.48	-0.35	12.16	7.81
Mean ± SE	0.38 ± 0.07	0.15 ± 0.14	12.54 ± 1.13	9.18 ± 1.61

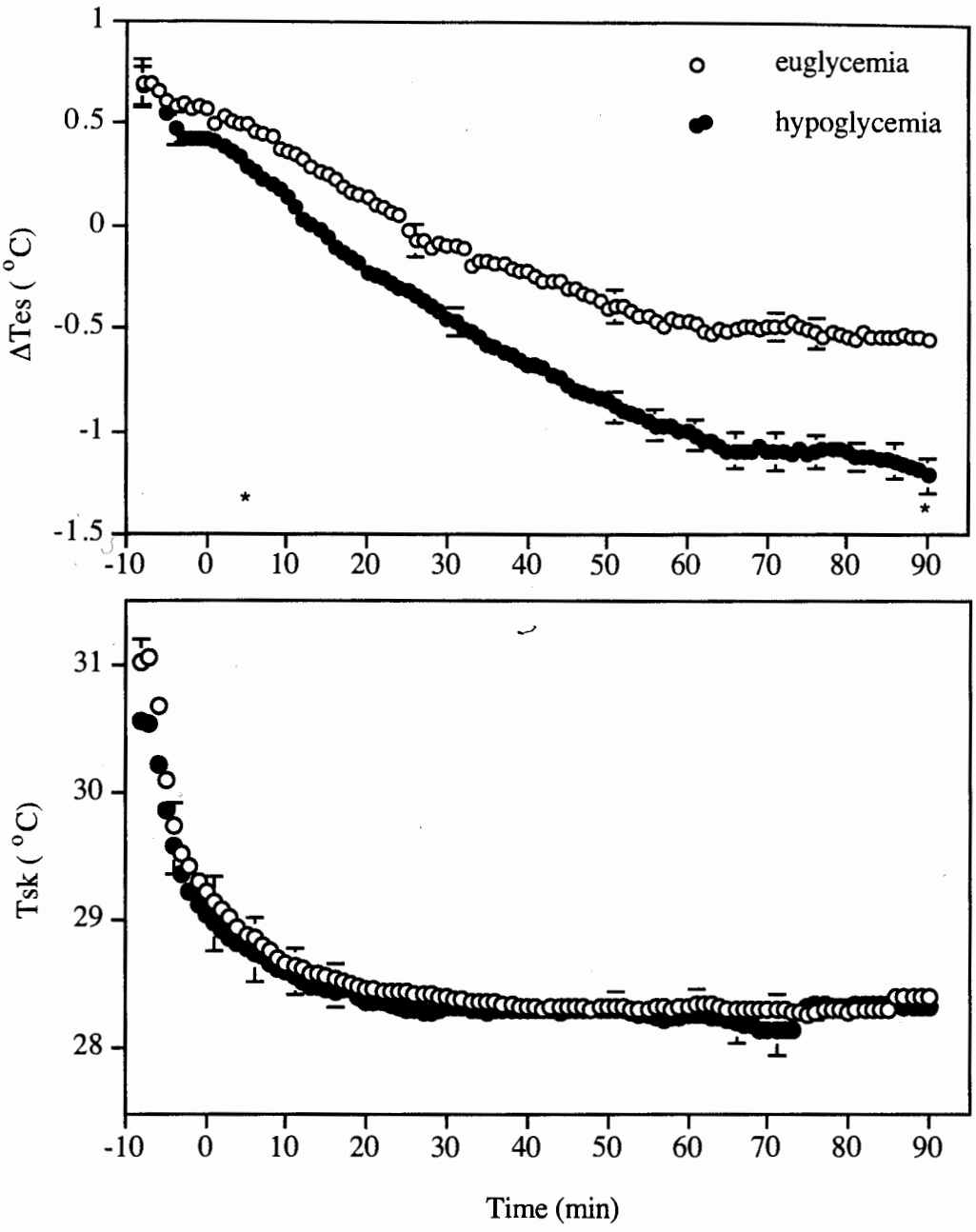

Fig. 4-1. Changes (mean ± SE) in esophageal temperature (ΔTes) from resting values (*top*) and the skin temperature response (Tsk, *bottom*), during the cooling post-exercise period, in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. *$p \leq 0.006$ from minute 5 onward.

