

The prolonged exercise of hill walking: physiological, metabolic and ergonomic aspects

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ABSTRACT

Hill walking is a popular recreational activity although it is likely to constitute a major physiological challenge to the participant. The aims of this thesis were to provide a comprehensive investigation into the prolonged exercise of hill walking. Firstly, the characteristic activities of such pursuits were considered in a questionnaire based study. Secondly, a range of physiological, metabolic and ergonomic aspects of hill walking was investigated in three field studies and one laboratory based study.

A questionnaire was designed to establish the characteristic activities of hill walkers. One hundred questionnaires were used for final analysis. Results indicate a typical distance covered of 18 – 26 km over 6 – 8 hours in duration and an ascent over 600 m. Significant relationships were found to exist between the incidence of injuries in walkers and a low energy intake during the walk. Despite the activity lasting a full day, quantification of typical energy intakes used for such hill walking events showed that, on average, they were not higher than normal reference energy intakes for the associated age groups.

On separate occasions, 13 subjects completed a self-paced hill walk over 12 km. During the first 5 km of the walk (100 to 902-m elevation), rectal temperature increased (36.9 ± 0.2 to 38.5 ± 0.4 °C). Rectal temperature decreased by ~ 1.0 °C during a 30-min stop for lunch, and it continued to decrease a further 0.5 °C after walking recommenced. The total energy intake from both breakfast and lunch (5.6 ± 0.7 MJ) was lower than the energy expended (14.5 ± 0.5 MJ; $P < 0.001$). Despite the difference between energy intake and expenditure, blood glucose concentration was maintained. The major source of energy was an enhanced fat oxidation, probably from adipose tissue lipolysis, reflected in high plasma nonesterified fatty acid concentrations.

The effect of age on responses to consecutive days of hill walking was investigated in seventeen male subjects who were divided into two groups. The nine subjects in group 1 constituted the younger group (< 29 years) whilst eight older subjects were in group 2 (> 52 years). Both groups completed 10 consecutive days of high-intensity hill walking. Energy expenditure (EE) was measured by the doubly-labelled water method. Blood and urine were sampled on alternative days to determine any changes in metabolism and hydration during the 10 days. Subjects also completed a battery of tests which included muscular strength (handgrip), jump performance, cognitive processing time and flexibility. The younger group remained hydrated whereas the older group became progressively dehydrated, indicated by a near 2-fold increase in urine osmolality concentration on day 11. This increased urine osmolality, in the older group, was highly correlated with impairment in vertical jump performance ($r = -0.86$; $P < 0.05$) and decreased cognitive processing time ($r = 0.79$; $P < 0.05$). Despite EE of ~ 21.5 MJ/day, body mass was well maintained in both groups. Both groups displayed a marked increase in fat mobilisation, reflected in significantly lowered pre-walk insulin concentrations and elevated post-walk glycerol and non-esterified fatty acid concentrations. Despite the dehydration and impaired performance in the older group, blood glucose concentrations were well maintained in both groups, probably mediated via the increased mobilisation of fat.

In a laboratory based simulation of a hill walk, eight men undertook three 450-min walks at intensities varying between 25-30% and 50-55% of $\dot{V}O_{2\text{ max}}$. In a balanced design the subjects were given breakfast, snacks and lunch containing carbohydrate (CHO), protein (Pr) and fat in the following amounts (g/70 kg body mass): mixed diet, 302 CHO, 50 Pr, 84 Fat; high-CHO diet, 438 CHO, 46 Pr, 35 Fat; high-fat diet, 63 CHO, 44 Pr, 196 Fat. The high-fat diet resulted in negative CHO balance (-140 ± 1 g) and a less negative fat balance (-110 ± 33 g) than the other two diets ($P < 0.05$). Plasma glucagon, non-esterified fatty acids, glycerol, and 3-hydroxybutyrate were highest with the high-fat diet ($P < 0.05$ vs. high-CHO), whilst plasma insulin was lowest after the high-fat diet ($P < 0.05$ vs. mixed and high-CHO). Subjective ratings of fatigue and appetite showed no differences between the three trials. Whilst diet influenced the degree of total CHO and fat oxidation, fat was the main source of energy in all trials.

In the final field study, 16 male subjects completed a strenuous 21-km hill walk with either a high (15 MJ) or low dietary energy intake (5 MJ), in random order. Generally, consumption of the low-energy intake led to a marked deterioration in performance compared with the high-energy dietary conditions. Adverse subjective responses were noticed in the majority of the conditions when subjects consumed the low-energy dietary provision. Although not statistically significant, there was a marked trend towards a lower rectal temperature during the low-energy intake condition. Furthermore, during the low-energy intake, mean blood glucose concentrations levelled off at the low-mid range of normoglycemia whereas, on the high-energy intake, they were significantly elevated compared with the low-energy intake. The maintained blood glucose levels were most probably mediated via an increased mobilisation of fat in the low energy group whereas in the high intake condition, fat mobilisation was suppressed and CHO utilisation was promoted. Whilst the impairment in the low-energy intake compared with the high-energy intake was somewhat moderate, this impairment may well be an influencing factor in susceptibility to both fatigue and injury whilst pursuing outdoor recreational activity.

From the studies described, the main conclusions are that the prolonged activity of hill walking has the potential to impose severe stress simultaneously upon several regulatory systems. High energy expenditures and dehydration are likely to be closely linked to the activity. Failure to provide enough fuel and fluid to sustain the activity may lead to a compromised ability to sustain the activity and an increased likelihood of injury. Older age participants may be particularly prone to both dehydration and an impaired ability to operate in the mountainous environment. An overall perspective of hill walking can only be considered from the unification of the epidemiological, laboratory-based and field-based studies described in this thesis.

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DECLARATION

I declare that the work presented in this thesis is entirely my own, with the exception of the following.

The urine catecholamines were measured at Liverpool Royal Hospital by Dr Norman Roberts and his staff. Professor Ian Macdonald and his staff measured the plasma catecholamines at Nottingham University Medical School.

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Some of the work reported in this thesis has already been presented at European conferences, and published in European and American Journals (see Appendix 1)

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LIST OF PUBLICATIONS AND COMMUNICATIONS

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO THESIS

Hill walking is a popular leisure time activity in many of the world's developed countries. It engages males and females over a large range of ages. Activity tends to be light to moderate in intensity but prolonged and so the varied terrain typical of hill walking may have overall high energetic consequences for the enthusiast. The nutritional requirements of hill walkers have not been studied systematically despite the potentially deleterious physiological and psychological consequences of activity sustained over a whole day, sometimes in adverse climatic conditions. Failure in providing enough exogenous nutritional intake to sustain the activity may have an impact on physical fatigue, deterioration in neuromuscular function, mental fatigue accompanying physiological strain (e.g. linked to hypoglycaemia) and thermoregulation.

Despite recognition of the problem of the prolonged stresses associated with hill walking by safety organisations, their ability to design educational material concerning these hazards is hindered by the inadequacy of experimental documentation of physiological process. What information that is available, relating to prolonged hill walking, derives from the pioneering work of Pugh (1966; 1967), supplemented by anecdotal descriptions of exposure incidents (Pugh, 1964; Kreider, 1967; Hunter, 1968; Ogilvie, 1977; Anderson 1994; Sharp, 2001). Unfortunately these studies and reports provide no real experimental documentation concerning the physiological, psychological or nutritional aspects to the hill walking scenario.

More recently, Weller et al. (1997a,b) investigated the effects of a cold, wet and windy environment on the physiological responses to prolonged (360 min) intermittent walking. These conditions were simulated in a climatic chamber on treadmill. During the last 240 min of the intermittent low intensity walking (<30% VO_2 peak), rectal temperature was lower, while VO_2 , the respiratory exchange ratio, blood lactate, plasma noradrenaline and adrenaline concentrations were higher, compared with those under a thermoneutral conditions. This study is useful in the sense that it is perhaps the only documented work in this area. However, the simulated environment, lack of load carrying and time span (360 min), may not be considered

representative of a typically, prolonged hill walk. Additionally no nutritional strategies or performance tests were investigated.

It is hoped that through this study a greater insight will be gained into the potential energetic, nutritional, physiological and metabolic demands of hill walking.

1.2 AIMS OF THESIS

The main aims of the thesis were as follows.

1. Identify the characteristic activities (intensity, duration, frequency, nutritional intake) of hill walkers.
2. Investigate the physiological and metabolic responses to a hill walking event
3. Investigate the energy expenditure of strenuous hill walking.
4. Examine the effects of high carbohydrate and high fat intakes on metabolic and subjective factors during prolonged walking in the laboratory.
5. Determine the effects of different energy intakes on metabolism, physiology and performance during a whole-day high intensity hill walk

The specific aims and hypothesis are outlined in each respective chapter.

It is envisaged that accomplishing these aims will lead to a greater understanding of the energetic, nutritional, physiological and metabolic demands of prolonged exercise, such as hill walking. The findings in these investigations should provide a basis for recommending guidelines for hill walkers, ramblers and other pursuits which entail light to moderate but prolonged energy expenditure. There are consequences also for other sustained activities, for example recreational cycling. The project will help to adumbrate the potential risks associated with fatigue of prolonged activity e.g. hypoglycaemia, subjective fatigue, loss of motor skills, decrease in muscle strength, drop in core temperature and loss of

thermoregulatory ability. The extent to which such changes can be offset by a systematic nutritional strategy will be established.

1.3 STRUCTURE OF THESIS

The studies within the thesis were separated into five distinct phases, which were conducted sequentially. The sequential construction of the thesis was necessary since the results of each helped to finalise the research protocol for the study to follow. The five phases of the thesis were as follows:

Phase 1 – Characteristic activities of hill walking (questionnaire)

Phase 2 – Physiological and metabolic responses to a hill walk

Phase 3 - Energy expenditure and metabolism during 10 days of high intensity hill walking

Phase 4 - Laboratory-based simulation under different nutritional conditions

Phase 5 - Field study of different energy intakes for hill walkers

The distinctive features of participation in recreational hill walking must be more closely studied to highlight the characteristic patterns, physiological and nutritional demands of such activities. Field-based research provides crucial information about the specific demands of the activity. In many sports this information can be supported by laboratory-based simulations. In the case of an activity such as hill walking in which the unique demands are related to terrain and climatic features which cannot readily be reproduced in the laboratory, the emphasis must be placed heavily upon field techniques. In recent years, the development of accurate, innovative and sophisticated techniques have provided reliable methods of obtaining information regarding the stresses of such activities in the field.

This thesis is structured so that the characteristic activities of hill walking have been investigated in Chapter 4. The physiological, metabolic and nutritional demands of

hill walking are more specifically studied in the research investigations in Chapters 5 - 8, as outlined above. Specifically, in Chapter 5, the physiological and metabolic responses to a strenuous 12-km hill walk were monitored. In Chapter 6, the findings of the initial field study (Chapter 5) were extended over 10 consecutive days of hill walking. The focus of Chapter 6 was on the effect of age on energy balance, metabolism, hydration and performance during 10 days of strenuous hill walking. In a laboratory-based study, described in Chapter 7, the potential benefits of macronutrient manipulation was considered over 7 h 30 min (35-km) of simulated walking. Finally, in Chapter 8, the physiological, metabolic and performance implications of different energy intakes were considered over a prolonged 21-km hill walk.

CHAPTER 2

THE PROLONGED EXERCISE OF HILL WALKING: PHYSIOLOGICAL, METABOLIC AND ERGONOMIC ASPECTS - A REVIEW OF THE LITERATURE

2.1 PHYSIOLOGICAL AND PHYSICAL ASPECTS DURING HILL WALKING

2.1.1 Demands

The prolonged duration of a typical hill walk places exceptional demands on the participants. The specific demands of hill walking tend to involve activity of varying intensity and duration, both of which are influenced by factors such as the physical fitness of the participant, dietary intake, back-pack weight and environmental weather conditions. Hill walkers can be caught unexpectedly and unprepared when rain and wind accompany outdoor activities in cool weather. Potential adverse conditions may further increase the physiological demands of such activity.

In addition to the obvious physiological demands of such hill walking events, the cognitive demands must also be considered. The cognitive demands involve the ability to navigate in adverse conditions, and the ability to make important decisions regarding serious survival and rescue considerations (Thomson and Haywood, 1996; Sharp, 2001).

2.1.2 Fitness

An important physiological measure is the maximal rate to consume oxygen. This function has been, albeit loosely, equated with aerobic fitness. Whilst aerobic fitness, as defined as maximal oxygen consumption ($\dot{V}O_2$ max), is related to performance in endurance events, it cannot be related so closely to hill walking. Since hill walking does not involve any performance trial, apart from the ability to complete a determined walk, the relevance of $\dot{V}O_2$ max in hill walkers is questionable. Indeed, one of the greatest endurance feats in the human was the walk to the North Pole over 48 days (Stroud et al., 1993). The $\dot{V}O_2$ max of the two subjects who completed this walk was not particularly high (53.6 and 58.1 ml/kg/min) compared with top class runners.

There is the important, but often overlooked fact that all humans are not physiologically equal. A calculated degree of physiological strain may be tolerable for the majority but could constitute an intolerable strain for others. Hill walkers habitually work at a range of 25 – 70 percent of their $\dot{V}O_2$ max (Pugh, 1966; Freeman

and Pugh, 1968). Exercise intensities (and associated energy expenditures) of this order are sufficient to prevent a fall in core temperature in wet-cold conditions with mean skin temperatures as low as 20°C (normal mean skin temperatures are approximately 30°C; Pugh, 1966). However, groups of people walking and climbing are not always well matched physically, and when conditions are such that mean skin temperatures falls to 25°C, there is a strong inclination to walk faster, thereby raising core temperature to a value that makes the low skin temperature tolerable (Pugh, 1966; Freeman and Pugh, 1968). In this way less fit persons may become exhausted, most likely coinciding with a decrease in body carbohydrate stores. In this sense, fitness may be important when less fit walkers pace themselves with other walkers who possess superior fitness levels. Since the majority of walkers pursue this activity in groups, the less fit walkers may be more susceptible to fatigue when exercising at a higher intensity compared to their fitter counterparts.

Whilst physical fitness is one component that may confer some advantage in the mountains, the experience of the participant cannot be overlooked. The experienced walkers will most probably maintain a steady pace, matched to their preferred level of intensity.

2.1.3 Intensity

As mentioned already, hill walkers habitually work at a range of 25 – 70 percent of their maximum oxygen uptake (Pugh, 1966; Freeman and Pugh, 1968). Pugh (1967) proposed that maintaining a $\dot{V}O_2$ of 2-2.5 l/min or 50-60% $\dot{V}O_2$ max would offset heat loss and combat the debilitating effects of the cold, wet and windy environment. Even though these observations were based on only three subjects, recent work by Weller et al. (1997a) supported Pugh's postulate. Both experiments were based in an environmental chamber in which subjects exercised on a cycle ergometer and treadmill, respectively. Pugh (1967) and Weller et al. (1997a) showed that when exercise metabolism is reduced, the increase in shivering may be insufficient to prevent a decrease in deep body temperature. It is not unreasonable to expect that the field conditions during hill walking would likely impose additional stresses not encountered during simulated conditions, such as stoppage of activity for fluid and

food intake (transiently altering the balance between heat production and heat dissipation), in conjunction with varying terrain and weather conditions.

Indeed, the varying terrain which may be encountered during a hill walk will directly alter the intensity of the walk. Furthermore, the intensity of the walk will be both subjectively and physiologically increased during adverse climatic conditions. Walking in deep snow, the use of crampons, and strong wet and windy weather will all increase the relative intensity of the walk. Likewise, dehydration may also add to creating a higher intensity (Adolf, 1947), possibly as a function of an increased core temperature (Sawka, 1992).

2.1.4 Weather and terrain

Both adverse weather and difficult terrain may create higher relative intensities during a walk, and hence cause physiological strain. Decreased thermal insulation of wet clothing may present a severe challenge to body temperature regulation, which can be compounded by fatigue associated with prolonged exercise such as hill walking (Pugh, 1966; Pugh, 1967; Thomson and Hayward, 1996; Noakes, 2000). Wet and windy weather has been shown to be the potentially most dangerous weather for the development of hypothermia (Freeman and Pugh, 1967). The problem of wet-cold hypothermia is recognised by the safety organisations. Nevertheless their ability to design educational material concerning this hazard is hindered by lack of knowledge of the physiological and psychological responses (Thomson and Hayward, 1996). The information that is available derives from the pioneering work of Pugh (1966a, 1966b, 1967), supplemented by subjective descriptions of exposure incidents (Pugh, 1966; Hunter, 1968; Strang, 1969; Kreider, 1991; Noakes, 2000). Furthermore, in adverse wet weather the likelihood of navigational errors and slips is increased (Sharp, 2001). Despite the potentially serious consequences of wet and windy weather, very hot weather also has its own dangers. Participants may become more susceptible to both dehydration and hyperthermia (Noakes, 1991).

2.1.5 Equipment and clothing

Whilst in the past, both poor clothing and equipment may have been potential factors in the increased physiological strain and likelihood of injury (Pugh, 1967), this lack of adequate equipment cannot be considered the case today. Indeed, recent analysis of

the Scottish Mountain Rescue Services indicates a decline in the amount of fatalities compared with previous years (Sharp, 2001). This favourable change may reflect many developments including improvements to clothing worn by casualties, their overall skill/awareness level, as well as improvements in rescue provision, particularly faster response times by the rescue teams as a result of mobile phone use (Sharp, 2001). The advances in equipment such as the mobile phone and global positioning systems may help to decrease further the fatalities in the mountainous environment.

In summary, the previous mentioned aspects of hill walking, namely the demands, physical fitness of the participant, intensity, weather, terrain, and equipment and clothing used are all closely interrelated. For example, whilst walking, the physiological demands (i.e., the intensity of the walk) will be influenced by the terrain, weather conditions, fitness of the participant and the subsequent clothing worn by the participant. Indeed, the increased clothing requirement in adverse weather will have a direct effect on the thermoregulatory function of the participant; this is discussed further in section 2.3.0. Hill walking represents a relatively unique form of activity in which the prolonged duration and varying intensities may place exceptional demands on the recreational participants. The antihomeostatic nature of such activity may have the potential to impose maximal stress simultaneously upon several regulatory systems.

2.2 INJURIES IN THE HILL WALKING ENVIRONMENT

The information regarding injuries in the hill walking environment is limited. The information is sparse due to the probable large number of accidents which take place, but are not reported to the police or rescue services and consequently limit the recording of such incidents. The information that is available is derived from studies by Anderson (1994) and Sharp (2001). Anderson (1994) examined Scottish mountain rescue incidents involving 878 casualties (injured only) over the five-year period 1989-1993. The more recent study by Sharp (2001) extended the findings of Anderson (1994); it constitutes one of the most comprehensive examinations of Scottish mountain incidents ever undertaken and one of the most exhaustive in the UK. This study yielded a total of 1027 incidents involving 1269 individual people (both injured and not injured).

Sharp (2001) reported a number of important findings regarding injuries in the mountainous environment. Data were recorded for each incident and every person on the following variables - gender, age, address (living in / out of Scotland), experience, occupation, group affiliation, month of the year, injury sustained (e.g. fatal, limb), activity involved (e.g. hill walking, abseiling, weather, terrain) contributory causes (e.g. poor planning) and the nature of the incident (e.g., lost, slipped). Some of the important findings of Sharp's (2001) study were:

1. Men are more likely than women to be involved in an accident, even when participation figures are accounted for. However, with increasing age women are more likely to be involved in an incident.
2. Over half of all casualties are classified as experienced.
3. There are incident high points in summer (August) with females and winter (February) with males.
4. The majority of incidents in the mountainous environment involve those who participate in hill walking.
5. Many incidents take place when the weather is not inclement and where the terrain is not serious - footpaths, dry and fine, walking uphill.
6. Slips and stumbles remain the prime causes of incidents. Older, experienced hill walkers (especially women) are especially prone.

7. Many slips result from poor concentration/distraction (especially with men). Almost half of these take place whilst walking uphill/flat.
8. Navigation, poor planning and timing, and group separation are associated with many incidents (especially with men).
9. A significant number of problems arise through absence of key items of equipment.
10. Those most 'at risk' belong to professional occupations.

A slip/stumble was the main (40%) underlying cause of the injuries sustained. Almost two-thirds of all slips resulted in a limb injury and over one-third of all fatalities resulted from a slip. The predominance of lower limb injury mirrors that found in other sports such as football, skiing, tennis and volleyball (Steinbruck, 1999). Fatal injuries accounted for around 14% of all casualties, which is a reduction of 4 % from the earlier study by Anderson (1994). Hypothermia (described below) accounted for 8 % of all casualties. Generally, the data indicated that slips tend to happen to those who: a) are experienced and lack concentration (especially men), c) wear poor footwear (especially women), d) misjudge where to place their foot (almost one-half), e) descend hills (two-thirds), f) are older, g) are female, and h) walk in summer.

Whilst the data from Anderson (1994) and Sharp (2001) provide a useful insight into the causes of injuries in the mountainous environment, they clearly lack any documented physiological, subjective or metabolic evidence. An insight into some of the relevant responses that are important in the safety of hill walkers, such as the likelihood of dehydration, impaired performance, and the ability to maintain glycaemia may be used to quantify the stresses of such activity. Also the possible effect that age may have on these responses may provide some additional information into the aetiology of the accidents occurring in the mountainous environment.

2.3 TEMPERATURE REGULATION DURING PROLONGED EXERCISE

2.3.1. Control of heat exchange

To maintain cell function, the core temperature of humans has to remain within a narrow band, though small variations occur with the menstrual cycle, circadian rhythm, fever and exercise. Two main mechanisms regulate core temperature and keep it at approximately 37°C. There are physiological or reflex mechanisms which are involuntary, and behaviour mechanisms which are voluntary.

Physiological mechanisms: The human body can self-compensate for small upward or downward variations in temperature through thermoregulatory control. Indeed, during hill walking in adverse climatic conditions, core temperatures of over 39°C may be regularly encountered (Pugh, 1967). The body's responses to this exercise-induced 'hyperthermia' are sweating and dilation of the peripheral blood vessels, i.e. mechanisms to lose heat. When a rest is taken during these adverse climatic conditions, rapid cooling and a decrease in core temperature will occur. The body responds to this decrease in core temperature by firstly constricting the peripheral vessels in an attempt to limit heat loss from the body. Once this vaso-constriction is maximized, core temperature can only be maintained by an increase in heat production, i.e., shivering, which is thought to be the major contributor to the cold-induced increase in heat production (Doubt, 1991). Shivering is an involuntary contraction and expansion of muscle tissue occurring on a large scale. This muscle action generates heat, but shivering at peak (maximal) rate is time limited, and cannot be sustained. This limitation to shivering may be due to fuel exhaustion, metabolite accumulation or some other component of 'muscle fatigue'. Regardless, shivering should be used as an immediate sign of imminent danger.

Behavioural mechanisms: These include all voluntary actions that make the individual thermally comfortable. In hot conditions, taking cold drinks, seeking the shade and air-conditioning are common; in cold climates extra clothing is worn, exercise is undertaken, and if possible the individual seeks shelter and warmth. These behavioural mechanisms give the greatest independence from the environment and permit survival in even the most demanding of climates. However, during increased intensities of walking, the clothing worn by the walkers will increase the evaporation required for the maintenance of thermal

balance. This increase creates a difficult balance for walkers; on one hand they require an increased insulation from clothing for protection from the environmental weather conditions. On the other hand, they need to keep the insulation relatively low in order to aid evaporative heat loss. The failure to achieve this balance will result in an increased heat strain leading to an increased likelihood of dehydration (section 2.4.2). From a thermoregulatory point of view this balance will always be difficult to achieve for the walker, especially when high insulation levels are required. Furthermore, when high insulation levels are required, a situation will arise in which the clothing will restrict airflow to the skin. This restriction will promote the increased saturation of water vapour from air close to the skin. Because effective evaporation is prevented if the vapour pressure gradient between the skin and the environment is low (Clark and Edholm, 1985), as in the case when high insulation is required, the ability of the body to evaporate water from its skin surface may become severely compromised. The significance of this decreased ability to evaporate sweat creates a paradoxical situation whereby the participant may initially, in adverse weather condition, be at risk from an increased heat strain.

2.3.2. Hypothermia

Hypothermia is a state in which there is a fall in body temperature, which occurs when the heat produced internally by the body no longer balances heat loss. The clinical condition of hypothermia may be produced by the exposure of an individual to the adverse weather that is associated with mountain environments. The lower the core temperature the greater the danger for the individual although the definition of hypothermia, using only the measurement of the core temperature, may mislead people into a false sense of security.

The average daily core temperature is around 37°C, with a circadian variation of about 0.6°C; the lower value is in the early morning and the peak is found in the evening. This variation, along with the effects of exercise, complicates the normal definition of hypothermia, which is defined as a condition where the core temperature is less than 35°C, although most authorities agree there is no distinct threshold. Therefore, when core temperature is above 35°C but below 37°C the person cannot be diagnosed as suffering from hypothermia but is obviously affected by the cold environmental conditions whether in air and or in water.

2.3.3. Development of hypothermia in the mountains

Hypothermia has long been recognized as a most serious condition, often with fatal consequences for hill walkers, fell runners and high altitude mountain climbers. The physiology and mechanisms of hypothermia have been well documented during immersion in cold water (Tipton and Golden, 1996). Research into hypothermia occurring in the mountainous environment has, at best, been limited. This is somewhat surprising when one considers the popularity of such mountainous pursuits.

Whilst walking in the mountains, participants normally operate between 30 to 55%, depending on the terrain, of their maximal oxygen uptake. When exercising at these levels, the body's heat production is generally sufficient to offset heat loss. In adverse conditions, due to the increased insulation of clothing and hence the decreased ability lose heat, rectal temperatures of over 39°C may be encountered (Pugh, 1967). As a result, once the walker, runner or mountain climber fatigues and starts to slow or stops walking altogether, the rate of heat production falls dramatically. This alone predisposes to the development of hypothermia. These processes, in adverse weather conditions, will be accelerated. The critical point for this heat loss to occur is on ceasing of activity when participants take a rest or stop for fluid/food intake, transiently altering the balance between heat production and heat dissipation.

Therefore, during activity in the mountains, improper management of energy expenditure and clothing leads to sweating and ultimately fatigue. Dehydration may be a consequence of this increased rate of sweating and, in addition to low fluid intakes, may increase this thermal stress. An attitude of competitiveness may keep the walker from admitting to his or her condition, and he or she may reach a rather severe state of exhaustion and wetness before calling for a rest stop (Pugh, 1966). At this point the individual's peripheral circulation will be dilated, so when the walker sits down for a rest, heat loss occurs rapidly. Sweating may cease, but the wet clothing continues to dry, chilling the vaso-dilated vessels of the skin and adding to the increase in heat loss.

Paradoxically, it would appear that the prevention of overheating is likely to be a major factor in the avoidance of hypothermia. Therefore, prevention of overheating, and hence hypothermia, requires proper management of energy expenditure, clothing, fluid intake and evaporation.

2.4. WATER BALANCE AND HYDRATION DURING PROLONGED EXERCISE

2.4.1 Regulation of water balance and hydration

Total body water is normally maintained with a small window of fluctuation on a daily basis by intake of food and drink and excretion of urine (Greenleaf, 1992). Mostly, water intake is related to habit rather than thirst, but after periods of deprivation, the thirst mechanism is effective at driving intake (Rolls et al., 1980; Philips et al., 1984; Engell et al., 1987). Hyperhydration is corrected by an increase in urine production and hypohydration by an increase in water intake via food or drink consumption initiated by thirst (Shirreffs, 2000). There are also water losses via the respiratory tract, the gastrointestinal tract and the skin but these normally represent only a relatively small fraction of the total body water loss (Clark and Edholm, 1985). During exercise, there is also a small amount of fluid lost through the sweat glands. The extent of this loss will vary from individual to individual, but a general example for a sedentary individual is outlined in Table 2.1.

Table 2.1. Daily body water input and output

Parameter	ml	Parameter	ml
<i>Daily water loss</i>		<i>Daily water intake</i>	
Kidneys (urine)	1500	Fluid	1300
Respiratory tract	400	Food	1000
Gastrointestinal tract	200	Cellular oxidation	300
Skin	500		
Total	2600	Total	2600

From Astrand and Rodahl (1986)

Water homeostasis depends on a balance between water intake, driven by factors affecting the sense of thirst, and water output, driven by factors affecting renal function (Mack et al., 1994). Of the components of water balance listed in Table 2.1, only two, that is fluid intake and urine volume, are controlled by homeostatic mechanisms responsive to the state of body water (Wrong, 2000). A water deficit produces increased osmolality of all body fluids, and this increase, via stimulation of

the thirst centre and the osmoreceptors, causes a sensation of thirst and the release of the antidiuretic hormone vasopressin (Robertson et al., 1976; Roberston and Berl, 1985). This hormone acts on receptors in the renal collecting tubule to cause increased water absorption and reduced urinary volume. When the body contains an excess of water, the reverse processes occur: reduced plasma osmolality inhibits thirst and the osmoreceptor centre, drinking ceases and, in the absence of vasopressin, water absorption in the renal tubules is reduced, and subsequent water diuresis is increased (Wrong, 2000).

Prolonged exercise will clearly increase the requirement for water intake in order to maintain hydration. The fluid requirements for such activities can be enormous. Sweat rates of between 1.0 to 2.5 l/h have been recorded in a range of endurance events (Sawka, 1992; Rehrer and Burke 1996); failure to match fluid intake to these large fluid losses will lead to dehydration. Sweat losses for various sporting and occupational activities are well categorized, but the variability is large because of the different factors that affect the sweating response (Rehrer and Burke 1996). Even at low ambient temperatures, high sweat rates are sometimes observed when the energy demand is high, as in a marathon run, so it cannot be concluded that dehydration is a problem only when the ambient temperature and humidity are high (Maughan, 1985).

2.4.2 Implications of dehydration for the hill walker

Adolph and associates (1947) were the first to communicate effectively the devastating effects that body water loss can have on physiological strain and exercise performance. Generally, body water deficits adversely influence exercise performance (Sawka and Pandolf, 1990). The critical water deficit and magnitude of performance decrement are related to the environmental temperature and exercise task; the warmer the environment the greater the potential for exercise decrements (Sawka and Pandolf, 1990).

Thermoregulatory and cardiovascular functions, as well as cognitive function are adversely influenced by body water deficits (Adolph et al., 1947; Ladell, 1955; Gopinathan et al., 1988; Sawka, 1992). For many complex tasks, both the mental decision-making and physiological functioning are closely related (Sawka, 1992). As

a result, dehydration probably has more profound effects on real-life tasks than solely physiological performance measures. In hill walking, dehydration may decrease thermoregulatory and cognitive functioning, which could impair decision making, leading to an increased susceptibility to fatigue and injury in the mountainous environment.

As mentioned previously, hill walkers may be particularly prone to dehydration due to a combination of factors such as the poor availability of water in the hills, high thermal stress from increased insulation from clothes, high fluid requirements to match the prolonged duration of such pursuits. Dehydration of as little as 1% of bodyweight can adversely affect performance; the capacity for prolonged exercise is reduced (Saltin, 1964; Craig and Cummings, 1966) and, more dangerously, temperature regulation is impaired (Ekholm et al., 1970; Wyndam, 1977; Nadel et al., 1983). Temperature regulation is perhaps one of the most important considerations for safety in the mountainous environment, yet due to the likelihood of dehydration, it is likely to be the function most compromised in the participant.

2.4.3 Fluid homeostasis during prolonged exercise

During exercise, alterations in plasma volume appear to be determined by postural changes and by the duration and intensity of the exercise (Harrison, 1985; Kargotich et al., 1998). Moderate to high-intensity exercise on a cycle ergometer causes haemoconcentration due to movement of water out of the vascular space (Greenleaf et al., 1977; Novosadova, 1977; Senay et al., 1980). If the exercise is prolonged and fluids are supplied orally, there may be some restoration of plasma volume (Ekelund, 1967; Hagan et al., 1980). The changes in plasma volume which accompany movement from the supine to the sitting or standing position occur rapidly and can account for the haemoconcentration reported in many exercise studies (Ekelund, 1967; Harrison, 1985).

Generally, prolonged walking causes haemodilution unless subjects become dehydrated (Adolph, 1947; Pugh, 1969; Dill et al., 1973; Williams et al., 1979; Milledge et al., 1982). This is true even at high environmental temperatures (Dill et al., 1973). During prolonged moderate to strenuous walking exercise, Milledge et al.

(1982) showed that the expansion of the plasma volume (25% increase) and extracellular fluid volume (4.4 % increase) over 5 consecutive days of hill walking is considered to be mainly due to activation of the renin-aldosterone system (Milledge et al., 1982).

Leiper et al. (1982) showed a calculated increase of 21% in plasma volume after 4 days of low intensity walking. In this study subjects walked 37 km each day for 4 consecutive days. The daily exercise intensity was consistent and was equivalent to 17% of the subject's maximal oxygen consumption. Leiper and co-workers provided further evidence that the haemodilution observed in prolonged exercise appears to be due to an actual increase in plasma volume. There were no urinary or faecal haemoglobin (Hb) losses, and serum bilirubin levels were consistently low, suggesting that increased erythrocyte destruction was not a major factor in producing this haemodilution. The studies mentioned (Milledge et al., 1982; Leiper et al., 1989) showed a disproportional increase in serum Na⁺ concentration during prolonged walking which is also consistent with an increased activation of the renin-aldosterone system, resulting in the retention of sodium and causing a shift of fluid into the circulation (Bozovic et al., 1967).

In summary, the increase in plasma volume which occurs during prolonged walking may be due mainly to a postural haemodynamic reflex activating the renin-aldosterone system (Pugh, 1969; Rowell, 1974; Harrison, 1985). In two previous studies of prolonged walking, increases in plasma volume and Na⁺ accumulation were still apparent for between 4 and 6 days post-exercise, although plasma renin and aldosterone activity had returned to near pre-exercise control levels within 24 h of cessation of the exercise (Williams et al., 1979; Milledge et al., 1982). These data suggest that other factors are involved in the haemodilution response to prolonged exercise.

2.5 NUTRITIONAL ASPECTS OF PROLONGED EXERCISE

2.5.1 Exercise and the energy reserves in the human body

The human body derives its energy requirements from a mixture of fat, carbohydrate (CHO) and protein, utilising non-esterified fatty acids (NEFA), glucose and amino acids from the circulation as well as the intramuscular stores. In situations where metabolic demands are elevated as in endurance exercise, CHO and fats are the main fuels that are metabolised by means of oxidative processes in muscle to produce the adenosine tri-phosphate (ATP) needed for skeletal muscle to contract. In contrast, the body's content of protein is only available as an energy store at the expense of loss of some functional protein. In other words, protein is not utilised as an energy reserve in the same way that CHO and fat are. The body's fuel reserves are summarized in Table 2.2.

Table 2.2. Energy stores in the human body

Fuel	Amount (typical in a 65-kg person)	Energy equivalent	Days supply if the only energy source
Carbohydrate			
Free glucose	12 g	0.2 MJ	0.02 = 30 min
Glycogen	450 g	7.65 MJ	0.77 = 18 h
Fat			
Triacylglycerol	15 kg	550 MJ	55
Protein	12.5 kg*	210 MJ	21

Assumptions: energy produced by biological oxidation is 17 kJ/g for CHO and protein, 37 kJ/g for fat; energy expenditure is 10 MJ/day. * Not all the protein can be utilised, so the numbers for protein are notational only (From Frayn, 1996, with permission).

The energy stores of CHO (~8 MJ) are small compared to the large stores of fat (550 MJ). The storage of glycogen in the liver and muscle accounts for only 1 -2 % of the total energy reserves of the body, whereas the triacylglycerol stored in adipose tissue accounts for about 80% of energy reserves, the remaining 18-19% being protein located in the muscle.

For prolonged exercise it would be of clear benefit to use the large fat stores over the small CHO stores. However, CHO becomes the preferred fuel at high exercise intensities (Jeukendrup and Jentjens, 2000) with a subsequent decrease in fat oxidation (Jones et al., 1980; Romijn et al., 1993). If the body were to use only its CHO store for energy, for example during a marathon, exercise would be limited for 80 - 90 min (Maughan et al., 1997). In contrast, if fat were the prime energy source, energy for up to 119 hours of continuous marathon running would be available (Newsholme and Leech, 1990). Hence, it is not the availability of the fat stores but the ability to mobilise fat from the available stores and the ability of the muscles to utilise this energy source that might limit endurance exercise. This ability to mobilise and utilise fat is dependent on several factors which are discussed in the next section (2.6.). Failure or lack of energy during an endurance event will result in a decreased performance time. Conversely, this energy production failure in the mountainous environment may compromise physiological safety leading to an increased susceptibility to fatigue, injury and possible hypothermia. Consequently, as with the endurance athlete, it would be desirable that the body derives its energy supply primarily from the large, unlimited fat stores rather than rely on the limited capacity of the glycogen stores. In the next section, the mobilisation and utilisation of these energy substrates during prolonged exercise are described.

2.5.2. Substrate mobilisation and utilisation during prolonged exercise

The transition from the resting to the exercise state is characterised by an increase in energy expenditure and an alteration in the use of metabolic fuels, carbohydrate and fat. Specifically, there is a greater dependence on carbohydrate oxidation for energy production during exercise at high intensities, compared to a greater dependence on fat as a fuel source at low exercise intensities (Romijn et al., 1993; Coyle, 1997). As mentioned previously, the important energy sources during endurance exercise are glucose stored as glycogen in muscle and liver, and fatty acids stored as triacylglycerol in muscle, adipose tissue and circulating lipoproteins. The relative contribution and absolute quantity of these energy substrates utilised during exercise depend on a number of factors including the intensity and duration of the exercise, training status, initial muscle glycogen content (which is related to the preceding diet

and exercise regimen), age and gender, environment and mode of exercise. How these factors affect substrate utilisation will be detailed in section 2.6.

2.5.2.1 Mobilisation and utilisation of carbohydrate during exercise

The general metabolic alterations that occur in carbohydrate metabolism during endurance exercise are similar to those which occur in the post-absorptive state, although there is a greater glucose requirement during exercise. Circulating blood glucose released from the liver by the pathways of glycogenolysis and gluconeogenesis represent a major source of glucose used for ATP production by contracting skeletal muscle during endurance exercise.

With the transition from rest to exercise, there is an initial acceleration of hepatic glycogenolysis to meet the increased glucose requirements of the contracting muscles. As exercise is extended in duration, the liver glycogen stores gradually become reduced, which results in an increased reliance on hepatic gluconeogenesis. The contribution of hepatic gluconeogenesis to the total hepatic glucose production increases from 5-15% in the first 50 minutes of exercise to 50% after several hours of exercise (Ahlborg et al., 1974; Coggan, 1991). This rise in gluconeogenic activity is accounted for by the increased supply of gluconeogenic precursors, particularly lactate which is released via the glycolytic pathway in exercising muscle (Ahlborg et al., 1974; Wahren et al., 1975), and an increase in gluconeogenic enzyme activity with exercise (Dohm et al., 1985). Additionally the contribution of gluconeogenesis and glycogenolysis to hepatic glucose production can vary according to the intensity of the exercise and the nutritional and training status of the individual. In some situations the rate of gluconeogenesis may fail to compensate for the decline in hepatic glycogenolysis, resulting in hypoglycaemia (a blood glucose concentration of < 3 mmol/l) and consequently fatigue (Felig et al., 1982). During exercise, the liver plays a dual role in providing glucose to the muscle at a rate equal to muscle uptake, and also in maintaining glucose homeostasis. For this reason, dietary manipulations that may evaluate pre-exercise liver glycogen stores may maintain blood glucose concentrations during endurance exercise, and therefore offset or delay fatigue. This would lead to an improvement in exercise performance.

Equally, muscle glycogenolysis provides another major source of glucose used for ATP production during endurance exercise. As in the liver, the magnitude of glycogenolysis in exercising muscle is dependent on factors including exercise intensity and duration, and the nutritional and training status of the individual.

Dietary manipulations that either increase the pre-exercise muscle glycogen concentration, and/or attenuate the rate of muscle glycogen degradation during exercise have the potential to delay the onset of fatigue and hence improve exercise performance (Bergstrom et al., 1967; Coggan and Coyle, 1986). This delay of fatigue and improvement of performance are clearly of benefit in the athlete. The value of such manipulations, at lower intensities of activity, may be less important.

2.5.2.2 Mobilisation and utilisation of fat during exercise

Fatty acids are present as triacylglycerol in adipose tissue, muscle and in circulating lipoproteins. During endurance exercise, these represent the three main sources of fatty acids used for ATP production by contracting skeletal muscle.

From a quantitative aspect, the main source of lipid energy during endurance exercise is from adipose tissue outside the cell. The fatty acids liberated in adipose tissue by means of lipolysis are transported bound to albumin for uptake and oxidation in the contracting muscle during exercised. It appears that the primary determinant of the rate of utilisation of NEFA derived from adipose tissue during exercise is the rate of mobilisation of NEFA from the adipose tissue (Hodgetts et al., 1991). This regulatory step depends on a number of factors including the activity of the enzyme hormone sensitive lipase (HSL) and hence the rate of lipolysis, the plasma transport of NEFA, and the rate of re-esterification of NEFA by the adipocyte. The capacities of these processes during endurance exercise have several limitations, which are subject to hormonal and substrate regulation.

With the transition from rest to exercise, there is a transient decrease in systemic plasma NEFA concentration as a result of a temporary imbalance between the rate of uptake of NEFA by the muscle and the rate of NEFA release from adipose tissue. This temporary delay is attributed to the decline in blood flow to adipose tissue during

the early stages of exercise, when blood is diverted to the active muscle. As the exercise is continued, lipolysis is increased and there is a large increase in the rate of appearance and availability of NEFA in the plasma. This increased rate of appearance and availability of NEFA is associated with suppression in the rate of re-esterification and a concomitant increase in adipose tissue blood flow (Wolfe et al., 1990; Hodgetts et al., 1991).

It is believed that the contribution of NEFA to the oxidative metabolism is largely dependent on the concentration of NEFA in the plasma during exercise (Hagenfeldt and Wahren, 1968, 1971). Indeed, during low intensity exercise at 25% $\dot{V}O_2$ max, for instance walking, it is presumed that plasma NEFA derived from adipose tissue is the exclusive source of energy substrate. This exclusive source of energy substrate is due to the close relationship between the rate of fat oxidation and the rate of plasma NEFA disappearance. This close relationship implies that at low exercise intensity, the rate of mobilisation of NEFA from adipose tissue is not a limiting factor. As exercise increases in intensity from low (25%) to moderate (65%), studies have shown that in endurance trained men there is a decline in the rate of appearance of plasma NEFA derived from adipose tissue, despite an increase in fat oxidation (Romijn et al., 1993). The increase in fat oxidation is believed to be accounted for by fatty acids derived from the intra-muscular stores (Romijn et al., 1993; Keins, 2000).

Results of several studies suggest that intra-muscular triacylglycerol stores represent a considerable portion of the total fat used during endurance exercise (Carlson et al., 1971; Froberg et al., 1971; Essen, 1977; Hurley et al., 1986; Jansson and Kaijser, 1987; Romijn et al., 1993; Martin et al., 1993; Philips et al., 1996). Estimates of intra-muscular triacylglycerol store use, calculated indirectly with isotopic tracer methods, indicate that non-plasma-derived fatty acids (presumably from intra-muscular triacylglycerol stores) provide >50% of the total fat oxidised during exercise (Martin et al., 1993) and muscle contraction (Dyck et al., 1998). Several studies in which muscle biopsies were taken before and after exercise indicated that intra-muscular triacylglycerol concentration declines by 25-40% after 1-2 h of moderate intensity exercise on a cycle ergometer, which could account for 60-75% of the total amount of fat oxidised (Carlson et al., 1971; Froberg et al., 1971; Essen, 1977; Hurley et al., 1986; Philips et al., 1996). In contrast, other researchers found that intra-muscular

triacylglycerol concentration decreases minimally or not at all after prolonged exercise and therefore does not contribute significantly to total energy production (Kiens et al., 1993; Wendling et al., 1996; Starling et al., 1997; Kiens and Richter, 1998; Bergman et al., 1999). The reason for the discrepancies between the studies is not clear but may be related to differences in exercise protocols, variability when measuring intra-muscular triacylglycerol concentrations in muscle biopsies (Bergman et al., 1999), and differences in the interval between the last exercise bout and the experimental trial.

Few researchers have evaluated the contribution of plasma triacylglycerols (TAG) to total energy production. The available data suggest that during resting conditions, plasma TAG may account for 5-10% of total fat oxidation (Ryan and Schwartz, 1965; Wolfe et al., 1985). There is also indirect evidence that only a small fraction of total energy production is derived from plasma TAG during exercise (Mackie et al., 1980; Kiens and Lithell, 1989; Turcotte et al., 1992). For example, Kiens and Lithell (1989) found that very low-density lipoprotein TAG uptake by skeletal muscle is negligible during exercise.

Klein et al. (1994) showed that lipolysis of adipose tissue TAGs, plasma fatty acid uptake and fatty acid oxidation increase progressively throughout a bout of exercise. After ~2 h of exercise, the rate of plasma fatty acid uptake becomes greater than the rate of fatty acid oxidation, suggesting that fatty acids released into plasma from adipose tissue can supply all fatty acids used by active muscle. Thus, as exercise duration increases, it is likely that the relative contribution of intra-muscular triacylglycerols to total fat oxidation declines and the contribution from plasma fatty acid increases.

Another potential source of fatty acids utilised for energy production is triacylglycerol present in triacylglycerol rich lipo-proteins, very low-density lipoprotein and chylomicron particles. The contributions of very low-density lipoprotein and chylomicron-TAG to energy expenditure during exercise remain unclear but are likely to play only a minor role (<5%) to energy production during prolonged exercise (Kiens and Lithell, 1989; Hargreaves et al., 1991; Kiens et al., 1993; Whitley et al., 1998).

Although branch chain amino acids released from the splanchnic bed can be oxidised by the working muscles (Felig and Wahren, 1971; Rennie et al., 1981; Wolfe et al., 1982), it is unlikely that this fuel can satisfy more than a small fraction of the energy requirement (<10%) even during prolonged exercise in excess of 2 h (Poortmans, 1984). Protein breakdown may play a more important role in supplying amino acids for gluconeogenesis (Felig and Wahren, 1971; Wasserman et al., 1988) and/or protein synthesis within the liver (Cararo et al., 1990). Formation of amino acids, de novo, may be important in nitrogen transport. Although amino acids utilised in this way are not a direct energy source, they are necessary for high metabolic rates to be sustained (Wasserman and Cherrington, 1996). The mobilisation of the body's stored TAG and glycogen is depicted schematically in Figure 2.2.

2.5.3 Regulation of substrate utilisation during prolonged exercise

2.5.3.1 Hormonal regulation

The balance of CHO and fat utilisation at rest and during exercise can partly be explained by activities of a number of regulatory hormones. These include plasma insulin, glucagon, catecholamines (noradrenaline and adrenaline), cortisol and growth hormone (Frayn, 1996).

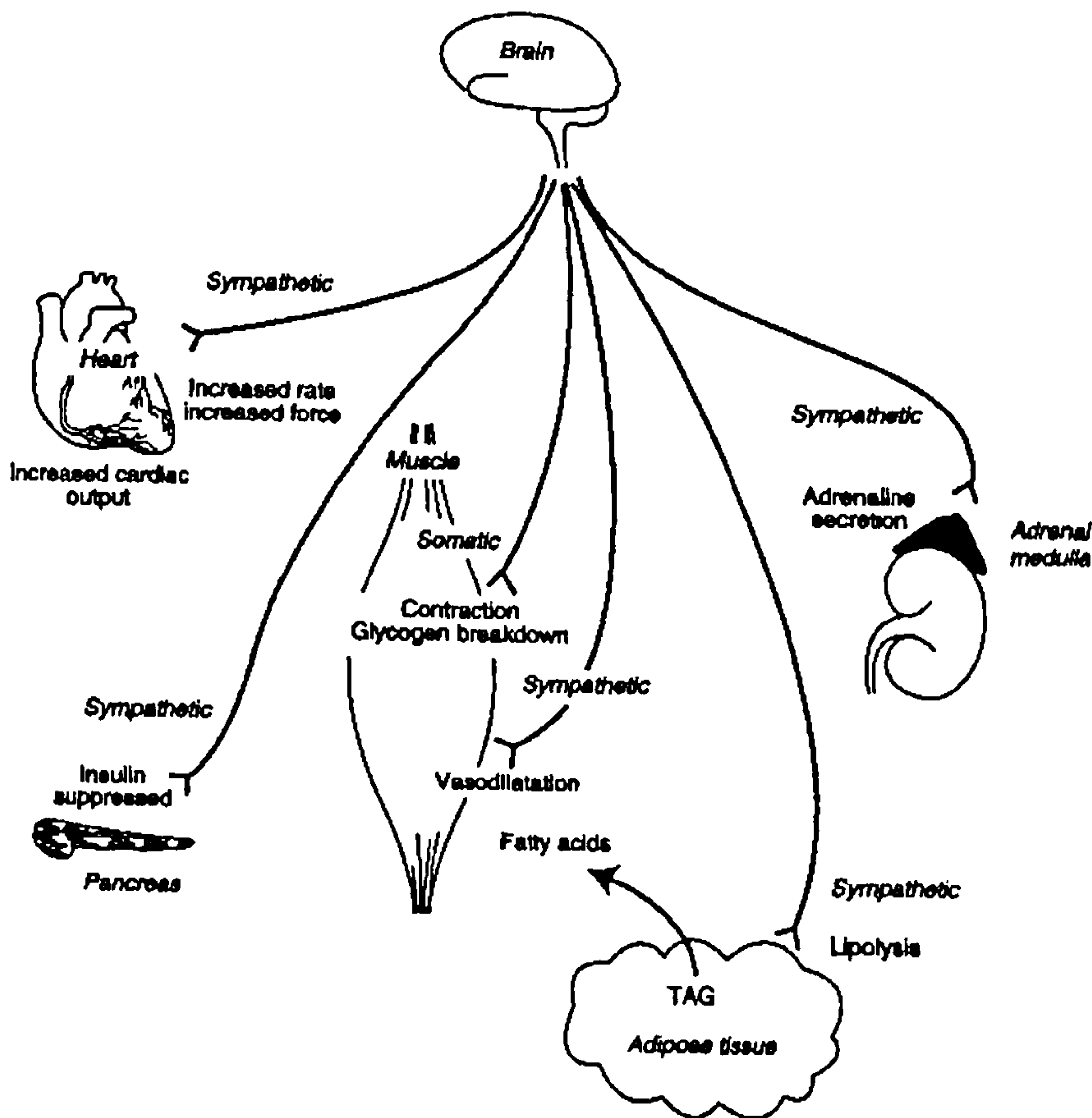


Figure 2.1 Co-ordination of metabolism by the nervous system during endurance exercise (From Frayn, 1996, with permission)

During exercise, there is normally a fall in plasma insulin and a reciprocal rise in plasma glucagon. The balance between these two pancreatic hormones is affected by the concentration of glucose in the plasma, whereby an increase in plasma glucose concentration will result in an increase in the ratio of insulin to glucagon and a decrease in plasma glucose concentration will decrease this ratio (Frayn, 1996). The fall in plasma insulin during exercise is brought about by α -adrenergic inhibition of insulin secretion via the increased concentration of circulating adrenaline (Arner et al., 1990).

An elevation of adrenaline during exercise has been shown to stimulate muscle glycogen breakdown due to an increase in glycogen phosphorylase activity (Jansson et al., 1986; Spriet et al., 1988). In the liver, the exercise-induced rise in the catecholamines, noradrenaline and adrenaline, is associated with an increase in hepatic glucose production during exercise via pathways of hepatic glycogenolysis

and gluconeogenesis (Kjaer et al., 1991). Increased adrenergic stimulation to the pancreas directly by adrenaline results in a decrease in plasma insulin and increase in glucagon during exercise. From the view of CHO metabolism, this increased ratio of glucagon to insulin potentiates the breakdown of liver glycogen during exercise. In the view of fat metabolism, the decreased insulin levels will promote the activation of hormone sensitive lipase, resulting in an increase in adipose tissue and intra-muscular lipolysis, although the effects on muscle hormone sensitive lipase appear to be weak (Arner et al., 1990). In this way, insulin appears to be the main hormone regulating lipolysis in promoting hormone sensitive lipase activity when concentrations are low, and inhibiting hormone sensitive lipase activity when concentrations are high (Arner et al., 1990; Holloszy and Kohrt, 1996; Fig. 2.2). Conversely, glucagon in low concentrations has been reported to inhibit the activity of lipoprotein lipase in the muscle, whereas high concentrations have been shown to promote the activation of lipoprotein lipase in muscle (Oscai, 1979; Fig. 2.2).

In relation to CHO metabolism, an increase in cortisol and growth hormone, associated with the response to exercise, appears to contribute a minor role in increasing hepatic glucose production by means of their action on gluconeogenic enzyme activity (Sellers et al., 1988). In addition the increase in cortisol may potentiate the activity of the catecholamines on glucose production via glycogenolysis in liver and muscle. In contrast to CHO metabolism, increases in the secretion of cortisol and growth hormone during prolonged exercise have a marked effect on stimulating fat metabolism (Hodgetts et al., 1991). These two hormones appear to promote activity of catecholamines and hence lipolysis in adipose tissue and muscle.

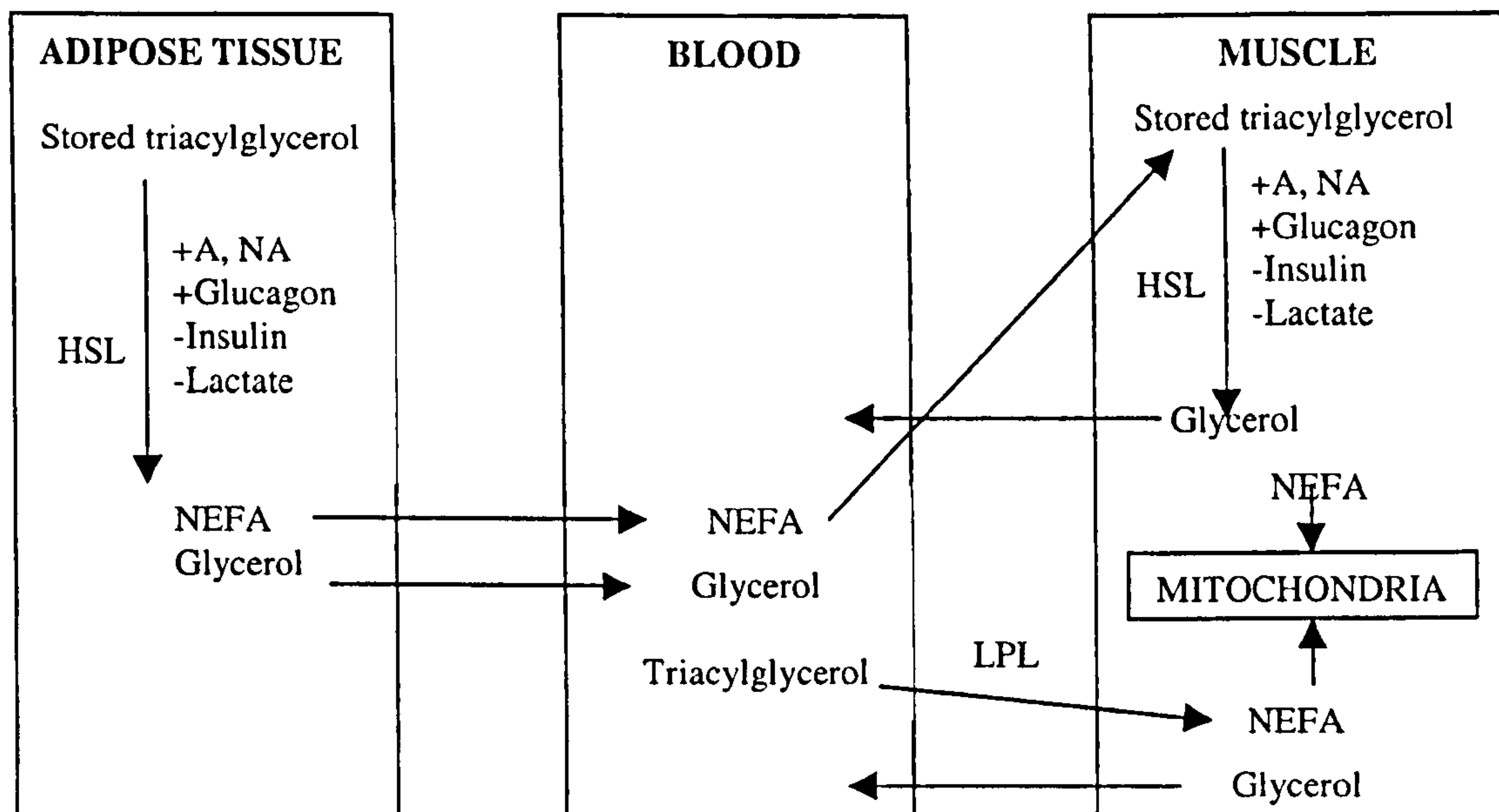


Figure 2.2. A schematic representation of the hormonal regulation of the source of fatty acids for oxidation in muscle. The scheme is taken in part from Sherman and Leenders (1995) and Frayn (1996).

2.5.3.2 The glucose-fatty acid cycle

The balance of CHO and fat utilisation at rest and during exercise can partly be explained by the glucose-fatty acid cycle. During the 1960s Randle and co-workers proposed the "glucose-fatty acid cycle," in which increased uptake and oxidation of FFA, due to elevated FFA levels, result in a citrate-mediated inhibition of PFK activity and glycolysis (Randle et al., 1963; Randle et al., 1964). The resultant increase in muscle glucose-6-phosphate levels inhibits hexokinase activity, glucose phosphorylation, and glucose uptake (Randle et al., 1964).

In subsequent years the traditional view of the glucose-fatty acid cycle has been opposed. In humans, increased FFA availability is associated with reduced glucose utilisation under resting conditions (Ferrannini et al., 1983), although no effect of FFA on plasma glucose oxidation has also been reported (Wolfe et al., 1988). During moderate-intensity (44% $\dot{V}O_2$ max) exercise, elevation of FFA had no effect on CHO oxidation (Favussin et al., 1986). Furthermore, a doubling of plasma FFA within the physiological range was associated with a 30-35% reduction in muscle glucose uptake

during 1 hour of knee-extension exercise at 80% of maximal work capacity (Hargreaves et al., 1995).

More recently, the opposite view has been proposed (Coyle et al., 1997), which is that CHO metabolism regulates fat oxidation during exercise. Coyle et al. (1997) demonstrated that hyperglycaemia and hyperinsulinaemia from glucose ingestion, equivalent to a 200-g CHO meal, substantially reduced long-chain fatty acid oxidation from plasma NEFA and intra-muscular triacylglycerol stores by 48% and 27% respectively, during 40 min of cycling at 50% $\dot{V}O_2$ max in six endurance trained men. The authors suggested that the preferred oxidation of CHO when both CHO and fat were made available to muscle is mediated in part by the active inhibition of fat oxidation. The mechanism operates by means of an accumulation of malonyl-CoA, a potent inhibitor of carnitine palmitoyl transferase-1, which consequently reduces the transport of long-chain fatty acids into the mitochondria. These observations and similar findings have been supported both at rest (Sidossis and Wolfe, 1996) and during exercise (Sidossis et al., 1997a; Sidossis and Wolfe 1997b).

2.6 SUBSTRATE MOBILISATION AND REGULATION DURING PROLONGED EXERCISE

Substrate mobilisation and regulation have been intensively investigated in the context of short-term exercise / endurance performance. In contrast, there is little information regarding substrate mobilisation and regulation over very prolonged exercise, as may be related to activity sustained over one or consecutive days. The nature of very prolonged exercise normally, but not always, necessitates the use of a lower intensity of exercise than that used in competitive performance events. A low intensity of exercise is especially the case in hill walking where the participants are more likely to be recreational, as opposed to elite performers.

2.6.1 Prolonged activity over one day

As mentioned earlier, prolonged full day activity is normally only sustained at low to moderate intensities, especially when pursued by recreational active people. Hence, the preferred source of fuel at this level of intensity is from the fat stores. Indeed, a number of studies sustained over a full day have indicated significant increases in fat mobilisation (Pugh, 1969; Kirkeby et al., 1977; Enger et al., 1980; Guezennec et al., 1984a; Loy et al., 1986; Greenhalf et al., 1987; Maughan et al., 1987)

Enger et al. (1980) measured changes in lipoprotein metabolism before and immediately after a 70-km cross-country ski race, over one day. They confirmed findings of previous studies showing that prolonged heavy exercise, over one day, is accompanied by major changes in fat metabolism (Carlson and Mossfeldt, 1964), and that this increased mobilization of fat may last for up to several days in duration (Kirkeby et al., 1977). Furthermore, Enger et al. (1980) showed that there was a marked increase in HDL-C, after the 70-km race which lasted for at least 4 days.

A number of investigations into the effects of ultra-long distance running and triathlons on biochemical levels have produced comparable results (Noakes and Carter, 1982; Sagnol et al., 1989; Nagel et al., 1992; Fournier et al., 1996). For example, after a 24-h running race, the lipid parameters cholesterol, triglycerides and LDL-cholesterol declined significantly whereas HDL-cholesterol increased significantly (Nagel et al., 1992). Fournier et al. (1996) measured various plasma hormones before, during and after a 110-km ultra-

marathon race. During the race, cortisol and beta-endorphins showed significant increases whereas testosterone decreased throughout the race.

When considering the energy stores in the human body, it is clear that the CHO stores cannot cover the energy pr nutritional demands of such prolonged events. The body must increase fat mobilisation to meet the demands of such exercise, and to prevent hypoglycaemia.

2.6.2 Prolonged activity over consecutive days of activity

Greenhaff et al. (1987) and Maughan et al. (1987) investigated changes in metabolism over 4 days of consecutive walking. These studies were based on a flat 37-km walk which corresponded to operating at 17 % of $\dot{V}O_2$ max. In the studies of Greenhaff et al. (1987), subjects either fasted or had unrestricted access to food, whereas the subjects studied by Maughan et al. (1987) consumed isoenergetic diets either high in CHO (~85% CHO) or low in CHO (2% CHO). In both studies, the pattern of substrate mobilization in response to prolonged walking exercise was reproducible between individuals and for the same individual on different occasions. In the presence of a mixed diet, with an energy intake calculated to meet total energy expenditure, there is little variation in the circulating levels of fuel substrates on successive days (Greenhaff et al., 1987). Conversely, this pattern can be altered if the dietary composition is changed even in the presence of a constant energy intake (Maughan et al., 1987): glycerol, NEFA and 3-OHB levels were all higher on the low-CHO diet compared with the high-CHO condition which resulted in a suppression of lipid mobilisation.

In earlier studies by Carlson and Fröberg (1967) and Marniemi et al. (1984), subjects completed a 500-km walk over 10 days and a 344-km walk over 7 days, respectively. In both studies prolonged walking on the flat was combined with very low energy intakes (~837 kJ/d). In the study of Carlson and Fröberg (1967), NEFA and glycerol concentrations peaked at day 6, then subsequently declined over the following 4 days (Fig. 2.3). Similarly, TAG concentrations decreased, attained a plateau and remained stable after the first 3 days with a trend for an increase on day 7 (Marniemi et al., 1984). Despite the low energy intakes in these studies (Carlson and Fröberg, 1967; Marniemi et al., 1984), there was an increased fat mobilisation and blood glucose concentrations were maintained (Fig. 2.3). The results of these studies indicate that despite low energy intakes and high physiological stress, the human body is remarkably efficient at altering its metabolism to

maintain blood glucose concentrations.

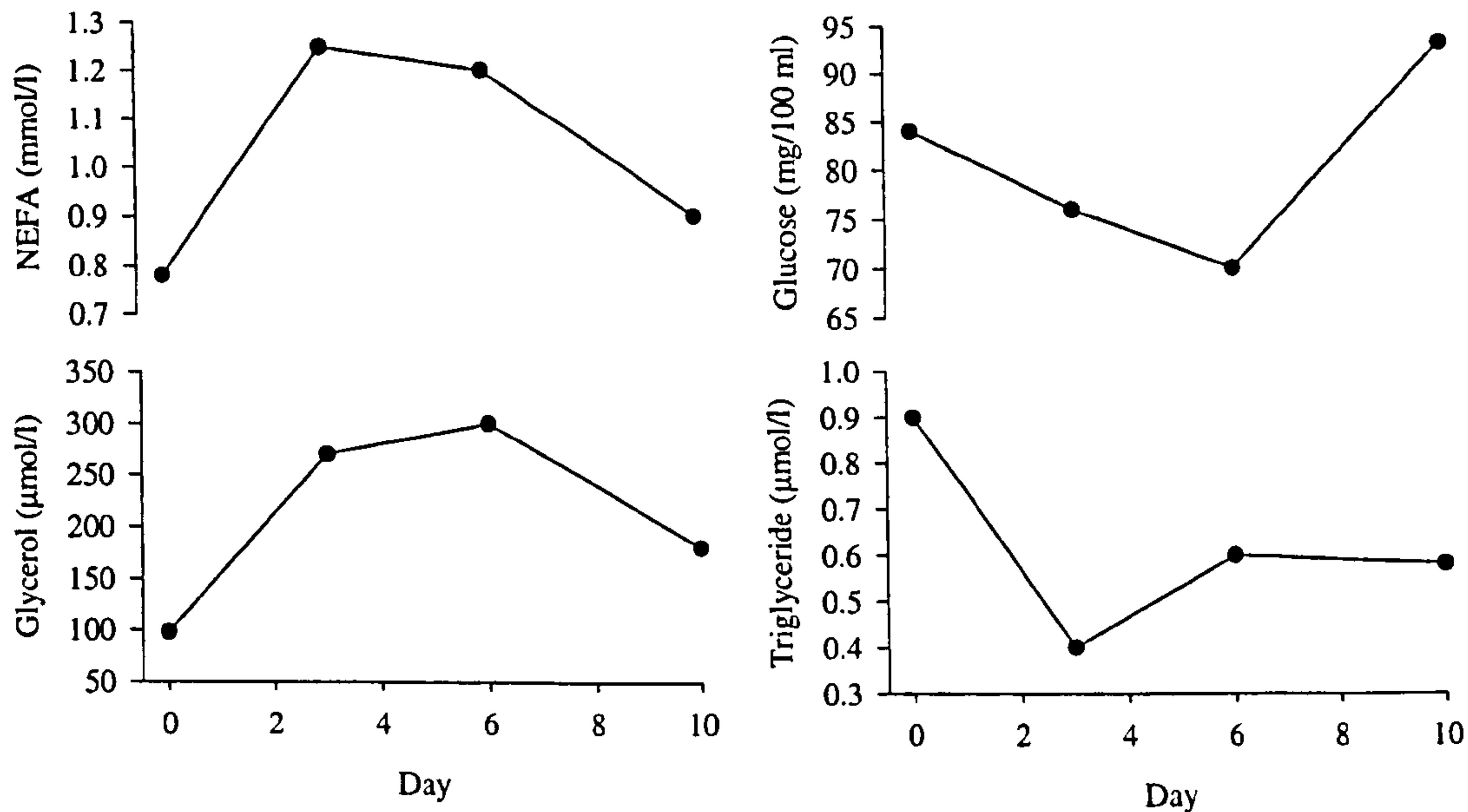


Figure 2.3. Concentration of NEFA, glycerol, glucose and triacylglycerol over 10 days of prolonged walking combined with low-energy intake. Mean values (Redrawn from Carlson and Fröberg, 1967)

An outstanding feat of human endurance was the 95-day Antarctic crossing reported by Stroud et al. (1997). On that journey, blood samples taken at 10-day intervals revealed mean end-of-day glucose concentrations of just 3.0 mmol/l and 2.8 mmol/l in both the subjects. Furthermore, on two occasions (days 70 and 95) both men were apparently grossly hypoglycaemic, with glucose values of 0.3 mmol/l. This hypoglycemia occurred despite CHO intakes that were quite reasonable (490 g/d). However, CHO metabolism was clearly disturbed towards the end of the expedition, with abnormally high insulin levels in the face of hypoglycaemia and elevated cortisol and growth hormone levels.

In summary, due to the relatively low intensity of sustained prolonged activity, the major metabolic adjustment the human body makes is an enhanced mobilisation of fat. With the exception of the most extreme events, even in spite of low energy intakes and hence large energy balance deficits the body seems to be able to defend its blood glucose levels via the mobilisation of fat. Clearly this makes good metabolic sense - the body utilises its almost limitless stores of fat in order to maintain the small stores of which glucose is the preferred fuel for the brain.

2.7 FACTORS AFFECTING SUBSTRATE MOBILISATION DURING PROLONGED EXERCISE

The precise contribution of each of the previous mentioned substrates to the metabolic demand of the working muscle will depend on a number of different variables relating to subject characteristics (e.g., fitness, nutritional state, age, sex, specific health deficits) and exercise condition (e.g., high altitude, temperature extremes). The most important determinants of the type and amount of fuel utilised are the duration and intensity of exercise (Wasserman and Cherrington, 1996). The impact of these two primary exercise parameters is summarised below. Furthermore, both the individual and environmental influences on substrate mobilisation are considered in section 2.7.2 and 2.7.3.

2.7.1 Exercise duration and intensity

During the transition from rest to moderate intensity exercise, the muscle shifts from using primarily circulating NEFAs to using a blend of NEFAs, extramuscular glucose, and muscle glycogen (Bergstrom et al., 1967; Wahren et al., 1971; Ahlborg et al., 1974). During the early stages of exercise, muscle glycogen is an important source of energy, with significant contributions also derived from circulating glucose and NEFA. As exercise is prolonged, hepatic glucose output becomes slightly less than glucose utilisation and blood glucose levels fall gradually. In contrast, the contribution of circulating NEFAs increases progressively with prolonged exercise (Ahlborg et al., 1974; Wolfe et al., 1990; Fig. 2.4).

The transition to a greater utilisation of circulating NEFAs during prolonged exercise is important, since it curtails the rate at which glycogen stores are depleted. Fats are the preferred fuel for long-term exercise, since differences in the degree of saturation between fatty acids and glucose suggest that more than twice as much energy is derived from 1 g of fat than 1 g of CHO. As a result, fatty acids are the most economical fuel for exercise of long duration from the standpoint of both its storage and metabolism.

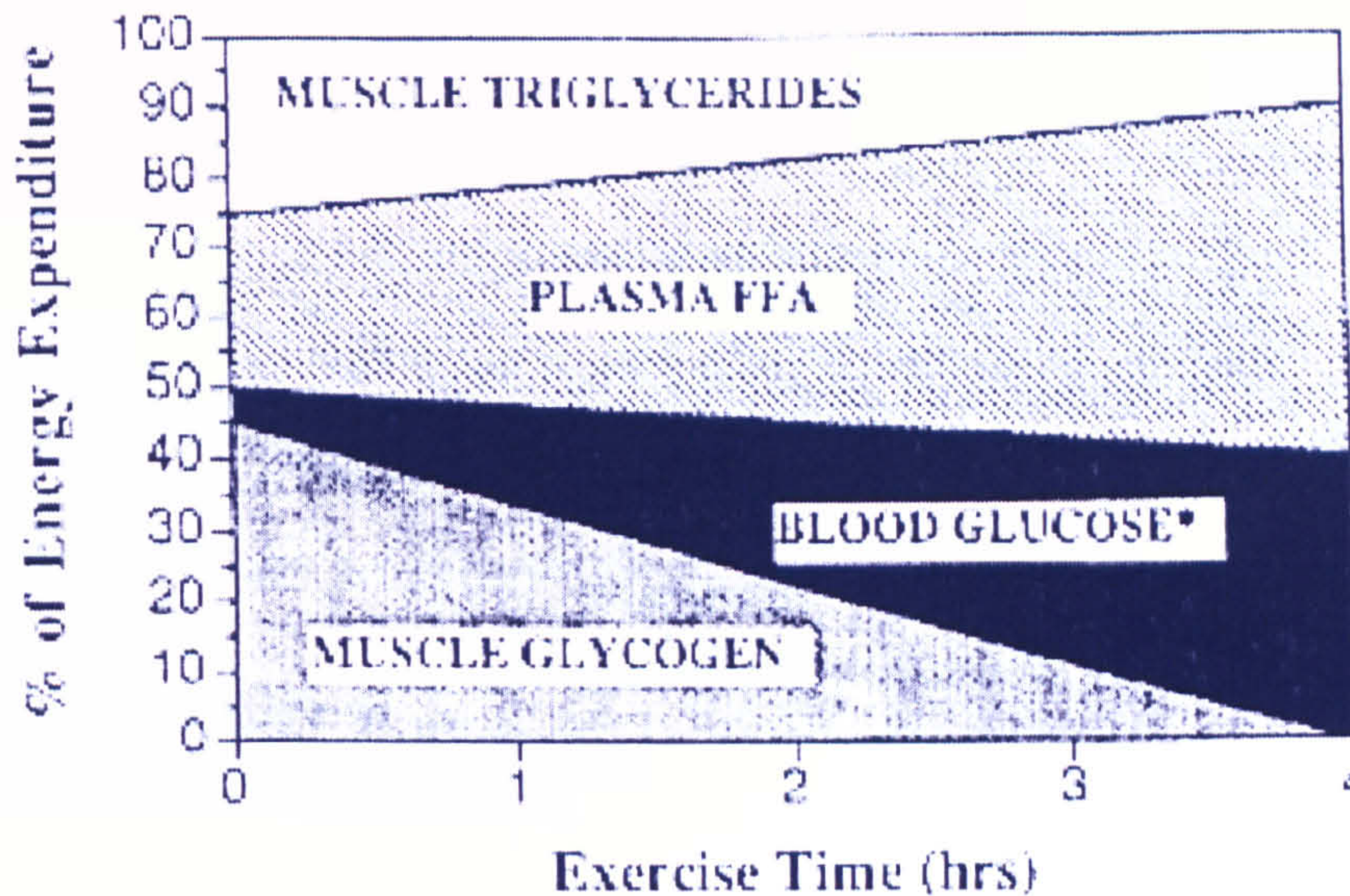


Figure 2.4. Percentage of energy derived from the four major substrates during prolonged exercise at 65-75% $\dot{V}O_2$ max (From Coggan and Coyle, 1991). Initially, approximately one half of the energy requirement is derived from CHO and the other half from fat. As muscle glycogen concentration declines, blood glucose becomes an increasingly important source of CHO energy for muscle. After 2 h of exercise, CHO ingestion is needed to maintain blood glucose concentration and oxidation.

With increasing intensity of exercise, the balance of substrate usage shifts to a greater oxidation of CHO (Hermansen et al., 1967; Karlsson and Saltin, 1970; Wahren et al., 1971; Romijn et al., 1993; Fig. 2.5). This change is due both to an increased muscle glycogenolysis and muscle glucose uptake from the blood. In contrast to the high rate of CHO metabolism during high intensity exercise, the utilisation of NEFAs by the working muscle is reduced (Jones et al., 1980). Although the capacity for BCAA oxidation is increased at higher intensities of exercise (Hood and Terjung, 1987), it is still unlikely that this substrate contributes significantly to energy production (Wasserman and Cherrington, 1996).

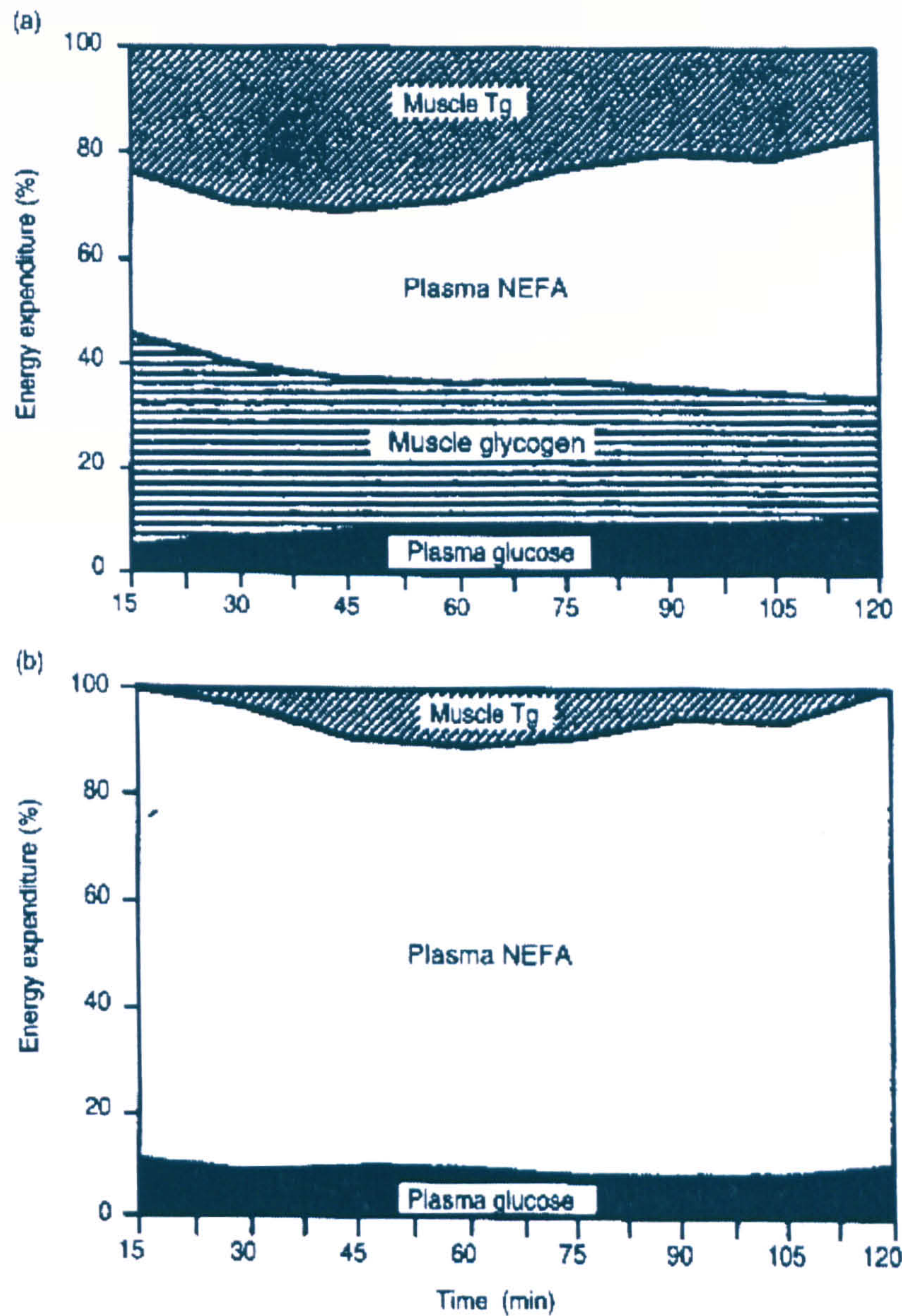


Figure 2.5. Utilisation of different fuels during exercise at two intensities. Reproduced from Romijn et al. (1993). The intensities of exercise are judged in relation to the maximal oxygen consumption of for the individual. Panel (a) shows exercise at 65% $\dot{V}O_2$ max; 2 h at 65% $\dot{V}O_2$ max is relatively heavy exercise. Panel (b) shows exercise 25% $\dot{V}O_2$ max; 2 h at 25% $\dot{V}O_2$ max is relatively light. (An elite marathon runner would maintain about 85% of $\dot{V}O_2$ max for 2 h 10 min). The figure shows the relative contribution to energy expenditure (total energy expenditure is taken in each case to be 100%, although it is 65/25 or 2.6. times greater in the top panel). The data were obtained by a combination of indirect calorimetry and use of

isotopic tracers to measure the whole-body turnover of glucose, glycerol and fatty acids.

2.7.2 Individual differences: gender and age

Relatively few research groups have compared the metabolic responses of males and females during exercise due, in part, to the difficulties in matching male and female subjects for $\dot{V}O_2$ max, training status, body composition, and exercise intensity. Some authors have observed that CHO oxidation and muscle glycogen utilisation during exercise are lower in female subjects than in male subjects (Nygaard et al., 1984; Tamopolsky et al., 1990), although some have shown no differences (Costill et al., 1979; Brewer et al., 1988).

For similar problematic reasons as highlighted in the differences among male and females, there is also a lack of research regarding the effects of age on substrate utilisation during exercise. Studies of the effects of age on substrate utilisation have shown lower muscle glycogen concentrations (Seals et al., 1984) and greater glycogen utilisation during sub-maximal exercise with increasing age (Meredith et al., 1989), which are both attenuated following endurance training. Equally, as ageing is associated with a change in body composition, specifically a decrease in muscle mass and an increase in body adiposity, it has been reported that lean men (9-15% body fat) have a greater ability to oxidise fat and thus reduce CHO oxidation during cycling exercise at 60% $\dot{V}O_2$ max compared to fatter men (20-25% body fat) (Keim et al., 1996).

2.7.3 Environmental influences

2.7.3.1 Environmental temperature

Heat stress increases muscle glycogen breakdown (Fink et al., 1975; Febbraio et al., 1994), muscle and blood lactate accumulation (Fink et al., 1975; Young et al., 1985; Febbraio et al., 1994), and blood glucose levels (Yaspelkis et al., 1993; Febbraio et al., 1994) during exercise. It has been suggested that these responses are due, in part, to reduced blood flow and oxygen delivery to contracting muscles (Fink et al., 1975);

however, the effects of heat stress on muscle blood flow are somewhat equivocal (Nielsen et al., 1990).

At lower environmental temperatures, it has been shown that during low-intensity exercise at 9°C muscle glycogen utilisation was increased compared with exercise at 21°C (Jacobs et al., 1985). This change was most likely due to shivering thermogenesis increasing glycogen use (Martineau and Jacobs, 1988). At higher exercise intensities, with increased metabolic heat production, no difference was observed in muscle glycogenolysis during exercise at the two temperatures (Jacobs et al., 1985).

2.7.3.2 Altitude

Generally, moderate exercise under hypoxic conditions is associated with increased glucose disposal (Brooks et al., 1991a; Copper et al., 1986), increased glycolysis (Green et al., 1992), and elevated blood and muscle lactate levels (Brooks et al., 1991b; Green et al., 1992). Following a period of altitude acclimatisation, glycogenolysis, glycolysis, and lactate accumulation are reduced (Green et al., 1992; Young et al., 1982). These adaptations are believed to reflect a number of possible changes with acclimatisation that include increased NEFA levels (Young et al., 1982), improved metabolic control (Green et al., 1992), and reduced adrenaline levels (Mazzeo et al., 1982). However, it should be noted that there is no evidence for any performance benefits of training at altitude (Milledge, 1996; Bailey and Davis, 1997). Indeed, the reduction in oxygen partial pressure with altitude increases fatigue and reduces $\dot{V}O_2$ max during events which are relatively aerobic and prolonged in nature.

2.8 HIGH CARBOHYDRATE VERSES HIGH FAT FEEDING

2.8.1 The effects of dietary manipulation on substrate metabolism

The majority of studies of the effects of high-CHO and high-fat diets have been conducted either at rest (Bobbioni-Harsch et al., 1997; Whitley et al., 1997) or in high-intensity exercise ($> 65\%$ maximal oxygen consumption) (Whitley et al., 1998; Burke et al., 2000; Carey et al., 2001), whereas few researchers have considered such dietary manipulations during prolonged low to moderate intensity exercise ($< 65\%$ oxygen consumption). The metabolic responses are resistant to dietary change in moderate-severe exercise (Whitley et al., 1998; Burke et al., 2000; Carey et al., 2001) but are susceptible to change at rest (Bobbioni-Harsch et al., 1997; Whitley et al., 1997). Although substrate turnover has been investigated over 4 h of cycling at approximately 30% of maximal oxygen uptake (Ahlborg et al., 1974), it is not known what happens during more sustained exercise at low to moderate intensity, with the addition of dietary manipulation. This is somewhat surprising as participation in recreational events such as prolonged ultra-endurance events (Heini et al., 1998), hill walking (Sharp, 2001) and recreational cycling are growing in popularity. More to the point, fat oxidation has the potential to meet a large proportion of the fuel requirements of exercise (Rauch et al., 1998; Rauch et al., 1999). Manipulation of macronutrients may be of some benefit to these activities.

2.8.2 High-CHO diet

Most authorities are in no doubt that utilisation of a high-CHO diet before exercise (Bergstrom et al., 1967; Karlsson and Saltin, 1971) and CHO ingestion during exercise (Coggan and Coyle, 1987) can enhance exercise performance for more than 2 h at levels at or above 70% $\dot{V}O_2$ max. A diet containing approximately 70% or more energy as CHO has become the conventional intake for competitors in events such as marathon running and this has been shown to improve run times. For example, Tsintzas et al. (1995) studied seven endurance trained runners completing three treadmill marathons in random order at 4-week intervals. The subjects ingested 3 ml/kg body weight of water, 69 g CHO/l solution or 55 g CHO/l solution immediately before the tests, followed by 2 ml/kg of the same solution at 5-km intervals thereafter.

Running times were 193.9 min for water, 192.4 min for the 69 g CHO/l solution and 190 min for the 55 g CHO/l solution. Clear beneficial effect of the order of 4 min on marathon times are enormous, and hence, most ultra-distance athletes have followed similar dietary regimens in the hope that improvements of a similar order will accrue during their longer periods of exertion. Nevertheless, it is by no means clear that this is optimal policy, for during very prolonged activity the maintenance of glycogen stores must become less relevant.

Ultra-distance events are likely to be performed at lower exercise intensities than shorter races such as marathons. Likewise, these intensities are likely to be even lower during hill walking activities. This should, therefore, permit an even higher dependence on fat oxidation than in shorter events. Indeed, such dependence is inescapable, for during a 100-mile (160 km) non-stop run, glycogen stores are likely to become severely depleted before even 20% of the race is over (Stroud, 1997). Again a comparison may be made by analogy to prolonged hill walking over consecutive days. For the majority of the distance, therefore, the muscles must utilise fat as their main substrate, and this will be true even if CHO containing foods are freely available (Stroud, 1997).

The maximal rate at which exogenous CHO can be absorbed and utilised is about 60 g/h, which could only provide about 1 MJ/h to support activity which may demand more than four times that much. Furthermore, in many of the prolonged activities, the nature of the event may limit exogenous energy intake.

There are two obvious macronutrient strategies by which fat oxidation might be improved: these are, either starvation or a period of adaptation to a high-fat diet. Studies of the former do not support its use to promote endurance (Loy et al., 1986; Coyle, 1997; Whitley et al., 1998), and in a study of nine male marathon runners who ran to exhaustion at a treadmill speeds equivalent to their marathon performance, times to exhaustion decreased by 44.7% following a 27-h fast (Nieman et al., 1987). The following section evaluates the current literature regarding the potential merits of high-fat feeding as opposed to the traditional diet high in CHO.

2.8.3 High-fat diet

The ability to transport, take up and oxidise NEFA is essential for meeting the metabolic demands of prolonged exercise as CHO cannot supply the required energy over such long periods. Even before researchers looked at the respiratory exchange ratios, it was known that fat was the most important fuel during sustained physical exercise. The Norwegian explorer Amundsen was reportedly one of the first to employ a high-fat diet. During one long exploration he consumed 350 to 400 g/day of pemmican, which contains ground beef with fat melted into it. This diet provided him with 60 to 70 % of his daily requirement (Huntford, 1979).

In relation to endurance performance the benefits of a high-fat diet, either a chronic adaptation or acute feeding, remain equivocal. Studies which have compared a high-CHO diet to a high-fat diet have yielded mixed results: a decreased performance (Galbo et al., 1979; Van Zyl et al., 1994; Helge et al., 1996), no change in performance (Pinney et al., 1993; Satabin et al., 1987; Helge et al., 1997; Whitney et al., 1998), or an improved performance (Lambert et al., 1994; Muoio et al., 1994; Putman et al., 1994; Pitsiladis et al., 1999). However, the general consensus seems not to support the use of high fat regimes (Kiens and Helge, 2000). The differences in the results most probably reflect the range of protocols and methods used (Kiens and Helge, 2000).

For the recreational walker, however, the potential advantage of a high-fat diet may lie in altering metabolism in favour of enhanced fat mobilisation and consequently preserving the small CHO stores. The evidence for the benefits of such dietary manipulation over prolonged recreational activity is limited. Arieli et al. (1985) investigated the effects of exogenous metabolic fuel intake on the prevention of sensations of fatigue during a 34-km march on the flat with a 25-kg back-pack, in both trained ($\dot{V}O_2$ max 52.3 ± 2.76 ml/kg/min) and untrained (40.6 ± 2.7 ml/kg/min) subjects. The subjects were divided into three dietary groups. One group had free access to sugar cubes (high-CHO), the second group was offered almonds (high-fat) and the third served as a control. In the untrained subjects, ingestion of almonds delayed the subjective sensation of exhaustion, while 50% of the controls and the sugar-consuming subjects complained of exhaustion. Whilst the data suggest that

ingestion of food containing fat (3.0-5.2 MJ, 77% energy from fat) delays exercise induced exhaustion or fatigue to a greater extent than does CHO ingestion (1.4-3.7 MJ ~99.9% energy from CHO), the results need to be treated with caution. The dietary intake was non-isoenergetic i.e., subjects gained more energy from the high-fat diet than the CHO. Besides, no subjective data were reported for the trained subjects, making any comparisons between the untrained and trained groups impossible.

In a study by Maughan et al. (1987), subjects walked for about 37 km per day for 4 days. On one occasion, CHO constituted about 3% of their total energy intake, on the other a high-CHO intake was followed. Although the utilisation of fat was increased (during the low-CHO regime) during this period, at no point did the subjects become hypoglycaemic and all subjects completed the exercise task (Fig 2.6). It was unfortunate that in the study of Maughan et al (1987) there were no subjective or performance measurements to gain an indication of the potential merits of the differing diets. Nevertheless it would appear that, similar to resting conditions, liver glycogenolysis and gluconeogenesis can match the CHO demands of this form of activity.

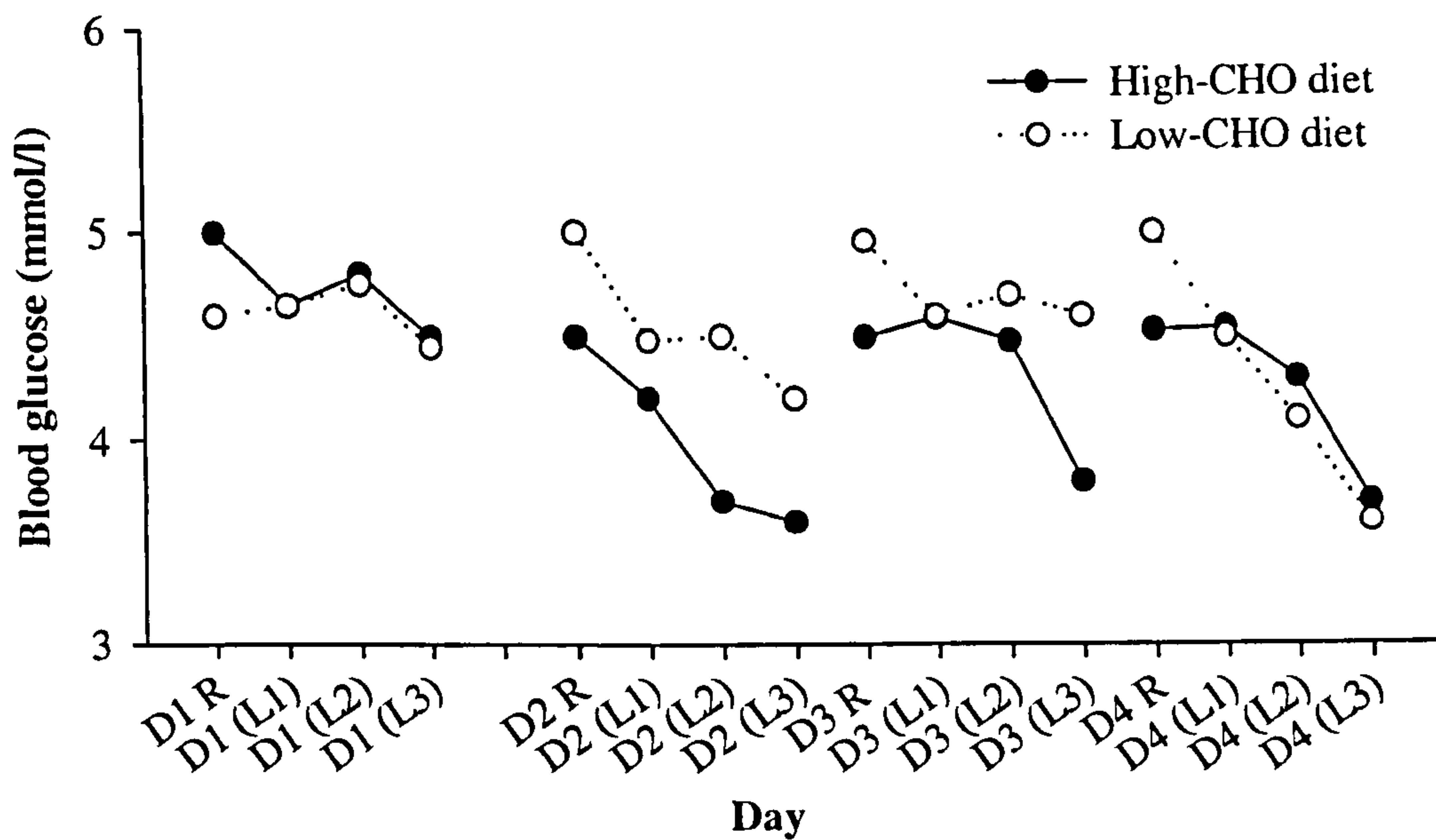


Figure 2.6. The effect of diet on the blood glucose response to prolonged walking on consecutive days (redrawn from Maughan et al., 1987, with permission). Mean values of blood glucose concentration before and during high-CHO (open circles) diets. R refers to the fasting pre-exercise sample each day and L1, L2, and L3 refer to samples obtained after completion of laps 1, 2 and 3, respectively. Each lap was approximately 13 km in distance and was covered at a constant speed.