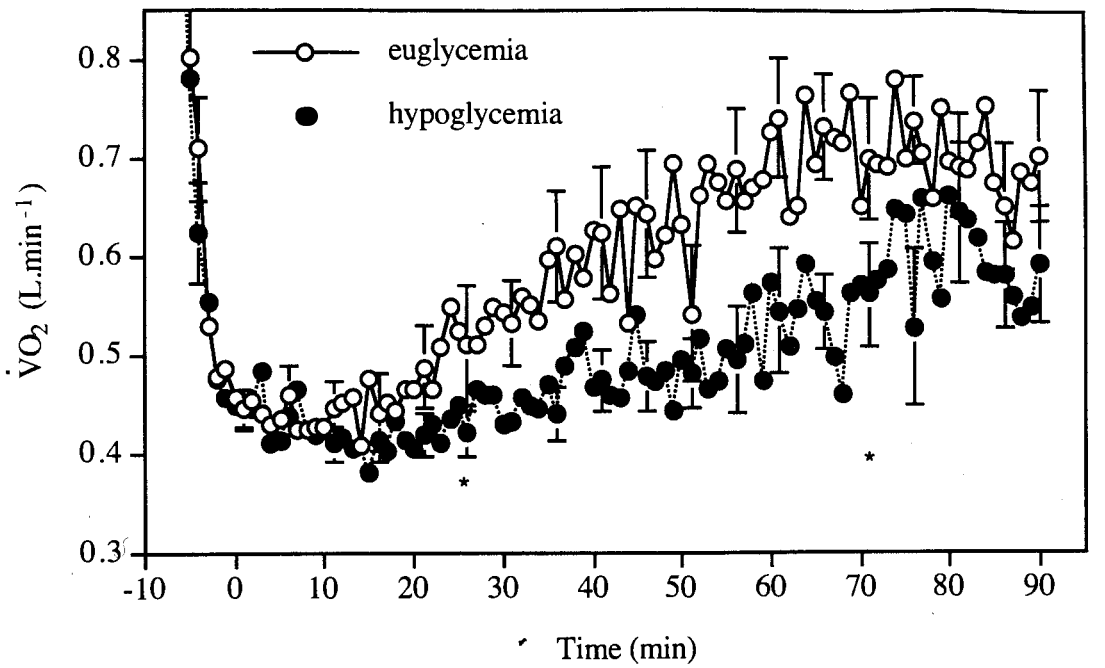

Fig. 4-2. Oxygen uptake ($\dot{V}O_2$) values (mean $\pm$ SE), during the cooling post-exercise period, in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. *p $\leq$ 0.004 from minute 25 to 70.

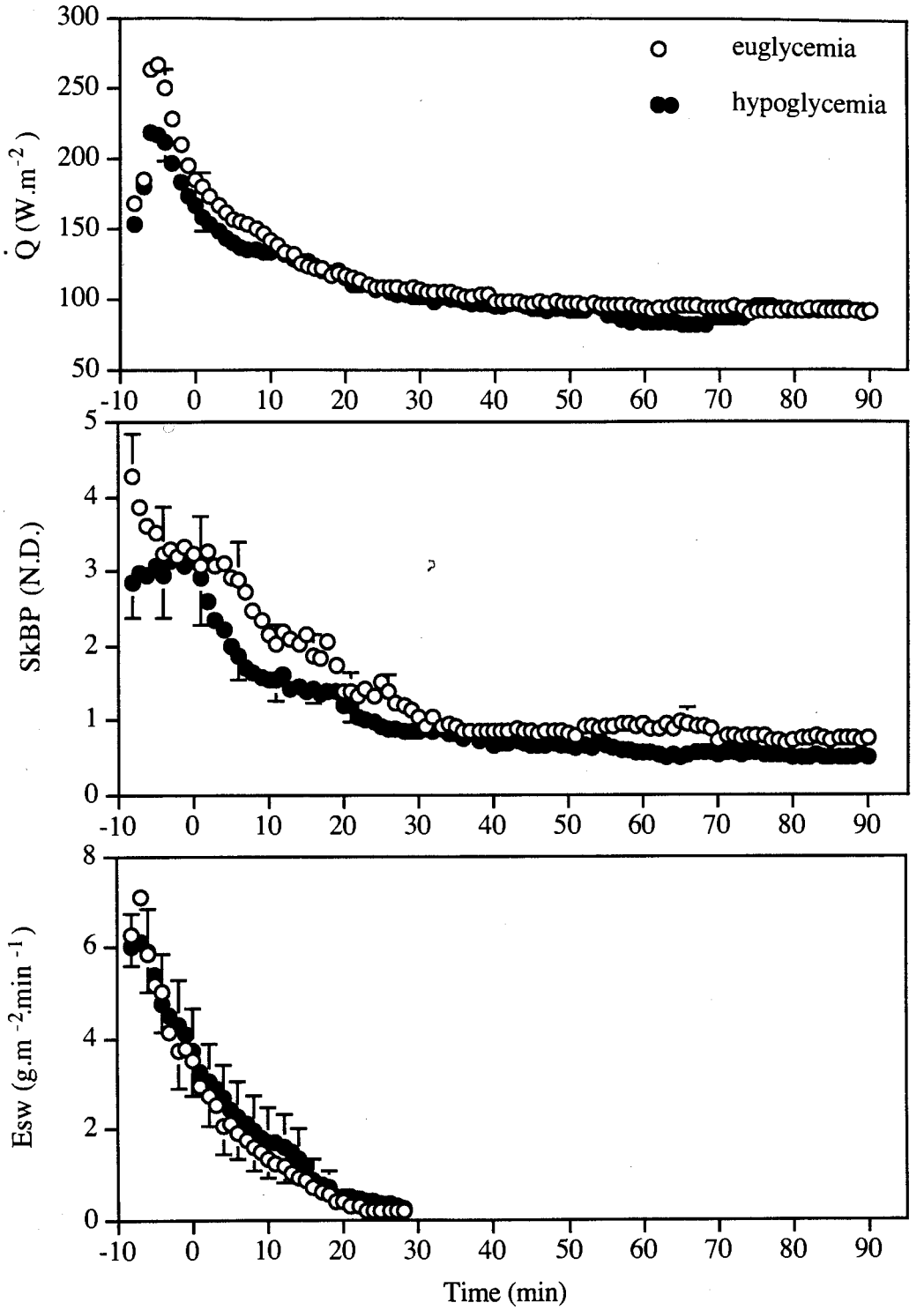

Fig. 4-3. Heat flux from the skin ($\dot{Q}$,*top*), skin blood perfusion (SkBP, *middle*) and sweat rate (Esw, *bottom*) values (mean ± SE), during the cooling phase, in the euglycemic (open circles) and hypoglycemic (closed circles) conditions.