In summary, fat contains more than double the amount of energy found in CHO: 1 g of fat yields 37 kJ whereas 1 g CHO contains only about 16 kJ (the calorific value of protein is similar lower). The greater increase in fat utilisation is not, apparently, without its limitations. The oxygen requirement for the oxidation of fat can be up to 16% greater than that required to produce the same amount of ATP from the oxidation of CHO. One litre of oxygen can oxidize glycogen and produce approximately 6.5 moles of ATP compared with 5.6 moles when palmitate is oxidized (Saltin and Gollnick, 1983). Consequently, a change towards fat oxidation should produce a higher cardiovascular stress (Newsholme, 1981; Sherman and Leenders, 1995). In previous studies in which the plasma NEFA has been elevated acutely by either a high fat meal or by Intralipid-heparin infusion, no effect on oxygen uptake or heart rate (HR) during exercise has been reported (Hargreaves et al., 1991; Vukovich et al., 1993; Pitsiladis et al., 1999). These studies indicate a negligible cardiovascular stress as a consequence of increased NEFA concentrations.

The uncritical assumption that providing approximately 70% of energy as CHO must be optimal because it has been proved as such in shorter endurance events is not entirely logical, and the best balance of dietary substrates for very prolonged activity remains unclear. Besides, in contrast to exercise at higher intensities where there is a need for a high-CHO intake to permit resynthesis of muscle glycogen between repeated exercise sessions, the lower intensities of hill walking may not require CHO replacement with this time scale. Since the hill walker is not concerned with an improvement in performance trials, perhaps the maintenance of energy balance over prolonged activity is the most important factor. Snacks and/or food high in fat content may be one such method by which energy-dense food can be easily consumed to meet these requirements. Furthermore, food dense in energy will also allow walker's to cut down on the amount of food that they need to carry.

2.9 ENERGY BALANCE DURING PROLONGED EXERCISE

2.9.1 Energy and substrate balance

Energy balance: Energy balance represents the difference between metabolizable energy intake and total energy expenditure. Adult humans generally maintain a balance between energy intake and energy expenditure. The energy store of the body does not fluctuate much, as the consistency of body weight and body composition shows, although there is often a trend towards an increased body mass and body fat with increasing age. The main component of the daily energy turnover (average daily metabolic rate) in the average subject is the energy expenditure for maintenance processes, usually called resting metabolic rate. The remaining components of average daily metabolic rate are the diet-induced energy expenditure and the activity-induced energy expenditure. Diet-induced energy expenditure is a fraction of ~10% of energy intake, depending on the macronutrient composition of the food consumed (Westerterp, 1998). Activity-induced energy expenditure is the most variable component of the daily energy turnover, ranging between an average of 25-35% of average daily metabolic rate up to 75% in extreme situations during heavy, sustained exercise.

A positive energy balance implies a gain in energy store in the form of CHO, fat or protein. Short-term day-to-day energy imbalance is mostly accommodated by rapid changes in CHO balance, whereas over a prolonged period of time positive energy balance is mostly expressed as fat storage since CHO stores are small (Abbott et al., 1988; Schrauwen et al., 2000). Conversely, exercise may promote a negative energy balance in a number of ways. Firstly, a negative energy balance may be favoured by the increased energy expenditure involved in the exercise itself. Furthermore, there is evidence that metabolic rate may remain elevated for some time after the exercise ceases, and this will increase the energy cost associated with a bout of exercise (Maughan, 1994). The thermogenic effect of food ingested during the post-exercise period may be potentiated, and this also will have a negative effect on overall energy balance. Exercise may also affect appetite and, hence, energy intake, and it is possible that hard exercise may reduce the amount of activity which the individual undertakes during the remainder of the day.

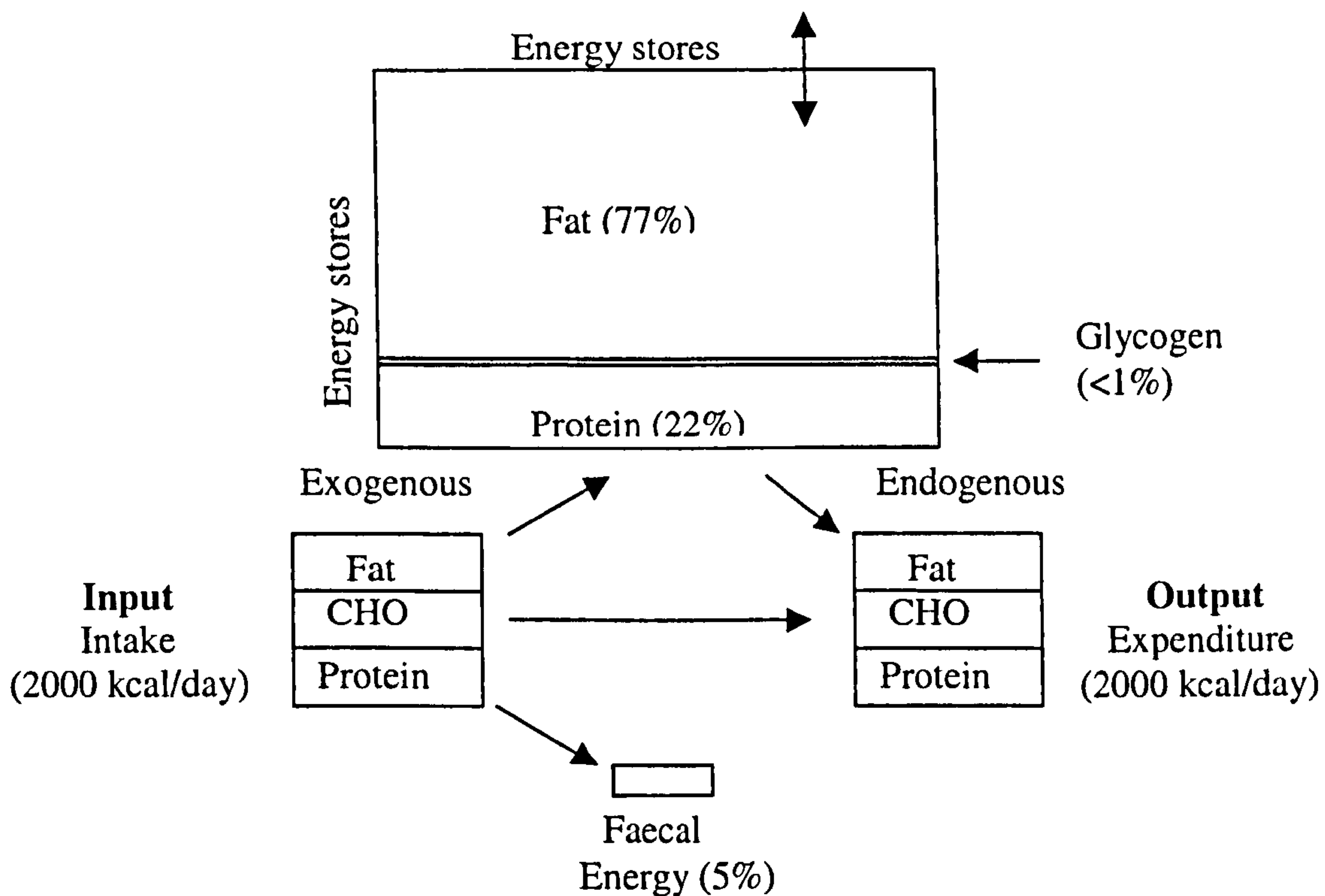


Figure 2.7. Energy stores (constituted mainly by fat stores) are shown as a proportion of the food intake (2000 kcal/day, mixed diet) in a 60-kg non-obese woman with 25% body fat. The total energy stored is about 90 times total daily energy intake: typical fat stores are 175 times total daily energy intake:, protein 133 times daily protein intake, and CHO only 1.3 times daily CHO intake (adapted from Schutz and Garrow, 2000).

Substrate balance: In resting conditions, there is a clear hierarchy in the maintenance of macronutrient balances, with CHO and protein having the highest priority (Abbott et al., 1988; Schrauwen et al., 2000). Fat oxidation, on the other hand, is only marginally influenced by fat intake during resting conditions. Prolonged exercise will generally lead to a negative energy balance due to difficulties in matching sufficient energy intake to the high-energy turnover as a consequence of the exercise. This negative energy balance will aid the promotion of fat oxidation if the exercise is of low to moderate intensity. At high intensity exercise carbohydrate (CHO) becomes the preferred fuel (Jeukendrup and Jentjens, 2000) with a subsequent decrease in fat oxidation (Jones et al., 1980; Romijn et al., 1993). Since fat oxidation is determined mainly by the difference between energy expenditure and CHO and protein oxidation

(and exercise intensity), fat balance is strongly correlated with energy balance (Schrauwen et al., 2000).

2.9.2 Energy intake and energy expenditure during prolonged exercise

An important aspect of the maintenance of energy balance during prolonged high intensity exercise is the adaptation of food intake to high-energy requirements (Westerterp, 2001). Edholm et al. (1955) showed that intake tended to be low on days when expenditure was very high and that the difference was made up some days later when expenditure was lower. Apparently, very high levels of exercise reduce appetite, and subjects need time to adjust their intake to increased energy requirement (Westerterp, 2001).

The capacity to eat and process food certainly limits the energy supply. The absorption in the small intestine is thought to be a limiting factor (Westerterp, 2001). The evolutionary design of the nutrient absorption system is adequate but not ideal (Diamond, 1991). Brouns et al. (1989) showed that athletes maintained energy balance during simulation of the 'Tour de France' in a respiration chamber on a conventional solid diet with a high CHO content and supplemented with 20 % enriched CHO liquid. The energy intake was 5-10 MJ/day too low when the same diet was available ad libitum without the supplement.

The upper limit of power output during activities can be increased when energy-dense, CHO-rich food is eaten during the exercise, a practice common in endurance sports. Energy-rich drinks make up a substantial proportion of energy intake in professional athletes (Westerterp, 2001). Thus, the upper limit of sustainable metabolic rate in professional athletes is about twice the upper limit in the general population (Westerterp, 2001a; 2001b). Two important contributors have been suggested for the upward shift in physical activity level (Westerterp, 2001). Firstly, endurance athletes have learned to ingest large amounts of food and incorporate a significant amount of CHO drinks into their diet. Secondly, they often follow a continuous pattern consisting of many small 'meals' at short intervals.

Studies of daily energy intakes and expenditures during very prolonged exercise are summarised in Table 3. Sjodin et al. (1994) reported a contribution of 16% of energy intake and 25% of CHO consumption by CHO-rich formulae in athletes sustaining physical activity levels of up to 4.5, over a 6-day training period. In the 'Tour de France' of 1984, athletes maintained energy balance at a physical activity level of 3.5-5.5 (Westerterp et al., 1986), aided by large amount of CHO-rich drinks and snacks. Body mass and body composition did not change significantly over the 6-day training and 23-day race.

Hoyt et al. (1991) made measurements on 23 marines during 11 days of severe-cold weather mountain training, achieving good agreement between estimates from isotopes of 20.6 MJ/day and estimates from intake-balance levels of 19.9 MJ/day (Table 3). Similar energy expenditure levels of 19.9 MJ/day were identified by Forbes-Ewan et al., (1989) in soldiers during 7 days of jungle warfare training and of 18.0 MJ/day by Jones et al. (1993) in ten soldiers on Arctic exercises. In all these military studies the men lost weight, sustaining energy deficits of between about 3 and 7 MJ/day with no obvious ill-effects. However, energy expenditures were relatively low in comparison with those that can be achieved by athletes, probably because the demands of military activities are less continuous. In contrast to elite athletes, none of the studies of soldiers during field training mentioned the use of energy-dense, CHO-rich liquid formulae during exercise (Forbes-Ewan et al., 1989; Hoyt et al., 1991; Jones et al., 1993: Table 2.3).

Table 2.3. Studies of daily energy intakes and expenditures during very prolonged exercise

Study	Type of activity	n	Energy intake (MJ/day)	Dietary composition (% of total energy)			Energy expenditure (MJ/d) measured using:		
				Protein	CHO	Fat	Energy balance	DLW	
Branth et al. (1996)	Offshore sailing race, 13 d	11	17.1	13	52	35	18 - 20	19.3	
Eden & Abernethy (1994)	1005 km running, 9 d	1	25.0	11	62	27	25	-	
Gabel et al. (1995)	3280 km cycling, 10 d	2	29.8	10	63	28	29.8	-	
Hoyt et al. (1991)	Military mountain training, 11 d	23	13.1	13	49	38	19.7	20.6	
Forbes Ewan et al. (1989)	Military jungle training, 7 d	4	16.9	-	-	-	23.3	19.9	
Jones et al. (1993)	Military Arctic training, 10 d	10	11	18	47	35	13.5	18.0	
Westerterp et al. (1986)	Tour de France, 20 d	4	24.7	15	62	23	25.0	33.7	
Sjodin et al. (1994)	Cross-country skiing, 7 d	4 males	25.7-36	13	58	28	-	25.4-34.9	
		4 females	15.7-20.4	13	58	28	-	15.1-20.2	
Stroud (1987)	South Pole expedition, 70 d	3	21	9	34	57	23.6-25.4	-	
Stroud et al. (1993)	North Pole expedition, 48 d	2	19.2	8	33	59	25.7-24.9	28.1-32.4	
Stroud et al. (1997)	Trans-Antarctic expedition:								
	Day 0 - 50	2	19.9	9	42	57	28.6-38.3	29.1-35.5	
	Day 51 - 95	2	22.2	9	42	57	24.1-26.8	18.8-23.1	
Trappe et al. (1997)	High intensity swim training, 5 d	5	13.1	11	68	21	-	23.4	

2.9.3 Physiological and metabolic implications of negative energy balance

When energy expenditure exceeds energy intake, a decrease in body mass will occur. Since prolonged activity is likely to result in such a negative energy balance, an important question is what are the implications of such an imbalance?

The information that is available suggests that sufficient energy intake to balance the high energy expenditure in endurance athletes during training has been shown to be of great importance for performance (Brouns et al., 1989). Furthermore, there is increasing evidence that a negative energy balance may have several adverse effects on health e.g., on the immune system (Chandra, 1990) as well as on sex hormones and bone mineralisation (Drinkwater, 1983; Calloway, 1987; Santora, 1987).

In the aforementioned studies of military personal, the men lost weight, sustaining energy deficits of between about 3 and 7 MJ/day with no obvious or reported ill-effects (Forbes-Ewan et al., 1989; Hoyt et al., 1991; Jones et al., 1993). Performance measures were not made during these studies. Guezennec et al. (1994) investigated physical performance and metabolic changes induced by combined prolonged exercise with three different energy intakes. Twenty-seven male soldiers were randomly assigned to three groups receiving either 7.6 MJ/day (low intake), 13.4 MJ/day (medium intake), or 17.6 MJ/day (high intake). They took part in a 5-day combat course of heavy and continuous physical activity (energy expenditure was estimated to exceed 21 MJ/day), with less than 4 h sleep per day. Maximal oxygen uptake and anaerobic performances were measured before and after the combat course. Whilst the soldiers on the medium and high energy intake maintained their measured performances, the 'low energy intake' group showed a significant decrease in $\dot{V}O_2$ max (8%) and in anaerobic power (14%). The data suggest that only a severe energy deficit leads to a small decrease in performance.

In summary, energy requirements of as much as 30 MJ/day may not be uncommon and they may be even greater in activities involving many muscle groups and work of 12 h/d or more (Stroud, 1997), which could feasibly be covered in a prolonged hill walk. Very high overall demands may occur, even if work levels are far lower than those sustained by elite athletes in traditional continuous events such as the marathon

or in staged ultra distance events such as the Tour de France. Hill walking is such an event which is both likely to attract large participation and entail a high energy expenditure. The high overall energy expenditure is due to the prolonged nature of the activity. An important and unique consideration is that hill walking is a recreational activity which attracts participants ranging in age and fitness level. The outcomes of such a prolonged event will likely entail a large deficit in energy balance. The implications of such an acute energy deficiency for the participants remain unclear.

3.0 SUMMARY

Hill walking represents a relatively unique form of activity in which the prolonged duration and varying intensities may place exceptional demands on the recreational participants. The antihomeostatic nature of such activity may have the potential to impose high stress simultaneously upon several regulatory systems. There is little information concerning the characteristic activities of hill walkers, such as 'typical' nutritional and fluid usage. Similarly, there has been no documented research about the physiological, metabolic and nutritional demands of such activities. In contrast to exercise at higher intensities where there is a need for a high-CHO intake to permit resynthesis of muscle glycogen between repeated exercise sessions, the lower intensities of hill walking may not require CHO replacement with this time scale.

In view of the lack of documented research surrounding the activity of hill walking, the present thesis was designed to address the following aims: i) to quantify the typical characteristic patterns commonly used by participants; ii) to establish the physiological and nutritional demands of such an event over one day and further investigate the potential merits of dietary manipulation and effects of differing energy intakes over this type of prolonged activity; iii) to investigate the physiological and nutritional demands of sustained activity on consecutive days of hill walking, and also the effect that age may have on these responses.

CHAPTER 3

GENERAL METHODS

3.1. ANTHROPOMETRY

3.1.1 Body Mass

The body mass of each subject during the studies was measured using calibrated balance scales accurate to 0.1 kg. During the morning measurements of body mass, subjects were instructed to record their nude body mass before consuming any food or beverages and after voiding. For the measurements post-walk, subjects were instructed to remove, using a towel, any noticeable sweat.

3.1.2 Body Height

The height of each subject was measured in the laboratory using a calibrated stadiometer (CMS Weighing Equipment Ltd, London). Measurements were recorded to the nearest 0.5 cm.

3.1.3 Techniques to estimate fat and fat-free mass

The percentage body fat for each subject was estimated from the measurements of skinfold thicknesses (Durnin and Womersley, 1974), using Harpenden skinfold callipers (John Bull, British Indicators Ltd, Leicester, UK). Skinfolds were measured at the standard biceps, triceps, subscapular, and supra-iliac sites on the right side of the body. For each reference location, the skinfold was picked up firmly between the thumb and forefinger and pulled slightly away from the underlying tissue. In doing this, care was taken not to include the underlying muscle in the skinfold measurement. The caliper jaws were then applied to the skinfold as the thumb and the forefinger were simultaneously removed, and a reading was taken after 2 or 3 s. The measurement was recorded to the nearest 0.1 mm and was performed three times at each reference site, by the same trained investigator, and a mean reading was calculated for each skinfold. The four skinfolds were then added together to give a total sum of skinfolds in mm. This value was then converted to percentage body fat using the tables from Durnin and Womersley (1974) taking into account the subjects age and gender.

To estimate changes prior to and immediately after the 10-day experiment (Chapter 6), fat-free mass was also estimated using the equation described by Wright and Wilmore (1974):

$$\text{FFM} = (40.99 + 1.0435 \times \text{body mass}) - (0.6734 \times \text{abdomen circumference})$$

Fat mass was calculated as body mass minus fat-free mass. Fat-free mass was assumed to be 27% protein and 73% water; fat mass was assumed to be 100% fat (Westerterp et al., 1995). Limb circumferences (calf, quadriceps, waist, abdomen and bicep) were estimated from three sequential measurements which were made before and after the experiment on each subject by the same investigator using a spring-loaded fibreglass anthropometric tape.

In addition, at the beginning of the 10-day experiment, fat-free mass was calculated as:

$$\text{FFM} = \text{TBW}/0.73,$$

where TBW is the total body water measured by isotope dilution (H_2^{18}O) and fat mass was calculated as the difference between body mass and fat-free mass. The method of calculation of TBW using by isotope dilution is described in section 3.3.1.3.

3.2 METHOD FOR DETERMINING MAXIMAL OXYGEN CONSUMPTION

3.2.1 Maximal exercise tests

In all studies, maximal oxygen uptake ($\dot{V}O_{2\max}$) was established by using a continuous incremental treadmill test to voluntary exhaustion (American College of Sports Medicine, 1986). After a 5-min warm up, all subjects started running at 10 km/h and this increased by 2 km/h every 2 minutes. After 2 minutes at 16 km/h, the speed did not increase further, whilst an incline was added of 2% every 2 minutes. Subjects were verbally encouraged throughout the test and continued until they reached volitional exhaustion. For the duration of the test, indirect calorimetry was performed using a previously calibrated on-line automated gas analyser (Metamax, Cortex Biophysik GmbH, Borsdorf Germany). Since a plateau in the $\dot{V}O_2$ -to-work relationship was not reached in all of the subjects tested, the highest aerobic power was expressed as peak $\dot{V}O_2$ ($\dot{V}O_{2\text{ peak}}$) and not $\dot{V}O_{2\text{ max}}$. Verification of $\dot{V}O_{2\text{ peak}}$ was confirmed using established physiological criteria as outlined by the American College of Sports Medicine (1986). These criteria for maximal aerobic performance include: forced mean expiratory volume, levelling off of oxygen uptake, respiratory exchange ratio above 1.15, and ratings of perceived exertion of 20 or heart rate at age-predicted maximal values (American College of Sports Medicine, 1986). The same equipment, criteria and procedures were used for all subjects.

3.3 METHODS FOR MEASUREMENT OF SUBSTRATE OXIDATION AND ENERGY BALANCE

3.3.1 Direct and indirect calorimetry

Measurement of energy metabolism over a period of time entails two basic approaches - direct calorimetry, and indirect calorimetry. Direct calorimetry measures total heat loss from the body; indirect calorimetry measures total energy production by the body. With the former, the subject is placed in a thermally isolated chamber, and the heat he/she dissipates (by evaporation, radiation, conduction and convection) is recorded accurately and measured precisely (Jequier, 1985). Despite its sophistication, the over-riding disadvantage of direct calorimetry is its inability to provide information concerning the relative contributions of different substrates (fat, carbohydrate and protein) to energy expenditure. Therefore, for the purpose of the present studies the alternative method of indirect calorimetry was used. This method has been shown to produce results which differ by only 0.3% from energy expenditure values calculated from direct calorimetry (Atwater and Benedict, 1903) and has been reported to be highly reproducible in measuring 24-hour energy expenditure and 24-hour macronutrient oxidation rates in adults (White et al., 1996).

During indirect calorimetry, the oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) are what are really measured. On the assumption that all the oxygen is used to oxidise degradable fuels and all the CO_2 thereby evolved is recovered, it is possible to calculate the total amount of energy produced (Ferrannini, 1988). Only indirect calorimetry and its extensions will be considered, as direct calorimetry is of limited practical interest in the present context of total energy output by free-living populations. Indirect, in contrast to direct, calorimetry does not involve the direct measurement of heat produced, but requires the measurement of physiological gases (oxygen and carbon dioxide) in expired air over a fixed period of time. It enables energy expenditure to be determined and also the rate of oxidation of the major energy yielding substrates i.e. carbohydrate and fat.

Within the work conducted for the present thesis, three different methods were used in the measurement of indirect calorimetry: i) stationary indirect calorimetry systems, ii) portable indirect calorimetry and, iii) the doubly-labelled water method. These different systems and their associated calculations are discussed below.

3.3.1.1 Indirect calorimetry: Stationary and portable systems

Stationary indirect calorimetry: During the laboratory based study indirect calorimetry was performed using an stationary automated gas analyzer (Exercise Tester, P.K. Morgan, Chatham, Kent, UK). This particular system contained fast response analysers for breath-by-breath analyses of mixed expired air ($\dot{V}O_2$ and $\dot{V}CO_2$) whilst the subject was at rest and during exercise.

This procedure required the subject to wear a nose-clip and breathing through a mouth-piece which was connected to a tube comprising of two one-way low-resistance valves. This arrangement allowed subjects to inspire air from laboratory, whilst expired air was analysed by means of the gas analysers. One common response to wearing a nose clip is mild hyperventilation, which may confound the gas collection measurement by increasing the $\dot{V}CO_2$ above the level of metabolic CO_2 production. To avoid the hyperventilation, the nose clip was placed upon the subject for approximately 2 min prior to gas collection. The gas analysers recorded gas exchange parameters, the most important being oxygen consumption, carbon dioxide production and respiratory exchange ratio. The results were printed out every 20 s. These data were then averaged and used to calculate rates of substrate oxidation and energy expenditure for each subject based on the formulae and assumptions described in section 3.3.1.2.



Plate 3.1. Photograph of gas measurements at rest during the laboratory based study.

Prior to its use, the on-line system was left on continuous stand by before being fully calibrated. The O₂ and CO₂ analysers were calibrated using 3-point calibration gases. The volume transducer was then calibrated using a known volume of air contained in a 3-litre syringe. After calibration, the system was programmed with the subjects' height and weight and environmental measurements. These environmental measurements included ambient temperature and barometric pressure in the laboratory prior to each period of testing. To maintain precision in the gas and volume measurement, the whole calibration procedure was repeated every 3-4 hours.

Portable indirect calorimetry: During the field based study (Chapter 5), continuous indirect calorimetry was performed on subjects throughout a 12-km hill walk using a portable automated telemetry gas analyzer (Metamax, Cortex Biophysik GmbH, Borsdorf Germany). Signals from the Metamax system were logged and subsequently retrieved at the end of the hill walk. This procedure allowed for continuous monitoring of $\dot{V}O_2$, ventilation and respiratory exchange ratio. The percentage contributions of the CHO and fat oxidation were estimated from non-protein (NP) $\dot{V}O_2$ and RER data. During the hill walk, CHO and fat oxidation rates were calculated and averaged over 10-min blocks at predetermined points in accordance with the other measurements. Total oxidation rates were then averaged for the whole hill walk. Energy expenditure was calculated from the averaged $\dot{V}O_2$ and $\dot{V}CO_2$ from the whole walk using the formulae described in section 3.3.1.2.

Prior to use, the Metamax system was calibrated using both calibrated gas and ambient air. The volume transducer was calibrated using a 3-litre syringe. To decrease any error, the system was re-calibrated during the hill walk when the subjects stopped for lunch. Due to technical problems during three of the walks, the respiratory gas exchange data were based on 10 subjects.



Plate 3.2. Photograph of the portable Metamax system for measurements of respiratory gas exchange.

3.3.1.2 Calculations to determine substrate oxidation and energy production using indirect calorimetry

To determine substrate oxidation over a period of time, the ratio of carbon dioxide produced to oxygen consumed was calculated. This is referred to as the respiratory exchange ratio (RER) when measured in expired air. The respiratory quotient (RQ) represents the ratio of V_{CO_2} to VO_2 at cellular level and differs for each substrate, whereas the RER represents the same ratio but measured from the lungs with expired gases. Thus, strictly speaking, the ratio of these two gases should be described as the RER, not the RQ.

In its simplest form, in the metabolism of carbohydrate as 1 g of glucose, the production of an amount of carbon dioxide equal to that of oxygen consumed in the results of the RER of 1.0 and the liberation of 15.6 kJ of energy. In contrast, when fat is mobilised as 1 g of a typical triacylglycerol, more oxygen is required, which results in an RER of 0.7 and the liberation of 39.4 kJ. The metabolism of 1 g of a standard protein on the other hand, gives an RER of 0.8 and an energy equivalent of 20.1 kJ. To summarise, Table 3.1 shows the volumes of oxygen (O_2) consumed and carbon

dioxide (CO₂) produced in oxidation of the three macronutrients. These values were taken from Frayn and Macdonald (1997).

Table 3.1. Oxidation of metabolic substrates¹

1 g glucose + 0.747 LO₂ = 0.747 LCO₂ + 0.6 g H₂O + 15.56 kJ: RER = 1.0

1 g protein + 1.031 LO₂ = 0.859 LCO₂ + 0.403 g H₂O + 0.311 g urea + 0.02 g ammonia + 0.021 g creatinine + 19.72 kJ: RER = 0.833

1 g fat + 2.023 LO₂ = 1.436 LCO₂ + 1.07 g H₂O + 39.63 kJ: RER = 0.71

¹Volumes of oxygen and carbon dioxide are given at standard temperature and pressure dry (STPD).

For the present studies, urinary urea or total nitrogen excretion was not measured. As has been done in similar prolonged exercise studies, it was assumed that protein oxidation contributed 12.5% of energy expenditure at rest and that exercise did not alter this relative rate of protein utilization (Weller et al., 1997a, 1997b; Ralph, 2000). The respiratory quotients of CHO, fat and protein were taken as 1.00, 0.704, and 0.83, respectively. Oxidation rates (g/min) were estimated for CHO, fat and protein, respectively, from Table 1. During the hill walk (Chapter 5), CHO and fat oxidation rates were calculated and averaged over 10-min blocks at predetermined points in accordance with the other measurements. Total oxidation rates were then averaged for the whole hill walk. In chapter 7, CHO and fat oxidation rates were determined prior to exercise, during lunch, and each 45 min of walking. The CHO and fat oxidation rates were determined as a mean for a 5-min period from 35 - 40 min into each exercise stage in accordance with the other measurements as a mean for a 5-min period. From each 45-min block, total oxidation rates were then averaged for the exercise protocol. Energy expenditure was calculated from the averaged $\dot{V}O_2$ and $\dot{V}CO_2$ from the whole protocol using the formulae of Elia and Livesey (1992).

Energy expenditure (kJ) = (15.913 x $\dot{V}O_2$) / 1000 + (5.207 x $\dot{V}CO_2$) / 1000 - (4.646 x N)

Since N was not measured the following formula was used:

$$\text{Energy expenditure (kJ)} = 16.32 \times \text{VO}_2 + 4.60 \times \text{VCO}_2$$

where VO_2 represents the volume of oxygen consumed in litres, VCO_2 the volume of CO_2 produced in litres and N the amount of urinary nitrogen excretion in grams. The error introduced by not measuring urinary urea or total nitrogen excretion is less than 5% (Weir, 1949; Frayn and Macdonald, 1997). The percentage contributions of the CHO and fat oxidation were estimated from non-protein (NP) $\dot{\text{V}}\text{O}_2$ and RER data, using the following formulas:

$$\% \text{CHO} = [(\text{RER}_{\text{NP}} - 0.707) \times (1 - 0.707)]^{-1}$$

$$\% \text{Fat} = 100 - \% \text{CHO}$$

An important point to consider is that the energy production is estimated by the indirect calorimetry measurements. These measurements are under the same set of assumptions as are utilised to calculate the rates of glucose, lipid, and protein oxidation, namely, on the basis of the stoichiometry of the oxidative reactions of these fuels. Any deviation from the assumed model will introduce an error both in the relative amounts of substrate oxidised and in the corresponding energy production rate (Ferrannini, 1988). Whilst the limitations and assumptions of indirect calorimetry must be considered, it still provides an extremely valuable 'tool' in the study of human metabolism.

3.3.1.3 Indirect calorimetry: the doubly-labelled water method and calculations

The use of doubly-labelled water for the assessment of free-living energy expenditure in humans was first reported by Schoeller and van Santen (1982), and the technique has been subsequently evaluated (Prentice, 1990; Speakman and Roberts, 1995; Schoeller and Delany, 1998). This method provides information on the total energy expended by a free-living subject for a period of 4-20 days, which is likely to reflect the normal energy requirement of the subject. The subject takes an oral dose of water containing a known amount of stable (non-radioactive) isotopes of both hydrogen and oxygen. The isotopes, ^2H (deuterium) and ^{18}O , mix with the normal hydrogen and oxygen in the body water within a few hours. As energy is expended in the body, CO_2 and water are produced. The CO_2 is lost from the body in breath, whilst the water is lost in breath, urine, sweat and other evaporations. As ^{18}O is contained in both CO_2 and water, it is lost from the body more rapidly than ^2H , which is contained in water but not CO_2 .

The difference between the rate of loss of ^{18}O and ^2H reflects the rate at which CO_2 is produced, which in turn can be used to estimate energy expenditure, if a value for the respiratory quotient is assumed. Figure 3.1 shows a plot of the change in concentrations of the two isotopes in body fluids, from which the rate of loss of these isotopes from the body fluid can be calculated (Westerterp, 1999).

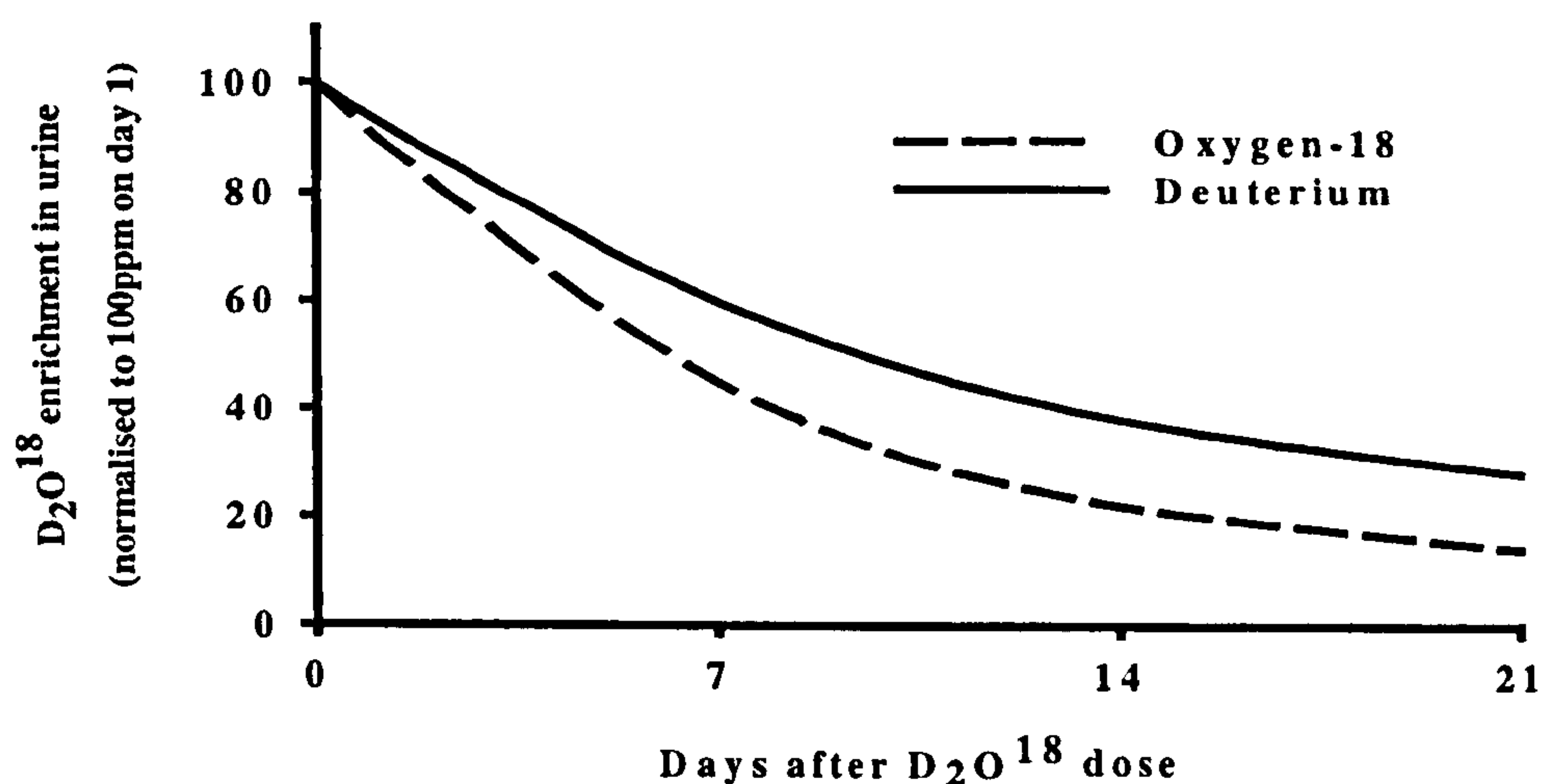


Figure 3.1. Decline of ^2H (deuterium, D) and ^{18}O in body fluids (urine, plasma or saliva) during a hypothetical doubly-labelled water experiment [from Westerterp, 1999, with permission].

An example of what has been learnt by using the technique is the level of total energy expenditure in all age-groups, including premature infants, hospitalised patients, children, obese subjects, pregnant and lactating women and elderly, for whom other methods might have serious problems. The applications of doubly-labelled water include the validation of techniques for the assessment of dietary intake and physical activity, the assessment of energy requirement, and the assessment of the effect of dietary and physical activity interventions, including its use with endurance athletes competing at the highest level.

Despite the clear advantages of the doubly-labelled water method, it is not without some disadvantages. These include; a) the high cost of the ^{18}O , and the specialised expertise required for the analysis of the isotope concentrations in body fluids by mass spectrometry; b) total EE is measured over about 4 to 21 days, so no knowledge is obtained about brief periods of peak expenditure; c) in field studies, because CO_2 production and not O_2 utilisation is being measured, some error is introduced if the respiratory quotient is not known. Nevertheless, the fact that the results provide the closest measure of free-living energy expenditure, makes the doubly-labelled water method an extremely valuable reference technique for validating estimates of energy requirements obtained by other methods.

Energy expenditure, water loss and physical activity level: Energy expenditure using the doubly-labelled water was measured according to Westerterp (1999). The estimated CV for EE_{DLW} was 7%, whereas water loss calculated by using the deuterium method, has an estimated CV of 7% (Westerterp et al., 1995). In the evening of day 0, subjects were given a weighted dose of a mixture of 99.84 atom% $^2\text{H}_2\text{O}$ in 10.05 atom% H_2^{18}O , such that ^2H and ^{18}O increased from baseline by ≥ 150 and ≥ 300 ppm, respectively. A background urine sample was collected in the evening of day 0. Additional urine samples were collected on day 1 (from the second void of the day and during the evening) and in the morning and evening of days 5 and 10, and in the morning of day 11. Isotope abundances in the urine samples were measured with an isotope-ratio mass spectrometer (Optima; VG Isogas). The calculation of energy expenditure from the rate of CO_2 production (rCO_2) is based on the relationship:

$$r\text{CO}_2 = \frac{k_o \times D_o - k_h \times D_h}{2 \times f_3} - \frac{f_2 - f_1}{2 \times f_3} \times r\text{Gf} \quad (1)$$

where k_o , D_o , k_h , and D_h are elimination rates and dilution spaces from ^{18}O and ^2H respectively. Factors f_1 , f_2 and f_3 are for fractionation of ^2H in water vapour (0.941), ^{18}O in water vapour (0.992) and ^{18}O in CO_2 (1.039), respectively, and $r\text{Gf}$ is the rate of isotopically-fractionated gaseous water loss. Then:

$$r\text{Gf} = 1.3 \times 1.77 \times r\text{CO}_2 \quad (2)$$

assuming that breath is saturated with water and contains 3.5% CO_2 fractionated breath water = $1.77 \times r\text{CO}_2$) and transcutaneous fractionated (non-sweat) water loss amounts to approximately 30% of breath water. Then:

$$r\text{CO}_2 = 0.455N \times (1.01 \times k_o - 1.04 \times k_h) \quad (3)$$

where N is the TBW calculated from the isotope dilution spaces ($(D_o / 1.01 + D_h / 1.04) / 2$) at the start of the observation period, corrected for the change over the observation period. The latter correction is calculated from the initial and final body mass of the subjects during the study, assuming the change of the body water volume is linear and proportional to the change in body mass. The CO_2 production was converted to energy expenditure using an energy equivalent based on the individual macronutrient composition of the diet. The energy expenditure from the doubly-labelled water was calculated over the same 10-day period during which subjects recorded their food intakes.

There are a number of additional advantages of using the doubly-labelled water methods. Some of these advantages include the calculation of the extent of any under-reporting of food intake or any under-eating from any weighed food intake measurements. Furthermore, the overall physical activity level of an activity can be calculated. These calculations are described further below.

The percentage under-reporting of food intake was calculated by using the energy expenditure calculated from the doubly-labelled water measurements as follows:

$$\text{Under-reporting} = [(EI - EE)/EE] \times 100\% \quad (4)$$

Where EI is the 10-day energy intake and EE is the 10-day energy expenditure calculated from the doubly-labelled water. The percentage under-eating was calculated from the change in body mass over the 10 days, assuming 1 kg of body mass to be 30 MJ (75% fat mass, 25% fat-free mass with 73% water) (Westerterp et al., 1995b; Goris et al., 2000)

$$\text{Under-eating} = [(\Delta \text{ body mass} \times 30 \text{ MJ}/10\text{d})/(EE)] \times 100\% \quad (5)$$

Physical activity level (PAL) was calculated by the ratio of the averaged daily EE_{DLW} to the estimated basal metabolic rate (*BMR*) (Westerterp, 1999; Goris et al., 2000).

Basal metabolic rate (BMR) values were estimated using an equation including age, sex, body mass and height (WHO, 1985);

$$\text{BMR (kJ/d)} = 64.4 \times \text{body mass (kg)} + 113 \times \text{height (m)} + 3000 \text{ (males)} \quad (6)$$

A recent study has indicated no differences between the estimation of BMR using this formula and by direct measurement (Goris et al., 2000).

3.4. BLOOD ANALYSES

3.4.1 Procurement and storage of blood samples

There are several methods and sites for blood sampling that can be used in the collection of samples for metabolite and hormonal analyses. The main sampling procedures entail the drawing of arterial, venous, arterialisised venous or capillary blood. Although arterial blood is perhaps the most appropriate for determining the concentrations of metabolites and hormones available to the cell, it is also the most invasive and potentially unpleasant for the subject. The main procedures used in the present thesis were predominately the procurement of venous blood and, for the laboratory based study, arterialisised venous blood sampling. The arterialisised venous blood sampling entails the collection of blood from the superficial vein on the dorsal surface of the hand or forearm. The hand is heated to increase peripheral temperature, which increases skin blood flow and effects the opening of arterial-venous anastomoses in the hand. This allows the blood to by-pass the capillary bed, resulting in a decreased transit time. This 'heated hand vein' technique has been reported to provide a valid alternative to arterial sampling and has been validated for a number of metabolites and hormones (McGuire et al., 1976; Liu et al., 1992).

3.4.2 Measurement of haematocrit, haemoglobin and plasma volume in whole blood

During exercise, changes in blood volume and plasma volume occur as a consequence of a net movement of water from the capillaries to the interstitial fluid space (Harrison, 1885; Fellmann, 1992). This net movement is a result of an increase in blood pressure within the capillaries, an increase in metabolic waste products and loss of water in sweat rate which occurs during exercise (Harrison, 1985; Fellmann, 1992).

The most precise methods of measuring plasma volumes in the steady-state are the plasma-albumin labelling techniques (^{131}I) or the Evan's blue-dye analysis. An assumption during these protein based methods is that the protein content remains constant during the measurement period. Consequently, these methods cannot be applied during short term changes in plasma volume because when plasma volume is rapidly changing, proteins are usually changing too (Fellmann, 1992). In addition, these methods are impractical for use in the field. These disadvantages can be

overcome by estimating plasma volume from the changes in haemoglobin and haematocrit concentrations.

To determine changes in blood volume during exercise, haemoglobin (Hb) is used as an endogenous marker, since no changes in the amount of Hb occur within the circulation over a short time period (Harrison, 1985). Therefore, any change in the concentration of Hb reflects a change in blood volume. Equally, haematocrit (Hct), or packed cell volume occupied by the erythrocytes, is used as a marker of changes in red cell volume and plasma volume during exercise. The procedures for measuring haemoglobin and haematocrit, and the estimation of plasma volume are described below.

Measurement of haemoglobin within whole blood

In all studies, the total amount of haemoglobin in whole blood was determined in duplicate using a Hemocue met-Hb system (Hemocue Ltd, Sheffield). The Hemocue system consists of a purpose-designed photometer and disposable microcuvettes containing dried reagents. The microcuvette serves as a pipette, reaction vessel and measuring curvette. Approximately 50 µl of blood is drawn into two Hemocue curvettes, taking care to avoid any air bubbles. The filled curvettes are then immediately placed into the curvette holder on the photometer and pushed into the measuring position. The chemical reactions involved in this procedure are as follows.

Test principle:

1. Haemoglobin + potassium ferricyanide \longrightarrow methaemoglobin
2. Methaemoglobin + potassium cyanide \longrightarrow cyanmethaemoglobin

The coloured cyanmethaemoglobin is then quantified photometrically at a wavelength of 540 nm in the photometer. The results are displayed when the end point of the reaction has been determined, usually between 15 and 240 s, depending on the haemoglobin concentration. The accuracy of the Hemocue was checked with a standard reference curvette before each set of measurements was made.

Measurement of haematocrit with whole blood

To determine haematocrit, whole blood was drawn into two plain micro-capillary tubes (L.I.P. Equipment and services Ltd, West Yorkshire, England), avoiding the introduction of air bubbles. The tubes were filled to approximately two-thirds of their length and were sealed with Critaseal (Gelman-Hawksley Ltd, England). The Critaseal is a clay type sealant that is placed at the blood free end of the capillary tube. The tubes were placed with the sealant end outermost in the opposite channels of a rotor in a micro-capillary centrifuge (Hawksley and Sons Ltd, Sussex, UK). The tubes were spun at 12 000 g for 3-5 min, and the percentage haematocrit was read using the Hawksley micro-haematocrit reader (Gelman-Hawksley Ltd, England). The percent haematocrit reading was averaged from the two micro-capillary tubes.

Measurement of plasma volume changes within whole blood

In all studies, the values for Hb and Hct concentrations at rest, prior to exercise, were used to calculate changes in blood volume (BV), plasma volume (PV), and red cell volume (CV). Measurements of Hb and Hct were made during every study when blood samples were procured. The formula used to calculate any changes in plasma volume were those of Dill and Costill (1974) and are shown below:-

Change in blood volume during exercise

$$BV_A = BV_B \times (Hb_B / Hb_A) \quad (1)$$

Change in red cell volume during exercise

$$CV_A = BV_A \times Hct_A \quad (2)$$

Change in plasma volume during exercise

$$PV_A = BV_A - CV_A \quad (3)$$

where the subscripts B and A represent the pre-exercise (B) and during exercise (A) volumes, and $BV_A = 100$.

In order to calculate the percentage change in BV, CV and PV with exercise the following formulae were used (where $CV_B =$ pre-exercise red cell volume and $PV_B = 100 -$ pre-exercise red cell volume).

Percentage change in blood volume with exercise

$$\Delta BV\% = 100 (BV_A - BV_B) / BV_B \quad (4)$$

Percentage change in red cell volume with exercise

$$\Delta CV\% = 100 (CV_A - CV_B) / CV_B \quad (5)$$

Percentage change in plasma volume with exercise

$$\Delta PV\% = 100 (PV_A - PV_B) / PV_B \quad (6)$$

The inter- and intra-assay CV's for Hb were $< 5.97\%$. The inter- and intra-assay CV's for Hct were $< 4.5\%$.

3.5. LABORATORY METHODS FOR MEASUREMENT OF METABOLITES AND HORMONES

For determination of plasma (or serum) metabolite and hormone concentrations, previously frozen samples were thawed, mixed thoroughly for a few seconds using a vortex mixer. All plasma or serum samples for each subject were analysed in the same assays to avoid inter-assay variations. All samples were performed in duplicate to give a mean value for each sample. Samples were repeated if the intra-assay coefficients of variation (CV) exceeded 10% between duplicates.

Only one study necessitated the use of serum samples. Serum samples were the preferred method of blood procurement in Chapter 5 because blood was sampled in the field where it was not possible to provide centrifuge facilities for plasma removal. During this study, all blood from the three sample points was procured by the same method. The remaining three studies all used plasma. The sample procedures for analysis, described below, were the same whether using serum or plasma samples.

3.5.1 Preparation and measurement of plasma NEFA and TAG

Measurement of plasma NEFA

Plasma NEFA concentrations were estimated using a commercially available kit (WAKO NEFA C kit, Alpha Laboratories Ltd., Eastleigh, Hampshire, UK), which utilised enzymatic methods adapted for an IL Monarch Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, Lancs.). The IL Monarch centrifugal analyser is shown in plate 3.3. The IL Monarch centrifugal analyser was used for all plasma metabolite assays. Standardised calibrations were followed prior to any analyses, described further below. The precision of all assays was monitored with commercially available quality control material (Boehringer Mannheim, Lews, UK).

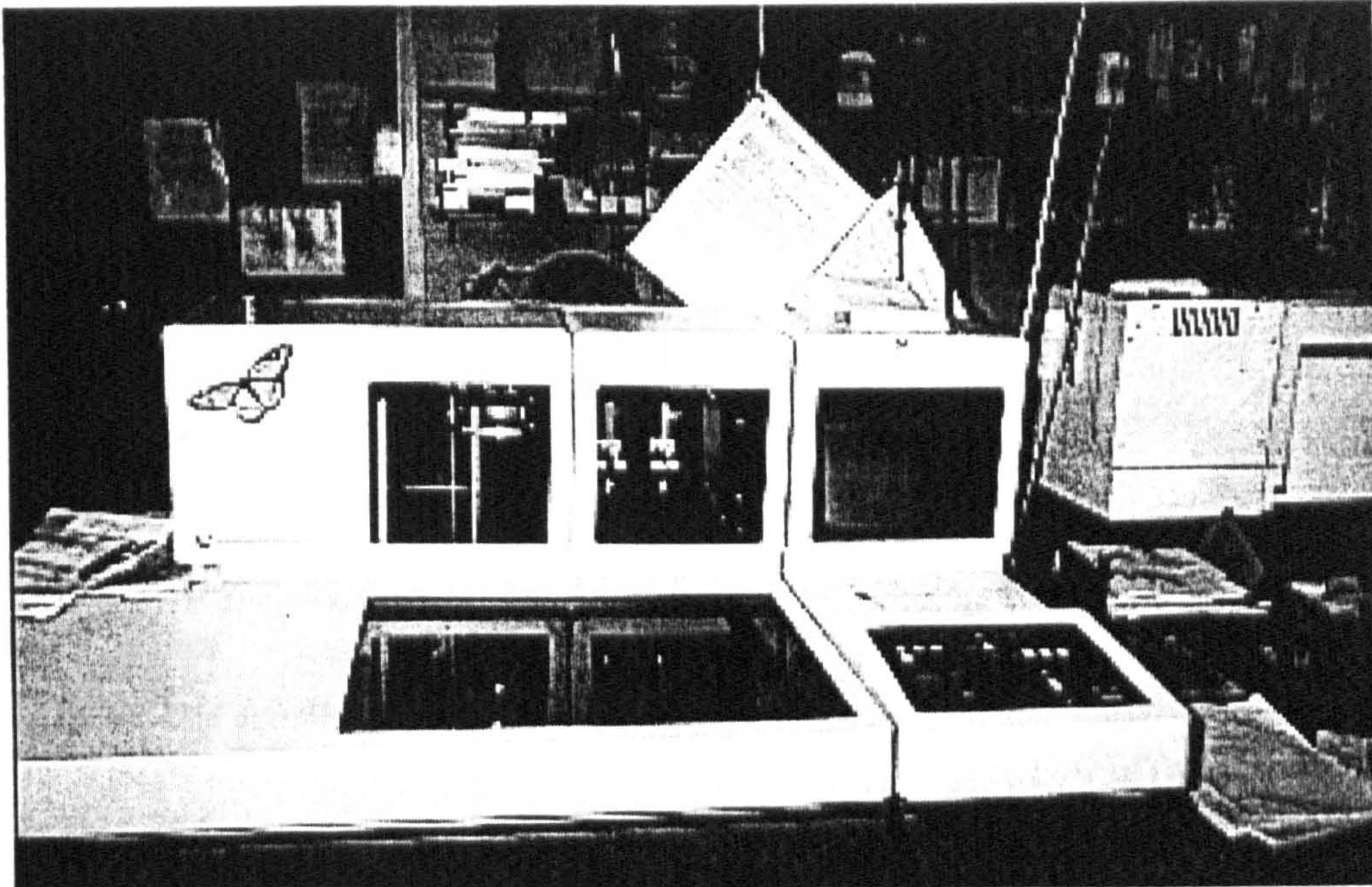
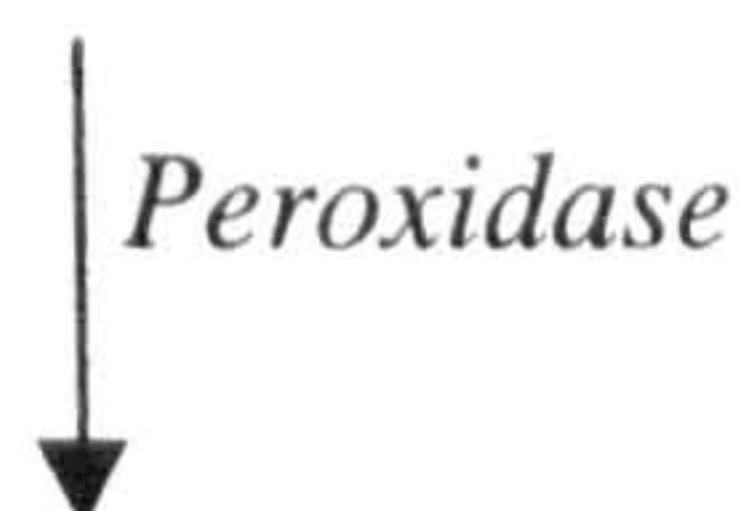
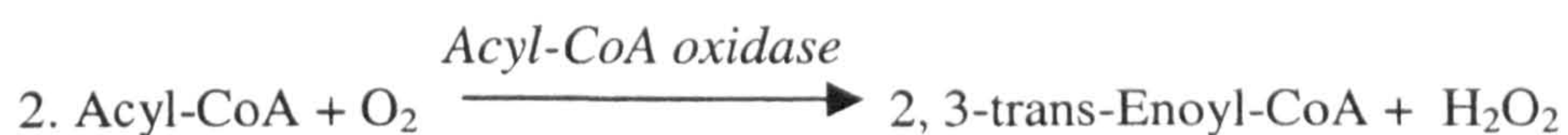
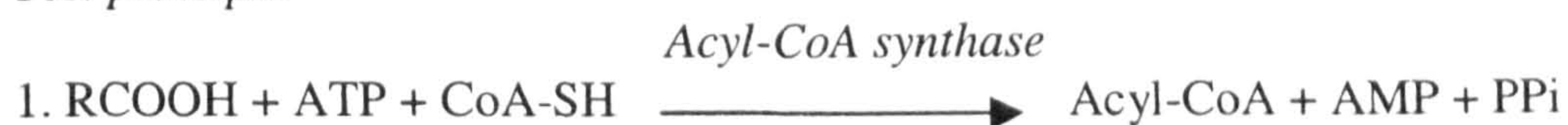


Plate 3.3. Photograph of IL Monarch centrifugal analyser.

The enzymatic reactions involved in the estimation of NEFA are as follows:

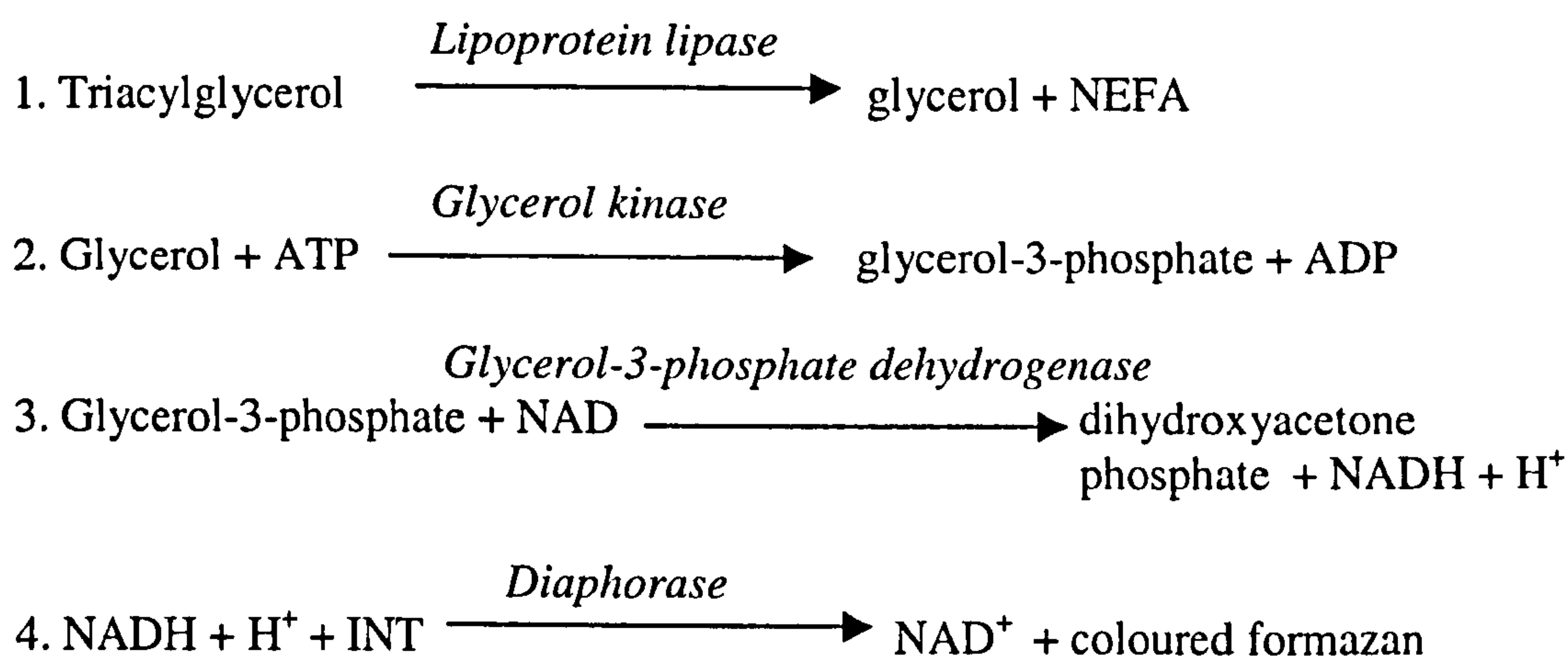
Test principle



The change in absorbance of the reaction mixture at 500 nm was measured and the reaction was linear for plasma NEFA concentrations up to 3 mmol/l. The inter-assay CVs were 3.4 % at 670 $\mu\text{mol/l}$ and 3.1 % at 1127 $\mu\text{mol/l}$. The intra-assay CV was calculated from the differences between duplicate samples and was < 10%.

Measurement of plasma TAG

Plasma TAG concentrations were estimated using an in-house method (Oxford Lipid Metabolism Group, The Radcliffe Infirmary, Oxford) of Humphreys et al. (1990) with correction for free glycerol. The enzymatic reactions involved in this procedure are as follows:

Test principle:

The maximum absorbance of the coloured formazan end product was at 500 nm. The increase in absorbance of the reaction mixture at 500 nm was directly proportional to the TAG concentrations of the sample up to 4 mmol/l (Humphreys et al., 1990).

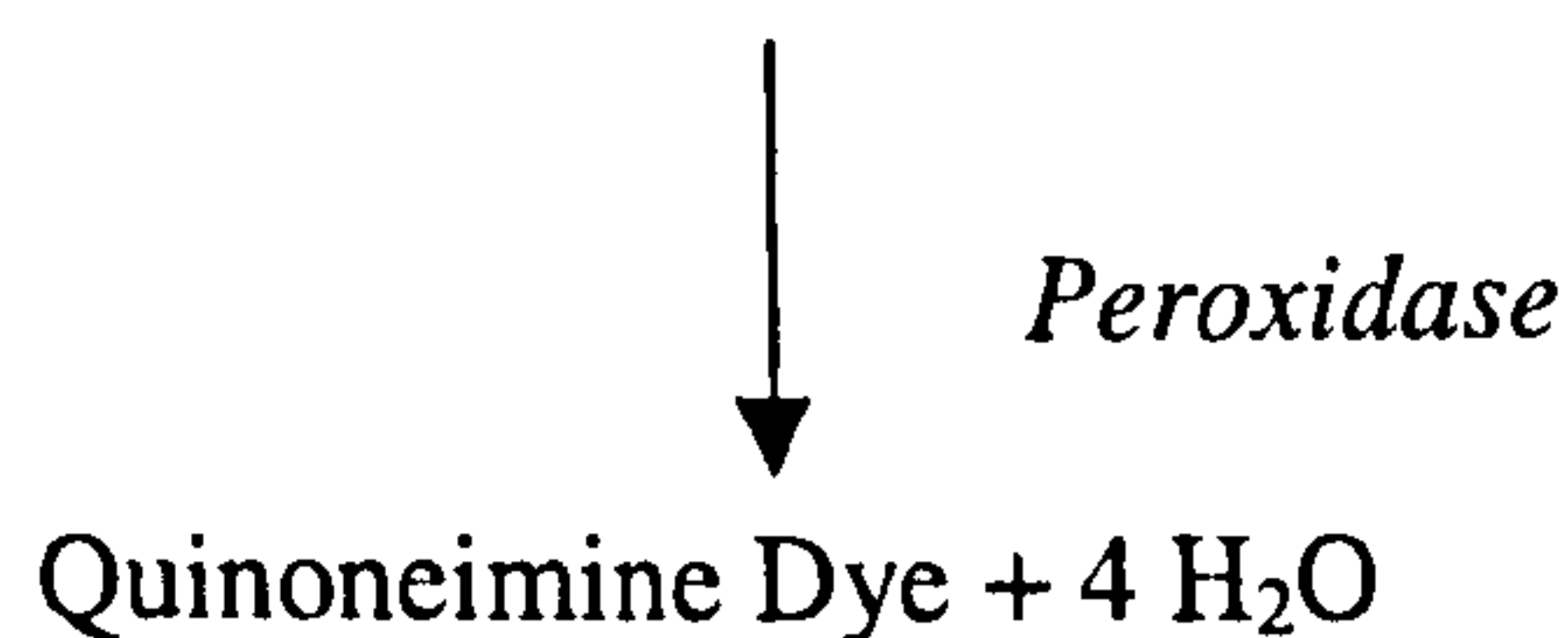
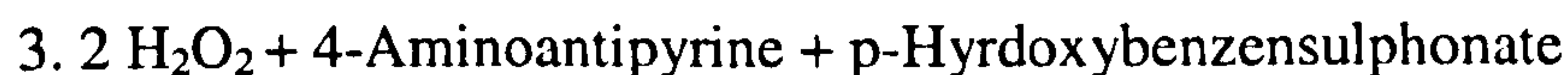
For the determination of free glycerol, the reaction was repeated with the omission of lipoprotein lipase. Total TAG concentration was calculated from the difference between total and free glycerol.

The methods were standardised against glycerol (Merck, Poole, UK), and the precision of the assay was monitored with commercially available control material (Boehringer Mannheim, Lews, UK). The inter-assay CVs were 2.8 % at 1568 $\mu\text{mol/l}$ and 3.5 % at 3816 $\mu\text{mol/l}$. The intra-assay CV was calculated from the differences between duplicate samples and was < 10%.

3.5.2 Measurement of plasma cholesterol, HDL and LDL cholesterol

Plasma cholesterol and HDL-cholesterol concentrations were estimated using a commercially available kit (Sigma Diagnostics kit, Sigma-Aldrich company Ltd., Diagnostics Division, Poole, Dorset, UK), which utilised enzymatic methods and was adapted for an IL Monarch Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, Lancs.). The enzymatic reactions involved in this procedure are as follows:

Test principle



The maximum absorbance of the coloured quinoneimine dye end product was at 500 nm. The increase in absorbance of the reaction mixture at 500 nm was linear with the cholesterol concentration of the sample up to 15 mmol/l. The inter-assay CVs were 2.5 at 2.1 mmol/l and 2.0 at 6.5 mmol/l. The intra-assay CV was calculated from the differences between duplicate samples and was less than 4.5 %.

Estimation of LDL-cholesterol

The total cholesterol, HDL-cholesterol and plasma TAG concentration were used to estimate LDL-cholesterol by means of the Friedewald equation (1972) as follows:

$$\text{LDL-C} = (\text{T-Chol} - ((\text{TAG}/2.2) + (\text{HDL-C})))$$

3.5.3 Preparation and measurement of plasma glycerol, lactate and 3-hydroxybutyrate

Preparation for plasma glycerol, lactate and 3-hydroxybutyrate

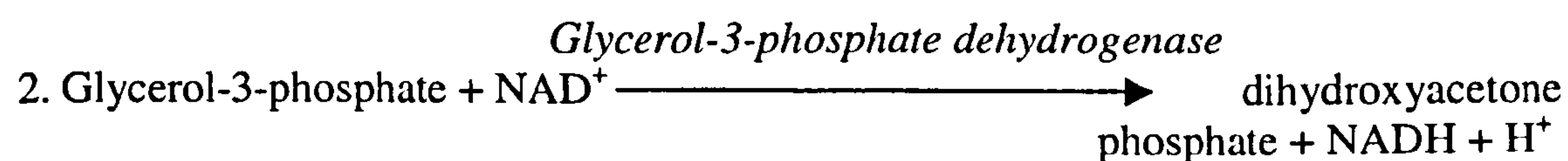
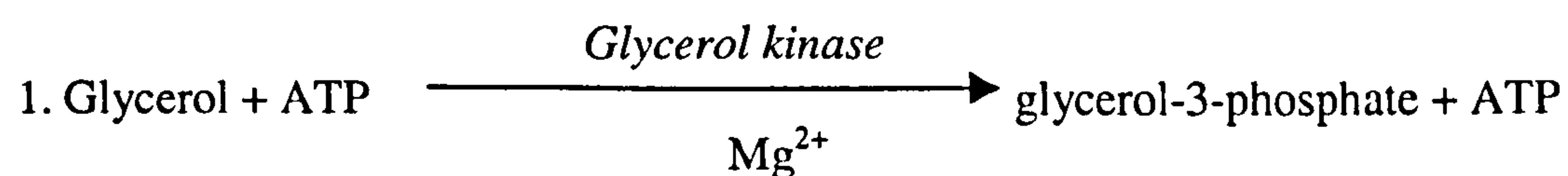
A deproteinised fraction for plasma glycerol, lactate and 3-hydroxybutyrate determination was prepared in duplicate as follows:

A portion of plasma (0.25 ml) was added to 500 μ l of cold perchloric acid (7% wt/vol) in a 1.5-ml eppendoff tube, as described by (Coppack et al., 1990). The sample tubes were thoroughly mixed and kept cold before centrifugation at 12 000 g for 5 min at 4°C. The clear supernatant from each tube was recovered and stored at -20°C for subsequent analyses of plasma glycerol, lactate and 3-hydroxybutyrate concentrations.

Measurement of plasma glycerol

Plasma glycerol concentrations were determined using the method of Humphreys and Frayn (1988). The enzymatic reactions involved in this procedure are as follows:

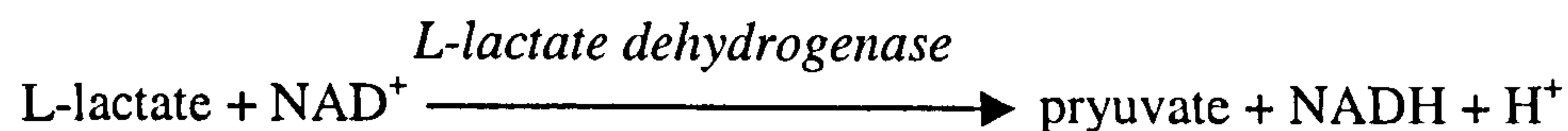
Test principle



The production of NADH was measured by fluorescence detection using a Monarch Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, Lancs.). The methods was standardised against glycerol made up in (4% wt/vol) perchloric acid. The inter-assay CVs were 6.8% at 42 μ mol/l and 5.1% at 87 μ mol/l. The intra-assay CV was calculated from the differences between duplicate samples and was < 10%.

Measurement of plasma lactate

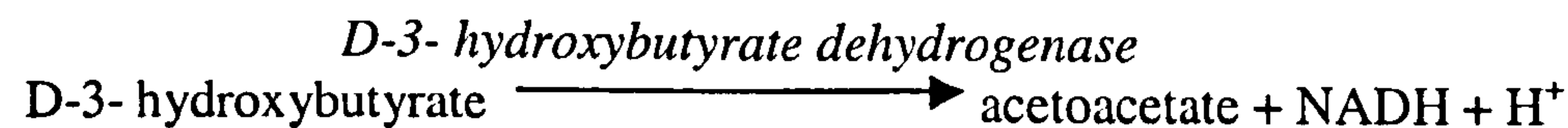
Plasma lactate concentrations were determined using the method of Humphreys and Frayn (1988). The enzymatic reactions involved in this procedure are as follows:

Test principle:

The production of NADH was measured by fluorescence detection. The method was standardised against L-lactate made up in 4% wt/vol perchloric acid at 0, 200 and 400 $\mu\text{mol/l}$. The inter-assay CVs were less than 10% at 237 $\mu\text{mol/l}$ and 1450 $\mu\text{mol/l}$. The intra-assay CV was calculated from the differences between duplicate samples and was < 10%.

Measurement of plasma 3-hydroxybutyrate

Plasma 3-hydroxybutyrate concentrations were determined using the method of Humphreys and Frayn (1988). The enzymatic reactions involved in this procedure are as follows:

Test principle

The production of NADH was measured by fluorescence detection. The method was standardised against D-3-hydroxybutyrate made up in 4% wt/vol perchloric acid at 0, 100 and 200 $\mu\text{mol/l}$. The inter-assay CVs were less than 8% at 25 $\mu\text{mol/l}$ and 43 $\mu\text{mol/l}$. The intra-assay CV was calculated from the differences between duplicate samples and was < 10%.

3.5.4 Measurement of plasma insulin, growth hormone, glucagon and cortisol

All samples for the hormone analysis were frozen according to the instructions of the manufacturers of the kit. They were then batch analyzed, as described below.

Measurement of plasma insulin

Plasma insulin concentrations were determined using a double-antibody radiomunnoassay (kit from Pharmacia and Upjohn, Milton Keynes, UK). The procedure involved insulin in the plasma competing with a fixed amount of ^{125}I -labelled insulin for the binding sites on specific antibodies (antiserum raised in guinea pig) during incubation. The bound and free insulin were separated by addition of a second antibody immunoabsorbent (an anti-guinea pig immunoglobulin G antiserum raised in sheep and bound to Sepharose) before centrifugation and decanting. The radioactivity in the pellet was measured using an automatic gamma counter (Wallac Multigamma 1260, EG & G Berthold, Milton Keynes, Bucks, UK). The radioactivity in the pellet was inversely related to the quantity of insulin in the sample, which was calculated using a 6-point calibration curve. The inter-assay CVs were 6.4% at 11.6 mU/l and 7.6% at 65.2 mU/l, and the intra-assay CV was less than 10%.

Measurement of plasma growth hormone

Plasma growth hormone concentration was determined by using a two-site immunoradiometric assay (kit from Pharmacia and Upjohn, Milton Keynes, UK). The procedure involved using two different antibodies in excess. During incubation, growth hormone in the plasma sample reacts with ^{125}I -labelled anti growth hormone antibodies (antiserum raised in rabbit) and unlabelled anti-growth hormone antibodies (raised in sheep, immunologically bound to Sepharose particles). The bound and free-anti-growth hormones ^{125}I -labelled were separated by centrifugation and decanting. The radioactivity in the pellet was measured using an automatic gamma counter (Wallac Multigamma 1260, EG & G Berthold, Milton Keynes, Bucks, UK). The radioactivity in the pellet was directly related to the concentration of growth hormone in the sample which was calculated using a 6-point calibration curve. The inter-assay CVs were 6.5% at 2.3 mU/l and 5.2% at 48.2 mU/l. The intra-assay CV was less than 10%.

Measurement of plasma glucagon

Plasma glucagon (kit from DPC Ltd, Llanberis, UK) was collected into potassium EDTA-containing tubes with 200 kIU aprotinin/ml blood (Trasylol, Byer PLC, Newbury, UK), and analyzed by using a double-antibody polyethylene glycol precipitation method. The procedure involved pre-incubation of the plasma sample with an anti-glucagon antibody (^{125}I -labelled glucagon), which then competes with glucagon in the sample for antibody sites. After incubation for a fixed time, separation of bound from free is achieved by the PEG-accelerated double-antibody method, followed by centrifugation. The precipitate containing the antibody-bound fraction was then counted using an automatic gamma counter (Wallac Multigamma 1260, EG & G Berthold, Milton Keynes, Bucks, UK), and plasma concentrations were read from a 5-point calibration curve. The inter-assay CVs were 5.1% at 100 pg/ml and 4.3% at 500 pg/ml, the intra-assay CV was less than 5%.

Measurement of plasma cortisol

Plasma cortisol concentrations were determined by using a solid phase radiomunnoassay (kit from Coat-A-Count, Diagnostic Products, Llanberis, Wales, UK). The procedure involved cortisol in the plasma sample competing with a fixed amount of ^{125}I -labelled cortisol for the binding sites on specific antibodies attached to the walls of a coated polypropylene tube. The bound and the free cortisol were separated by decanting. The radioactivity in the pellet was measured using an automatic gamma counter (Wallac Multigamma 1260, EG & G Berthold, Milton Keynes, Bucks, UK). The radioactivity in the pellet was directly related to the concentration of cortisol in the sample which was calculated using a 6-point calibration curve. The inter-assay CVs were 7.4% at 133 nmol/l and 5.1% at 758 nmol/l, the intra-assay CV was less than 6%.



Plate 3.4. Photograph of the automatic gamma counter used in the RIA measurements.

3.5.5 Measurement of urine and plasma catecholamines

Urine adrenaline, noradrenaline, dopamine and creatinine concentrations were analysed at The Royal University Hospital, Liverpool by Dr Norman Roberts and his staff using an in-house method (Department of Clinical Chemistry, The Royal University Hospital, Liverpool). This method was based on the organic solvent technique of Smedes et al. (1982) and was modified using cation-exchange isolation (Macdonald et al., 1985). The adrenaline, noradrenaline, dopamine and creatinine concentrations were analyzed by using high performance liquid chromatography with electrochemical detection.; the inter- and intra-assay CV's were < 6.5%.

The plasma adrenaline and noradrenaline concentrations were analysed at The Royal University Hospital, Nottingham, by Professor Ian Macdonald and his staff using an in-house method, similar to that described above (Ian Macdonald, personal communication, February, 2002). The inter- and intra-assay CV's were < 4.3%.

3.6. METHODS FOR THE ESTIMATION OF HYDRATION STATUS

3.6.1 Urine osmolality

Although there is no 'gold standard' method to assess hydration status, measurements of urine osmolality provide one of the optimum methods available (Sherriffs, 2000).

Urine osmolality is determined from a small urine collection. The samples were stored at 4°C until ready to be batched analysed. The osmolality was then determined in triplicate by the use of the freezing point depression method (Advanced Micro-osmometer (model 3300), Vitech Scientific Ltd, West Sussex). Prior to use, the osmometer was calibrated using three standard reference samples. A urine osmolality greater than about 850 mosmol/kg H₂O could reasonably be used as indication of a hypohydrated state ((Shirreffs and Maughan, 1998; Shirreffs, 2000).

The inter-assay CVs were 2.3% at 50 mOsm/kg and 3.6% at 290 mOsm/kg and 3.4 % at 850 mOsm/kg. The intra-assay CV was less than 7.4 %.

3.6.2 Other methods

A number of other methods can be used as an indicator of hydration status. In the short term, less than 8 hours, change in body mass is one useful method. Subjects weigh themselves nude prior to exercise then repeat the measurement after a pre-determined time. The loss in body mass will be a general indication of water loss sustained during the activity. Other methods include urine indices, including the volume, colour, conductivity, specific gravity, have been investigated as hydration status markers. Similarly, blood born indices, including haemoglobin concentration and haematocrit, plasma osmolality and sodium concentration, plasma testosterone, adrenaline, noradrenaline, cortisol and atrial natriuretic peptide concentrations, have been investigated as hydration status markers (Shirreffs and Maughan, 1998; Shirreffs, 2000). The blood borne indices have the disadvantage of being more invasive than the urinary measures described and in an exercise setting some respond not only to hydration stresses but also to exercise. As incorporated in the present thesis, urine osmolality rather than specific gravity was the preferred index of urine concentration used as the measure of hydration status because, unlike the specific gravity measure, it is not influenced by solutes such as urea, glucose and protein (Jacobs et al., 1990).

3.7. HEART RATE AND THERMOREGULATORY MEASUREMENTS

3.7.1 Measurements of human physiological and thermal responses

Heart rate was recorded by means of short-range radio telemetry (PE3000 Sports Tester, Kempele, Finland). Recordings were calculated every minute and subsequently averaged over 5-min blocks.

Participants inserted a rectal temperature probe to a depth of 10 cm beyond the anal sphincter. Skin temperature was assessed by the placement of temperature thermistors on the chest, forearm, thigh and shin. Thermistors and the rectal probe were connected to a data logger (Squirrel meter 1000, Grant Instruments, Cambridge, UK) which recorded data every 6 min. On the walks a rest period of approximately 1 – 3 min was allowed every time thermal measurements were made, and 10-30 min for lunch. All subjects carried a lightweight water-proof backpack that contained thermal logger to allow continuous recording of rectal and skin temperatures, respectively.

Rectal temperature (T_{rec}) and skin temperature (T_{sk}) were monitored continuously. Data were recorded every 6 min with the data logging system.

Mean T_{sk} (\bar{T}_{sk}) was estimated by the formula of Ramanathan (1964):

$$\bar{T}_{sk} (^{\circ}\text{C}) = (0.3 \times \text{chest}) + (0.3 \times \text{arm}) + (0.2 \times \text{thigh}) + (0.2 \times \text{shin}).$$

Mean body temperature (\bar{T}_b) was estimated by the method of calculation used by Bittel (1987):

$$\bar{T}_b = x \times T_{rec} + (1 - x) \times \bar{T}_{sk},$$

where x represents the cold weighting coefficient of 0.67.

Thermal balance: All thermal balance data (rates) are expressed in units of W/m^2 . The rate of heat gained or lost (heat debt) from the body mass ($\pm S$) was computed from the equation of Burton (1935):

$$\pm S = \Delta \bar{T}_b \times \text{mass} \times c_{\text{tissues}} \times SA^{-1},$$

where $\Delta \bar{T}_b$ is the change in \bar{T}_b during each exercise period (from the preceding resting value to the end of the exercise period), c_{tissues} is the average specific heat of the body tissues ($1.29 \text{ W} \times \text{kg}^{-1} \times ^\circ\text{C}^{-1}$), and SA is the body surface area. The thermal balance was calculated and averaged every 2 km of the walk.

3.7.2 Measurements of thermal environment

Environmental air, dry and wet bulb temperatures and velocity were recorded with a digital sling psychrometer thermo-hygrometer and a kestrel vane anemometer, respectively. Wind chill index was calculated from the air temperature and velocity by the equation of Nishi and Gagge (1977):

$$K_o = (33 - t_a) (10v^{0.5} - v + 10.45),$$

where K_o is the cooling power of the environment in $\text{kcal/m}^2/\text{h}$, t_a the ambient temperature ($^\circ\text{C}$) and v represents the air velocity in m/s .

3.8. METHODS FOR THE MEASUREMENT OF PSYCHOLOGICAL VARIABLES

3.8.1 Self-rating mood questionnaires for rating of mood

The Profile of Mood State (POMS) was measured using 65 ratings each on a 5-point rating scale. The scales are factored into six mood scores; depression/dejection, tension/anger, anger/hostility, confusion/bewilderment, fatigue/inertia and vigour/activity (McNair et al., 1992). In addition, during the laboratory based experiment, various subjective ratings were recorded prior to breakfast, immediately after breakfast, during all the rest breaks, prior to and immediately after lunch, and immediately post-exercise. During these time points subjects were asked to complete ratings of "hunger", "fullness", "satiety", "thirst", "nausea", "strength of appetite", "desire to eat" and "fatigue". These ratings were assessed by a 100-mm visual analogue rating scale labeled from "not at all" to "extremely". The nature of these rating scales, their manner of use and their validity in relation to food consumption have been described previously (DeCastro and Elmore, 1988; Hill and Blundell, 1990). A number of the same subjective ratings were used in a similar manner to that above during the Scotland study.

The format of the mood questionnaire and of the use of the visual analogue scales is illustrated in Appendix 2.

3.8.2 Borg scale for rating of perceived exertion during exercise

To determine the subjects' perception of fatigue during the field and laboratory studies, Borg's (1970) perceived exertion grading scale was used. The scale consists of 15 grades from 6 to 20, where a rating of perceived exertion (RPE) grade of 6 indicates the subject feels the work load to be very, very light, whereas a RPE of 20 indicates the subject feels the workload to be very, very hard. During the field studies, subjects were asked to rate their RPE from the start of the walk until the lunch stop, and from the lunch stop until the completion of the walk. Similarly, during the laboratory study subjects rating their RPE at the end of during the last 5 min of each 45-min block of exercise.

3.9. METHODS FOR THE MEASUREMENT OF PSYCHOMOTOR VARIABLES

A range of performance tests were incorporated during this thesis as described below. All subjects were fully familiarised with the use of the equipment and test, and each test was performed three times in a balanced fashion. Furthermore, the same investigator carried out the supervision of all tests. The reliability and test-retest reproducibility of these tests have been recently described (Rinne et al., 2001).

3.9.1 Grip and leg strength and jump tests (motor function)

Motor function was assessed by means of a handgrip dynamometer (Taki, Narragansett, Japan). In addition, muscular power was assessed by means of a leg dynamometer (Takei Scientific Instruments Compant Ltd, Tokyo, Japan). Vertical jump (anaerobic power) performance was assessed in the ability to perform a maximal jump from an electronic force platform and also the maximal jump a subject could make from a stationary line.

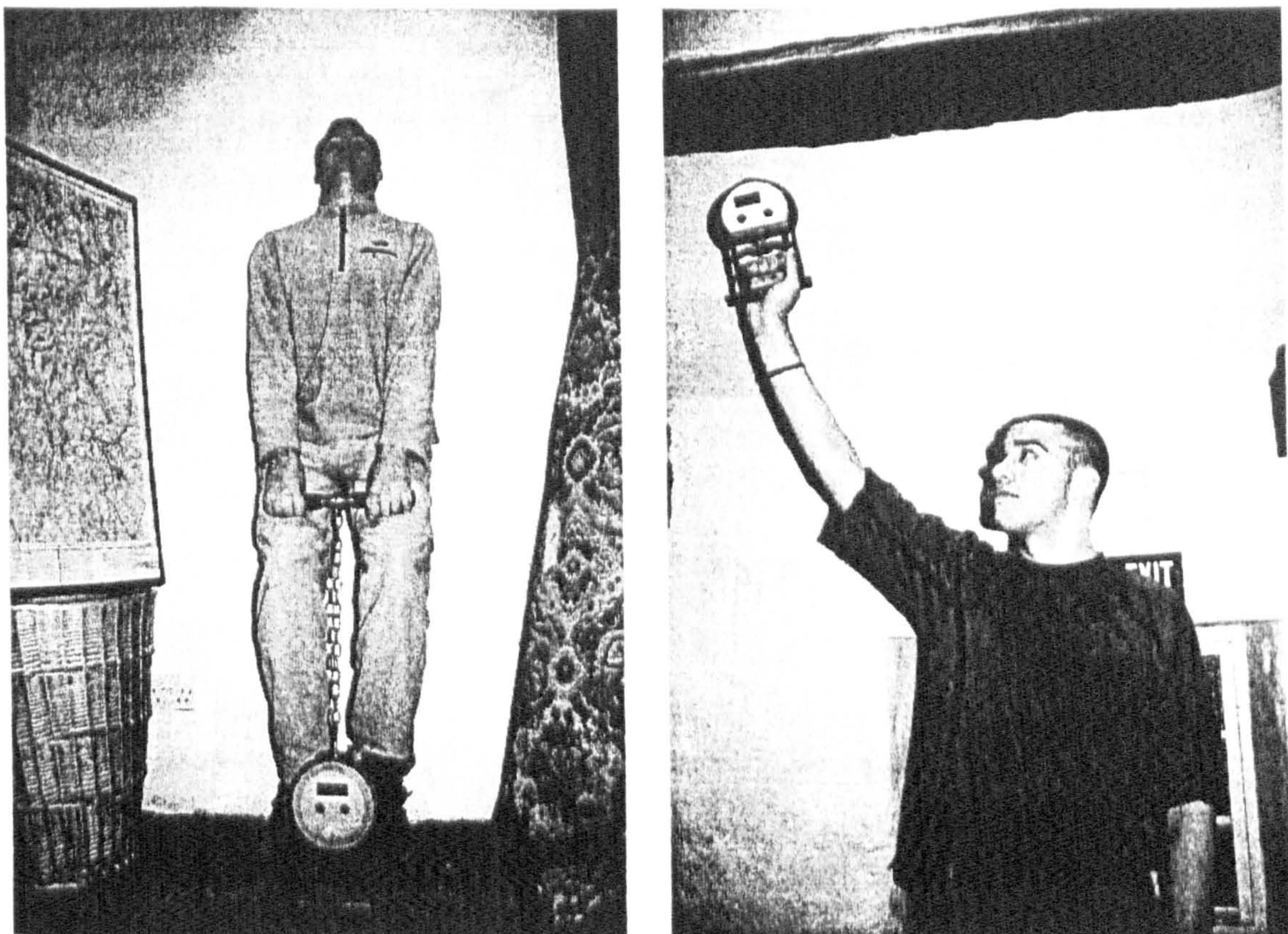


Plate 3.5. Photograph of subjects carrying out handgrip and leg dynamometer tests.

3.9.2 Choice reaction time tests (Cognitive function)

Reaction time tests (Hick's Law) - 1, 2, 4 and 8-choice reaction time for a finger response - were assessed on a laptop computer. The 1 and 2-finger reaction time test was considered to be a perception task, whereas the 4 and 8-choice reaction time was considered to be a decision task (Schmidt, 1982).

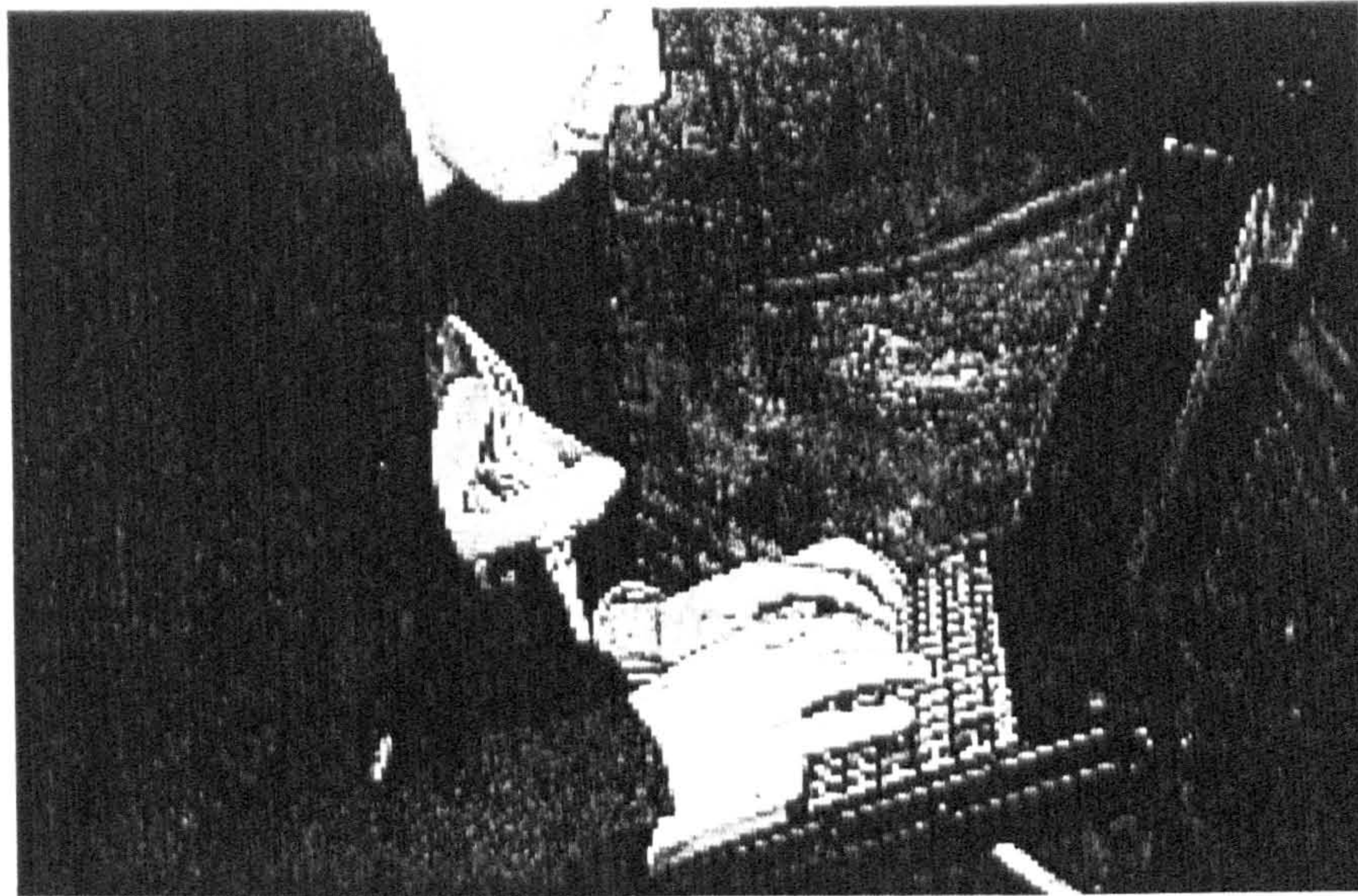


Plate 3.6. Photograph of subject carrying out reaction time tests in the shelter during a 30-min rest of lunch (Chapter 5).

3.9.3 Flexibility

Flexibility was measured using conventional 'sit and reach' test following guidelines by the American College of Sports Medicine, (1991). Participants sit and bend their trunk forward with their knees straight. The distance reached with the tip of their middle finger on a scaling box was recorded.

3.9.4 Balance

Dynamic balance: (Tandem walking forwards and backwards): Subjects walked along a line 6 m long. Subjects placed one foot in front of other with heel and toe of their shoes touching and walking as fast as possible without making mistakes or side touches. The best result, time in seconds, of three trials was recorded.

Static balance: Standing on one foot. Subjects stood on one foot with eyes open and arms relaxed by sides. Heel of opposite foot was placed against medial side of supporting leg at the level of the knee joint and keeping the thigh rotated outwards. Balance time was measured in seconds with a stopwatch; the upper limit for the trial was 60 seconds.

Static balance: Standing on a narrow bar. Participants stood with one foot on a narrow bar (width 2 cm, height 5 cm, length 50) with the other foot free for one minute. The stopwatch was stopped every time the subject touched the floor with the free foot and restarted when the balance position is re-achieved. The participants were allowed to use their arms at their sides for balance. The number of restarts was registered.



Plate 3.7. Photograph of subject carrying out one of the static balance tests.

3.9.5 Kinaesthetic differentiation: (Standing broad jump):

Distances of 50, 75, and 100 cm, marked by line, were used in this test. Each jump started at a line, with the subjects jumping the distances landing on the exact mark. The accuracy of the jumps was assessed according to the participants' heel which should be placed as close to the line as possible. Distance was recorded from the determined distance line.

3.9.0 PILOT WORK

3.9.1.1 Preparation and analysis of test meals

Pilot work was conducted on the meals used in chapter 7 and the final field study (chapter 8), as described below.

3.9.1.1.1 Laboratory based study (Chapter 7)

For the laboratory based study of prolonged exercise, three isoenergetic diets containing different proportions of fat and CHO were prepared. The ingredients and the nutritional composition of the three meals were initially taken from two primary sources: 'The composition of foods' (Holland et al., 1991) and from Whitley (1998). The ingredients and nutritional composition of the diets were then verified according to the commercial product. Furthermore, the diets were determined in relation to a 70-kg reference person. The nutritional composition of the three diets, which included breakfast, snacks and lunch, was calculated on Excel version 9 spread-sheets which allowed different proportions of ingredients to be calculated for different body weights amongst subjects.

For the three diets the following ingredients were used. The constituents for the high-CHO diet were chosen to typify an athlete's breakfast, comprising cornflakes, puffed rice, skimmed milk, banana, white toast, jam, flavoured low-fat yoghurt, and orange juice. The snacks included high-CHO products such as raisins and apricots. Lunch was comprised of bread, jam, banana, flavoured low-fat yoghurt, and orange juice. The constituents of the high-fat diet were chosen to typify a breakfast cereal, comprising oats, coconut, almonds, raisins, honey, sunflower oil, banana, double cream and milk. Snacks during the high-fat manipulation comprised products such as coconut and almonds, lunch included bread and cheese sandwiches with additional margarine, and ice cream with a small amount (50 ml/70 kg body mass) of long-chain triacylglycerol emulsion drink (Calogen; Scientific Hospital Supplies Group UK Ltd, Liverpool). The mixed meal incorporated the same isoenergetic nature of the high-CHO and high-fat diets. The macronutrient intake for the mixed diet was within the normative values for the general population (Ralph, 2000). All the meals had similar

proportions of simple sugars relative to total carbohydrate, and similar saturated:unsaturated fatty acid values. All ingredients were purchased from Holland and Barrett Health food store and Tesco supermarkets and the amount of each ingredient was dependant on the subject's body mass.