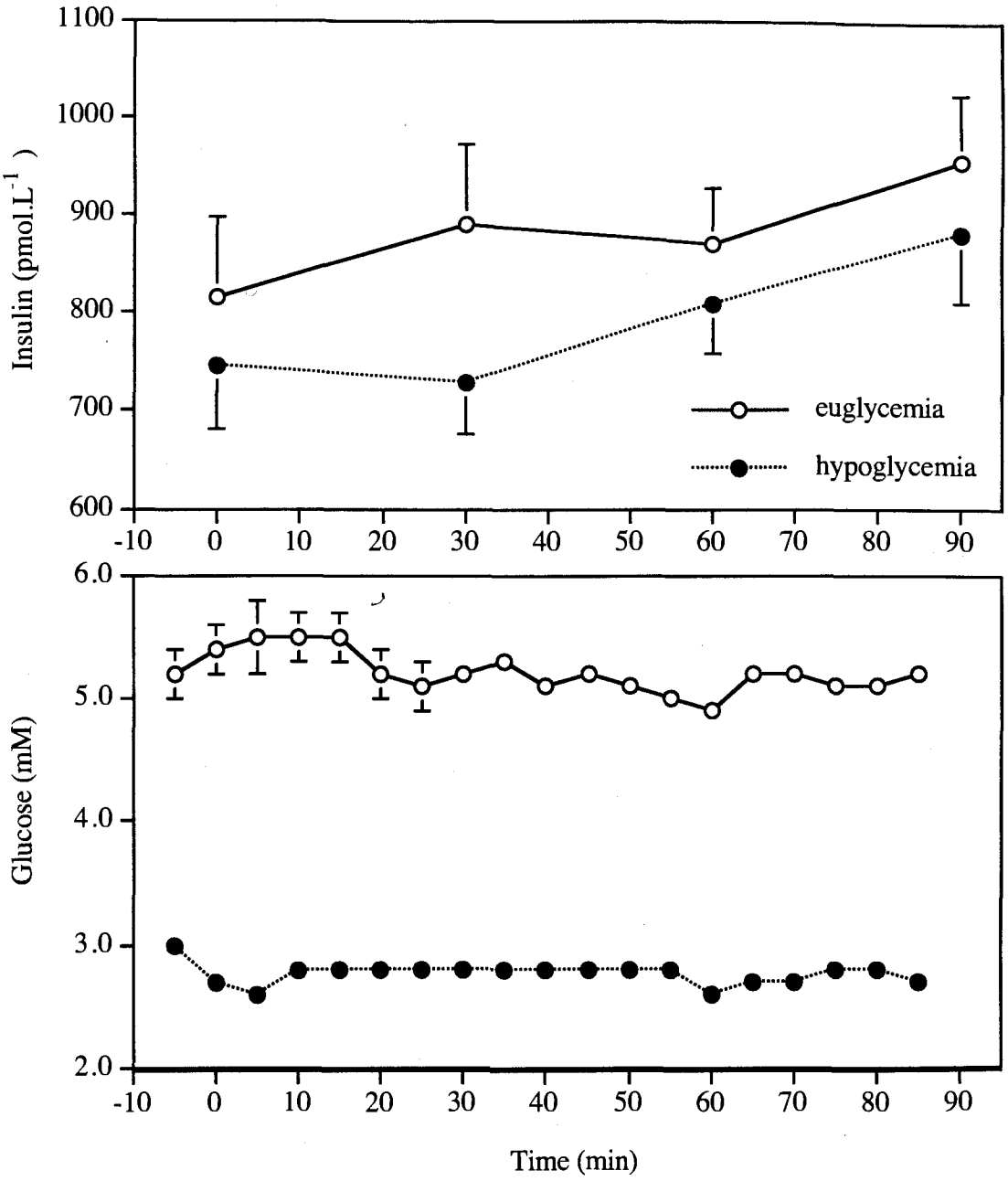

Fig. 4-4. Plasma insulin (*top*) and glucose (*bottom*) values (mean ± SE) during the cooling post-exercise period in the euglycemic (open circles) and hypoglycemic (closed circles) conditions.