Prior to the main study, a pilot "taster session" was conducted on four volunteers from The Research Institute for Sports and Exercise Sciences, Liverpool John Moores University, Liverpool. Both the high-CHO and high-fat diets were tested for appearance, taste, texture, palatability and any feeling of bloatedness and gastrointestinal discomfort following the meals and snacks. This "taster session" enabled minor adjustments to be made before the main period of testing.

3.9.1.1.2 Field study (Chapter 8)

Prior to the main field study, pilot work was conducted on three subjects over two days. Each subject consumed a high (~10 MJ), medium (~5 MJ) or no food during a strenuous 21-km walk. A range of snacks and sandwiches was used to formulate the required dietary intake. Subjects were asked to give feedback on the palatability and appropriateness of the snacks. This pilot session enabled refinements to be made to the dietary intake. One of the changes was the use of self-sealed waterproof sandwich bags to prevent all the food from becoming wet!

3.9.2 Questionnaire design (Chapter 4)

After the initial questionnaire was designed, it was distributed to 10 subjects who were experienced hill walkers. Subjects were asked to give as much feedback as possible as to the design of the questionnaire, including such factors as the ease in which they could respond to the questions and clarity of the questions posed. On the basis of this beneficial feedback, the questionnaire was further modified to address the comments provided. The questionnaire is shown in Appendix 3.

3.9.3 Initial field study (Chapter 5)

Pilot work into selected walk and associated measurements was completed on five separate occasions prior to the main study. The initial visits were for the experimenter to practise the logistics of the walk. These logistics included the equipment required and to refine any navigational issues on the walk prior to leading subjects around. These proved to be of great benefit as the majority of the walks were in very severe weather. Any errors in navigation could have led to a serious compromise in safety. In the next three pilot days, the practicalities of the associated measurement were considered. Again this proved very worthwhile and emphasised the need to modify the equipment to make it practical for the use in the field. Some of these considerations were quite simple: spare equipment for the procurement of blood samples, tape to secure all the wires from the data logger and on-line respirometer so that they did not cause the wires to pull out in the strong winds, spare clothing and survival equipment.

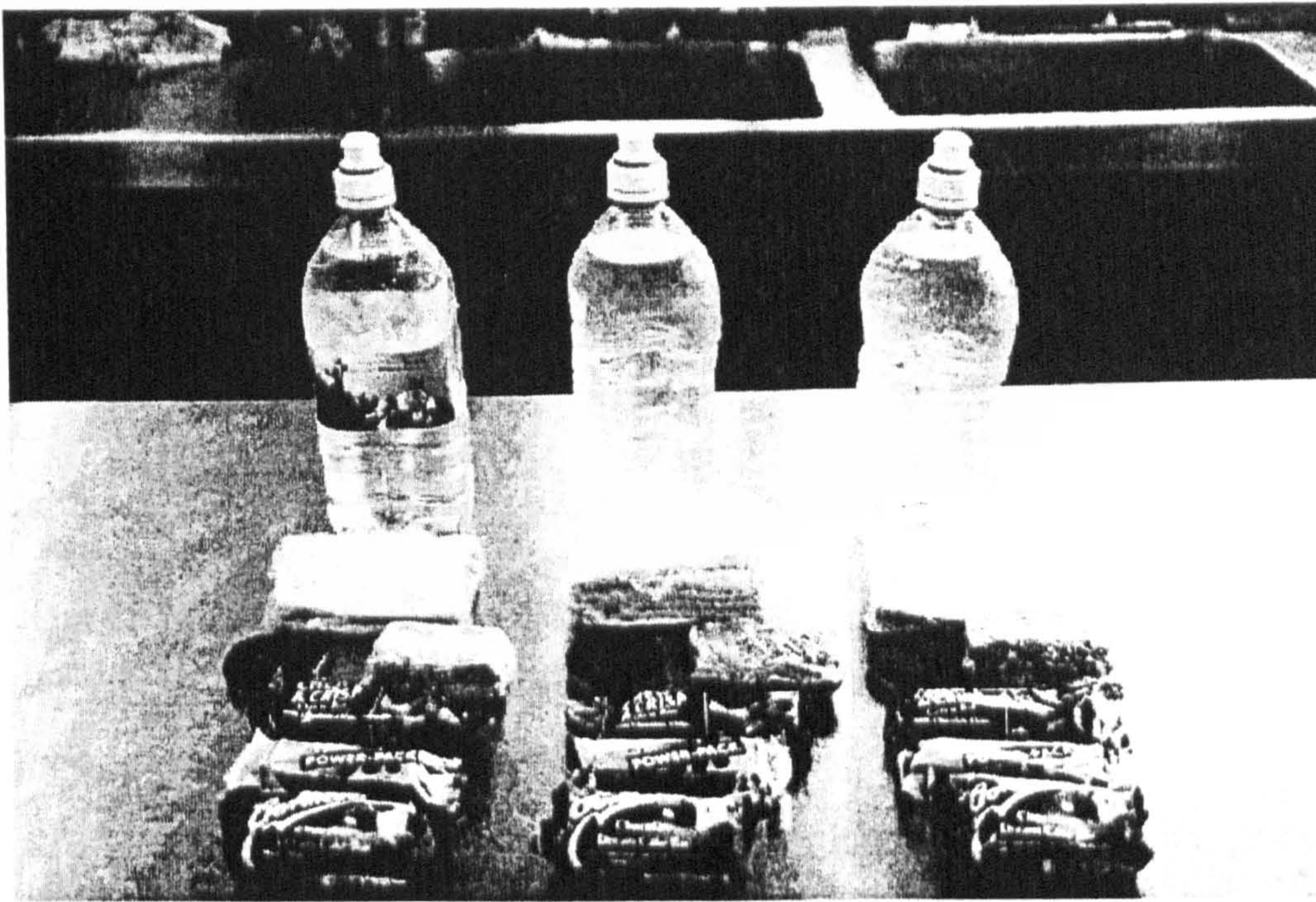


Plate 3.8. Appearance of the high-energy dietary intake.

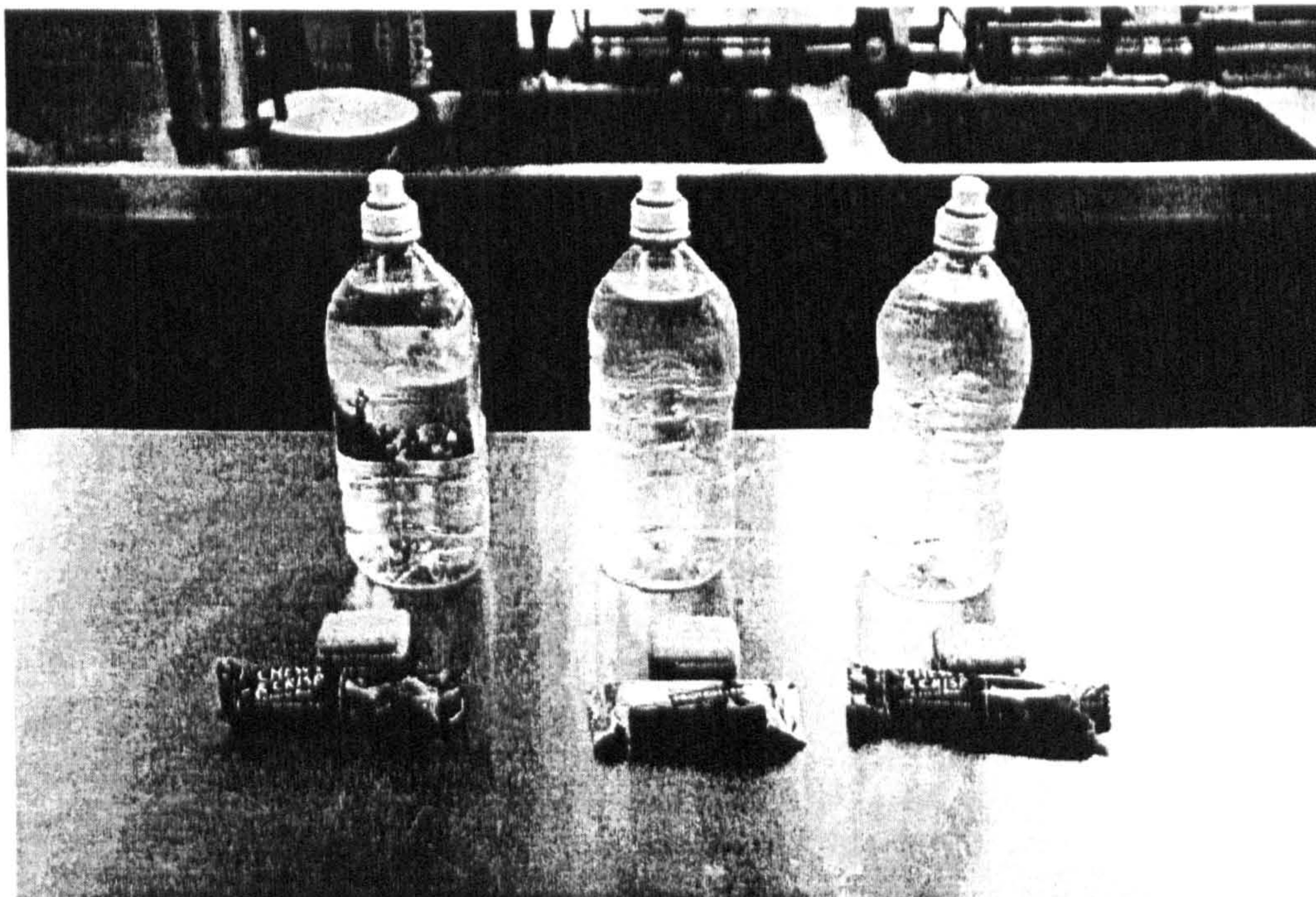


Plate 3.9. Appearance of the low dietary intake.

3.9.4 Laboratory based study (Chapter 7)

Pilot work in the design of the study was also conducted at Withington Hospital, Manchester. The pilot work enabled practical adjustments to the full-day exercise protocol in order to ensure subject comfort and compliance. Also, the pilot work

served as a beneficial way of familiarising the experimenter and medical doctors with the techniques to be performed in the main study.

3.9.5 Field study (Chapter 8)

Prior to the main field study, pilot work was conducted on three subjects over two days. Each subject consumed a high (~10 MJ), medium (~5 MJ) or no food during a strenuous 21-km walk. Prior to starting the walk and immediately upon completion, finger prick blood samples were taken for blood glucose concentrations and a urine sample for the analysis of urine osmolality. Likewise, subjects recorded their nude body mass prior to starting the walk and immediately upon completion. Fluid intake was allowed ad libitum. The results showed the blood glucose concentrations were marginally better maintained in the subjects who consumed the high intake compared with the low. Most interesting, however, was the observation that subjects lost between 3 and 6 kg in body mass. This loss in body mass could only be accounted for by the failure of the fluid intake to match the high rates of sweat loss which was likely incurred. This loss of body mass, dehydration, was also reflected in a near two-fold increase in urine osmolality concentrations. On the basis of this pilot study it was decided to control for fluid intake. The required fluid intake was calculated to be approximately 400 ml /h.

3.9.1 SUBJECT INFORMATION AND INFORMED CONSENT

The Human Ethics Committee of Liverpool John Moores University approved all studies reported within this thesis. In addition, for the laboratory based study, carried out in Withington Hospital Manchester, also had ethical approval granted by the South Manchester Medical Research Ethics Committee. During each experimental study, subjects were recruited by the means of local advertisement in Liverpool John Moores University and local walking clubs. All subjects were volunteers and were fully informed of the nature, purpose and possible risks associated with the study. Subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained.

CHAPTER 4

CHARACTERISTIC ACTIVITIES AND INJURIES OF HILL WALKERS

4.1 INTRODUCTION

Hill walking is one of the most popular recreational pursuits, yet there is a lack of information regarding the characteristic activities of such events. The prolonged duration of a typical hill walk places exceptional demands on the participants. The specific demands of hill walking tend to involve activity of varying intensity and duration, both of which are influenced by factors such as the physical fitness of the participant, dietary intake, back-pack weight and environmental weather conditions. The injuries occurring in the mountainous environment only receive attention in serious incidents involving the rescue services and consequently limit the recording of such incidents (Sharp, 2001).

In the present study, recreational hill walkers completed a questionnaire detailing characteristic patterns, injury, and typical nutritional and fluid intake for such activities. Information on the characteristic patterns such as distance, intensity, frequency, back-pack weight and typical rest and lunch breaks, was collected in order to gauge, firstly, the 'typical' activity for hill walkers and, secondly, to investigate whether these activity characteristics may be a factor in the incidence of injury. Questions relating to diet and fluid intake were also incorporated to enable the calculation of typical energy and fluid intakes, which are commonly used by hill walkers.

The aim of the questionnaire was, initially, to establish characteristic patterns of hill walkers and incidence of injury. A further aim was to quantify 'typical' fluid and energy intakes which are used for such activities. It was then hoped to establish any possible relationships between the various factors under analysis.

4.2 METHOD

4.2.1 Questionnaire content: A retrospective approach to data collection was adopted in this study. A questionnaire was designed to cover three main areas of interest. Initial questions were posed to establish the physical attributes and possible reasons for participation of the subject group. The three main sections of the questionnaires then followed, consisting of (a) hill walking activity details, (b) injury / accidents, and (c) typical fluid and nutritional habits. Details of the hill walking activity were sought in 10 questions regarding the distance, duration, intensity, frequency, back-pack weight and typical rest and lunch breaks. Questions in the injury section referred to the amount and type of injury/accident sustained, in addition to the location of the accident in relation to the hill walk. The final portion of the questionnaire contained questions to establish the typical fluid and nutritional intakes, used by walkers. This enabled the calculation of energy intakes using a nutritional data analysis system and standard food tables (Holland et al., 1991). Furthermore in this final section, questions concerning the timing of nutritional intake both prior to, and immediately after the hill walk were addressed.

4.2.2 Questionnaire distribution: The questionnaire was distributed, by mail, to over 500 members of two hill walking clubs in the UK, the Wayfarers and the Rucsac Clubs. Altogether 114 hill walkers responded to the questionnaire. From the 114 questionnaires, 14 were discarded due to failure in providing adequate responses to a number of the questions posed.

4.2.3 Statistical Analysis: Descriptive statistics were initially carried out for a general analysis. Frequencies and chi-squared analysis served this purpose. The final statistical procedure employed was either a Spearman's rank order correlation coefficient, when the data did not meet with parametric assumptions, or a Pearson's correlation coefficient for the analysis of parametric data.

4.3 RESULTS

4.3.1 Physical characteristics and reasons for participation: In total, 91 male and 9 female subjects completed the full questionnaire. In comparing the male and female hill walkers, not many variables were significantly different and thus were combined for most of the analysis. The age distributions of the subjects were, 44%, over 60 years; 24%, 50 - 59 years; 20%, 40 - 49 years; and 12% under 40 years of age. The physical characteristics of the subjects were body mass (range) 72.8 (47.1 - 102.1) kg, height 1.76 (1.61 - 1.9) m. The ratings of fitness, health and well-being as reasons for participation in hill walking are summarised in Fig. 4.1, illustrating that the majority of walkers considered fitness, health and wellbeing as been important or very important reasons for participation.

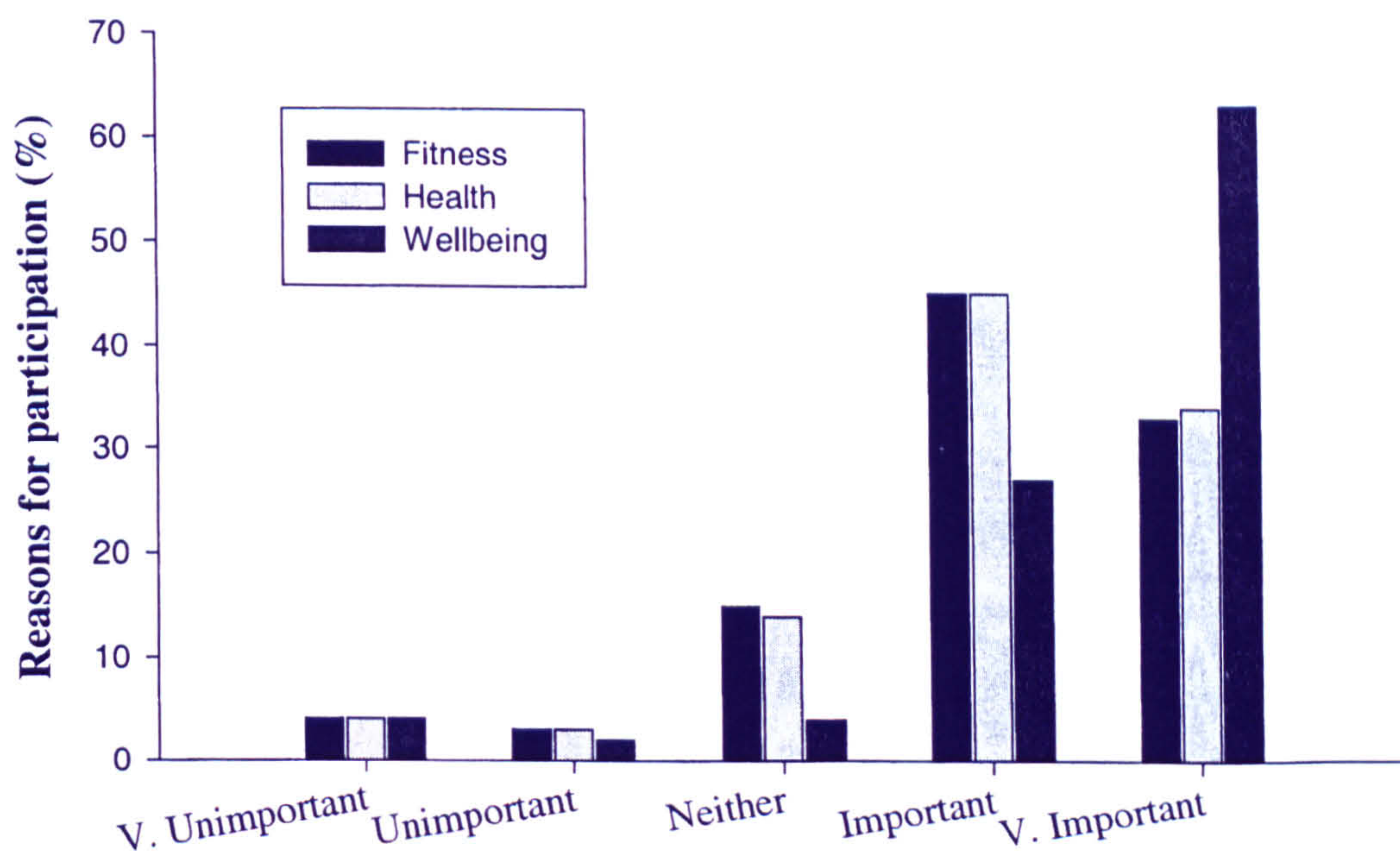


Figure 4.1. Reason for participation in hill walking (%) n = 100.

4.3.2 Hill walking activity details: When asked about their experience, 75% of the subjects had over 25 years of active hill walking participation, indicating the experience of the group. On average, subjects spent 57 (10 - 200) days per year out participating in hill walking, generally (67%) above 600 m in altitude. The average

distance and duration of the walks are shown in Fig. 4.2, illustrating that approximately 50% of the subjects walk between 18 – 26 km over 6 – 8 h.

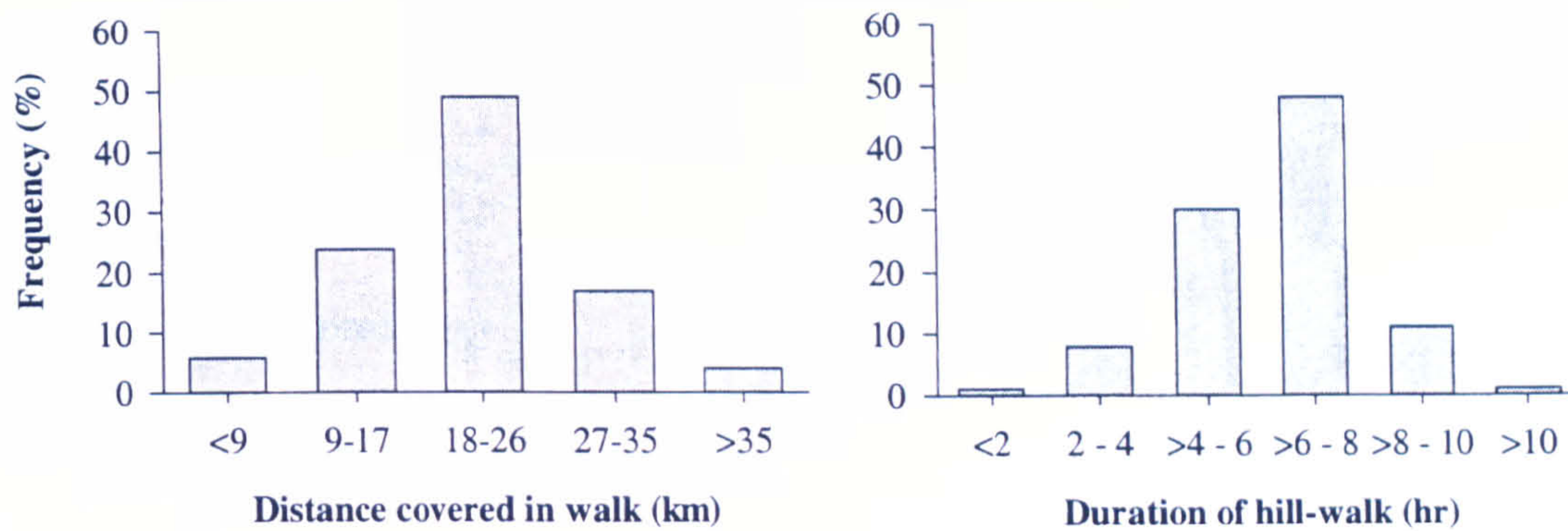


Figure 4.2. Typical duration and distance covered in a hill walk.

Overall, subjects described their particular intensity of walking as, very light (1%), light (4%), moderate (39), difficult (49%) or very hard (7%). On average, 90% of the subjects reported carrying a back-pack weight of 14 kg or less.

4.3.3 Incidence of injury / accidents during hill walk: The incidence of injury / accidents during hill walks was 35%. The incidence (% of subjects reporting the injury at least once) of many specific commonly occurring injuries was recorded in the questionnaire. Since many of the injuries occurred more than once, over different periods of time, the frequency of each injury is reported. In total, from the 35 subjects reporting injuries, there was 81 reported incidents of which 67% occurred at end of the walk, 25% near the middle and only 8% near the beginning of the walk. There was a mix of reported weather conditions with incidences occurring as frequent in summer as in winter walking conditions. The majority of the incidences (83%) occurred during descent. All of the reported injuries did not involve the aid of the mountain rescue services and consequently limit the recording of such incidents. Table 4.1 identifies the type and frequency of injuries, indicating that knee and ankle are the most commonly occurring problem.

Table 4.1. Type and frequency of injuries sustained during hill walking.

Injury	Incidence (Frequency)
Knee (fractures, sprains, and ligament damage)	22
Ankle (fractures, sprains, and ligament or tendon damage)	31
Feet (fracture)	3
Facial (cuts and bruising)	5
Back (muscular)	1
Wrist (fracture)	2
Calf (muscular)	4
Finger (fracture)	3
Other (General bruising from a slip / rock fall)	10

4.3.4 *Typical fluid and nutritional habits:* The frequency distribution of the typical fluid intakes on a hill walking day is highlighted in Fig. 4.3. Generally, 96% of subjects drank less than 1 litre prior to going out for a hill walk, 89% and 78% reported having between 0.25 - 1.5 litres during and following the post walk, respectively. The typical type of fluid used at each stage is given in Table 4.2. In addition to the fluid intake, the amount of water contained in the food consumed was also calculated to give a more accurate measure in the calculation of the total fluid intake.

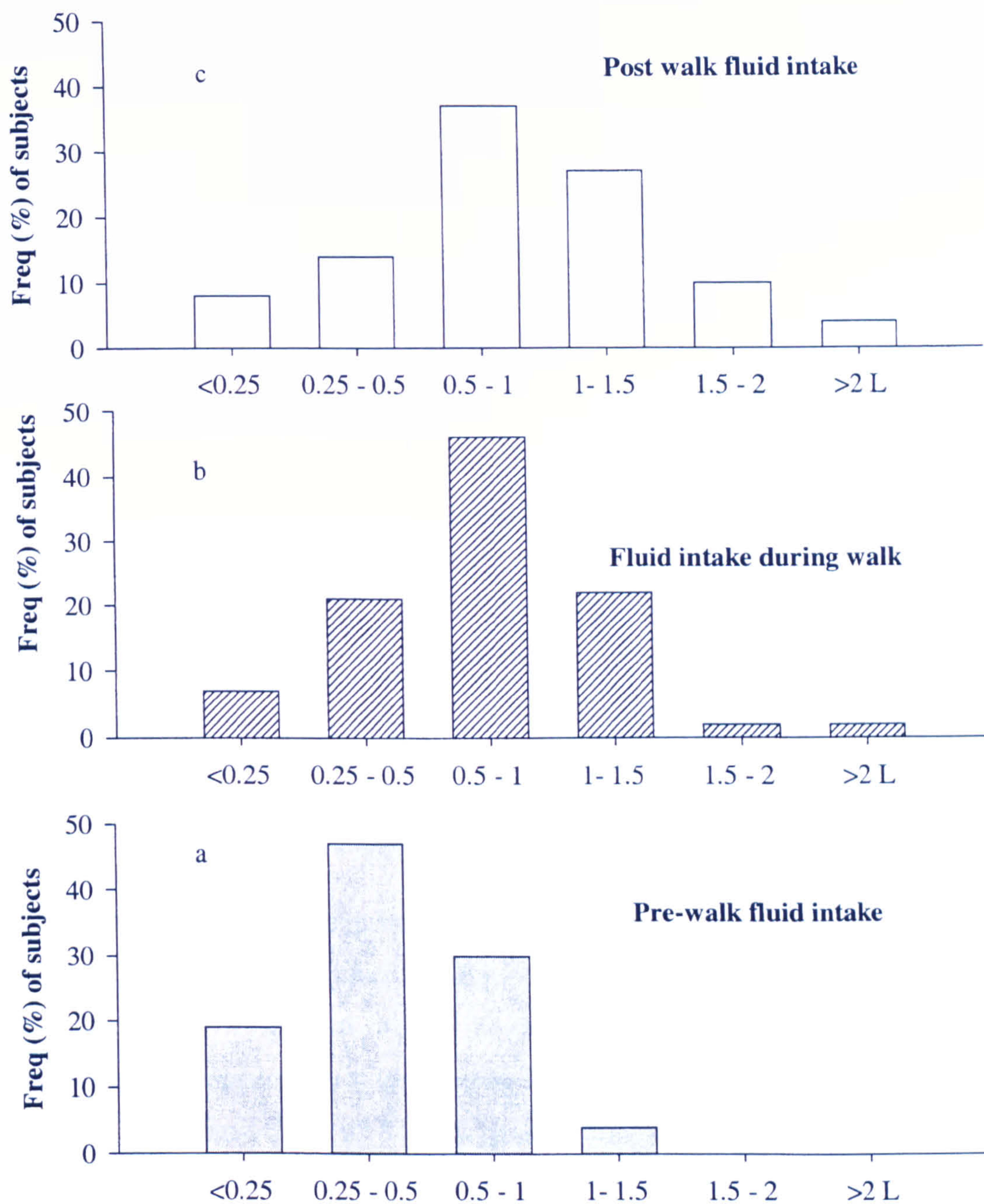


Figure 4.3. Distribution of typical fluid intake used a) prior to walking, b) during the walk and c) upon completion of walk.

Subjects were asked to list the type of fluid which normally constitutes their total fluid intake. Generally, walkers used a combination of drinks. Tea or coffee in addition to water was the most popular of the fluids consumed both prior to, and during the walk. The main preferences for fluid after completion of the walk were predominately tea, water and alcoholic drinks.

In addition to typical fluid intake and types, subjects were asked to give as much detailed information as possible about their typical nutritional intakes used during a walking day. This information was divided into the nutritional intakes used prior to the walk, during the walk and those for post-walk (Table 4.3). Generally, the total energy intake is within the population mean intake guidelines for the age of subjects, sufficient to meet normative requirements (Ralph, 2000). The comparable energy intakes used by the walkers monitored in this questionnaire and the reference population indicates that the energy intakes that walkers commonly use are insufficient to match the increase in energy expenditure as a consequence of the walk.

Table 4.2. Average energy and macronutrient intake on a hill walking day. Values are mean \pm SD.

Stage of walk	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
Pre-walk	2018 \pm 908	16 \pm 9	16 \pm 10	73 \pm 34
During walk	3566 \pm 1402	26 \pm 12	34 \pm 18	118 \pm 51
Post-walk	4943 \pm 1482	49 \pm 36	49 \pm 21	142 \pm 72

The macronutrient intake expressed as a percentage of energy intakes at the different stages of the walk is illustrated in Fig. 4.4. There was a high intake of CHO for breakfast (74%) with equal intakes of protein and fat. During the walk, however, protein intake remained similar whereas fat intake increased to 36 % of the total intake, CHO decreasing to 45 %. This general pattern of increased fat with a decrease in CHO intakes is similar to the intake post-walk. The timing of the food intake, relative to starting the walk was -81 min (-10 to -120 min) compared with consumption of food post-walk of +92 min (+25 to +180 min).

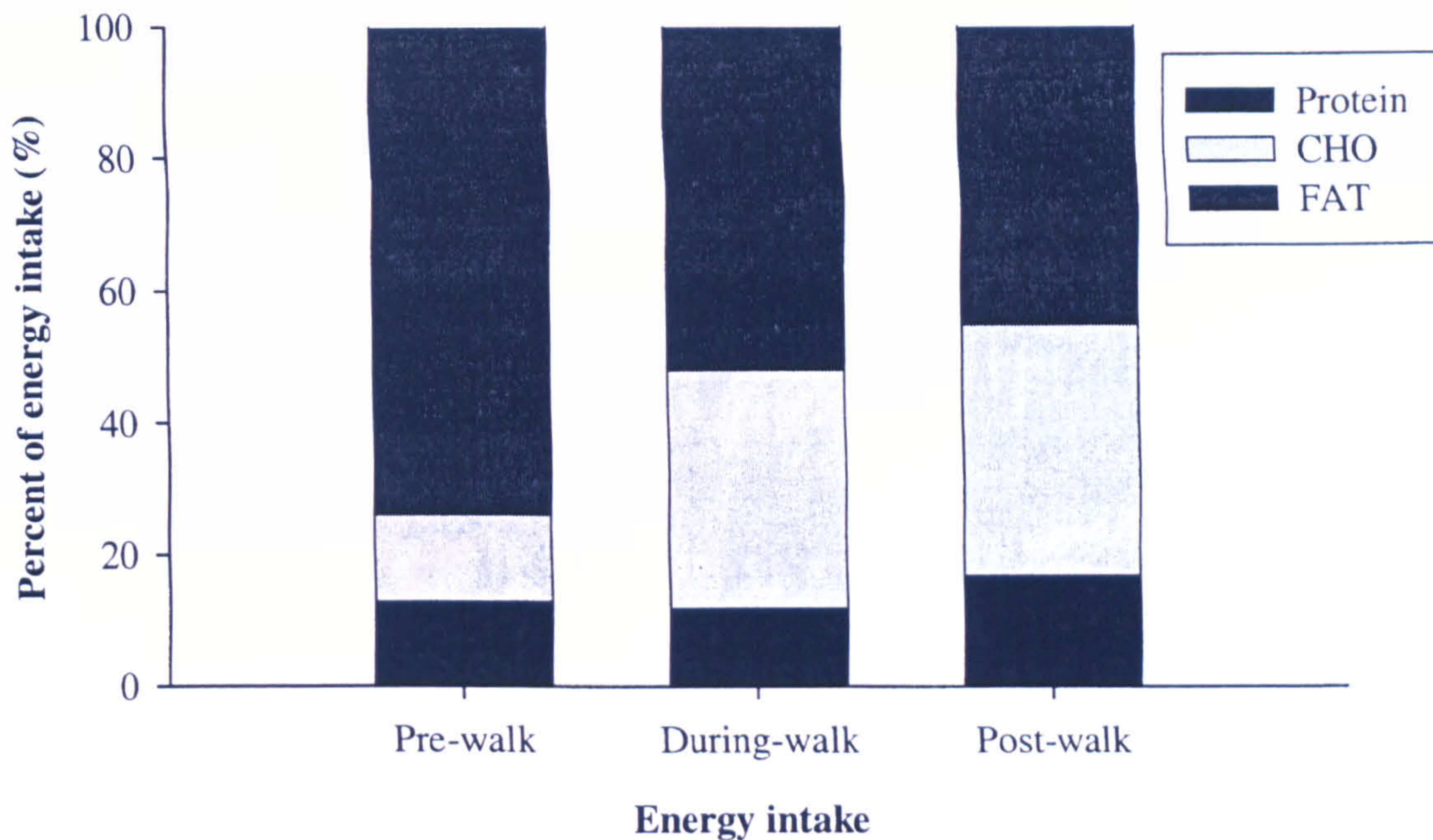


Figure 4.4. Distribution of macronutrient intake prior, during and following a hill walk.

4.3.4 Correlation Analysis: The final analysis carried out generated a correlation matrix of all variables in order to establish any relationships which might exist across the questionnaire. The only physical attribute to correlate to any other variables was the age group of the subjects (Table 4.3). Age was positively correlated to years of hill walking experience ($r = 0.44$, $P < 0.01$), reasons for participation, injuries sustained ($r = 0.49$, $P < 0.01$) and to the energy intake prior to walking ($r = 0.44$, $P < 0.05$). Negative correlations were found to exist between age and the distance of walk ($r = -0.36$, $P < 0.01$), back-pack weight ($r = -0.26$, $P < 0.01$) and fluid intake during the walk ($r = -0.41$, $P < 0.01$).

Table 4.4 gives the correlations relating to the characteristic activities of hill walking. Some positive correlations were found to exist between the duration of the walk and the intensity ($r = 0.25$, $P < 0.05$), distance ($r = 0.56$, $P < 0.01$) and back-pack weight carried ($r = 0.36$, $P < 0.05$).

Table 4.3. Correlations related to physical characteristics and reasons for participation.

	<u>Physical Characteristics</u>	<u>Reason for participation</u>		
	Age (yr)	Fitness	Health	Well-being
Years of HW (yr)	0.44**			
Fitness			0.90*	
Health				
Well-being		0.49*	0.50*	
Distance of HW (km)	-0.36**			
Back-pack weight (kg)	-0.26**			
Lunch stop			-0.28**	-0.26*
Injuries (freq)	0.49**			
Fluid during (l)	-0.41*			
Pre- Energy Intake (kJ)	0.44*			

Table 4.4. Correlations related to some of the characteristic activities of hill walking.

	<u>Characteristic hill walking activities</u>								
	HW (years)	Alt (m)	Freq (days)	Duration (h)	Intensity	Distance (km)	Bp (kg)	Rest (min)	Lunch (min)
Age	0.44*		-0.22*			-0.36**	-0.26**		
HW						-0.25*			
Fitness									-0.28**
Health									-0.26*
Alt				-0.24*	-0.43**				
Freq				-0.24*					
Duration		-0.24*			0.25*	0.56**	0.36*		
Distance					0.56**	0.38**			
BP					0.36*				
Injuries		0.37**	-0.41*						
Fluid during	-0.21*	-0.22*			0.32*	0.33*			
EI during	-0.21*			0.24*	0.29*				
Evening meal									-0.24*

The correlations relating to injuries sustained during the hill walks are given in table 4.5, indicating positive correlations between injuries and age ($r = 0.49$, $P < 0.05$) and walking at altitudes above 600 m ($r = 0.37$, $P < 0.05$). Negative relationships were found to exist between injuries and the frequency of hill walks ($r = -0.41$, $P < 0.05$) and that of the energy intake during the walk ($r = -0.26$, $P < 0.05$). In other words there was a greater incidence of injuries occurring in older subjects, walking above 600 m, in the less frequent walkers, and in walkers consuming a lower energy intake during the walk.

Table 4.5. Correlations related to injuries occurred during a hill walk.

	Injury
Age (yr)	0.49*
Altitude (m)	0.37*
Frequency	-0.41*
Amount of injuries	0.64**
Energy Intake during (kJ)	-0.26*

Various correlations relating to fluid and energy intake are given in table 4.6. Some interesting correlations were found to exist between an increase in age and a decrease in fluid intake during the walk ($r = -0.41$, $P < 0.05$) and also between a lower energy intake during the walk and the incidence of injury ($r = -0.26$, $P < 0.05$). Also, the harder the intensity and longer the distance of the walk, then, generally, the greater the fluid and energy intakes during the walk.

Table 4.6. Correlations relating to fluid and nutrition intakes.

	<u>Fluid intake (l)</u>			<u>Energy Intake (kJ)</u>			<u>Time of intake</u>	
	Pre-	During	Post-	Pre-	During	Post-	Prior	Post-
Age		-0.41*						
HW		-0.21*						
Alt		-0.22*			-0.21*			
Frequency								-0.25*
Duration				0.24*	0.24*			
Intensity		0.32*			0.29*			
Distance		0.34*			0.24*			
Injuries					-0.26*			-0.24
Fluid intake (pre)			0.43**					
Fluid intake (during)			0.27*	0.24*	0.24*			
Energy intake(during)			0.24*					
Energy intensity (post)								0.21*

DISCUSSION

The main findings of the present study are firstly, the characteristic patterns of recreational hill walkers indicate a normal distance covered of 18 – 26 km over 6 – 8 hours in duration and predominately over 600 m in elevation. Secondly, the pattern of injuries highlights the prevalence of knee and ankle damage, generally occurring near the end of the walk and predominately when moving downhill. Additionally, relationships were found to exist between the incidence of injuries occurring in older subjects, walking above 600 m, in the less frequent walkers and in walkers consuming a low energy intake during the walk. Finally, the quantification of typical energy intakes used for such hill walking events show that, on average, they are not higher than the normal reference energy intakes for the different age groups. Whilst clearly not causality linked, there was a relationship between low energy intakes and the incidence of an injury occurring during the hill walk. Additionally, there was a negative relationship between age and fluid intake during the walk.

4.4.1 Injuries: The high occurrence of injuries, predominantly during the downhill section is an important consideration. The observation that the injuries are occurring during the activity is different from other non-contact sports in which the injuries normally occur during training, for example orienteering and running (Creagh et al., 1992; Maughan and Miller, 1983). In the current activity there is little strenuous training on normal terrain. It is thought that injuries and pain which occur during down-hill walking are caused primarily by high loads on the joints of the lower extremities (Blake and Ferguson, 1993; de Loes, 1995). These injuries and pain will potentially be increased by the carrying of additional weight in the form of a back-pack (Jacobson et al., 1997). Furthermore, carrying a back-pack, clearly a necessity in the mountainous environment, will decrease both lateral stability and balance (Jacobson et al., 1997). The knee joint, in particular, receives higher loads during downhill walking. Kuster et al. (1995) reported significantly increased peak ground forces (+38%), peak knee flexion moments (+117%) and peak knee muscle power (+490%) during downhill walking, on a treadmill, at a decline of 11° compared with normal walking. These results indicate that downhill walking is stressful for different knee joint structures (Kuster et al., 1994). Importantly, however, is the consideration that the terrain encountered during a mountainous hill walk is liable to be up to a 35°

incline and/or decline, or steeper if scrambling, suggesting the loads received may be exceptionally higher.

An even higher incidence of injury was reported to the ankle, than that of the knee, including fractures and tendon damage. Excessive uphill or down-hill walking or running places increased stress upon the achilles tendon and ankle joint (Creagh et al., 1998a,b). When descending steep terrain, the forefoot strikes with greater force because it has further to drop and more time to accelerate (Mortensen, 1999; Grampp et al., 2000). Furthermore, traversing difficult, uneven terrain where the foot constantly must invert and evert is not only liable to risk a sprained ankle, but also potential micro-trauma to the achilles. Additionally, hill walking normally requires the use of shoes which are excessively stiff at the ball of the foot and do not allow the shoe to flex adequately, increasing the stress on the achilles tendon as the calf muscles work harder to lever the entire foot and heel off the ground.

One potential aid to combat the stress to the lower extremity joints during up and down-hill walking is the use of walking poles. Recent laboratory research indicates that the use of two walking poles during down-hill walking increases the maintenance of static balance and lateral stability (Jacobson et al., 1997), reduces external and internal loads on the knee and hip joints (Schwameder et al., 1999) and allows improved breathing and efficiency owing to an upright posture during uphill walking (Knight et al., 1998). Any reduction in the loads incurred during down-hill walking and an improved efficiency during up-hill walking will benefit all hill walkers, especially walkers with lower extremity injuries.

4.4.2 Fluid and nutrition intake: Both hypohydration and negative energy balance have a negative effect on physical performance as well as overall health (Calloway, 1987; Santora, 1987; Chandra, 1990; Shirreffs, 2000). The impact of hypohydration and the failure to provide sufficient energy intake for hill walking in the mountainous environment may lead to an increased exertional fatigue, susceptibility to hypothermia, and an potential for increased incidence of injury. Therefore, the question that needs to be addressed is what are the fluid and energy needs for hill walking? This very question is the focus of Chapters 5 and 6.

The estimated energy intake from the present study provides additional evidence that hill walkers are operating at a marked negative energy balance. Furthermore, the typical characteristics of hill walking suggest that the walkers, on average, walk between 18 - 26 km - this distance could potentially elicit a higher energy expenditure than that of 14 .5 MJ recorded over 12 km (reported in chapter 5), depending on the severity of the terrain. As mentioned there was small, but significant relationship existing between subjects who had lower energy intakes and the incidence of an injury occurring during the hill walk. Although not cause and effect, a lower energy intake may lead to an increase in fatigue which could possibly be an factor in increasing an individual's susceptibility to injury. This safety consideration would over-rule the health-benefits of such exercise used for weight-reduction purposes.

In the present study fluid intake prior to, and during the walk was similar to that previously reported (in Chapter 5), suggesting the need for greater fluid requirements to maintain hydration during hill walking pursuits. Potential reasons for this fluid deficit include high sweat losses, blunted thirst - especially in older people (Sawka and Montain, 2000), cold-induced diuresis, increased respiratory water losses, conscious under drinking, and poor availability of water (Freund and Sawka, 1995; O'Brien et al., 1998). In addition, there was a negative correlation between age and fluid intake during the walk. There is some evidence in the literature suggesting that older adults (>55 years) have a reduced thirst sensation, less ability to concentrate urine, and reduced potential to dissipate body heat (Kenny and Fowler, 1988; Mack et al., 1994; Kenny, 1995).

The major conclusions from the present study are:- firstly, there is a high prevalence of lower limb injuries sustained predominately during downhill walking, nearing the end of the walk. Secondly, the typical energy intakes used during a walk are probably inadequate to balance the high-energy turnover of such prolonged activity. This potential negative energy balance may one factor which increase exertional fatigue and leads to an increased susceptibility of injury in the mountainous environment.

The main practical recommendations generated from this questionnaire analysis are:-

- 1) The use of walking poles may confer some additional protection against lower limb injuries during downhill walking and may increase efficiency during uphill sections.

- 2) Walkers should take more food and/or foods with higher energy content to help increase energy intake and provide a measure of protection if the walk becomes unexpectedly prolonged.

Future research is needed to further investigate the effects of operating at a negative energy balance on the susceptibility to injury and fatigue in the mountainous environment.

CHAPTER 5

PHYSIOLOGICAL AND METABOLIC ASPECTS OF A HILL WALK

5.1 INTRODUCTION

The prolonged duration of a typical hill walk places exceptional demands on the participants. The specific demands of hill walking incorporate activity of varying intensity and duration, both of which are influenced by factors such as the physical fitness of the participant, dietary intake, backpack weight, environmental weather conditions and severity of the terrain. Besides, hill walkers can be caught unexpectedly and unprepared when rain and wind accompany outdoor activities in cool weather. Decreased thermal insulation of wet clothing presents a serious challenge to body temperature regulation, which can be compounded by fatigue associated with prolonged exercise such as hill walking (Pugh, 1966; Pugh, 1967; Thomson and Hayward, 1996; Noakes, 2000).

The problem of wet-cold hypothermia is recognised by the search and rescue organisations world-wide. Nevertheless their ability to design educational material concerning this hazard is hindered by lack of knowledge of the physiological and psychological responses (Thomson and Hayward, 1996). The information that is available derives from the pioneering work of Pugh (Pugh, 1966a; Pugh, 1966b; Pugh, 1967), supplemented by anecdotal descriptions of exposure incidents (Pugh, 1966; Hunter, 1968; Strang, 1969; Kreider, 1991; Noakes, 2000).

Pugh (1966) proposed that maintaining a $\dot{V}O_2$ of 2-2.5 l/min or 50-60% maximum oxygen uptake ($\dot{V}O_{2\text{max}}$) would offset heat loss and combat the debilitating effects of the cold, wet and windy environment. Even though these observations were based on only three subjects, recent work by Weller et al. (1997a) supported Pugh's postulate. Both experiments were based in an environmental chamber, subjects exercised on a cycle ergometer and treadmill, respectively. Pugh (1967) and Weller et al. (1997a) showed that when exercise metabolism is reduced, the increase in shivering may be insufficient to prevent a decrease in deep body temperature. Weller et al. (1997a) reported that rectal temperature (T_{rec}) and the metabolic responses to an initial 120-min phase of exercise at approximately 60% of peak $\dot{V}O_2$ were not influenced by the cold stress of a wet and windy environment. However, during a subsequent 240-min phase of exercise at 30% $\dot{V}O_2$ peak, T_{rec} was lowered by 0.6°C, whereas the following were elevated: $\dot{V}O_2$ (25%), the proportion of carbohydrate (CHO) oxidised, and the venous concentrations of lactate, glucose, noradrenaline, and adrenaline.

The studies reviewed have been limited to simulated conditions. Consequently the influence of a 'typical' day's hill walk on a range of physiological and metabolic variables, in the field, has not been established. Given such limitations, the aim of the current study was to investigate selected responses to a typical hill walking event in order to gauge the overall physiological and metabolic strain. The field conditions would likely impose additional stresses not encountered during simulated conditions, such as stoppage of activity for fluid and food intake (transiently altering the balance between heat production and heat dissipation), in conjunction with varying terrain and weather conditions. Furthermore, we aimed to quantify both the energy cost of such activities and relevant responses that are important in the safety of hill walkers, such as the potential thermal stress, impaired psychomotor performance and the ability to maintain glycaemia. This type of study may be important in adding to the mostly anecdotal information regarding exposure and recreational activities. Due to the continuously changing intensity of activity, it is unlikely that individuals will be able to operate at or above 50-60 % $\dot{V}O_{2\max}$, the $\dot{V}O_2$ 'cut-off' point described Pugh (1967) for combating heat loss during a hill walk. Whilst Pugh's postulate may have an element of truth, it would depend on factors such as favourable ambient temperature, the clothing worn, terrain and the physiological capabilities of the participants. The first hypothesis, therefore, is that the $\dot{V}O_2$ cut-off is not a realistic component of hill walking. Secondly, it was hypothesised that a seemingly 'normal' hill walk in possible adverse, but not uncommon, conditions leads to a significant physiological, psychomotor, and metabolic stress on the body.

5.2 METHODS

5.2.1 Subjects: Thirteen subjects, 11 male and 2 female, participated in this study which was reviewed and approved by the Human Ethics Committee of Liverpool John Moores University. The subjects gave written consent to participate in the study after they had been fully informed of the nature, purpose and possible risks associated with the study. The physical characteristics of the subjects are shown in Table 1. The majority of the subjects were active and experienced hill walkers. Experiments were conducted from January through March. Percentage of body fat (%fat) was estimated as described in section 3.1.3. Fitness level was established by using a continuous incremental treadmill running test to exhaustion (American College of Sports Medicine, 1986) as described in section 3.2.

Table 5.1. Physical characteristics of the 13 subjects.

	Age, year	Mass, kg	Height, m	BMI, kg/m ²	Surface Area, m ²	Body Fat, %	$\dot{V}O_{2\text{ peak}}$ ml/kg/min
Mean	25.6	72.5	1.8	22.7	1.9	17.2	58.9
SE	1.9	2.9	0.03	1.1	0.05	2.4	2.1
Range	18-32	55-82	1.7-1.9	18.3-26.7	1.6-2.16	9.9-30.4	47.7-67.0

Protocol and procedures: On separate occasions subjects completed a 12-km (8 mile) hill walk. The course varied in elevation from 100 m to 902 m above sea level and consisted of a range of gradients and terrain typical of a mountainous hill walk. A caravan was used as a temporary field laboratory and for living accommodation, and was located at the start and end of the hill walk. Subjects woke each morning between 05:00 and 05:30 hours and completed the preliminary experiments prior to the hill walk, shown in Figure 1. Self-paced walking began each day between 07:00–08:00 hours. Prior to the walk and upon its completion, the subjects weighed themselves nude. Subjects were permitted fluid and food *ad-libitum*. They selected their own food and fluids for the walk, which were pre-weighed prior to the walk. The energy gained

from CHO, fat and protein was subsequently determined using standardised food tables (McCance et al., 1991). Following the initial weighing the participants inserted a rectal temperature probe to a depth of 10 cm beyond the anal sphincter. Skin temperature was assessed by the placement of temperature thermistors on the chest, forearm, thigh and shin. Thermistors and the rectal probe were connected to a data logger (Squirrel meter 1000, Grant Instruments, Cambridge, UK) which recorded data every 6 min. On the walk a rest period of approximately 1 – 3 min was allowed every time thermal measurements were made, and 30 min for lunch (Figure 5.1). During the hill walk, respiratory gas exchange measures were obtained with a portable telemetry system (Metamax, Cortex Biophysik GmbH, Borsdorf Germany). All subjects carried a lightweight water-proof backpack that contained the Metamax system and thermal logger to allow continuous recording of respiratory gas exchange and rectal and skin temperatures, respectively. The loaded pack weighed 9.5 kg, which is consistent with a hill walking scenario.

Continuous respiratory gas exchange, thermal and IIR measurements

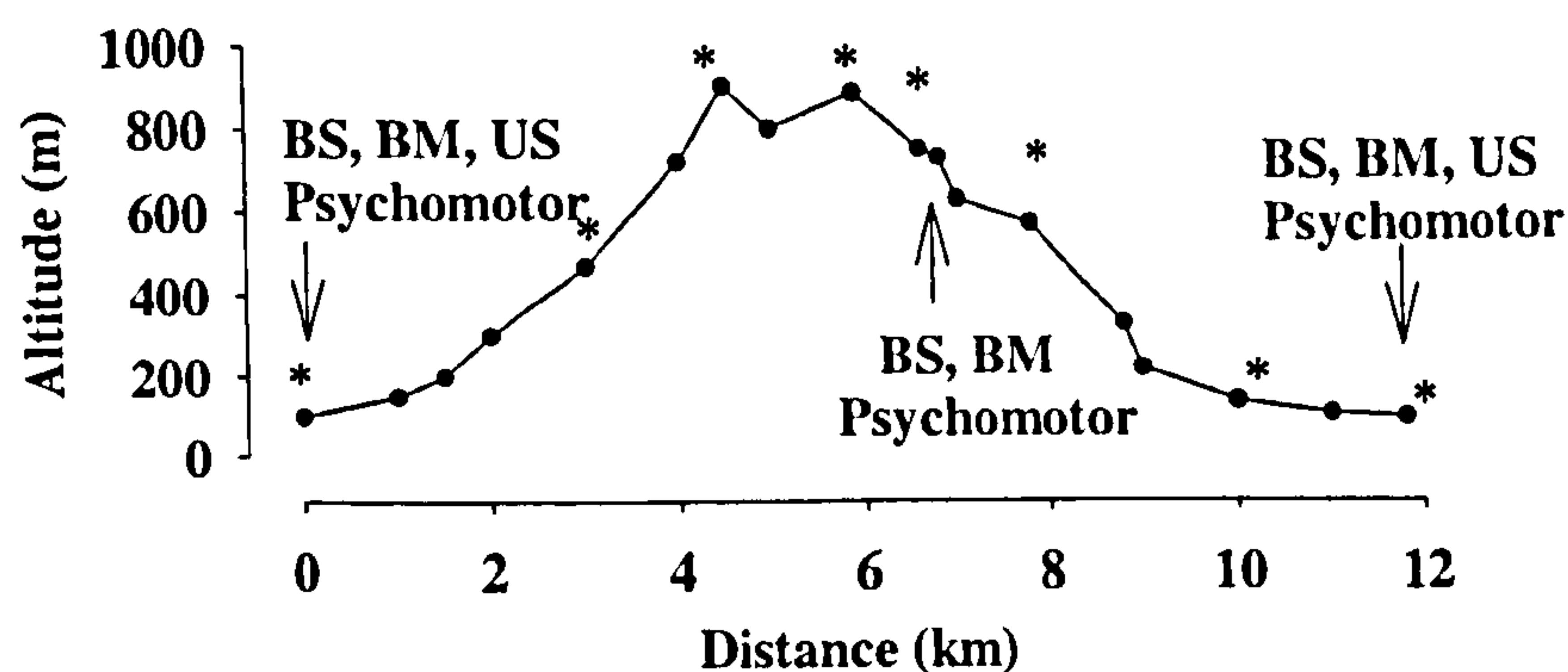


Figure 5.1. Illustrated profiles of hill walk and associated measurements. BS, blood sample, BM, nude body mass, US, urine osmolality, Psychomotor, profile of mood state, ratings of perceived exertion, cognitive function (reaction time) and grip strength measurements. *Wind chill index measurements.

5.2.2 Measurements and Analysis:

5.2.2.1 Temperature and heart rate: The measurements of rectal temperature (T_{rec}), skin temperature (T_{sk}) and heart rate are as described in section 3.7.1. Mean skin temperature, mean body temperature and thermal balance were measured and calculated as described in section 3.7.1.

During one of the experiments the data logging system malfunctioned. Furthermore, one subject was not willing to use a rectal probe and therefore the Trec data were based on 11 subjects and the \bar{T}_{sk} data were based on 10 subjects.

5.2.2.2 Environmental measurements: Measurements of the environmental conditions (wind speed, dry and wet bulb temperatures), and calculation of the wind chill index are given in section 3.7.2.

5.2.2.3 Indirect calorimetry: Continuous assessment of respiratory gas exchange was performed by the use of a portable telemetry system. Signals from the Metamax system were logged and subsequently retrieved at the end of the hill walk. This procedure allowed for continuous monitoring of $\dot{V}O_2$, ventilation and respiratory exchange ratio. Energy expenditure and the percentage contributions of the CHO and fat oxidation were estimated from non-protein (NP) $\dot{V}O_2$ and RER data as described in section 3.3.1.1. and 3.3.1.2.

Prior to use, the Metamax system was calibrated using both calibrated gas and ambient air. The volume transducer was calibrated using a 3-litre syringe. To decrease any error, the system was re-calibrated during the hill walk when the subjects stopped for lunch. Due to technical problems during three of the walks, the respiratory gas exchange data were based on 10 subjects.

5.2.2.4 Psychomotor measurements: The Profile of Mood State (POMS) and overall ratings of perceived exertion (RPE) (Borg, 1970) were measured as described in section 3.8. Equally, reaction time (cognitive function) and grip strength (motor function) were measured as described in section 3.9.2 and 3.9.1, respectively. The subjects were fully familiarised with the use of the equipment.

5.2.2.5 Blood and urine sampling and analysis: Blood samples were taken and stored as described in section 3.4.1. and as illustrated in Figure 5.1. The venous blood samples (5 ml) were drawn from a superficial forearm vein with minimum stasis, then immediately placed in a vacuum flask containing ice. The concentrations of the metabolites blood glucose, serum NEFA and TAG, glycerol, lactate, 3-OHB were measured as described in sections 3.5.1-3.5.3. Equally, the concentrations of the hormones insulin and cortisol were measured as described in section 3.5.4. Furthermore, the concentration of haemoglobin and percentage haematocrit in whole blood were measured in order to calculate changes in

plasma volume. These methods and calculations are given in section 3.4.2.

Urine was collected during the rest day prior to the hill walk, in which subjects performed no exercise, and during the hill walk at the following times; 08:00–13:00, 13:01–18:00 and 18:01–20:00 h. From these collections, a 5-ml mixed sample was removed. Urine adrenaline (Adn), noradrenaline (Noradn), creatinine and dopamine concentrations were then analysed as described in section 3.5.5. Index of dehydration was determined in triplicate using urine osmolality, as described in section 3.6.1. For the urine osmolality, a 5-ml sample was produced after the first void of the day, and then from the first sample after the walk.

5.2.2.6 Statistical Analysis: Variables are presented as means \pm standard error (SE). Data were initially tested for normality, before being analysed by repeated-measures analysis of variance (ANOVA). The ANOVA results were corrected by the Huynh-Feldt ϵ -adjusted degrees of freedom when the violation to sphericity was minimal (>0.75) and the Greenhouse-Geisser correction used when sphericity was violated (<0.75), and significant condition and condition-time interactions were identified (Field, 2000). Post hoc tests (Honestly Significantly Different) were performed to isolate any significant differences. Student's paired t-tests ascertained between-condition differences when a variable was measured once. Statistical significance was set at $P \leq 0.05$ for all statistical tests.

5.3 RESULTS

5.3.1 Exercise duration: All subjects completed the 12-km hill walk. The mean (range) duration for the hill walk was 348 (245 - 490) min. The differences in the time to complete the walk were due mainly to variations in weather conditions and terrain. Both cold, wet and windy weather, and deep snow underfoot led to an increased time to complete the hill walk.

5.3.2 Energy balance: Energy intake and energy expenditure during the hill walk are given in Table 5.2. Total energy intake was lower than total energy expended during the hill walk in all subjects. Carbohydrate, fat and protein comprised 65, 25 and 10%, respectively of all food consumed, in contrast to 47, 42 and 11%, respectively of fuels oxidised. The energy intake from both CHO and fat was lower than the amount oxidised ($P < 0.001$), leading to the lower energy intake relative to expenditure ($P < 0.001$). The relatively high energy expenditure of 14.5 ± 0.5 MJ reflects the high energetic cost of hill walking, even when pursued over a relatively short duration. The negative energy balance is also shown by a decrease (72 ± 2 to 70 ± 2 kg, $P < 0.05$) in nude body mass as a consequence of the walk.

Table 5.2. Total energy, carbohydrate (CHO), fat and protein intake vs. total energy expenditure, CHO, fat and protein oxidation's during the hill walk.

El, MJ	EE, MJ	CHO, g	CHO _{ox} , g	Fat, g	Fat _{ox} , g	P, g	P _{ox} , g
5.6 ± 0.7	$14.5 \pm 0.5^{***}$	232 ± 30	$432 \pm 16^{***}$	37 ± 4	$162 \pm 6^{***}$	41 ± 4	95^1

Values are means \pm SE; n = 10. EI, energy intake; EE, energy expenditure; CHO, Fat and P, carbohydrate, fat and protein intake; CHO_{ox}, Fat_{ox}, P_{ox}, amount of carbohydrate, fat and protein oxidised. Significant differences between total energy, CHO and fat intake compared with total energy expenditure, CHO and fat oxidation during the hill walk: *** $P \leq 0.001$ for expenditure / oxidation vs. intake. ¹, it was assumed that protein oxidation contributed 12.5% of energy expenditure at rest and that exercise did not alter this relative rate of protein utilisation.

5.3.3 *Thermoregulatory and environmental data:* The mean (range) of the recorded environmental data for air velocity, air temperature and wind chill index was 2.8 (0.1 - 10.4) m/s, 6.4 (-1.3 - 13.2) °C and 520 (176 - 1239) kcal/m²/h, respectively. These figures highlight the variability in the weather conditions over the period of testing. Five of the walks were completed in cold, wet and windy weather. The surface conditions on the walks tended to vary with the weather. Snow and ice were regularly encountered, along with high winds, as reflected by a high wind chill index; these factors represent walking in very demanding climatic conditions.

The rise in \bar{T}_{rec} as is illustrated in Fig. 5.2C is a typical response during both exercise and the initial stages of cooling, due to peripheral vasoconstriction decreasing the return of cooled blood from the periphery. Also reflected at this point were the apparent decreases in \bar{T}_{sk} (Fig 5.2A). Mean body temperature was maintained relatively well during the hill walk, until the stop for lunch at mid-walk (Fig 5.2A). A loss in \bar{T}_{b} was evident at this point, before a subsequent rise when walking commences again.

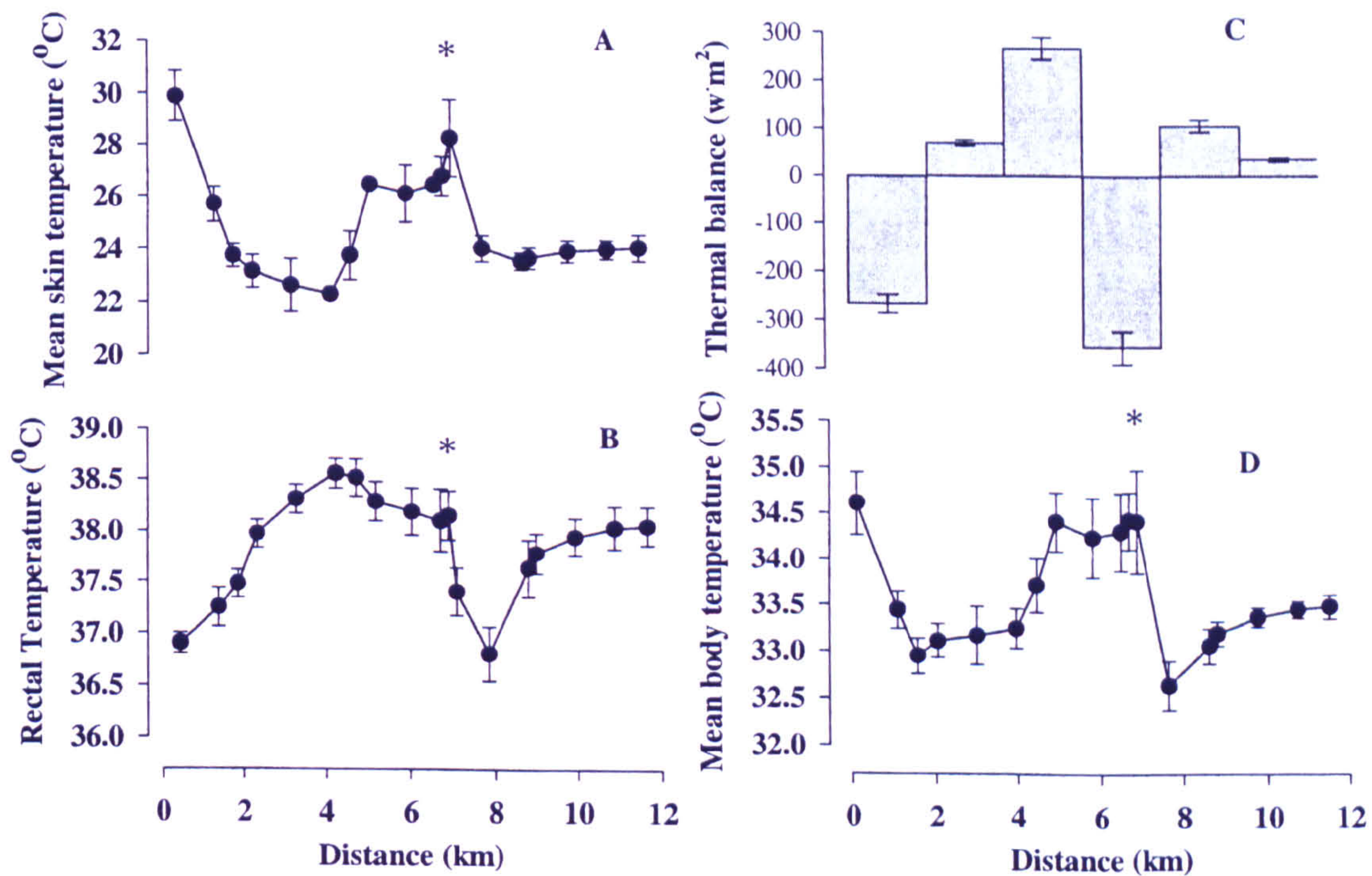


Figure 5.2. Thermoregulatory responses during the hill walk. * denotes the point at which a 30-min rest was taken for lunch. Mean (SE), n = 11).

Fig. 5.3. illustrates an apparent temperature 'afterdrop.' This drop was reflected in a further decrease in T_{rec} after walking recommenced. Mean skin temperature showed a typical rise during the 30-min rest at the mid-point of the walk, followed by a subsequent decrease when walking commenced (Fig. 5.3). Thermal balance demonstrated an initial decrease during the first 2-km walk, before gaining a positive balance (Fig. 5.2B). A marked negative balance ($-356 \pm 23 \text{ w/m}^2$) was observed during the lunch stop, and for the subsequent 2 km after this before gaining a positive balance again.

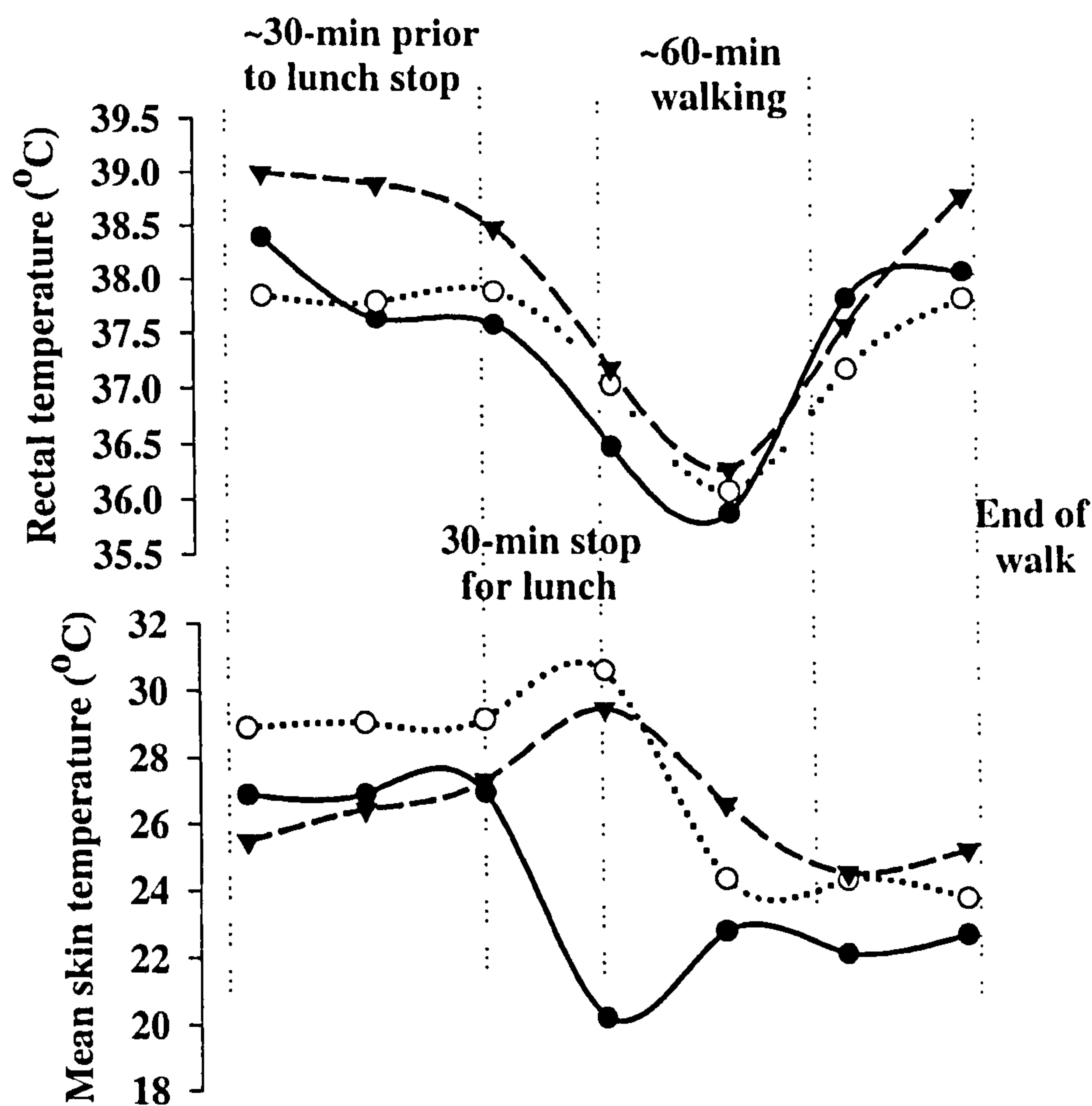


Figure 5.3. The apparent temperature 'afterdrop' that was evident in 3 subjects after the 30-min stop for lunch. Each corresponding line represents an individual subject.

During the ~30-min stop at mid-walk, three subjects showed a pronounced shivering response after about 15 min of rest. Rectal temperature fell $\sim 1.0^\circ\text{C}$ from the level observed during the pre-lunch walking. Prior to the lunch stop, two of these subjects

showed early signs of exposure; symptoms included stumbling, withdrawal from voluntary conversation, slowing down in pace. Decreases in thermal balance were also observed at this time. The majority of the subjects complained of feeling 'very cold' and wanting 'to speed up' during the period after the lunch stop: these subjective impressions usually lasted ~15-45 min, depending on the weather conditions.

5.3.4 Psychomotor performance: The POMS profile showed an expected rise in tension and confusion prior to the walk ($P < 0.05$) and an increase in fatigue ($P < 0.05$) post-walk relative to both before and at mid-walk. Overall RPE values from the start of the walk to the lunch stop, and the final part of the walk were 15 ± 2 and 13 ± 3 , respectively. There was a small decrease in grip strength (45.4 ± 2.7 to 43.5 ± 2.8 kg/m²) from pre-walk to post-walk ($P < 0.01$). Any changes in reaction time were less evident. The only significant changes in reaction time were evident in 'one-finger' reaction time ($P < 0.01$) and in the recorded errors (4-finger) ($P < 0.05$, not shown), both following completion of the walk. However, the normal circadian variation of an accelerated reaction time from morning to afternoon was not evident (not shown).

5.3.5 Respiratory gas exchange and HR responses: During the first ~5 km of ascent, RER increased from 0.82 ± 0.03 at base to 0.89 ± 0.02 . During the descent, RER gradually fell to less than 0.84 ± 0.02 for the final 5 km of the walk (Fig. 5.4). The RER changes are reflected in the oxidation rates of CHO and fat shown in Fig. 5.4. During ascent both CHO and fat oxidation increased. After the first 4 km, CHO oxidation decreased for the duration of the walk, with fat oxidation remaining elevated (Fig. 5.4).

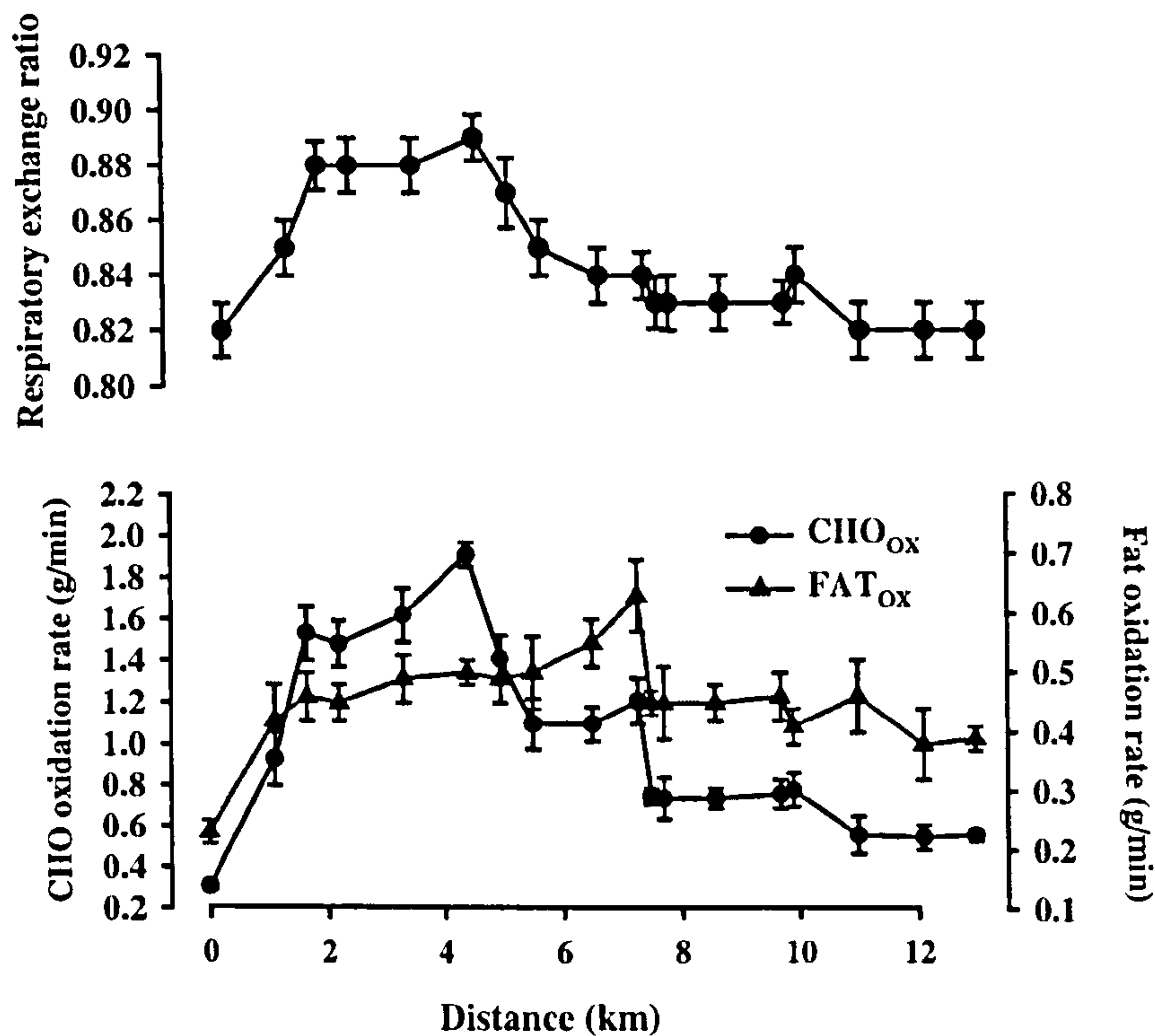


Figure 5.4. Respiratory exchange ratio and CHO and fat oxidation rates during the hill walk. Values are means \pm SE, $n = 10$.

Increases in the $\dot{V}O_2$ and heart rate were evident during the rise in altitude over the first 4.5 km of the hill walk (Fig. 5.5D, C). From ~ 3 km until the descent of the hill walk (~ 6 km), subjects were operating at $\sim 50\%$ $\dot{V}O_{2\text{ peak}}$ with an average HR of 148 ± 8 (beats/min); during the descent this value fell to approximately 25~40% of $\dot{V}O_{2\text{ peak}}$ with HR averaging 126 ± 5 (beats/min) (Fig 5.5.D, C).

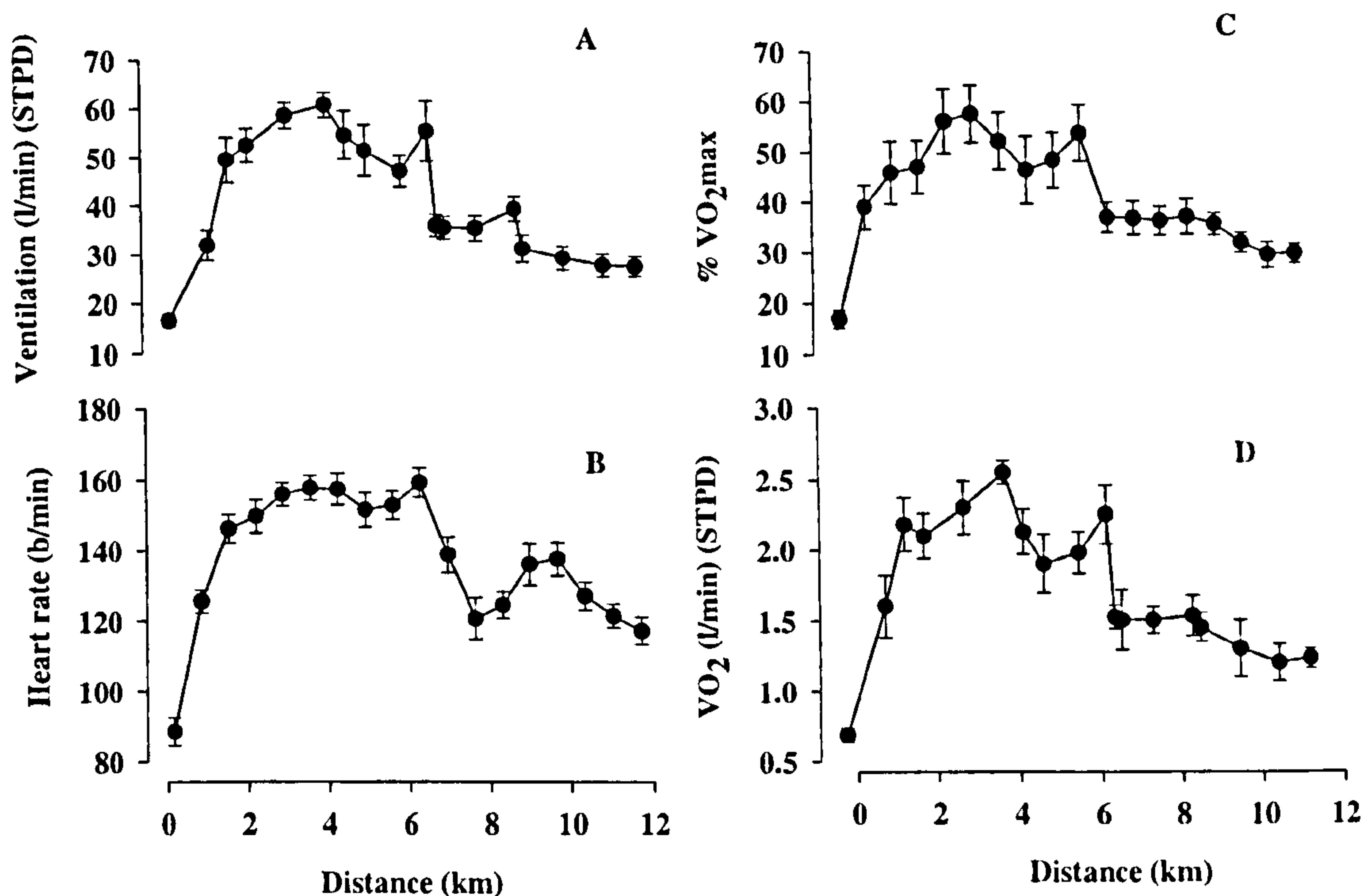


Figure 5.5. Respiratory gas exchange and heart rate results from the hill walk.

5.3.6 Blood and urine constituents: There were no significant changes in plasma volume during the walk. Consequently, circulating concentrations of substrates have not been corrected for haemo-concentration. Table 5.3 gives concentrations of the blood constituents. The energy metabolites 3-OHB, lactate, glycerol, NEFA increased from pre-walk to mid-walk ($P < 0.001$). In contrast, there was no change in TAG concentrations. Insulin increased significantly whereas cortisol decreased significantly ($P < 0.01$) post-walk, relative to both pre-walk and mid-walk values.

Table 5.3. Whole blood and serum constituents prior to, during and after the hill walk.

	Lactate, mmol/l	Glucose, mmol/l	Glycerol, μmol/l	NEFA, μmol/l	TAG, μmol/l	3-OHB, μmol/l	Insulin, mU/l	Cortisol, nmol/l
Pre	0.85±0.1	4.8±0.1	93±10	455±83	957±73	65±32	6.7±0.9	683±45
Mid	1.5±0.2 ^{##}	4.7±0.1	262±24 ^{***}	1757±144 ^{***}	989±49	332±68	4.8±0.7	627±96
Post	0.78±0.1	5.1±0.2	133±24	868±164 ^{**}	1073±110	89±68 ^{**}	12±2 ^{**}	326±50 ^{**}

Values are means ± SE; n = 13. Significant differences between pre- to mid-walk: ## P < 0.001; Significant differences mid, relative to both pre and post walk: *** P < 0.001. Significant differences between pre- to post-walk: ** P < 0.01; *** P < 0.001.

Table 5.4 gives the concentrations of the urine catecholamine collections. Generally, the hill walk led to a marked elevation in urinary adrenaline and noradrenaline when compared to the rest day. In addition, urine osmolality increased pre-walk to post-walk (603 ± 86 to 744 ± 71 mosmol/kg/H₂O, $P < 0.05$).

Table 5.4. Urinary catecholamine concentration during a rest day and monitored hill walk.

	<u>Rest</u>			<u>Exercise</u>		
	<u>08:00-13:00</u>	<u>13:01-18:00</u>	<u>18:01-20:00</u>	<u>08:00-13:00</u>	<u>13:01-18:00</u>	<u>18:01-20:00</u>
Nadn/CT (nmol/mmol)	27 (3)	25 (2)	2 (2)	43 (11)	43 (11) [#]	26 (3) [#]
Adn/CT (nmol/mmol)	4 (1)	4 (1)	5 (1)	11(4) ^{*#}	8 (2)	5 (1)
DOP/CT (nmol/mmol)	156 (17)	134 (11)	176 (22)	143 (22)	163 (37)	209 (86)
Nadn (nmol/l)	232 (29)	293 (51)	124 (22)	583 (165) [#]	737 (264) [#]	294 (48) [#]
Adn (nmol/l)	32 (6)	50 (18)	34 (11)	186 (92) [#]	144 (62)	55 (8)
DOP (nmol/l)	1406 (109)	1603 (304)	1090 (160)	1882 (348)	2621 (844)	1669 (309)
CT (mmol/l)	10 (1)	12 (2)	7 (2)	14 (2)	15 (2)	12 (2)

Values are means \pm SE; n = 10. Nadn/CT, noradrenaline / creatinine; Adn /CT, adrenaline / creatinine; DOP/CT, dopamine / creatinine. * Significantly different from exercise ($P < 0.05$) as a function of time (08:00-13:00 vs 18:01-20:00). # Significantly different from rest ($P < 0.05$) as a function of time.

5.4 DISCUSSION

The main finding of this study was that a seemingly normal outdoor hill walk in adverse, but not uncommon, conditions led to a significant physiological stress on the body. Despite these stresses, which included dehydration, high thermal stress and marked negative energy balance, subjects demonstrated only slight impairment in some of the measured psychomotor tests throughout the walk. Furthermore, the hill walk significantly altered the hormonal and metabolic milieu. In spite of the large difference between energy intake and expenditure, a normal blood glucose level was maintained. The major source of energy, in the monitored walk, was an enhanced fat oxidation probably from adipose tissue lipolysis.

5.4.1 Physiological responses: Pugh (1967) described a $\dot{V}O_2$ 'cut-off' point, above which individuals exercising in a cold, wet and windy environment would not experience any influence on the physiological responses to exercise i.e., drop in core temperature, mental impairment, extreme fatigue and exhaustion. Below this point there would be an obligatory increase in energy expenditure and subnormal Trec and muscle temperatures. Weller et al. (1997a) suggested that the cut-off point is likely to depend on factors such as clothing insulation, body morphology, mass and body fatness (Toner and McArdle, 1989) and may account for the random nature of the hypothermic casualties described by Pugh (Pugh, 1964; Pugh, 1967). The present observations highlight the variability in $\dot{V}O_2$ in response to the hill walk which is likely to depend on such factors as terrain, gradient, weather condition, backpack weight, exercise intensity, preceding diet and thermal stress. It was only during the high intensity part of the walk that subjects reached this cut-off point. This 'cut-off' point was clearly variable. Since the hill walkers in this study walked at their own pace, it could be cautiously concluded that hill walkers do not consistently operate at, or above this 'cut-off' level.

Weller et al. (1997a, 1997b) demonstrated heat loss was greater in low intensity ($\sim 30\%$ $\dot{V}O_{2\text{ peak}}$) than high intensity ($\sim 60\%$ $\dot{V}O_{2\text{ peak}}$) walking, illustrating how the rate of heat production during high intensity exercise will offset heat loss to the environment more effectively than low intensity exercise. This observation has important implications for hill walking in that during the low-intensity phase of a hill walk, e.g., walking downhill, navigation, and so on, an increased heat loss relative to heat production may be

experienced. The factors already mentioned, along with individual variation in effective cold stress under given environmental conditions, may lead to a compromise in the ability to operate safely in the mountainous environment.

The observations of the present study regarding the thermal stresses involved in a hill walking event have provided some novel results. The results show generally higher values for the Trec profile than described in previous studies (Thomson and Hayward, 1996; Weller et al., 1997a, 1997b); this increase is most likely due to the elevated thermal insulation from the protective clothes worn by the hill walkers, causing a decrease in heat loss, which will subsequently increase Trec. The maintenance of normal core temperature during cold stress depends on the subject's ability to generate enough heat to offset heat loss to the environment (Weller et al., 1997b; Noakes, 2000). This clearly was not a problem for the hill walkers due to high levels of prolonged exercise intensity.

When the subjects stopped for lunch and measurements mid-walk for approximately 30 min, the exercise hyperthermia was cancelled out by the decreased heat production and increased heat loss through conduction and radiation. The initial physiological responses to cold exposure to maintain core temperature in the cold are peripheral vasoconstriction to reduce heat loss and shivering to generate heat. Although shivering was not quantified directly, pronounced shivering was noted in four of the subjects. Once peripheral vasoconstriction is maximised, core temperature can only be maintained by an increased heat production, i.e., shivering, which is thought to be the major contributor to the cold-induced increase in heat production (Doubt, 1991). The core temperature continued to fall after subjects began walking following lunch. This temperature 'after-drop' has been reported in a number of studies of cold water immersion (Golden and Hervey, 1977; Noakes, 2000), but to my knowledge, has not been reported in circumstances such as the present. Even though Trec did not drop below 36°C, this after-drop may describe the reason for hill walkers slipping into the first stages of hypothermia after stopping for a rest. The suggested mechanisms for the after-drop are at present subject to controversy (Golden and Hervey, 1977; Collins et al., 1982; Lloyd, 1986; Webb, 1986). The extent of this after-drop was also reflected by the high negative thermal balance ($-356 \pm 23 \text{ w m}^2$). Weller et al. (1997a) reported similar values for thermal balance after 240-360 min of walking in a simulated cold, wet and windy environment with subjects wearing a minimum of clothing. The high negative thermal balance highlights the thermal stress placed on the human body,

even when apparently prepared for the prevailing weather conditions

One possible explanation for this continued drop in Trec may be the decrease in exercise intensity (and hence metabolic heat production) after lunch when the subjects were walking downhill. Studies of responses to immersion in cold water have shown Trec to continue to fall after immediate removal from cold water, even when subjects are actively re-warmed (Lloyd, 1986). The extent to which this after-drop can be limited by an increase in exercise intensity in this scenario merits further research.

5.4.2 Psychomotor response: The unremarkable changes in the psychomotor tests demonstrate that, despite serious physiological stress, the subjects demonstrated normal motor control during the walk. The small decrease in grip strength of ~3% suggests that, over the monitored walk, motor function was near normal, despite potential cooling of the peripheral tissues. Likewise, the reaction time tests, used to gain an indication of both perception and decision making ability, showed no change.

5.4.3 Energy Balance: The recorded energy expenditure of 14.5 MJ highlights the high energetic cost of the 12-km hill walk, and is comparable to the 12 MJ energy expenditure in the studies of Greenhaff et al. (1987) and Maughan et al. (1987). These latter studies were based on a flat 37-km walk which corresponded to operating at 17 % of $\dot{V}O_2$ max, with unrestricted access to either a mixed or CHO diet, respectively. In contrast, subjects in the present study operated at approximately 50 % of $\dot{V}O_2$ max during the first 5-km on the predominately uphill section and at ~30 % during the final 7-km which was on the down-hill section of the walk. This suggests that the main determinant of energy expenditure, in a hill walk is the relative difficulty of the walk, in terms of the gradient and terrain, both of which will contribute to a greater level of exercise intensity. Additionally, Maughan et al. (1987) demonstrated that a weight loss of 2 kg over their 4-day walk was apparent in the group who had a low CHO intake, but was not observed in the group ingesting a high CHO diet. They concluded that the loss of body weight was a consequence of the gradual use of the hepatic and muscle glycogen stores and loss of associated water. In the present study over one day, the weight loss also averaged 2 kg, with subjects consuming a mixed diet. The normal whole-body muscle glycogen pool amounts to some 400 g (Hedman, 1957). Water is stored in association with this glycogen in a ratio of approximately 3-4 g water per gram of glycogen (Olsson and Stalin, 1970; Bergström and Hultman, 1972), suggesting that the observed body weight loss of 2 kg, in

the present study, may reflect a small decrease in whole-body glycogen level. Supporting this postulated decrease in whole-body glycogen was an observed negative CHO balance of 200 g. In addition, this negative CHO balance of 200 g would equate to some 800 g loss of body water, suggesting a high element of dehydration which was also reflected by the increased urine osmolality concentrations. The fundamental difference in the relative intensities of the walk, compared to that of Maughan et al. (1987) may largely explain the magnitude of the weight loss in the present study. However, subjects in the studies described (Greenhaff et al., 1987; Maughan et al., 1987) had unrestricted access to food, whereas in the present study they supplied their own food which they considered appropriate for the conditions. The unrestricted food may have attenuated any potential negative energy balance in the aforementioned studies. In the present study, the negative energy balance, the failure to provide enough fuel for the exercise duration and intensity, could well have led to compromises in both physiological and psychological functioning if the duration was more prolonged. This suggestion generates important considerations for the hill walker with regard to nutrient intake both prior to and during the walk. For example, since the subjects were clearly in negative energy balance, a suggestion is that they should take more food and/or foods with higher energy content to help prevent this imbalance from occurring and provide a measure of protection if the walk becomes unexpectedly prolonged.

5.4.4 Metabolic responses: The measurements made at the mid-point of the walk showed an enhanced lipolysis, demonstrated by an almost 4-fold increase in NEFA concentrations accompanied by high glycerol and 3-OHB concentrations. Fatty acids delivered from adipose tissue are the predominant fuel for sustained exercise at moderate intensity (Ahlborg et al., 1974; Keins et al., 1993; Coyle, 1995). There is usually a surge in plasma NEFA concentrations shortly after exercise, presumed to reflect a continued high rate of lipolysis when muscle NEFA uptake has suddenly diminished (Hodgetts et al., 1991), and this may have been responsible for some of the elevation in NEFA concentration observed. The stimulus for lipolysis during exercise is mainly adrenergic (Arner et al., 1990), reinforced by decreased insulin concentrations. It is likely that the former stimulus was greater in our subjects than in many exercise studies because of the adverse conditions. Even though large variations were present in urine catecholamine concentrations, the results clearly indicate a general stress response to the hill walk when compared to the previous day.

In contrast, NEFA, glycerol and 3-OHB concentrations were considerably lower at the end of the walk than mid-way. The food intake mid-walk is likely to have influenced the pattern of lipid mobilisation during the final part of the walk. Ahlborg and Felig (1976) and Krzentowski et al. (1984) showed that CHO feeding prior to or during mild intensity prolonged exercise, decreased the amount of energy derived from fat oxidation and increased proportionally the amount of energy derived from blood glucose. However, in the present study, the decreased NEFA, glycerol and 3-OHB concentrations following the walk coincided with no evident decreases in fat oxidation assessed by indirect calorimetry. This maintained fat oxidation might be accounted for by fatty acids derived from the body's intramuscular stores (Romijn et al., 1993). The relatively high circulating plasma insulin levels recorded at the end of the walk ($\sim 12 \text{ mUI}^{-1}$) would be expected to lead to a decrease in adipose tissue lipolysis, whereas intramuscular lipolysis is not so readily inhibited by insulin (Bolinder et al., 2000; Hagström-Toft et al., 2001).

Hypoglycaemia, which would affect both fatigue and the shivering response (Haight and Keatinge, 1973), was not observed in this study at any time. The data indicate that the liver was able to meet glucose requirements by a combination of glycogenolysis and gluconeogenesis, supplemented by the mid-walk carbohydrate intake.

In summary, the pattern of substrate mobilisation and utilisation is likely to vary according to the intensity and duration of the walk, level of fitness, environmental conditions and preceding diet of the participant. It is apparent that the energy expenditure during the hill-walk exceeded energy intake, apparent in both total CHO and fat oxidation rates for the walk. The large energy expenditure observed served to highlight the high energetic cost of such hill walking events, even when completed over a relatively short duration. The observations generate important implications for hill walkers with regard to nutritional strategies for preventing some of the potential detrimental effects of operating at a marked negative energy balance. The negative energy balance may lead to a compromise in physiological function and safety if activity is performed over a prolonged period. Nevertheless, despite the physiological stress and the difference between energy intake and expenditure, blood glucose was maintained. The major source of energy, in the monitored hill walk, was an enhanced fat oxidation probably from adipose tissue lipolysis.

It is evident that more research on this topic is desirable. Future research should extend the findings of this study into hill walking events over a longer duration. Furthermore, there is

little in the literature with respect to repetitive high-intensity hill walks completed over 1-2 weeks. Finally, the optimal fluid and nutritional strategies need to be determined for activity of this form.