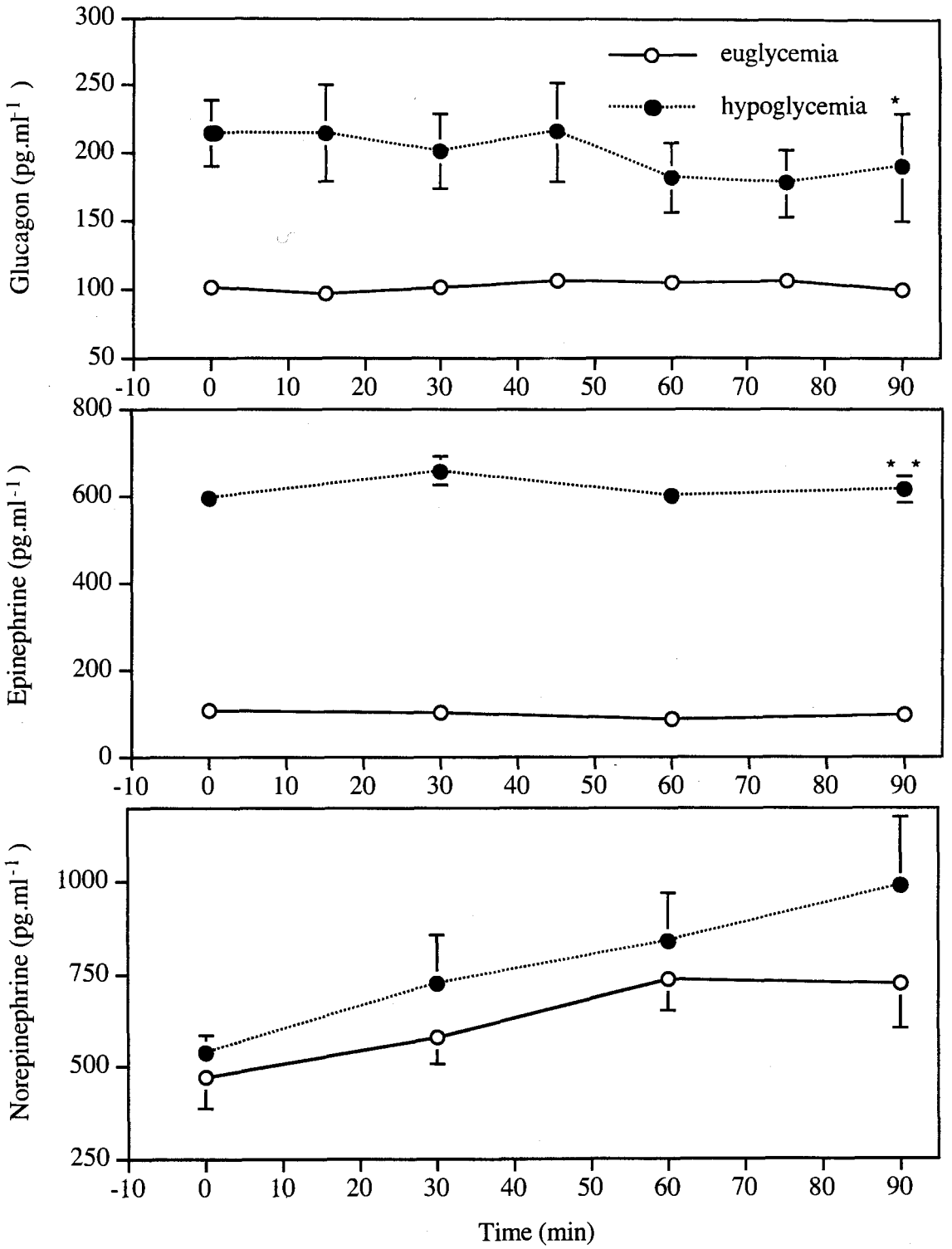

Fig. 4-5. Plasma glucagon (*top*), epinephrine (*middle*) and norepinephrine (*bottom*) values (mean ± SE) during the cooling post-exercise period in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. *$p \leq 0.05$, **$p \leq 0.007$.

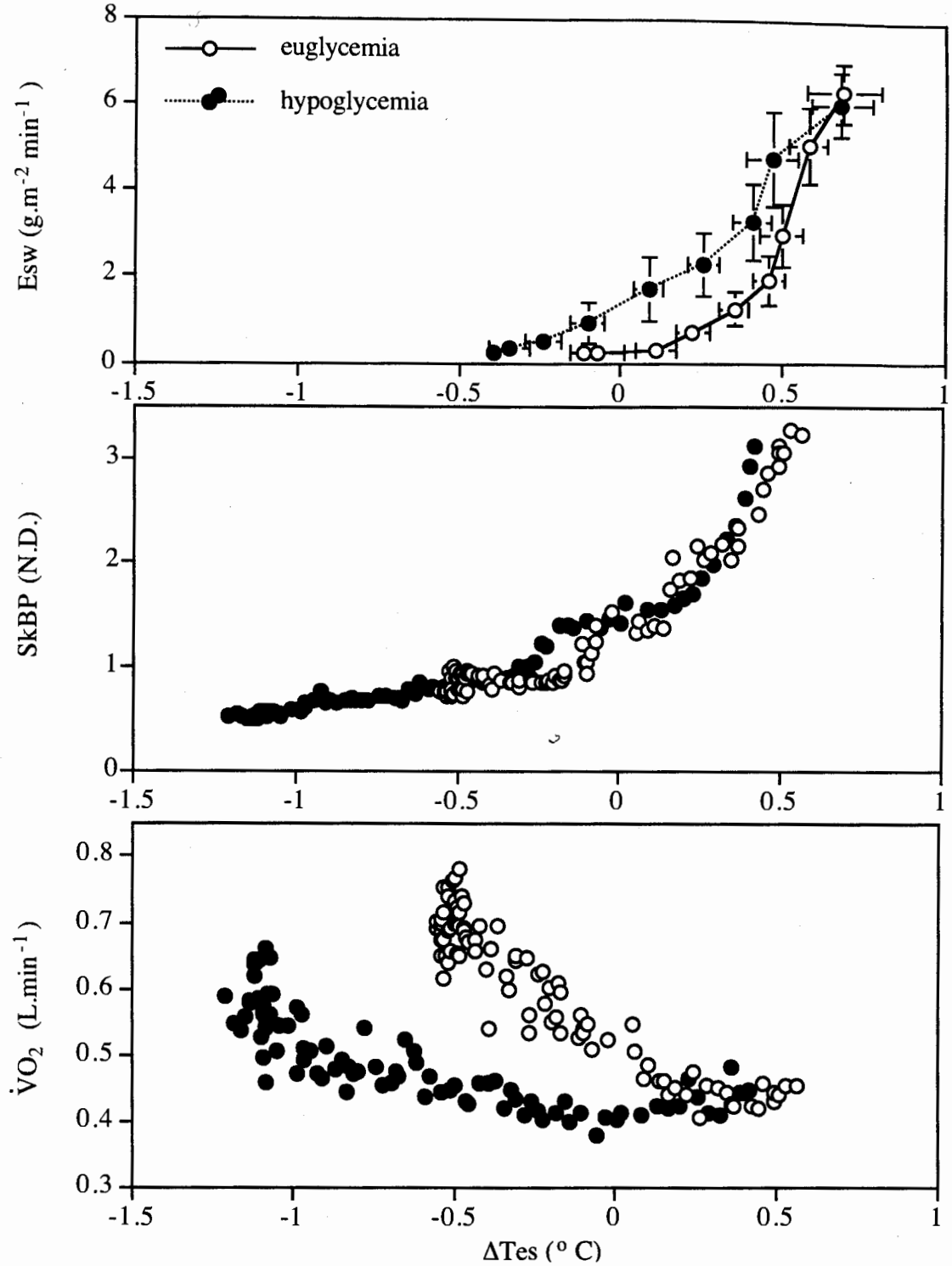

Fig. 4-6. Oxygen uptake ($\dot{V}O_2$, *top*), skin blood perfusion (SkBP, *middle*) and sweat rate (Esw, *bottom*) as a function of (ΔTes), during the cooling post-exercise period in the euglycemic (open circles) and hypoglycemic (closed circles) conditions.

CHAPTER FIVE: THERMAL PERCEPTION DURING HYPOGLYCEMIA

INTRODUCTION

Thermoregulatory behaviour is very important for survival in aggressive environments, since the autonomic responses *per se*, in most homeothermic species are inadequate to prevent core cooling to fatal levels (Cabanac, 1972). Behavioral thermoregulatory responses are initiated when the organism perceives that it is no longer indifferent to the thermal environment. Hence, thermal perception or thermal comfort is a reflection of the organisms level of indifference to the environment, and relies on peripheral and central thermal perception. Thermal perception or comfort ratings have been used extensively to study behavioral thermoregulation in humans (Corbit, 1970).

The effect of hypoglycemia on thermal perception and consequently on thermoregulatory behaviour in humans remains to be elucidated. Hypoglycemia inhibits shivering thermogenesis during cold exposure (Haight and Keatinge, 1973; Gale *et al.*, 1981) and immersion hypothermia (Chapter four of present thesis), whereas it does not seem to affect heat loss autonomic responses activated during exercise-induced mild hyperthermia (Chapter three of present thesis). In view of the established effects of hypoglycemia on autonomic thermoregulatory responses, it was considered of interest to investigate whether hypoglycemia also compromises thermal perception. Namely, whether hypoglycemia would modify thermal perception so that behavioral responses would promote heat loss and thus further core cooling during hypothermia and whether hypoglycemia would affect thermal perception during hyperthermia. This would, from a practical perspective, constitute crucial information for individuals predisposed to hypoglycemia due to pathological disorders or prolonged physical activity. For those individuals exposure to thermally extreme environments could be detrimental in case hypoglycemia affects their thermal perception.

The present study examined the effects of hypoglycemia on thermal perception in human subjects to exercise-induced mild hyperthermia and immersion-induced mild hypothermia.

METHODS

The present data were collected during the two phases (exercise and cooling) of the studies described previously (Chapters three and four) which were approved by the Simon Fraser University Ethics Review Committee. The exercise phase included 5 minutes of pre-exercise rest and the 20 min exercise session, whereas the cooling phase included 90 minutes of cooling.

Subjects

All subjects were physically active with similar experiences in outdoor activities and exposure to heat and cold.

Protocol

Subjects participated in two trials, as described previously (chapters three and four); they were rendered hypoglycemic in one (blood glucose at 2.8 mM) whereas in the other trial blood glucose was maintained at euglycemic levels (5.0 mM), using the glucose clamp technique (DeFronzo *et al*.., 1979). During the experiment the subjects were immersed to the chest in 28 °C water. Resting values were recorded during the initial 5 minutes of immersion while the subject was sitting on an underwater cycle ergometer. This was followed by a 20 minute exercise session performed on the underwater cycle ergometer at 50 % of the subject's maximal work rate which induced an elevation in core temperature and sweating stimulation. Ratings of thermal perception during the exercise phase were recorded during the last minute of the resting period and immediately after the completion of the exercise session. The increase of core temperature induced by exercise allowed the examination of the effects of hypoglycemia on thermal perception during mild hyperthermia. In the cooling phase the subject remained seated on the cycle ergometer after the completion of exercise, for an additional

99 minutes. During this period core temperature decreased to mildly hypothermic levels. The effects of hypoglycemia on thermal perception during the development of hypothermia were examined during the cooling phase of the study. Ratings of thermal perception during the cooling phase were recorded every 10 minutes.

Each subject was familiarized and given similar instructions prior to the experiment about the scale used for the thermal perception vote. During the experiment, subjects rated their perception of thermal comfort by indicating to the appropriate numerical vote on the scale presented to them at the time intervals described above.

Measurements

Thermal perception vote (TPV). The subjective thermal perception was rated based on a 21 point scale, in which +10 and -10 corresponding to the indications: very, very hot and very, very cold, respectively. In between these two remote indications the scale included the following ratings: +8: very hot; +6: hot; +4: moderately hot; +2: warm; 0: neither warm nor cold (neutral); -2: cool; -4: moderately cold; -6: cold and -8: very cold, as suggested by Enander *et al.*, (1979).

Esophageal temperature (Tes, ^{o}C). A YSI 702 (Yellow Springs Instruments, Ohio, USA) thermistor probe was used for the assessment of esophageal temperature. The probe was inserted through the nostril at a length determined from sitting height (Mekjavic and Rempel, 1990).

Oxygen uptake ($\dot{V}O_2$, $L.min.^{-1}$). Oxygen uptake was determined from the inspired minute ventilation and the analyses of O_2 and CO_2 content of the mixed expired gases as it has been described previously (Chapters three and four).

RESULTS

Skin temperature (Tsk) exhibited an increase during the exercise phase from 30.10 ± 0.20 and 30.16 ± 0.15 °C at the last minute of rest to 30.82 ± 0.2 and 30.51 ± 0.14 °C at the end of exercise during the euglycemic and hypoglycemic conditions, respectively. Tsk was relatively stable after the first 8-10 minutes of the cooling phase for both experimental conditions. Therefore the TPV values recorded during the first 8-10 miutes of cooling were not included in the present analysis. Core temperature was expressed as the relative change of Tes from the average resting values (ΔTes, °C), since resting Tes was similar in the two experimental conditions (36.57 ± 0.08 and 36.43 ± 0.07 °C for the euglycemic and hypoglycemic conditions, respectively). Results are expressed as mean ± SE.

Tes in relation to TPV

Cooling phase

The TPV responses recorded during the cooling phase were plotted in relation to the corresponding ΔTes, for each subject and experimental condition (euglycemia, hypoglycemia) separately (Fig. 1). In general, subjects felt colder as core temperature decreased. Regression analyses indicated that for all but one subject, TPV was related to ΔTes in a linear manner in both experimental conditions ($p \leq 0.05$; Table 1). The slope values obtained from the regression analyses were compared between the two experimental conditions using a paired t-test. They were substantially lower ($p \leq 0.02$) in the hypoglycemic compared to the euglycemic condition (2.6 ± 0.2 and 4.3 ± 0.5 TPV.$^{\circ}C^{-1}$, respectively).