CHAPTER 6

ENERGY BALANCE, METABOLISM, HYDRATION AND PERFORMANCE DURING 10 DAYS OF STRENUOUS HILL WALKING: THE EFFECT OF AGE

6.1 INTRODUCTION

The prolonged duration of a typical hill walk places exceptional demands on the participants. The specific demands of hill walking tend to involve activity of varying intensity and duration, both of which are influenced by factors such as the physical fitness, dietary intake, backpack weight and environmental conditions (Thomson and Haywood, 1996). Despite the popularity of hill walking and the increasing problem of accidents in the mountainous environment, the ability of the safety organisations to design educational material concerning this hazard is hindered by lack of knowledge of the physiological and psychomotor responses to such events, often pursued over consecutive days. The information that is available derives from the pioneering work of Pugh (Pugh, 1964; Pugh, 1966a; Pugh 1966b; Pugh, 1967), supplemented by descriptions of exposure incidents (Pugh, 1966b; Hunter, 1968; Strang, 1969; Kreider, 1991; Noakes, 2000).

The previous study (reported in Chapter 5) into the energy cost of a 12-km hill walk demonstrated a high energy expenditure of 14.5 MJ for the walk (recorded via continuous measurement of respiratory gas exchange by means of indirect calorimetry). In this study, food and fluid were allowed *ad-libitum*; nevertheless, subjects became dehydrated and lost, on average, 2 kg in body mass. Despite the high energetic cost of the walk, dehydration, and serious physiological stress, the subjects demonstrated little change in psychomotor control during and after the walk. Furthermore, in spite of the difference between energy intake and expenditure, blood glucose and triacylglycerol concentrations were maintained. The major source of energy was enhanced fat oxidation, probably from adipose tissue lipolysis (Chapter 5).

Thermoregulatory and cardiovascular functions, as well as cognitive function, are adversely influenced by body water deficits (Adolph, 1947; Ladell, 1955; Gopinathan, 1988; Sawaka, 1992). For many complex tasks, both the mental decision-making and physiological functioning are closely related (Sawka, 1992). As a result, dehydration probably has more profound effects on real-life tasks than solely physiological responses. Healthy older subjects may be more prone to dehydration than their younger counterparts (Sawka and Montain, 2000; Kenney and Chiu, 2001),

possibly via a blunted thirst sensation leading to a reduced fluid intake (Sawka and Montain, 2000; Kenney and Chiu, 2001). In hill walking, dehydration may decrease thermoregulatory and cognitive functioning, which could impair decision making, leading to an increased susceptibility to fatigue and injury in the mountainous environment.

It was aimed to extend the previous investigations, as outlined in Chapter 5, into a hill walking event to cover 10 consecutive days of walking. Furthermore, there have been no studies which have considered the effect of age on the potential stress of such activities. It was aimed therefore to quantify some relevant responses that are important in the safety of hill walkers, such as the likelihood of dehydration, impaired performance, and the ability to maintain glycaemia and also the possible effect that age may have on these responses. This type of study may be important in adding to the mostly anecdotal information regarding exposure and recreational activities.

Based on the initial study (Chapter 5) it was predicted, firstly, that due to the large energy cost of such events, subjects would have difficulties in maintaining body mass during sustained activity over several days. Secondly, due to the envisaged physiological stress, significant alterations in metabolism, hydration and performance may become apparent throughout the 10 consecutive days of hill walking. Finally, it was anticipated that the older subjects may experience a higher strain and impairment than the young, possibly as a consequence of lower physical fitness and a blunted thirst response which may impair their ability to rehydrate effectively.

6.2 METHODS

6.2.1 Subjects: Seventeen male subjects were divided into two groups according to their age: group 1 [younger; age (mean \pm SD) 24 ± 4 years ($n = 9$)] and group 2 [older; 56 ± 4 years ($n = 8$)]. The study was reviewed and approved by the Human Ethics Committee of Liverpool John Moores University. The subjects gave written consent to participate in the study as described in section 3.9.1. The physical characteristics of the subjects are shown in Table 6.1. The majority of the subjects were active and experienced hill walkers.

Table 6.1. Physical characteristics of the subjects

	Older	Younger
Age, years	56.4 ± 3.7	24.4 ± 2.9
Height, m	1.78 ± 0.04	1.78 ± 0.1
Body mass, kg	76.3 ± 11.8	75.8 ± 7.4
BMI, kg/m^2	24.6 ± 2.3	22.9 ± 2.1
TBW, litres	42.0 ± 4.9	43.3 ± 4.7
Fat-free mass, kg	57.6 ± 6.7	59.3 ± 6.5
Fat mass, kg	17.9 ± 7.4	12.8 ± 2.9
$\dot{V}O_{2 \text{ peak}}$, ml/kg/min	46.7 ± 7.3	56.4 ± 6.9

BMI, body mass index; TBW, total body water. Mean values \pm SD, based on 8 subjects in the older group and 9 in the younger group. Refer to methods for calculations and assumptions.

6.2.2 Protocol: Both groups completed 10 consecutive days of high-intensity hill walking during the month of April in the Scottish highlands. The distance varied between 10 – 35 km in distance and up to 1345 m in elevation above sea level, consisting of a range of gradients and terrain typical of a mountainous hill walk. The ascent and distance covered in the 10-day experiment were approximately 12 km and 180 km, respectively. The experimental design is outlined in Fig 6.1. Living accommodation was provided for the subjects, and was located close to the hill walks. Subjects woke each morning between 05:30 and 06:30 hours and completed

the preliminary experiments prior to the hill walk (Fig 6.1). Self-paced walking began each day between 08:30 and 09:30 hours. The subjects selected their own food and fluids for the walk, which were pre-weighed prior to starting.

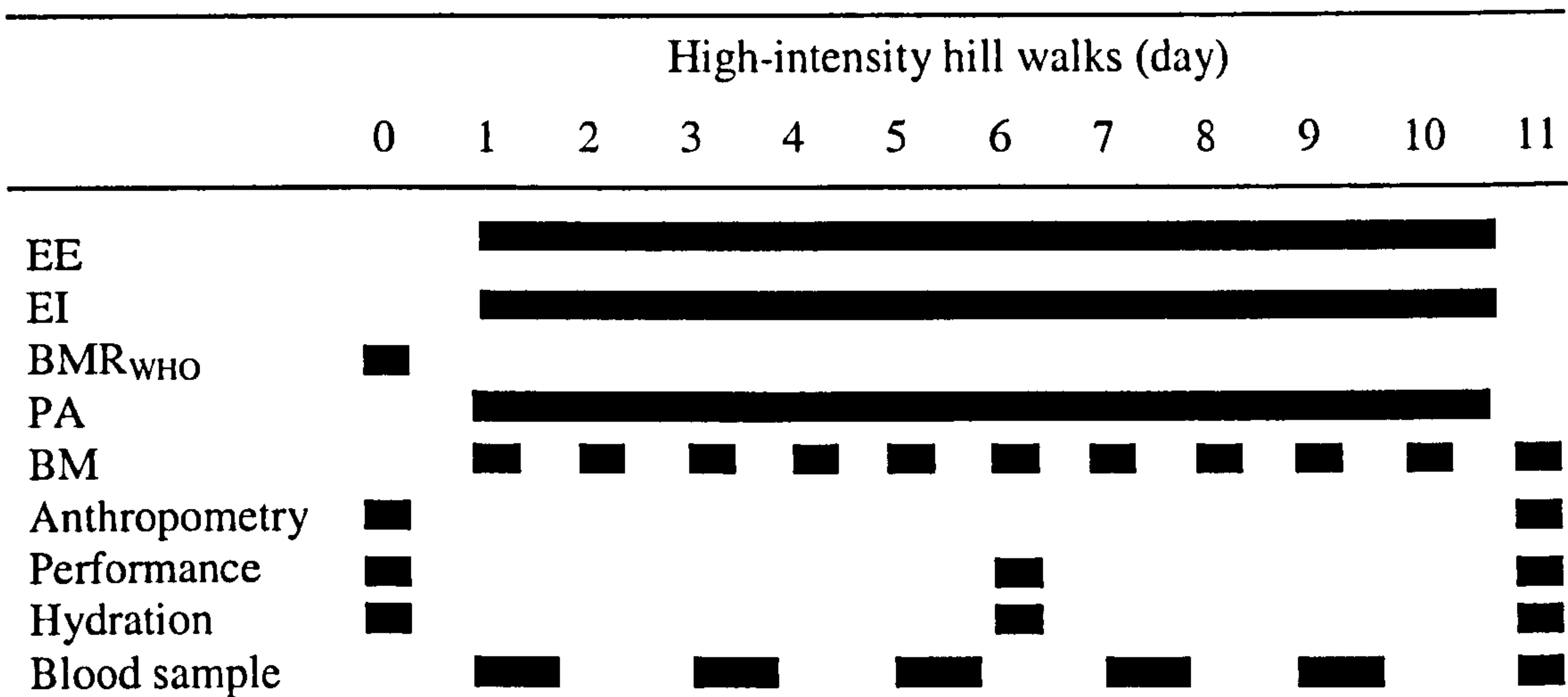


Figure 6.1. Summary of experimental design. █, AM collection; █, AM and PM collections; EE, Energy expenditure; EI, Energy intake, weighted food and fluid intake; BMR_{WHO}, basal metabolic rate, estimated using a formula for age, height and weight (FAO/WHO/UNU, 1985); PA, physical activity assessed using triaxial accelerometers; BM, Nude body mass, recorded, after voiding, first thing every morning; Anthropometry, limb circumference and skinfold measurements; Performance, tests of grip strength, flexibility, vertical jump (muscular power), and reaction time (cognitive function); Hydration, urine collection for the assessment of urine osmolality and perception of thirst; Blood sample, 9-ml venous blood sample.

6.2.2.1 Food and water intakes: Weighed food and water intakes were measured with a 10-day dietary record. Subjects received instructions on how to keep a food record. Food and water was allowed ad-libitum. The data on the food records were used to calculate intakes of total energy, protein, fat, carbohydrate (CHO) and alcohol with a computer programme based on food tables (CompEat, Version 5, Grantham, UK). Total water intake was calculated from reported food and water intakes and the calculated amount of metabolic water. The amount of metabolic water was estimated from protein, fat, and CHO intake derived from the 10-day food record. The oxidation of protein, fat, CHO gives 0.41, 1.07, and 0.6 ml water/g, respectively (Fjeld et al., 1988).

6.2.2.2 Energy expenditure, water loss and physical activity level: Energy expenditure, water loss and physical activity levels were estimated as described in section 3.3.1.3. In addition, the percentage of under-reporting of food intake and under-eating during the 10-days were calculated as described in section 3.3.1.2.

6.2.2.3 Basal metabolic rate (BMR) measurements: BMRs were estimated as described in section 3.3.1.2.

6.2.2.4 Daily physical activity: Physical activity over the 10-day interval was registered using a tri-axial accelerometer (Tracmor; Philips Research, Eindhoven, Netherlands), consisting of three uni-axial piezoelectric accelerometers, attached to the lower back of the subjects with an elastic belt. The accelerometer incorporates software which calculates the sum of the rectified and integrated acceleration curves from the antero-posterior, medio-lateral and longitudinal axis of the trunk. The time period for integration was set at 1 min. Subjects were instructed to wear the accelerometer during waking hours, except during bathing and showering.

6.2.2.5 Body composition: Energy balance was checked by measuring changes in body mass each day. Subjects were instructed to record their nude body mass each morning before consuming any food or beverages and after voiding, with calibrated balance scales accurate to 0.1 kg. On day 0 total body water (TBW) was measured by isotope dilution (H_2^{18}O) as described in section 3.3.1.3. To estimate changes prior to and immediately after the experiment fat-free mass was also estimated using a equation as described in section 3.1.3.

Percentage body fat was estimated as described in section 3.1.3. Limb circumferences (calf, quadriceps, waist, abdomen and bicep) were estimated from three sequential measurements which were made before and after the experiment on each subject by the same investigator using a spring-loaded fibreglass anthropometric tape.

6.2.2.6 Physical fitness: Maximal oxygen uptake was estimated as described in section 3.2.1.

6.2.2.7 Hydration and performance: In the morning, prior to walking, on days 1, 6 and 11, subjects provided a urine sample for the analysis of urine osmolality in order to assess hydration status, as described in section 3.6.1. Subjects rated their perception of thirst was assessed using a 100-mm visual analogue rating scale labeled from "not at all" to "extremely". The nature of this rating scale, its use and validity in relation to food consumption have been described previously (Dufaux et al., 1986; Hill and Blundell, 1990). Furthermore, care was taken to ensure that both age groups interpreted the scales in a similar manner.

Likewise, in the morning, prior to walking, on days 1, 6 and 11 subjects completed a battery of psychomotor performance tests which included choice reaction time (cognitive processing time), grip strength (motor function), flexibility and vertical jump (anaerobic power) tests. These psychomotor tests are described fully in section 3.9. The subjects were fully familiarised with the use of the equipment, and each test was performed three times in a balanced fashion.

6.2.3 Analytical methods: Blood samples were taken and stored as described in section 3.4.1. A portion (20 μ l) of blood was used immediately for the measurement of haemoglobin and haematocrit in order to calculate changes in plasma volume. These methods and calculations are given in section 3.4.2. The concentrations of the metabolites plasma NEFA and TAG, glycerol, lactate, 3-OHB, glucose, plasma cholesterol and HDL-cholesterol were measured as described in sections 3.5.1-3.5.3. Equally, the concentrations of the hormones insulin, cortisol and growth hormone were measured as described in section 3.5.4.

6.2.4 Statistical analysis: Data were initially tested for normality, before being analysed by repeated-measures analysis of variance (ANOVA) with age as a between group factor. The ANOVA results were corrected by the Huynh-Feldt ϵ -adjusted degrees of freedom when the violation to sphericity was minimal (>0.75). The Greenhouse-Geisser correction used when sphericity was violated (<0.75), and significant condition and condition-time interactions were identified (Field, 2001). Post hoc tests (Honestly Significantly Different) were performed to isolate any significant differences.

Student's paired t-tests ascertained between-condition differences when a variable was measured once. A Pearson's correlation coefficient was used to establish any relationships between variables. Statistical significance was set at $P \leq 0.05$ for all statistical tests.

6.3 RESULTS

6.3.1 Exercise duration and conditions: All the young subjects completed the hill walks apart from on day 9 when one of the subjects had to rest due to fatigue and injury. Likewise, one of the older subjects had difficulties in completing a number of the walks and did not manage to complete the full distances on a number of the days; since this subject was an outlier in most of the blood metabolic data, it was decided to exclude him from the analysis of the blood parameters. The duration for the hill walks ranged between 6 - 11 h. The differences in the time to complete the walk were due mainly to variations in weather conditions and terrain. The surface conditions on the walks tended to vary with the weather. Snow and ice were regularly encountered, along with high winds; these factors represent walking in very demanding climatic conditions.

6.3.2 Energy balance, PAL, water loss, under-reporting and under-eating: Values for EI, EE_{DLW} , PAL, water loss, water intake and %under-reporting during the 10 days are presented in Table 6.2. The high EE_{DLW} of 21.4 ± 3.2 and 21.7 ± 2.8 MJ/day for the two groups reflects the very high energetic cost of such hill walking events. There was a higher incidence of under-reporting of food intake in the older group when compared with the young ($P < 0.05$). The reported intake was lower than the measured energy expenditure. This underreporting was approximately half due to under-eating and the other half due to underrecording. The body mass decreased on day 4, in both groups, then remained stable throughout the 10 days with a mean body mass loss of -0.9 ± 2.2 and -1.1 ± 1.1 kg in the older and younger group, respectively. The body mass loss was significant only in the younger group (Day 11 vs. Day 1; $P < 0.05$; Fig 6.2.). The energy equivalent of the body mass loss was 2.7 ± 6.6 MJ/day and 3.3 ± 3.3 MJ/day (1 kg body mass was assumed to be 30 MJ; (Westerterp et al., 1995) in the older and younger groups, respectively. The recorded water intake plus the water from food was 2.9 ± 0.4 l/day and 4.0 ± 1.0 l/day. These values were significantly different from the measured water loss of 4.7 ± 0.7 l/day and 5.8 ± 1.0 l/day in the older and younger groups, respectively ($P < 0.01$).

Table 6.2. Energy intake (EI), energy expenditure (EE), basal metabolic rate (BMR), physical activity (PA), physical activity level, water intake, metabolic water values, water loss, and percentage of underrecording in the different age groups¹.

	Older	Younger
EI (MJ/day)	15.3 ± 1.8 (12.7 – 17.5)	19.2 ± 3.8 (13.2 – 24.5)*
EE _{DLW} (MJ/day)	21.4 ± 3.2 (16.8 – 25.7)	21.7 ± 2.8 (17.8 – 25.4)
² BMR _{WHO} (MJ/day)	7.8 ± 0.6 (7.1 - 8.7)	7.4 ± 0.5 (6.8 - 7.9)
PA (counts/day)	18.7 ± 5.8 (11.6 - 26.8)	19.6 ± 3.2 (14.7 - 23.2)
PAL	2.8 ± 0.9 (2.3 - 3.3)	2.9 ± 0.4 (2.5 - 3.7)
Water intake (l/day)	2.4 ± 0.4 (2.1 – 3.2)	3.5 ± 0.9 (2.2 – 4.6)*
Metabolic water (l/day)	0.5 ± 0.1 (0.4 – 0.6)	0.6 ± 0.1 (0.4 – 0.8)
Water loss (l/day)	4.7 ± 0.7 (3.4 – 5.3)	5.8 ± 1.0 (4.8 – 7.4)*
Under-reporting (%)	-27.5 ± 11 (-46.2 – -10.5)	-13.0 ± 10 (-30.3 – -0.7)*

¹ Mean values ± SD (range in parentheses), based on 8 subjects in the older group and 9 in the younger group. DLW, doubly labelled water method; ²BMR_{WHO}, basal metabolic rate estimated with an equation including age, sex, body mass and height (FAO/WHO/UNU, 1985). PA, triaxial accelerometer assessed physical activity. PAL, physical activity level. Refer to methods for calculations and assumptions. * ($P < 0.05$) denotes significant between group differences.

6.3.3 Daily physical activity: The daily accelerometer readings showed that the intensity of the activity was maintained throughout the 10 days. Due to the large individual variations in accelerometer output, there were no significant group or time differences in the physical activity levels (Fig. 6.2).

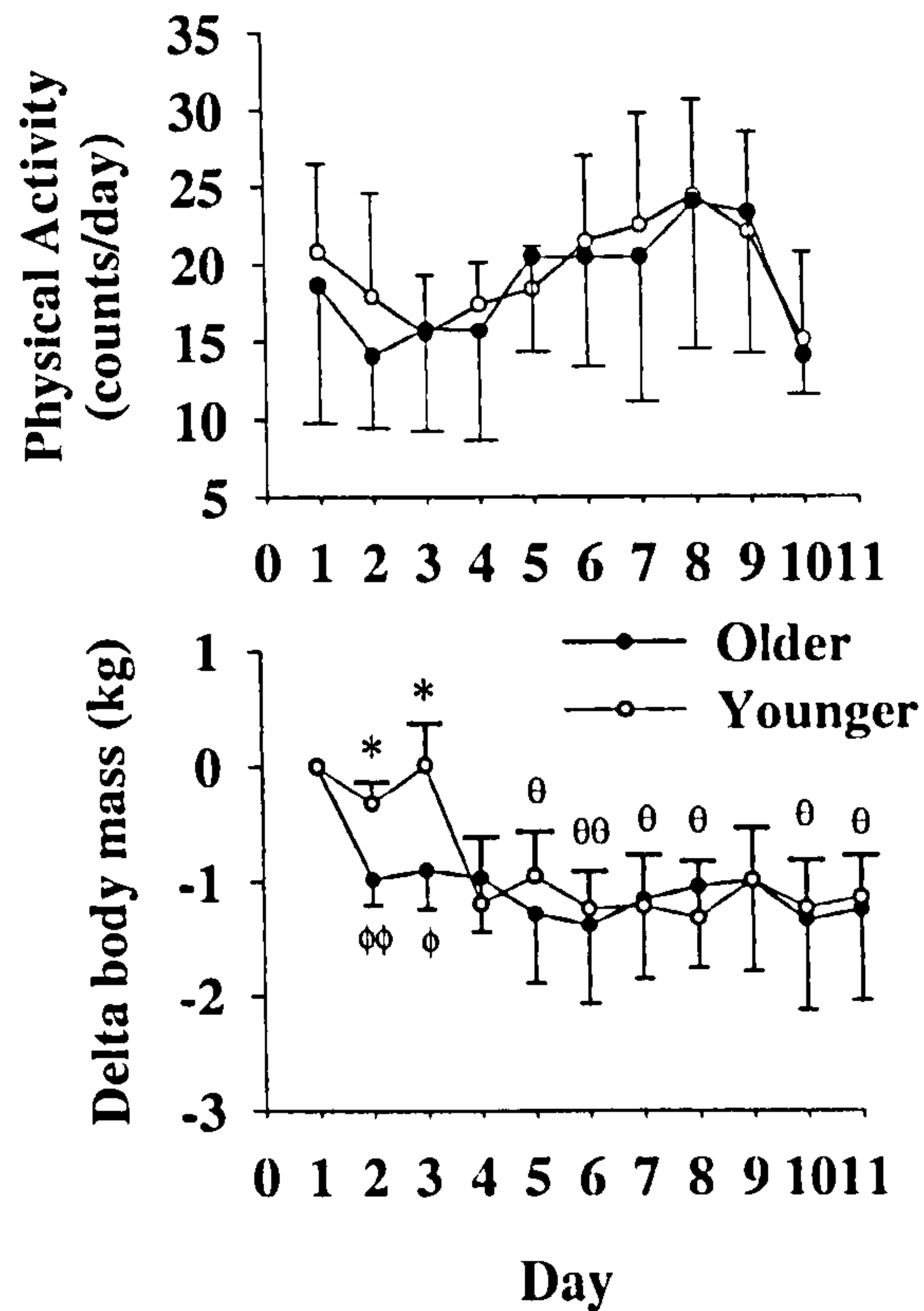


Figure 6.2. Physical activity levels and change in body mass throughout the experiment. Values are mean \pm SD based on 8 subjects in the older group and 9 in the younger group. Symbols ϕ and θ denote significant differences ($P < 0.05$; ϕ and θ , $P < 0.01$) from day 1 as a function of day in the older and younger groups, respectively. * ($P < 0.05$) denotes significant between group differences.

6.3.4 Body composition: Changes in body composition are given in Table 3. Body fat, estimated from skinfold thickness, decreased by 1.3 ± 1.2 (%) and 2.0 ± 1.5 (%) in the older and younger group ($P < 0.05$), respectively. There were no significant differences in estimated FFM or FM in either group (Table 6.3).

Table 6.3. Change in anthropometric parameters

	Older	Younger
Body mass, kg	-0.9 ± 2.2	-1.1 ± 1.1 ^θ
Fat-free mass, kg	0.7 ± 2.9	-0.7 ± 2.2
Fat mass, kg	-1.9 ± 2.5	-0.3 ± 1.5
Body fat, %	-1.3 ± 1.2 ^φ	-2.0 ± 1.5 ^θ

Values are mean ± SD based on 8 subjects in the older group and 9 in the younger group. Symbols ^φ and ^θ denote significant differences from day 1 as a function of day in the older and younger groups, respectively ($P < 0.05$). Refer to methods for calculations and assumptions.

6.3.5 Blood measurements:

Metabolites: Results from the plasma lipid measurements are shown in Figs 6.3, 6.4, and 6.5. During the morning samples, plasma TAG decreased significantly (30 - 60%) during the first five days to reach a plateau before rising back to normal on day 11 (Fig. 3), with no significant between group differences (statistic in Fig. 6.3).

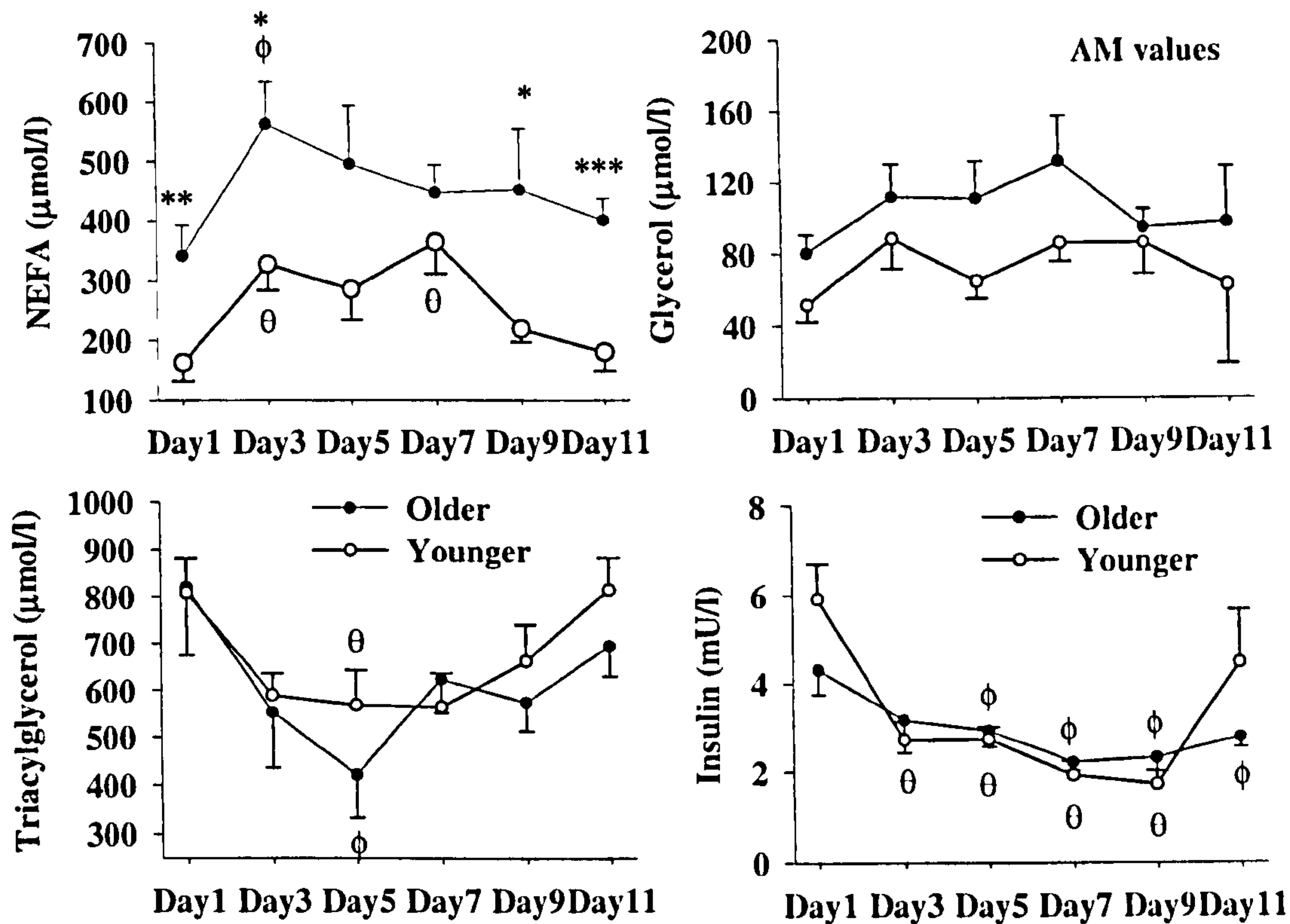


Figure 6.3. Hormonal and metabolite changes during the 10-day hill walk. Values are mean \pm SD based on 7 subjects in the older group and 9 in the younger group. Morning (AM) values only. Symbols ϕ and θ denote significant differences ($P < 0.05$) from day 1 as a function of day in the older and younger groups, respectively. *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$) denotes significant between group differences.

Conversely, when measured after the walks, plasma TAG concentration declined significantly throughout the experiment, in both groups (Fig. 6.4). In general, morning NEFA concentration remained elevated during the first seven days before returning to normal. The older group had significantly higher circulating NEFA levels when compared with the young (statistics in Fig. 6.3). Although there was a trend for higher glycerol concentrations in the morning samples compared with baseline, in both groups, this did not reach significance ($P = 0.093$). Both NEFA and glycerol showed marked increases, in each group, when measured upon completion of the walks. Likewise, 3-OHB remained significantly elevated both upon completion of the walks and relative to baseline values in the older group (Table 6.6). The younger

group showed significantly higher 3-OHB concentration upon completion of the walk but their resting values returned to normal by the end of the experiment (Table 6.6).

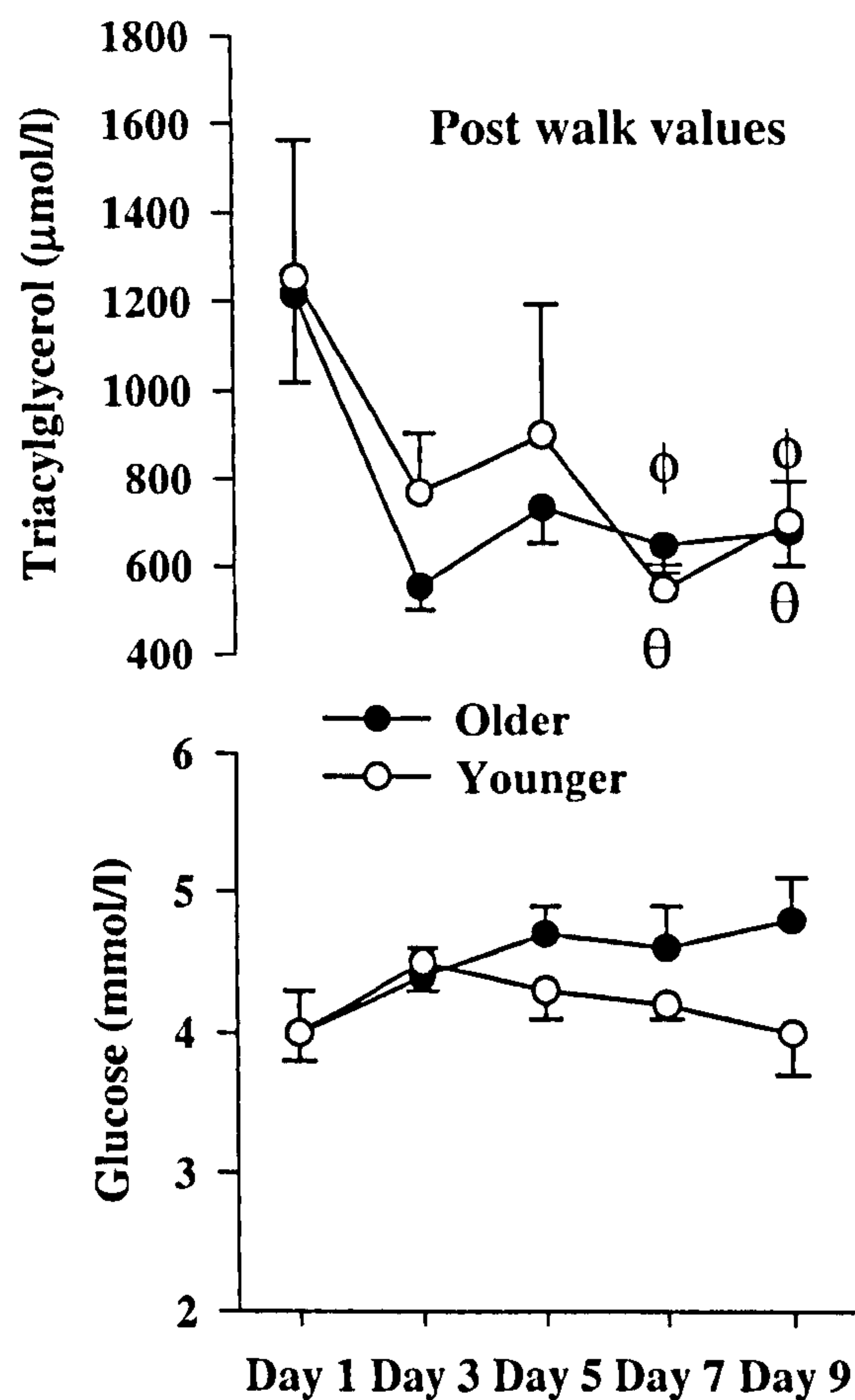


Figure 6.4. Changes in triacylglycerol and glucose immediately upon completion of the walks. Values are mean \pm SD based on 7 subjects in the older group and 9 in the younger group. Post walk (evening) values only. Symbols ϕ and θ denote significant differences ($P < 0.05$) from day 1 as a function of day in the older and younger groups, respectively. No between group differences were present.

Blood glucose was maintained throughout the study; although there was a trend for a lower glucose level in the younger compared to the older group on day 7 and day 9, this failed to reach significance ($P = 0.096$, day 7; $P = 0.052$, day 9; Fig. 4). Furthermore, plasma lactate demonstrated unremarkable changes throughout the experiment with no between group differences, apart from day 1 (Table 6.6).

Table 6.6. 3-OHB and lactate concentrations measured during the 10 days of high intensity hill walking.

	3-OHB, $\mu\text{mol/l}$		Lactate, mmol/l	
	Older	Younger	Older	Younger
Day 1 AM	28 \pm 5	31 \pm 6	0.9 \pm 0.3*	0.5 \pm 0.1
PM	349 \pm 44 ^{†††}	197 \pm 83 [†]	1.1 \pm 0.3	0.7 \pm 0.3
Day 3 AM	189 \pm 21 ^{φ*}	66 \pm 19 ^θ	0.7 \pm 0.2	0.6 \pm 0.2
PM	215 \pm 65	64 \pm 26	0.8 \pm 0.1	0.6 \pm 0.2
Day 5 AM	138 \pm 39 ^{φ*}	52 \pm 10	0.7 \pm 0.2	0.6 \pm 0.4
PM	392 \pm 91 [†]	458 \pm 200 [†]	1.8 \pm 0.8	0.8 \pm 0.3
Day 7 AM	144 \pm 35 ^φ	113 \pm 13 ^{θθ}	0.7 \pm 0.2	0.6 \pm 0.2
PM	309 \pm 74 [†]	95 \pm 40	0.9 \pm 0.3	0.6 \pm 0.1
Day 9 AM	245 \pm 137 ^φ	60 \pm 13 ^θ	0.7 \pm 0.1	1.0 \pm 0.4
PM	325 \pm 70	189 \pm 46 [†]	1.0 \pm 0.4	0.8 \pm 0.5
Day 11AM	128 \pm 24 ^{φφ}	30 \pm 9	0.7 \pm 0.1	0.5 \pm 0.1

Morning (AM) blood sample, prior to any food intake; Evening (PM) blood sample, with in 20 min of completion of walk. Values are mean \pm SD based on 7 subjects in the older group and 9 in the younger group. Symbols ϕ and θ denote significant differences ($P < 0.05$) from day 1 as a function of day in the older and younger groups, respectively. ^{†††} ($P < 0.001$), ^{††} ($P < 0.01$), [†] ($P < 0.05$) denotes significant differences between AM and PM values as a function of time. ^{***} ($P < 0.001$), ^{**} ($P < 0.01$), ^{*} ($P < 0.05$) denotes significant between group differences.

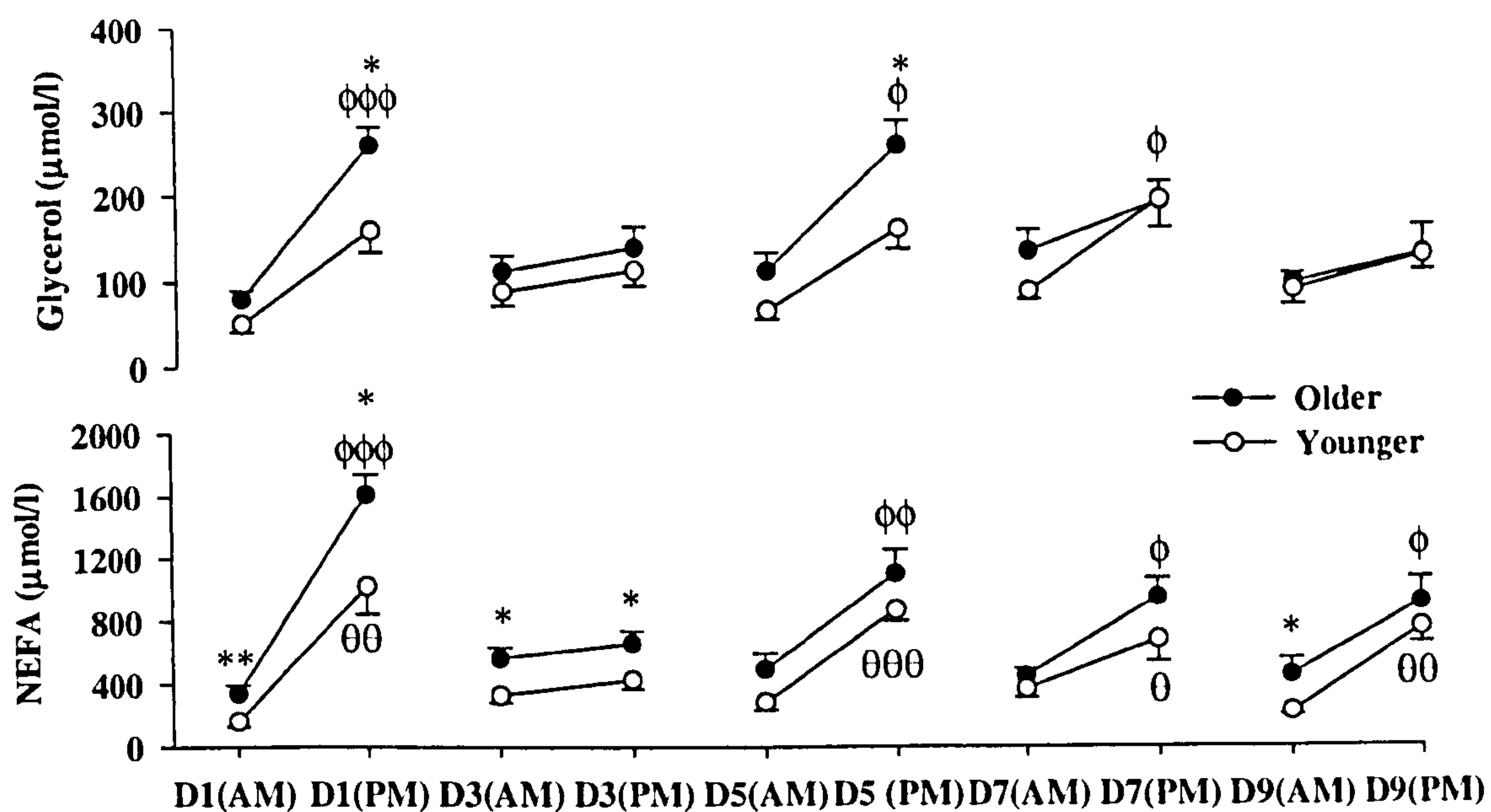


Figure 6.5. Metabolite changes during the 10-day hill walk. Values are mean \pm SD based on 7 subjects in the older group and 9 in the younger group. D, day; Morning and evening values. Symbols ϕ and θ denote significant differences (ϕ/θ , $P < 0.05$; $\phi\phi/\theta\theta$, $P < 0.01$; $\theta\theta\theta$, $P < 0.001$) between AM versus PM values in the older and younger groups, respectively. * ($P < 0.05$; ** $P < 0.01$) denotes significant between group differences.

Hormones: Morning plasma insulin remained significantly lowered throughout the 10 days compared with baseline with no between group differences (statistics in Fig. 6.3). The older group showed higher plasma cortisol concentration levels on days 1, 5 and 9, when compared with the younger group. However, apart from day 1, a similar trend in the cortisol levels was apparent between both groups, higher in the morning and lower in the evening samples (statistics in Fig 6.5). Furthermore, apart from a small increase in the evening from the morning samples on day 1 and day 9, plasma growth hormone demonstrated unremarkable changes throughout the experiments (Fig. 6.6).

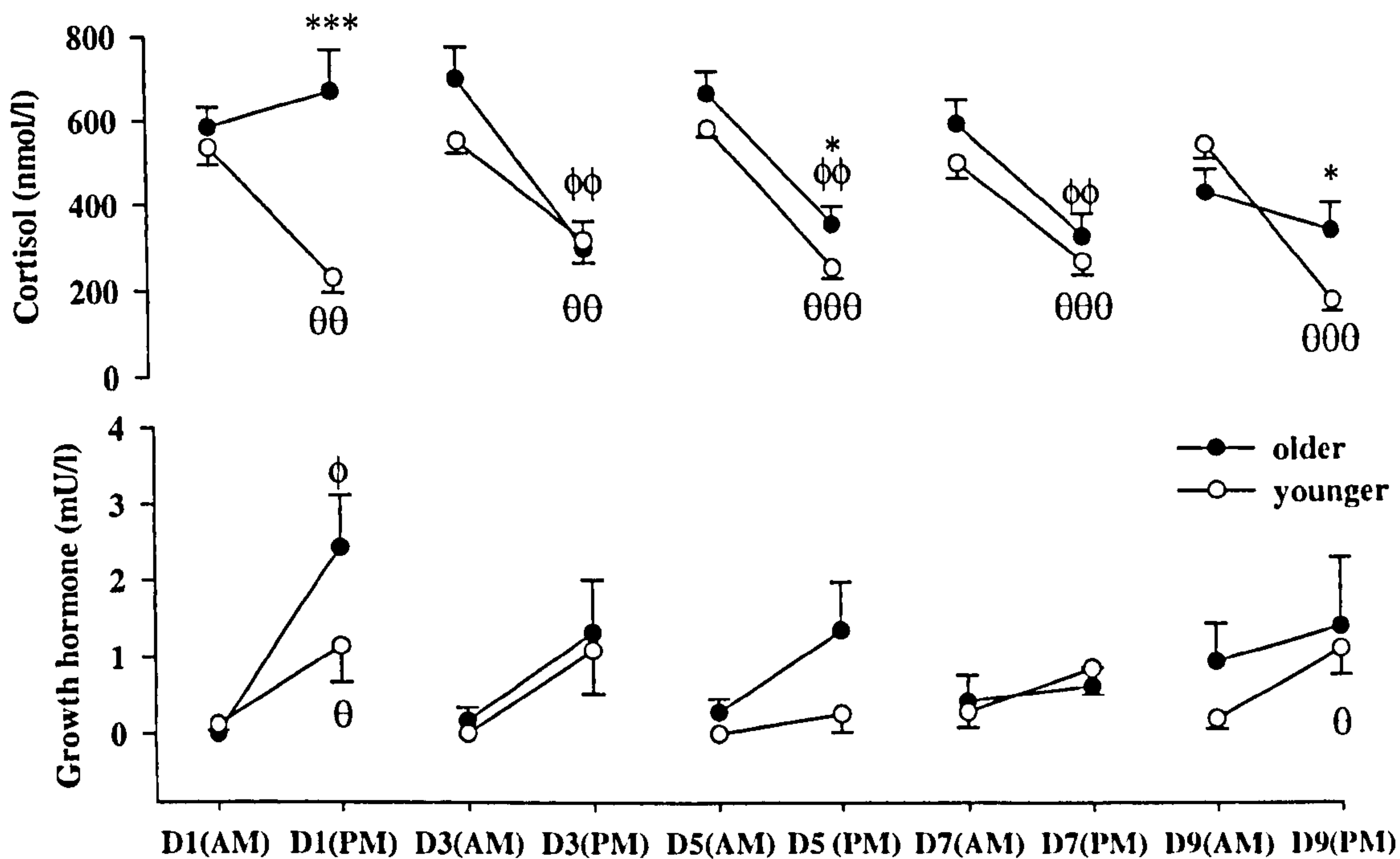


Figure 6.6. Hormonal changes during the 10-day hill walk. Values are mean \pm SD based on 7 subjects in the older group and 9 in the younger group. D, day; Morning and evening values. Symbols ϕ and θ denote significant differences ($\phi/0$, $P < 0.05$; $\phi\phi/\theta\theta$, $P < 0.01$; $\theta\theta\theta$, $P < 0.001$) between AM and PM values in the older and younger groups, respectively. *** ($P < 0.001$), * ($P < 0.05$) denotes significant between group differences.

Cholesterol: Table 6.4 displays the changes in T-cholesterol, HDL-C, and LDL-C over the 10 days. The older group had higher initial levels for T-Cholesterol and estimated LDL-C. The HDL-C values, however, were identical between the two groups. Nevertheless, the older group showed a greater decrease in both T-cholesterol and LDL-C when compared with the young. Likewise, the older group showed a greater increase in HDL-C ($P < 0.05$), after the 10 days, whereas no significant difference was evident in the younger group.

Table 6.5. Plasma cholesterol, LDL-C and HDL-C immediately prior to and after the 10 days of hill walking.

	<u>Older</u>			<u>Younger</u>		
	Day 1	Day 11	Diff (%)	Day 1	Day 11	Diff (%)
T-Chol, mmol/l	5.8 ± 0.9**	4.3 ± 0.8 ^{φφ}	-25 ± 11	4.2 ± 0.8	3.8 ± 0.7 ^θ	-10 ± 11
¹ LDL-C, mmol/l	4.0 ± 0.7**	2.5 ± 0.6 ^{φφ}	-26 ± 12*	2.7 ± 0.7	2.2 ± 0.6 ^θ	-4 ± 13
HDL-C, mmol/l	1.2 ± 0.2	1.5 ± 0.2 ^{φφφ*}	38 ± 15*	1.2 ± 0.2	1.3 ± 0.3	18 ± 9

T-Chol, total cholesterol; LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol. ¹ Calculated using the Friedewald et al. (1972) formula. Values are mean ± SD based on 7 subjects in the older group and 9 in the younger group. Symbols ^φ and ^θ denote significant differences from day 1 as a function of day in the older and younger groups, respectively (^{φφ} $P < 0.01$, ^{φφφ} $P < 0.001$; ^θ $P < 0.05$, ^{θθθ} $P < 0.001$). ** $P < 0.01$, * ($P < 0.05$) denotes significant between group differences.

In the older subjects, the decreases in T-cholesterol and LDL-C were negatively related to PAL (Fig. 6.6) i.e., the subjects with the lower PAL values had the greater changes in T-cholesterol ($r = 0.79$, $P < 0.05$) and LDL-C ($r = 0.74$, $P < 0.05$). Conversely, in the younger group there were strong negative relationships between the decreases in T-cholesterol and LDL-C and PAL (Fig.7); i.e. as PAL increased, so did the decreases in T-cholesterol ($r = -0.74$, $P < 0.05$) and LDL-C ($r = -0.86$, $P < 0.01$). These correlations persisted when changes in lipid concentrations were corrected for changes in plasma volume (Dill and Costill, 1974).