Exercise phase

TPV values obtained during the exercise phase were plotted in relation to the corresponding ΔTes (Fig. 2), in a similar manner as described for the cooling period. TPV

values increased with the increase in core temperature resulting from exercise. However, since TPV was recorded only twice during this phase (last minute of rest and following the completion of exercise) only two points were used to determine the slope of the ΔTes-TPV relation (Table 1). There was no difference in the slope values between the two experimental conditons (5.3 ± 1.4 and 5.8 ± 1.2 TPV.$^{o}C^{-1}$, for the euglycemic and hypoglycemic conditions, respectively, n=6).

Comparison between cooling and exercise

The slope values obtained during cooling were compared with those obtained during exercise in each experimental condition separately, by using a paired t-test. No difference was observed in the slope values between the two phases during euglycemia whereas they were lower ($p \leq 0.05$, Table 1) during cooling compared to exercise in the hypoglycemic condition.

TPV in relation to $\dot{V}O_2$

To establish whether there was a correlation between thermoregulatory shivering and thermal perception, TPV was plotted in relation to $\dot{V}O_2$ in a similar manner as described above for the TPV with ΔTes. In general, more negative TPV values were reported (subjects felt colder) as $\dot{V}O_2$ increased. However, regression analysis of the TPV-$\dot{V}O_2$ plots indicated that this relation was linear ($p \leq 0.05$) for 6 subjects in the euglycemic condition and for only three in the hypoglycemic. Similar results were obtained by fitting a logarithmic function in the TPV-$\dot{V}O_2$ plots.

DISCUSSION

The present findings demonstrated that hypoglycemia (2.8 mM) decreases the sensitivity of thermal perception during the progression to mild hypothermia. This was indicated by the decrease in the slope of the TPV-ΔTes relation observed in the

hypoglycemic compared to the euglycemic condition during the cooling phase (Fig. 1, Table 1). In contrast it was shown that hypoglycemia does not affect the sensitivity of thermal perception during the progress of exercise-induced mild hyperthermia. This was based on the observed lack of difference in the slopes of the TPV-ΔTes relation between the euglycemic and hypoglycemic condition in the exercise phase (Fig. 2, Table 1). However, conclusions based on the observations from the exercise phase should be reached with caution considering that only two points were used to determine the slope of the TPV-ΔTes relation during this phase. Finally the differentiation of the effects of hypoglycemia on the sensitivity of thermal perception during mild hypothermia from that during exercise-induced mild hyperthermia was further supported by the observed difference in the slopes of the TPV-ΔTes relation observed in the cooling in comparison to the exercise phase observed in the hypoglycemic but not in the euglycemic condition.

The important contribution of both skin and core temperatures on thermal sensation and thermoregulatory behavior has previously been established (Corbit 1970; Cabanac 1972, 1981). In the present study skin temperature was maintained relatively stable during the cooling phase. Therefore any change in TPV during cooling could be attributed to changes in core temperature. During the exercise phase, skin temperature increased by an average of 0.4 °C above resting values, in both experimental conditions. It has been shown that young people are able to discriminate differences in temperature between two objects of about 1 °C (Collins *et al.*, 1981). Assuming that this evidence applies to the circumstances of the present study then the changes in Tskin observed during exercise most likely did not affect overall thermal perception. Therefore changes in TPV observed during the exercise phase can be attributed to changes in core temperature. Although insufficient data points (TPV) were recorded during the exercise phase to assure linearity in the ΔTes-TPV relation, linearity was assumed based on previous evidence (Winslow and Herrington, 1949).

The neuronal mechanisms underlying the observed effects of hypoglycemia on thermal perception cannot be determined based on the present results. However, it has been shown that thermal stimulation of the anterior hypothalamus exerts autonomic as well as behavioral thermoregulatory responses in the rat (Satinoff, 1964). These observations probably allow the assumption that a similar neuronal arrangement is responsible for thermal perception and thermoregulatory behavior as has been proposed for autonomic thermoregulatory responses. It may be speculated that since hypoglycemia did not affect thermal perception during exercise-induced hyperthermia, the warm thermal inputs from the skin and the central regions as well as the central processing of thermal information related to warm perception was not affected by hypoglycemia. In contrast, the decrease in sensitivity of thermal perception observed during hypoglycemic hypothermia suggests that hypoglycemia affected either the cold peripheral and central thermal inputs and/or the central processing of thermal information related to cold perception. Assuming that thermal perception drives thermoregulatory behavior the present results probably indicate separate regulation for thermoregulatory behavior in response to mild hypothermia than to exercise-induced mild hyperthermia.

The parallel effects of hypoglycemia on autonomic thermoregulation and thermal perception as observed in the present study, probably indicates a similarity in the underlying neuronal mechanisms regulating physiological and behavioral thermoregulation. Thus, if thermal perception and consequently thermoregulatory behavior is controlled even partly by thermosensitive hypothalamic neurons then the effects of hypoglycemia on thermal perception during hypothermia could be explained in a similar manner as the effects of hypoglycemia on shivering thermogenesis were explained in chapter four, namely, based on the findings of Silva and Boulant (1984). According to these findings, only half of the thermosensitive neurons which were tested in the anterior hypothalamus were affected by low glucose media whereas the other half were not. It could be speculated that if thermal perception during hyperthermia is

controlled by the thermosensitive neurons which are not affected by low glucose media then this could explain the present finding that hypoglycemia does not affect thermal perception during exercise-induced mild hyperthermia.

Dissociation between shivering and thermal perception to cold

It was shown in chapters three and four that hypoglycemia affected the thermoregulatory autonomic responses activated during mild hypothermia but not those activated during exercise-induced mild hyperthermia. It is interesting to notice the parallel effect of hypoglycemia on thermal perception with that on autonomic thermoregulation. Namely, cold defence autonomic mechanisms and sensitivity of thermal perception were affected by hypoglycemia during hypothermia, whereas, heat defence autonomic mechanisms and thermal perception were not affected by hypoglycemia during exercise-induced mild hyperthermia. Assuming that thermal perception is expressed as thermoregulatory behaviour, this observation supports the notion that physiological and behavioral temperature regulations are very well coordinated in achieving control of body temperature (Satinoff, 1964). However, an association between autonomic thermoregulatory responses and thermal perception could be suggested as an alternative explanation to this parallelism observed in the present study. Thus, it may be questioned whether the autonomic thermoregulatory responses (shivering, sweating) originate the thermal perception in a sense that subjects feel more cold simply because they shiver more or feel warmer because they sweat more. However, it was observed in the present study that TPV was much better correlated with Tes than $\dot{V}O_2$, which reflects shivering thermogenesis, during the cooling phase. Although this observation is not a direct proof of a dissociation between shivering and thermal perception during hypothermia, it supports the notion that autonomic responses are not the major component of thermal perception. This suggestion is in agreement with previous observations (see Cabanac, 1981).

The inhibitory effect of hypoglycemia on the sensitivity of cold perception during hypothermia is the finding with the most practical importance in the present study. It indicates that an individual exposed to cold while in a hypoglycemic state will be less prone to detect body-core cooling than while being normoglycemic. As a result appropriate behavioral protective measures against hypothermia will not be taken. Considering that hypoglycemia also inhibits shivering thermogenesis (Haight and Keatinge, 1973; Gale *et al.*, 1981; Chapter four present thesis) it is obvious that hypoglycemia may potentiate the development of hypothermia. Individuals prone to hypoglycemia such as patients with endocrine disorders, diabetic patients receiving high doses of insulin, as well as healthy individuals undergoing strenuous and prolonged physical activity especially when it is combined with undernutrition or alcohol consumption, could be potential victims of hypothermia for the reasons described above.

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Table 5-1. The slopes of the TPV-ΔTes relation (TPV.$^{o}C^{-1}$) for both experimental conditions during the cooling (*left*) and the exercise (*right*) phases.

	Cooling phase		Exercise phase	
Subject	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
1	3.13	1.58	7.63	6.15
2	2.04	3.35	1.37	4.98
3	5.99	2.38	10.89	8.08
4	6.99	3.20	4.39	4.56
5	6.67	2.35	4.53	9.66
6	4.58	2.50	~	~
7	3.41	3.49	~	~
8	3.05	3.22	~	~
9	3.50	1.84	~	~
10	3.45	2.42	3.01	1.53
Mean + SE	4.28 ± 0.54	2.63 ± 0.21 *	5.30 ± 1.4	5.83 ± 1.17

The slopes of the ΔTes *versus* TPV relation in the cooling and exercise phases, *$p \leq 0.02$ from the comparison of the slopes (using paired t-test) between the euglycemic and hypoglycemic conditions during the cooling phase.

~ missing data

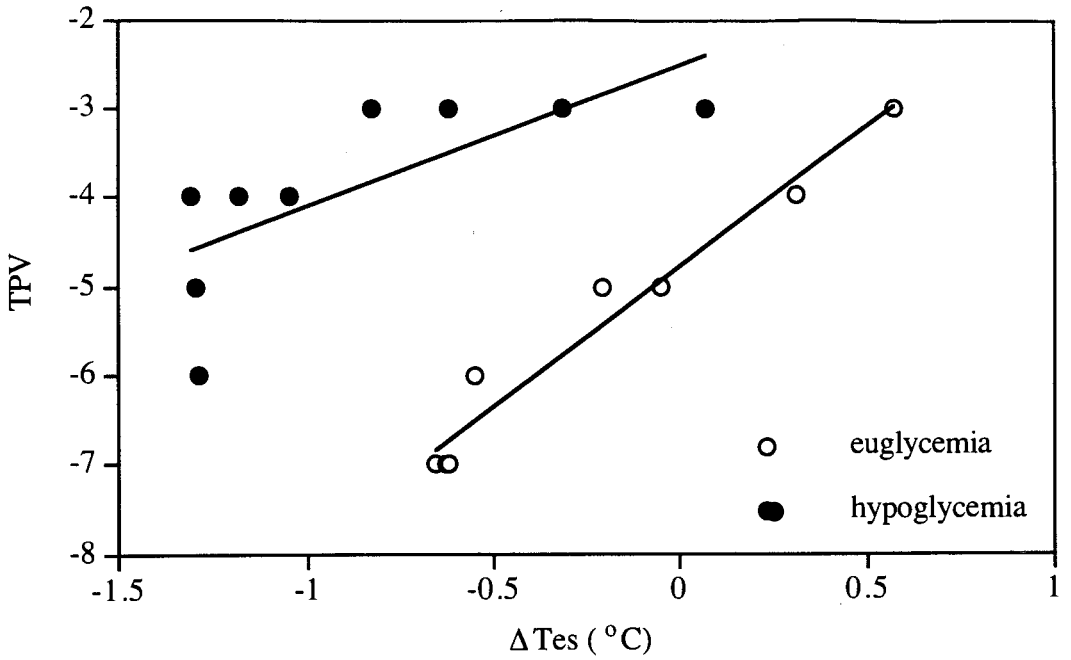

Fig. 5-1. TPV in relation to ΔTes of a representative subject during the cooling phase. The slope of the regression line indicates decreased sensitivity of thermal perception during the development of hypoglycemia in the hypoglycemic (closed circles) compared to the euglycemic (open circles) condition. These data are from the same subject as in Fig. 2.

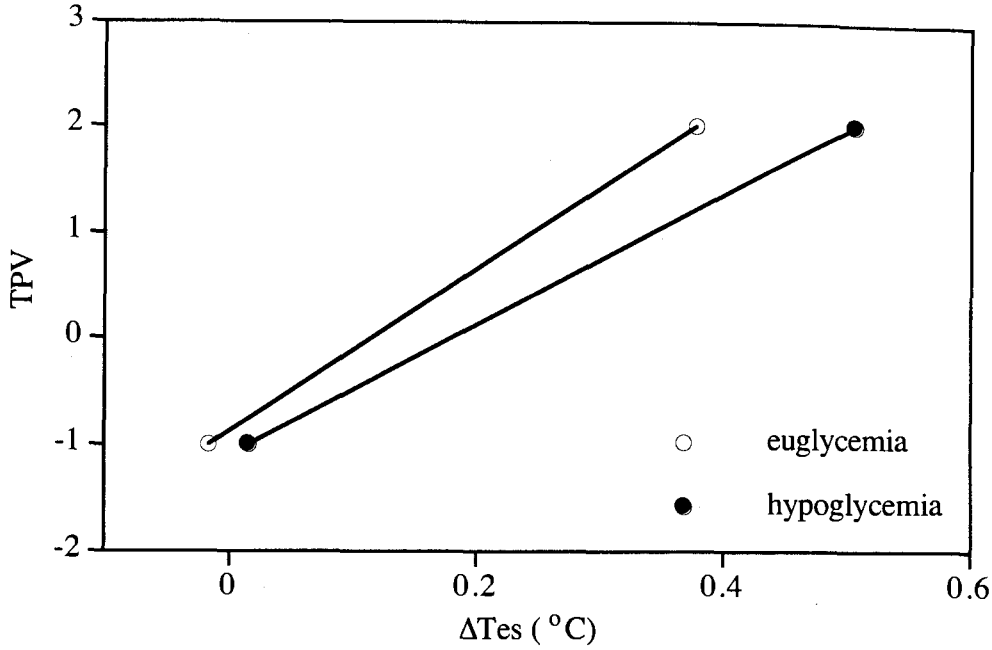

Fig. 5-2. Data from a representative subject showing the ΔTes - TPVrelation and the line connecting the two data points recorded during the exercise phase in the euglycemic (open circles) and hypoglycemic (closed circles) conditions. These data are from the same subject as in Fig. 1.

CHAPTER SIX: CONCLUSIONS AND PRACTICAL IMPLICATIONS

GENERAL CONCLUSIONS

The present study assessed some aspects of the interaction between two homeostatic mechanisms, that of thermoregulation and glucoregulation. It was shown that hypoglycemia (2.8 mM) does not totally abolish shivering but rather shifts the core temperature threshold for shivering to lower values by approximately 0.6 °C. The core temperature threshold values for initiation of sweating during exposure to exercise-induced mild hyperthermia and that of cessation of sweating during recovery from exercise were not significantly decreased by hypoglycemia. Similarly, hypoglycemia did not exert any effect on the core temperature threshold value for passive vasodilation. Hypoglycemia did not affect the gain of sweating and cutaneous passive vasodilation when these responses were activated by exercise-induced mild hyperthermia or during recovery from hyperthermia following the end of exercise. The assymetry of the effects of hypoglycemia on the core temperature threshold values for the different thermoregulatory responses examined in the present study seems to suggest separate regulation of these responses.

Interestingly, subjective thermal perception and autonomic thermoregulatory responses were affected in a parallel manner by hypoglycemia. Namely, sensitivity of thermal perception during the development of hypothermia was affected by hypoglycemia and so was shivering, whereas thermal perception was not affected during exercise-induced mild hyperthermia, similarly to sweating and passive vasodilation.

The effects of hypoglycemia on shivering and thermal perception during hypothermia seem to support Gellhorn's (1938) hypothesis, whereas the lack of effect on thermal perception, sweating and passive vasodilation during exercise-induced mild hyperthermia, do not. It is likely, that elimination of the glucose or energy requiring processes is the first priority of physiological systems during hypoglycemia rather than the decrease in body temperature.

PRACTICAL IMPLICATIONS

The present results, beyond assessing some aspects of the interaction between thermoregulation and glucoregulation, also offer practical insight applicable to special populations. Hypoglycemic individuals would have difficulties maintaining their core temperature, if they were exposed to a high heat loss environment. Their autonomic shivering response would be initiated at a lower core temperature thus predisposing them to hypothermia, and their perception to cold will be impaired. The impaired thermal perception may further exaggerate core cooling.

Hypoglycemia could be encountered by patients suffering from endocrine disorders related to glucose regulation, insulin dependent diabetics with poorly controlled blood glucose levels, as well as healthy individuals undergoing prolonged strenuous exercise or any type of physical activity. Hypoglycemia could be more acute in the latter case, if physical activity is combined with undernutrition and especially alcohol consumption. Many cases have been reported of fishermen thrown overboard or escaping from a sinking vessel, after having worked relatively hard throughout the day. Similarly skiiers and hikers have been trapped or lost after a day of intense physical activity. Such individuals are potential victims of hypothermia due to their cold exposure. However, hypoglycemia resulting from the victim's physical activity prior to the accident may exacerbate the development of hypothermia. Treatment of such victims should also include restoration of their blood glucose levels. This would allow them to regain shivering thermogenesis and thus enhance rewarming via endogenous thermogenesis. Similar suggestions apply to hypothermic victims who belong to populations that are more succeptible to hypoglycemia, such as, patients with endocrine disorders or diabetics who have received larger than the adequate amount of insulin.

GLOSSARY

Tes :	Esophageal temperature
ΔTes :	Relative change of esophageal temperature from resting value
Tsk :	Skin temperature
$\dot{V}O_2$:	Oxygen uptake
$\dot{V}O_2$ max:	Maximum oxygen uptake
$\dot{V}_I$:	Ventilation (inspired)
HR :	Heart rate
RER :	Respiratory exchange ratio
$\dot{Q}$:	Heat flux from the skin
SkBP :	Skin blood perfusion
Esw :	Sweat rate