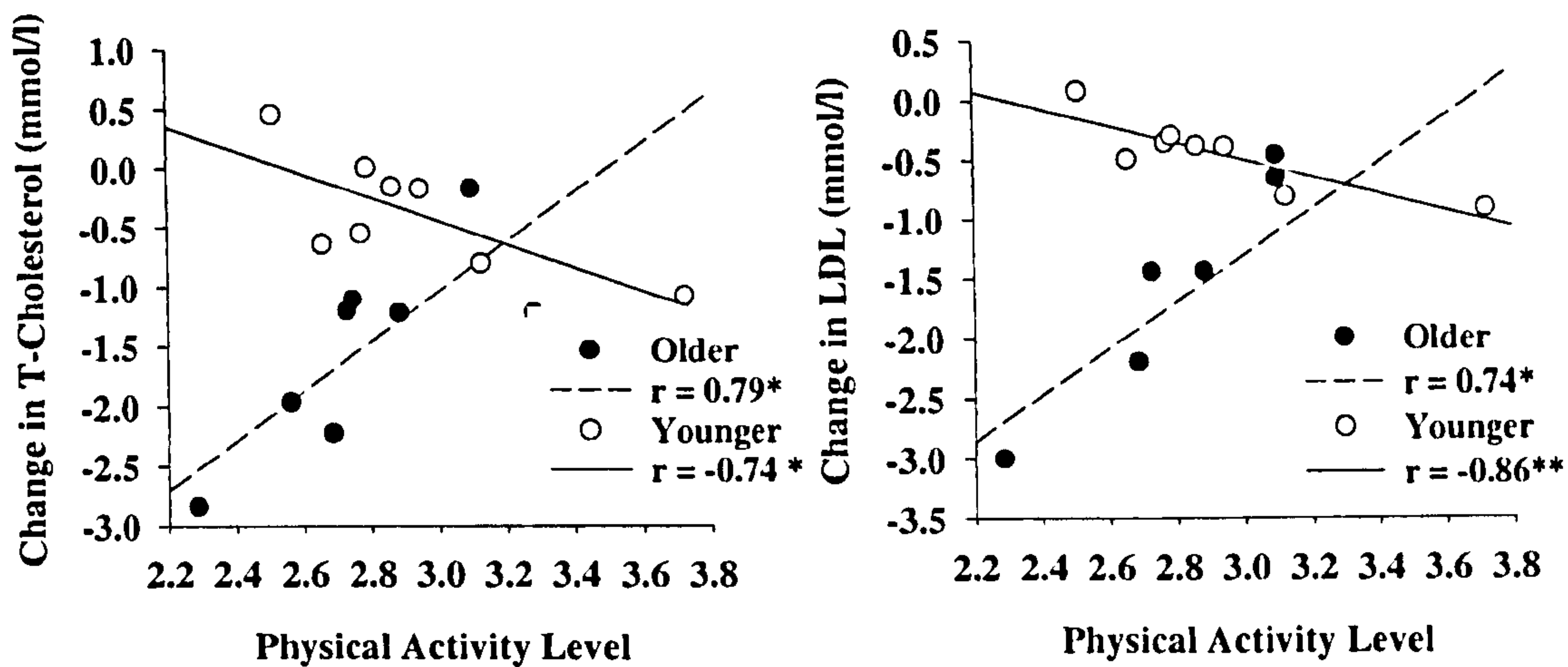


Figure 6.7. Relationship between physical activity levels and change in T-Cholesterol and LDL-C. ** ($P < 0.01$), * ($P < 0.05$) denotes significant relationships.

6.3.6 Hydration and performance: The older group demonstrated a marked increase in dehydration on days 6 and 11, relative to day 1 ($P < 0.05$; day 11) whereas the younger group remained hydrated throughout the 10 days (Fig. 6.7). Furthermore, the older group had lower perceptions of thirst compared with the younger group ($P < 0.05$, day 11; Fig. 6.7).

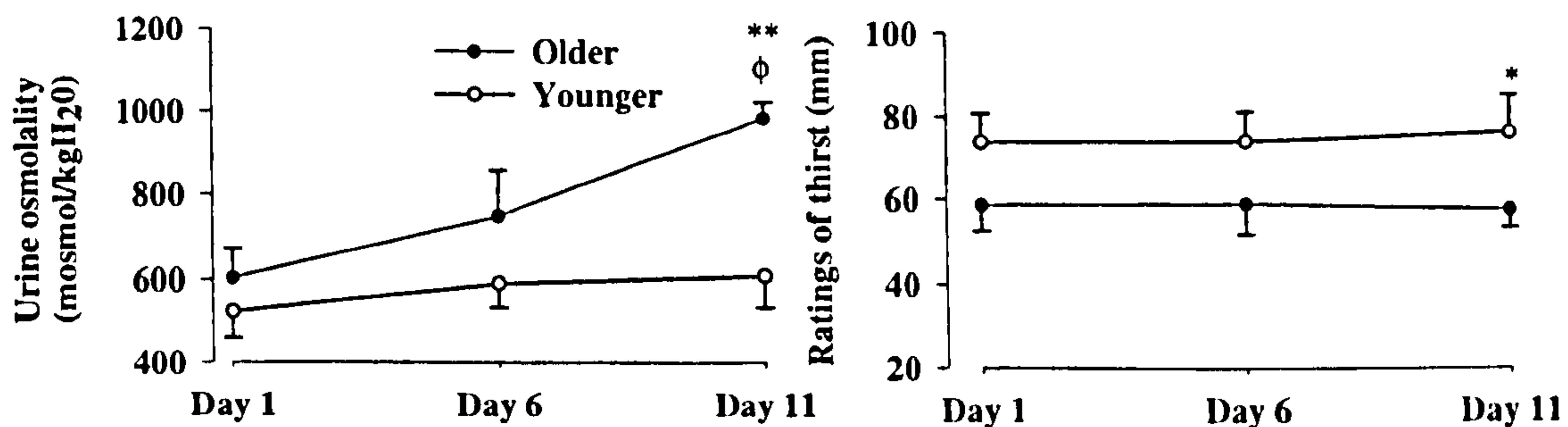


Figure 6.8. Changes in urine osmolality and ratings of thirst. Values are mean \pm SD based on 8 subjects in the older group and 9 in the younger group. Symbols ϕ denote significant differences ($P < 0.05$) from day 1 as a function of day in the older group. ** ($P < 0.01$), * ($P < 0.05$) denotes significant between group differences.

Table 6.5 gives the results for the psychomotor responses throughout the 10 days of walking. On the whole, the younger group attained higher levels in all the measured psychomotor tests when compared with the older group. Both groups showed a marked slowing of choice reaction time after the 10 days of walking. Grip strength

remained unchanged on day 11 in both groups, compared with day 1. Flexibility did not change in the older group but showed a progressive increase in the younger group, whereas the vertical jump performance showed a progressive decrease in the older group, whilst it was maintained in the younger group.

Table 6.6. Psychomotor performance during the 10 days of high intensity walking.

	Day 1		Day 6		Day 11	
	Older	Younger	Older	Younger	Older	Younger
Grip strength (kg/m ²)	41 ± 3	52 ± 3*	42 ± 4	49 ± 2* ^θ	43 ± 3	49 ± 4
Flexibility (cm)	14 ± 6	22 ± 3*	14 ± 4	25 ± 3** ^θ	15 ± 7	27 ± 4* ^θ
Vertical jump (cm)	34 ± 4	39 ± 3*	30 ± 3	42 ± 2***	27 ± 2 ^{φφ}	38 ± 3**
Reaction time (ms)	717 ± 30	531 ± 29*	----	----	793 ± 42 ^φ	583 ± 47** ^θ

Values are mean ± SD based on 7 subjects in the older group and 9 in the younger group. Symbols ^φ and ^θ denote significant differences ($P < 0.05$) from day 1 as a function of day in the older and younger groups, respectively. *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$) denotes significant between group differences.

The impact of the dehydration in the older group becomes apparent when the psychomotor tests are considered (Fig. 6.9). There was a strong relationship between the increase in urine osmolality from day 1 to day 11 (i.e., progressive increase in dehydration) to both a slowing in choice reaction time ($r = 0.79$, $P < 0.05$) and decreased vertical jump performance ($r = -0.86$, $P < 0.05$; Fig. 6.9).

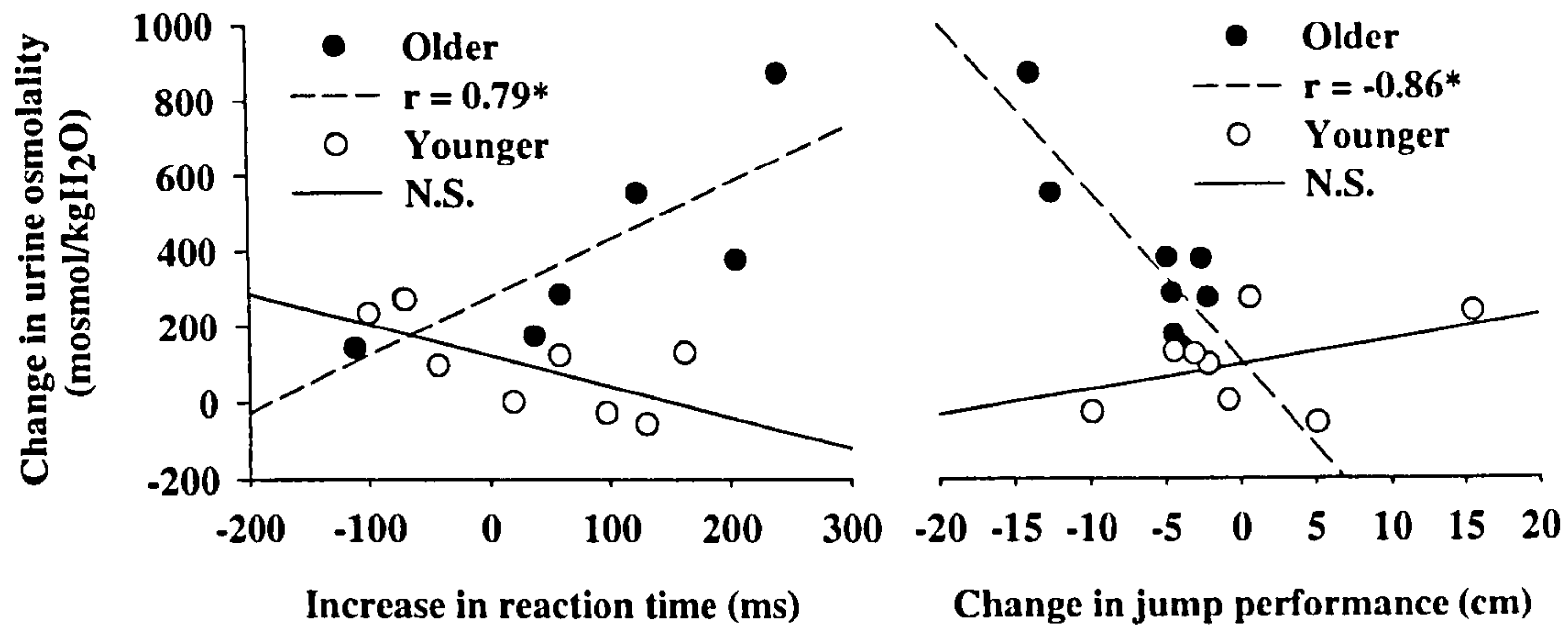


Figure 6.9. Relationship between change in choice reaction time and jump performance (anaerobic power) and hydration (urine osmolality). * ($P < 0.05$) denotes significant relationships.

6.3 DISCUSSION

The present study has yielded a number of important findings. Firstly, despite the very high EE and physiological stress, body mass was maintained in both groups. Secondly, the demanding nature of the walks was reflected in the impairment in some of the measured psychomotor tests throughout the 10 days. The impairment was more noticeable in the older subjects who also became progressively dehydrated during the 10 days. Finally, the hill walks significantly altered the hormonal and metabolic milieu in both groups. The major hormonal and metabolic perturbation, in both groups, was an enhanced fat mobilisation, reflected in lowered plasma insulin and high plasma NEFA, glycerol and 3-OHB concentrations. Despite the high EE, blood glucose levels were well maintained in both groups. The maintained blood glucose levels were probably mediated via the marked fat mobilisation. Enhanced fat mobilization should make it easier to maintain blood glucose by decreasing carbohydrate oxidation and promoting gluconeogenesis (Ahlborg et al., 1974; Marniemi et al., 1984).

6.4.1 Energy balance: The high EE values observed in our study reflect the very high energetic cost of such hill walking events. Despite the high EE, body mass was relatively well maintained in both groups, via high energy intakes. Comparable to the present study, Dressendorfer et al. (1982) reported energy intake values of 20.2 MJ/day in marathon runners during a 20-day 500-km road race. Also, one of the highest energy intake levels of 20-25 MJ/day reported in Maine lumbermen (Wood and Mansfield, 1904) is comparable to the present study. Indeed, only the measured EEs of 25.4 MJ/day over 22 days in the in the Tour de France (Westerterp et al., 1986), 15.1 - 34.9 MJ/day in elite cross country skiers during intensive training (Sjodin et al., 1994) and of 25.7-32.5 MJ/day during an arctic expedition (Stroud et al., 1993) reached higher values than those of the present study.

Physical activity levels or average daily multiples of BMR are commonly used to classify occupational work levels as light ($1.55 \times \text{BMR}$), medium ($1.78 \times \text{BMR}$), or heavy ($2.11 \times \text{BMR}$). The work levels in this and other DLW studies on heavy work consistently exceed $2.10 \times \text{BMR}$. The average multiple of BMR over the entire 10 days of this experiment of approximately 2.8 was similar to that measured over 7 and

11 days in highly-trained soldiers training for jungle warfare ($2.5 \times$ BMR and $2.8 \times$ BMR, respectively; Forbes-Ewan et al., 1989; Hoyt et al., 1991), over 3.5 days in trained amateur cyclists in a study comparing room respirometry to the DLW method ($2.6 \times$ sleeping metabolic rate; Westerterp et al., 1988), and over 21 days in elite female athletes during rigorous training ($2.8 \times$ BMR; Haggarty and McGaw, 1988). The multiple in the present study is higher than that of humans climbing Mt Everest ($2.2 \times$ BMR; Westerterp et al., 1992), but falls short of both elite cross-country skiers during high intensity training (Sjodin et al., 1994) and the extreme rates measured over 22 days in the Tour de France ($4.3 - 5.2 \times$ BMR; Westerterp et al., 1986).

In light of the high EE values and subsequent PAL in both age groups, subjects were close to the limits of body mass maintenance (Westerterp et al., 2001). The important and novel consideration in the present study is that the activity was monitored during recreational activity, and not with elite performers in extreme situations.

6.4.2 Metabolic responses: The measurements made upon completion of the walks showed an enhanced lipolysis, demonstrated by up to a 4-fold increase in NEFA concentrations accompanied by high glycerol and 3-OHB concentrations. Fatty acids delivered from adipose tissue are the predominant fuel for sustained exercise at moderate intensity (Ahlborg et al., 1974; Kiens et al., 1993; Coyle, 1995). There is usually a surge in plasma NEFA concentrations shortly after cessation of exercise, presumed to reflect a continued high rate of lipolysis when muscle NEFA uptake has suddenly diminished (Hodgetts et al., 1991). This may have been partially responsible for the elevation in NEFA concentration observed in the samples post-walk. The stimulus for lipolysis during exercise is mainly adrenergic (Arner et al., 1990), reinforced by decreased insulin concentrations, as supported in the present study. It is also likely that the former stimulus was also greater in our subjects than in many exercise studies because of the adverse conditions and physiological stress.

The decrease in TAG concentration and enhanced fat mobilisation are comparable to values reached in earlier studies after about 1-3 days of prolonged exercise and fasting (Carlson and Fröberg, 1967; Enger et al., 1980; Marniemi et al., 1984). In the studies of Carlson and Fröberg (1967) and Marniemi et al. (1984) subjects completed

a 500-km walk over 10 days and a 344-km walk over 7 days, respectively. Both studies combined prolonged walking on the flat with very low energy intakes (~837 kJ/day). In the study of Carlson and Fröberg (1967), NEFA and glycerol concentrations peaked at day 6 then subsequently declined over the following 4 days. Similarly, TAG concentration decreased, attained a plateau and then remained stable after the first 3 days with a trend for an increase on day 7 (Marniemi et al., 1984). Despite the low energy intakes in these studies (Carlson and Fröberg, 1967; Marniemi et al., 1984), both the maintained blood glucose concentration and the pattern of fat mobilisation were remarkably similar to those of the present study.

In the present study, the significantly lowered TAG levels on day 5 in the morning samples had returned to normal by day 11. In agreement with the previous study, described in Chapter 5, TAG concentrations remained unchanged after the first day of walking. During the consecutive days the TAG concentrations were significantly lowered when sampled upon completion of the walks, coinciding with the increased concentrations of circulating NEFA, 3-OHB and glycerol.

Hypoglycemia, which would affect both fatigue and the shivering response (Haight and Keatinge, 1973), was not observed in this study at any time. The data indicate that the liver was able to meet glucose requirements by a combination of glycogenolysis and gluconeogenesis, supplemented by carbohydrate intakes consumed throughout the 10 days. Since glucose was not measured during the walks, the possibility of transient hypoglycemia at particular stressful times during the walks cannot be ruled out. Taking the changes in metabolism collectively, the results from the present study are comparable to those of earlier studies in which both similar prolonged exercise and low energy intake were combined (Carlson and Fröberg, 1967; Enger et al., 1980; Marniemi et al., 1984). The results of these studies indicate that despite low energy intakes and high physiological stress, the human body is remarkably effective at altering its metabolism to maintain blood glucose concentrations.

The changes in T-cholesterol, LDL-C and HDL-C are consistent with the notion that exercise can favourably alter the lipid markers of cardiovascular disease risk by augmenting the reverse cholesterol transport pathway (Grandjean et al., 2000). The

mechanisms by which this reverse cholesterol transport pathway occurs are somewhat unclear, although aerobic exercise may influence blood lipid profiles by modifying the activities of intravascular enzymes and transfer proteins (Berg et al., 1994; Durstine and Haskell, 1994). Elevations in the activities of lipoprotein lipase (LPLa) and lecithin: cholesterol acyltransferase (LCATa), have been shown after both exercise training (Dufaux et al., 1986; Keins and Lithell, 1989) and after 5 days of military field training (Lithell et al., 1984). In addition, a reduction in the concentration of cholesterol ester transfer protein (CETP), which is closely related to CETP activity (CETPa), has been demonstrated after exercise training (Seip et al., 1993). Greater LPLa or LCATa brought about by exercise training may reduce TAG concentration and facilitate an increase in HDL-C (Tasken and Nikkila, 1981). Similarly, an exercise induced suppression of hepatic TAG lipase activity or CETPa may slow the catabolism of HDL particles, thereby enhancing the accumulation of cholesterol in all HDL subfractions (Fielding and Fielding, 1991; Bleicher and Lacko, 1992).

The greater decrease in T-cholesterol and higher increase in HDL-C in the older group compared with the young, is an interesting finding. In addition, strong negative relationships between PAL and the change in T-cholesterol and LDL-C were evident in the younger group. However, the reverse was evident in the older subjects; the subjects with the lower PAL values had greater changes in T-cholesterol and LDL-C. Two possible mechanisms, which may explain the different changes in the cholesterol metabolism in the different age groups, are possible. Firstly, a common postulation is that a threshold for energy expenditure, rather than a specific exercise intensity or duration, may be critical for inducing changes in HDL-C (Grandjean et al., 2000). The caloric threshold seems to vary directly with the functional capacity of the subjects (Crouse et al., 1995; Ferguson et al., 1998), and is thought to alter lipid concentrations primarily by inducing increases in LPLa (Lithell et al., 1984; Oscai et al., 1992). Secondly, differences in daily dietary intake can influence the effect of a single exercise session on plasma lipid activity (Grandjean et al., 2000). For example, increased carbohydrate ingestion can reduce LPLa and HDL-C and increase TAG concentration (Lithell et al., 1984). Conversely, increased cholesterol or fat intake can elevate cholesterol concentrations in all the lipoprotein fractions (National Cholesterol Education Program, 1994; Grandjean et al., 2000).

With the present results it is not possible to differentiate between the effects of the dietary intake and those of some potential threshold for 'caloric' expenditure for inducing changes in HDL-C. Upon closer inspection of the dietary intake it was found that there were no significant differences in CHO ingestion on days 8, 9 and 10 between the groups. Conversely, fat intake was significantly elevated in the younger group, compared with the older subjects, on days 8, 9 and 10. Although an increased fat intake may elevate cholesterol concentrations in all the lipoprotein fractions (National Cholesterol Education Program, 1994; Grandjean et al., 2000), it should not decrease HDL-C; however, these data need to be treated with caution due to the significant under-reporting of food intake in the older group (Table 6.2). Likewise the possibility that favourable changes in cholesterol transport may be modified by the ages of the participants, and their differing physical activity levels from those of their younger counterparts, cannot be ruled out. Although this is a potentially important clinical finding, there is no additional evidence in the literature to support this postulation. Further work in this area is required to establish whether age has an influence on the potential 'energy' cut-off or optimum 'functional capacity' levels, and whether this elicits favourable changes by reversing cholesterol transport.

6.4.3 Hydration and performance: Water loss, calculated from TBW and ^2H turnover rates, was not covered by water input. When the effects of dehydration and TBW alterations are considered, the older subjects especially were regarded to be in negative water balance. The impact of the dehydration incurred becomes apparent when the psychomotor tests are considered (Fig. 6.8). There was a significant relationship between the increase in urine osmolality from day 1 to day 11 (i.e. progressive increase in dehydration) and both the slowing in choice reaction time and the decreased vertical jump performance. The reasons for the dehydration in the older subjects are unclear, but the high sweat losses, blunted thirst (especially in older subjects (Sawka, 1992), cold-induced diuresis, increased respiratory water losses, conscious under-drinking and poor availability of water in the field (Freund and Sawka, 1995; O'Brien et al., 1998), may be contributory factors. When challenged by fluid deprivation, a hyperosmotic stimulus, hypovolaemia, or exercise in a warm environment, older adults exhibit a decreased thirst sensation and reduced fluid intake (Kenny and Chiu, 2001). However, in natural environments both the amount and

pattern of fluid intake are governed by the amount and timing of food intake (De Castro, 1988; De Castro, 1991) and there is no apparent difference with age (De Castro, 1992; Kenny and Chiu, 2001). Enough fluid is consumed with meals to maintain adequate fluid balance, and under stress-free conditions the renal response is sufficient to maintain this balance (Kenny and Chiu, 2001). Although there must be a clear discrepancy between the fluid intake between the two age groups, it is not possible to locate whether this fluid deficiency is occurring predominantly during the walks or during the periods of food intake at rest.

In agreement with the present study, active muscle blood flow during and after exercise using a small-muscle mass (such as hand-gripping), is well preserved in healthy older humans (Jasperse et al., 1994). Nevertheless, during exercise utilising a large mass of muscle, previous studies have shown that healthy older subjects had a lower whole-leg blood flow and vascular conductance compared with younger subjects during and after exercise (Wahren et al., 1974; Procter et al., 1998). This potential decrease in blood flow to a large muscle mass may be one explanation for why the older subjects became compromised in their ability to perform tests such as the vertical jump, which engages a large muscle mass.

The finding that the older group had both higher levels of dehydration and impaired psychomotor functioning and jump performance tests, is an important consideration. Both the decrease in choice reaction time and the decreased ability to employ a large muscle mass may impair decision-making abilities (e.g., leadership and navigational decisions). This impaired functioning may lead to an increased incidence of injury in the mountainous environment. Furthermore, both increasing age and dehydration lead to a decrease in thermoregulatory and cardiovascular functioning (Sawka, 1992; Sawka and Montain, 2000). Hill walkers can be caught unexpectedly and unprepared when rain and wind accompany outdoor activities in cool weather (Pugh, 1967). Decreased thermal insulation of wet clothing presents a serious challenge to body temperature regulation, which can be compounded by fatigue associated with prolonged exercise such as hill walking (Pugh, 1966b; Pugh, 1967; Thomson and Hayward, 1996). The present results suggest that the challenge to normal body temperature regulation may be increased in older participants. Taking the observations collectively, due to the marked dehydration and impairment of

psychomotor performance, the older age walkers may be more susceptible to fatigue and injury, and in adverse weather conditions the risk of hypothermia in the mountainous environment must be considered.

In conclusion, despite the high EE, blood glucose levels were well maintained in both groups, probably mediated via an enhanced fat mobilisation. Additionally, this study is the first to provide evidence that older participants, in part due to dehydration, may become compromised in their ability to operate in the mountainous environment. Further work and recommendations to both participants and the rescue services are clearly warranted.

CHAPTER 7

METABOLIC AND APPETITE RESPONSES TO PROLONGED WALKING UNDER THREE ISOENERGETIC DIETS: A LABORATORY BASED STUDY

7.1 INTRODUCTION

During prolonged exercise if energy intake fails to match energy expenditure, a negative energy balance will occur. This negative energy balance will aid the promotion of fat oxidation if the exercise is of low to moderate intensity. At high-intensity exercise, carbohydrate (CHO) becomes the preferred fuel (Jeukendrup and Jentjens, 2000) with a subsequent decrease in fat oxidation (Jones et al., 1980; Romijn et al., 1993).

The majority of studies have been concerned with the effects of high-CHO and high-fat diets either at rest (Bobbioni-Harsh et al., 19970; Whitley et al., 1997) or in high-intensity exercise ($> 65\%$ maximal oxygen consumption) situations (Whitley et al., 1998; Burke et al., 2000; Carey et al., 2001), whereas few authors have considered such dietary manipulations during prolonged low to moderate intensity exercise ($< 65\%$ oxygen consumption). The metabolic responses are resistant to dietary change in moderate-severe exercise (Whitley et al., 1998; Burke et al., 2000; Carey et al., 2001) but are susceptible to change at rest (Bobbioni-Harsh et al., 19970; Whitley et al., 1997). Although substrate turnover has been investigated over 4 h of cycling at approximately 30% of maximal oxygen uptake (Ahlborg et al., 1974), it is not known what happens during more sustained low to moderate intensity exercise, with the addition of dietary manipulation. This is somewhat surprising as there is a growing popularity in participation of recreational events such as prolonged ultra-endurance events (Hawley et al., 1998), hill walking (Sharp, 2001) and recreational cycling. More to the point, fat oxidation has the potential to meet a large proportion of the fuel requirements of exercise (Rauch et al., 1998; Rauch et al., 1999). Manipulation of macronutrients may be of some benefit to these activities, and in furthering our understanding of metabolic regulation during prolonged activity.

Since dietary manipulation has a marked effect on metabolism, it is reasonable to expect similar effects on perception of appetite and satiety. Food consumption usually suppresses hunger and inhibits further eating for a given period of time (Cotton et al., 1994). Because fat and CHO are known to undergo different rates of digestion (Horowitz

and Klein, 2000), the nutrients are likely to have differing effects on appetite and satiety, especially with the addition of exercise.

The two previous field studies, described in Chapter 5 and 6, have indicated that hill walkers may have difficulties in maintaining energy balance over one day of walking, but can rectify and maintain this energy balance over more prolonged periods by means of an increased energy intake. Since the vast majority of recreational hill walkers may pursue the activity over one day, the macronutrient composition of their energy intake is of important consideration. Although the previous field studies provided a means to collect more ecologically valid data, due to climatic conditions, differences in insulation and preferred pace and nutritional intakes, they lacked the controlled conditions that may be monitored in laboratory. The present study incorporated a comprehensive laboratory-based investigation in order to evaluate any potential benefits of macronutrient manipulation over prolonged periods of walking. This laboratory-based study provided a means to investigate diet manipulations per se during prolonged walking, without the influencing factors of weather, varying pacing and nutritional intakes, which would be impossible to control in a field-based study.

Therefore, the present study was designed with three primary aims: a) to investigate the effect of isoenergetic dietary manipulation on substrate balance and oxidation during prolonged walking, b) to identify the extent to which these dietary manipulations will alter the metabolic and hormonal milieu and, c) the extent to which the dietary strategies may affect indicators of performance (heart rate and ratings of fatigue / perceived exertion) and perceptions of appetite and satiety.

It was hypothesized that due to the prolonged relatively low intensity exercise (7 h walking between 25% and 55% of maximal oxygen uptake), the metabolic response is susceptible to dietary manipulations. It was further hypothesized that at this intensity of exercise, a high-fat diet is associated with decreased ratings of fatigue and perceived exertion mediated indirectly through the sparing of CHO and an enhanced fat utilisation.

7.2 METHODS

7.2.1 Subjects: Eight moderately trained male subjects participated in this study. All the subjects were active hill walkers with two of the subjects being 'club' level runners. Subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. The study was approved by the Human Ethics Committee of Liverpool John Moores University and the South Manchester Medical Research Ethics Committee (further details in section 3.9.1). Their physical characteristics (mean \pm SD) were: age 26 ± 3 years; height 1.8 ± 0.1 m; body mass 74 ± 4 kg; body mass index 20 ± 2 kg/m²; body fat 17 ± 2 ; $\dot{V}O_2$ peak 60 ± 4 ml/kg/min.

7.2.2 Experimental design: Subjects made an initial visit to the laboratory for familiarisation with the testing equipment. During this visit percentage of body fat (%fat) and maximal oxygen uptake were estimated as described in sections 3.1.3 and 3.2.1, respectively. In balanced design the subjects then attended the laboratory on three occasions. All subjects were studied 1 day/week during 1 calendar month. Subjects completed a 2-day dietary and physical activity diary recorded before each of the three trials, and were asked to keep their diet and activity the same before each test day. In this way, variations in diet and exercise before the three trials were minimized.

7.2.3 Protocol: In a balanced design, subjects were asked to fast from 20:00 hours and then to consume one of the three test diets. The test diets encompassed breakfast, two snacks and lunch, containing total carbohydrate (CHO), protein (Pr) and fat (F) in the following amounts respectively (g/70 kg body mass): mixed diet, 302 CHO, 50 Pr, 84 F; high-CHO diet, 438 CHO, 46 Pr, 35 F; high-fat diet, 63 CHO, 44 Pr, 196 F (Table 1). All diets were isoenergetic, containing 8940 ± 128 kJ/70 kg body mass, and were of similar appearance. Food was consumed at breakfast 90 min prior to the exercise, during a 5-min rest at 90 min and 355 min into the exercise protocol, and also during a 45-min rest for lunch at 235 min (Fig. 7.1).

Table 7.1. Total composition of test meals*

	High-Fat Meal	Mixed Meal	High-CHO Meal
Fat (g)	196	84	35
% Saturated	40	39	37
%Energy	81	36	14
CHO (g)	63	302	438
% Simple	48	56	54
% Energy	11	54	77
Protein (g)	44	50	46
% Energy	8	10	9
Total energy, kJ	9113	8743	8963

*All meals are based on a 70-kg reference person and were adjusted for each individual according to body mass. Subjects were given a cup of decaffeinated tea or coffee and water with each meal. All the meals had similar proportions of simple sugars relative to total carbohydrate, and similar saturated: unsaturated fatty acid values. For further details about individual constituents for breakfast, snacks and lunch, see table 7.2.

The subjects rested in the laboratory from 07:00 - 07:30 hours and then consumed one of the isoenergetic diets at 07:30. All subjects consumed breakfast within 20 min. Likewise, the isoenergetic snacks were consumed during a 5-min rest at 90 min and 355 min into the exercise protocol, and also during a 45-min rest for lunch at 235 min. Lunch was consumed within 25 min, by all subjects. The composition of the breakfast, snacks and lunch is displayed in Table 7.2.

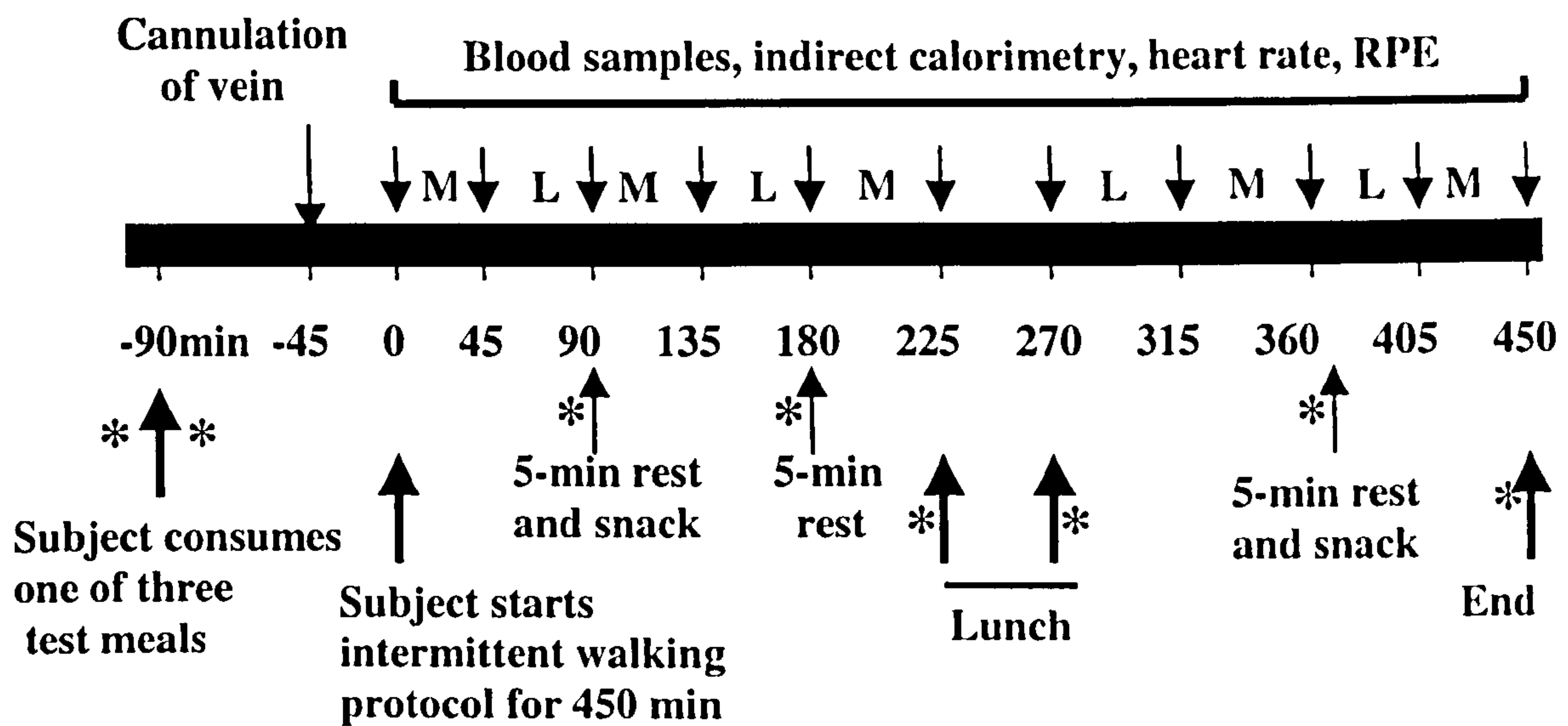


Figure 7.1. Test protocol for each exercise trial. Subjects consumed water ad-libitum. RPE, ratings of perceived exertion; M, moderate intensity walking at 50-55% $\dot{V}O_2$ max; L, low intensity walking at 25 – 30 % $\dot{V}O_2$ max; *administration of appetite questionnaires.

Table 7.2. Composition of breakfast, snacks and lunch meals.

	Breakfast			Snacks ¹			Lunch		
	Fat	Mixed	CHO	Fat	Mixed	CHO	Fat	Mixed	CHO
Fat (g)	70	30	8	21	6	2	84	42	23
% Energy as fat	80	35	9	86	29	1	80	40	22
CHO (g)	32	108	174	3	37	50	25	120	164
%Energy as CHO	15	57	85	5	68	95	10	47	65
Protein (g)	9	14	12	5	3	2	25	30	30
% Energy as protein	5	8	7	9	6	4	10	13	11
Total energy (kJ)	3291	3061	3234	923	857	894	3978	3968	3943

¹ Snacks were given in morning and afternoon. All the meals had similar proportions of simple sugars relative to total carbohydrate, and similar saturated: unsaturated fatty acid values.

Thirty minutes after the consumption of breakfast, a retrograde cannula was placed in a large vein draining the hand. The hand was then warmed throughout the study in a heated box to provide arterialized blood (McGuire et al., 1976). The cannula was kept patent with slow saline infusion (0.9% NaCl). Indirect calorimetry was performed using an online automated gas analyzer (Exercise Tester, P.K. Morgan, Chatham, Kent, UK). At 09:00 hours subjects began the intermittent walking protocol consisting of nine 45-min walking stages at either moderate or low intensity. The low and moderate intensity walks were estimated to correspond to 25-30% and 50-55%, respectively of $\dot{V}O_{2\text{ peak}}$. Subjects completed, a moderate followed by a low intensity walk and then rested for 5 min in which an isoenergetic snack was consumed. This was again repeated before another 5-min rest period, with no food intake. One final walk at moderate intensity was completed before a 45-min lunch break. The exercise following consisted of a low and moderate intensity walk, a 5-min rest in which an isoenergetic snack was consumed (same as previous), followed by a final low and moderate intensity walk (Fig. 7.1). The total distance walked was 35 km.

Blood samples (10 ml), indirect calorimetry, heart rate (Polar Sports Tester, Polar Electro, Kempele, Finland), and ratings of perceived exertion (RPE) (Borg, 1970) were obtained at rest and between every 35-45 min of the 9 walking stages and rest break for lunch, as described in sections 3.4.1, 3.7.1, and 3.8.2, respectively. Furthermore, ratings of fatigue and various appetite ratings (see below) were recorded prior to breakfast, immediately after breakfast, during all the rest breaks, prior and immediately after lunch, and immediately post-exercise (as described in section 3.8.1).

7.2.4 Design of diets: The constituents for the high-CHO diet were chosen to typify an athlete's breakfast, comprising cornflakes, puffed rice, skimmed milk, banana, white toast, jam, flavoured low-fat yogurt, and orange juice. The snacks included high-CHO products such as raisins and apricots. Lunch was comprised of bread, jam, banana, flavoured low-fat yogurt, and orange juice.

The constituents of the high-fat diet were chosen to typify a breakfast cereal, comprising oats, coconut, almonds, raisins, honey, sunflower oil, banana, double cream and milk. Snacks during the high-fat manipulation comprised products such as coconut and almonds, lunch included bread and cheese sandwiches with additional margarine, and ice cream with a small amount (50 ml/70 kg body mass) of long-chain triacylglycerol emulsion drink (Calogen; Scientific Hospital Supplies Group UK Ltd, Liverpool). The mixed meal incorporated the same isoenergetic nature of the high-CHO and high-fat diets. The macronutrient intake for the mixed diet was within the normative values for the general population (Ralph, 2000). All the meals had similar proportions of simple sugars relative to total carbohydrate, and similar saturated: unsaturated fatty acid values (Tables 1 and 2).

7.2.5 Calculation of energy and substrate balances: Energy expenditures and the percentage contributions of the CHO and fat oxidation were estimated from non-protein (NP) $\dot{V}O_2$ and RER data, as described in section 3.3.1.1 and 3.3.1.2

7.2.5 Subjective measurements: Various subjective ratings were recorded prior to breakfast, immediately after breakfast, during all the rest breaks, prior to and immediately after lunch, and immediately post-exercise. These subjective ratings and their measurements are described in section 3.8.1.

7.2.6 Analytic methods: Blood samples were taken and stored as described in section 3.4.1. A portion (20 μ l) of blood was used immediately for the measurement of haemoglobin and haematocrit in order to calculate changes in plasma volume. These methods and calculations are given in section 3.4.2. The concentrations of the metabolites plasma NEFA and TAG, glycerol, lactate, 3-OHB and glucose were measured as described in sections 3.5.1-3.5.3. Equally, the concentrations of the hormones insulin, cortisol, growth hormone and glucagon were measured as described in section 3.5.4. Plasma adrenaline and noradrenaline concentrations were analyzed as described in section 3.5.5.

7.2.7 Statistical procedures: Variables are presented as means \pm standard deviation (SD). Data were initially tested for normality, before being analyzed by repeated-measures analysis of variance (ANOVA). The ANOVA results were corrected by the Huynh-Feldt ϵ -adjusted degrees of freedom when the violation to sphericity was minimal (>0.75) and the Greenhouse-Geisser correction used when sphericity was violated (<0.75), and significant condition and condition-time interactions were identified (Field, 2000). To summarize the data not shown graphically, and to obtain post hoc comparisons between the dietary conditions, responses were assessed as total area under the curve (AUCs) over the 450-min protocol. The AUC was divided by the total exercise time to give an average value for the 450-min exercise period. Post hoc tests (Honestly Significantly Different) were performed to isolate any significant differences. Student's paired t-tests ascertained between-condition differences when a variable was measured once. Statistical significance was set at $P \leq 0.05$ for all statistical tests.

7.3 RESULTS

7.3.1 Energy Intake: Mean values and SDs for the three macronutrients during the 2 days before each trial (expressed as percentage of total energy intake) were 60.6 ± 8.4 CHO, 12.1 ± 4.1 protein, and 27.3 ± 8.1 fat. Mean daily energy intake was 11146 ± 1130 kJ/day. There were no significant differences in either the macronutrient intake or daily energy intake among the three trials. Furthermore, all subjects reported low levels of physical activity prior to each trial.

7.3.2 Energy expenditure, substrate oxidation and balances: For all trials, energy expenditure exceeded energy intake leading to a marked negative energy balance, which was the same throughout the trials (Fig 2). In the high-fat trial, the respiratory exchange ratio (RER) was significantly lower both before and during exercise compared with the other two diets, reflecting an increase in the proportion of fat oxidised (Fig. 7.2; Table 7.3). When comparing the three diets, fat balance was least negative on the high-fat trial (-110 ± 33 g) and mixed trials (-164 ± 14 g), and most negative on the high-CHO (-185 ± 10 g). In contrast, CHO balance was positive on the high-CHO (42 ± 36 g) and mixed trials (5 ± 6 g) but negative on the high-fat trial (-140 ± 31 g). Furthermore, the high-fat diet resulted in a higher total fat oxidation and when compared with the CHO diet (306 ± 63 g vs. 221 ± 34 g, $P < 0.05$), whereas the high-CHO diet resulted in an enhanced CHO oxidation when compared with the high fat trial (396 ± 26 g vs. 203 ± 10 g, $P < 0.05$), respectively (Table 7.3). However, as shown in Fig 7.3, when the total oxidation rates were expressed as a percentage of non-protein energy expenditure, after the high-CHO diet, the CHO and fat oxidation represented $44 \pm 17\%$ and $56 \pm 15\%$ respectively, of the non-protein derived energy expenditure; for the mixed diet oxidation of these substrates were $35 \pm 23\%$ and $65 \pm 16\%$ of the energy. Finally, in the high-fat diet, CHO and fat oxidation accounted for 23 ± 13 and $77 \pm 34\%$ of the non-protein derived energy expenditure. Taking the observations collectively, the mixed diet showed a metabolic response in between that of the high-CHO and high-fat diets.

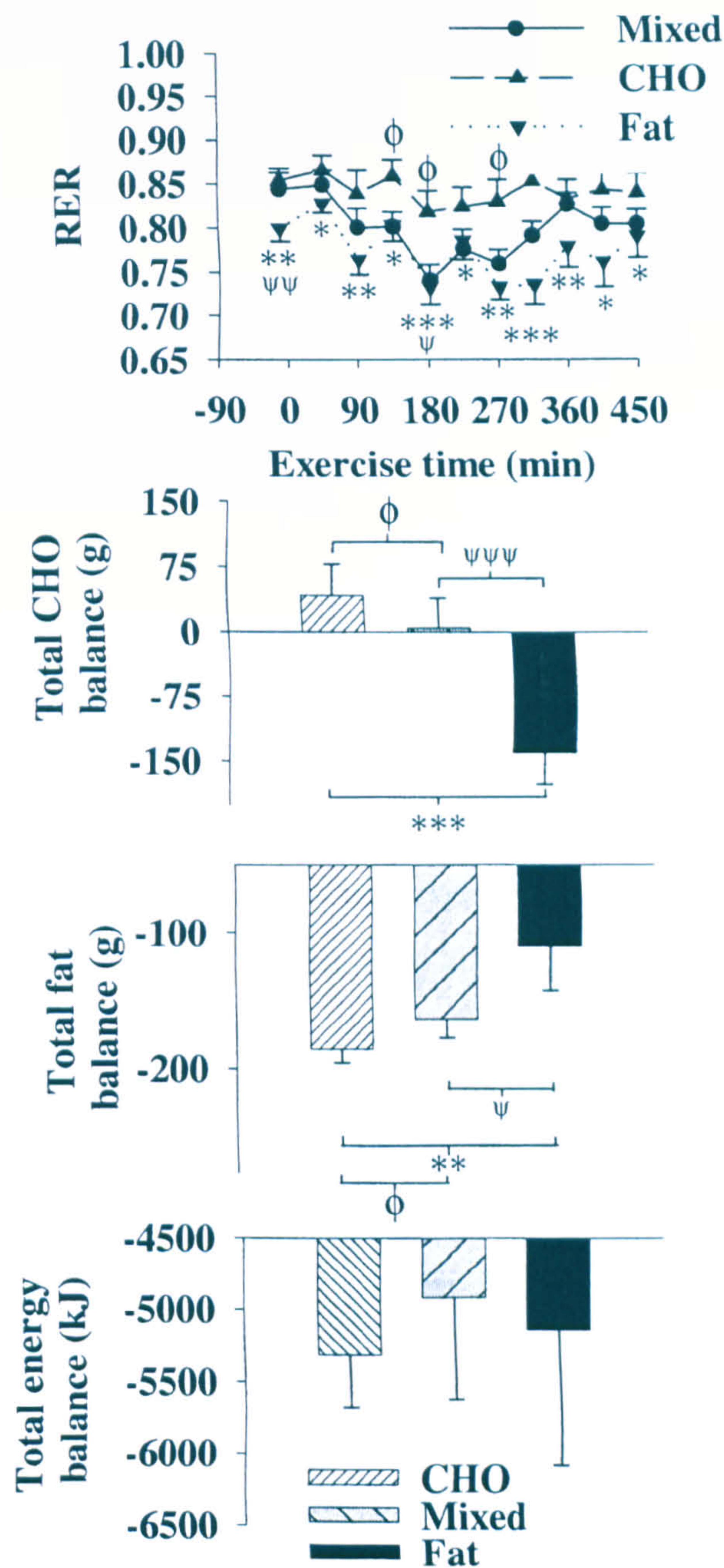


Figure 7.2. Respiratory exchange ratio (RER), total CHO, fat and energy balance during the 450-min exercise protocol after the 3 different diets. Values are means (SD); n = 8 for all 3 meals. * significant differences between the high-fat diet and the high-CHO diet (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). ϕ significant differences between the high-CHO and the mixed diet (ϕ $P < 0.05$). ψ significant differences between the high-fat and the mixed diet (ψ $P < 0.05$, $\psi\psi\psi$ $P < 0.001$).

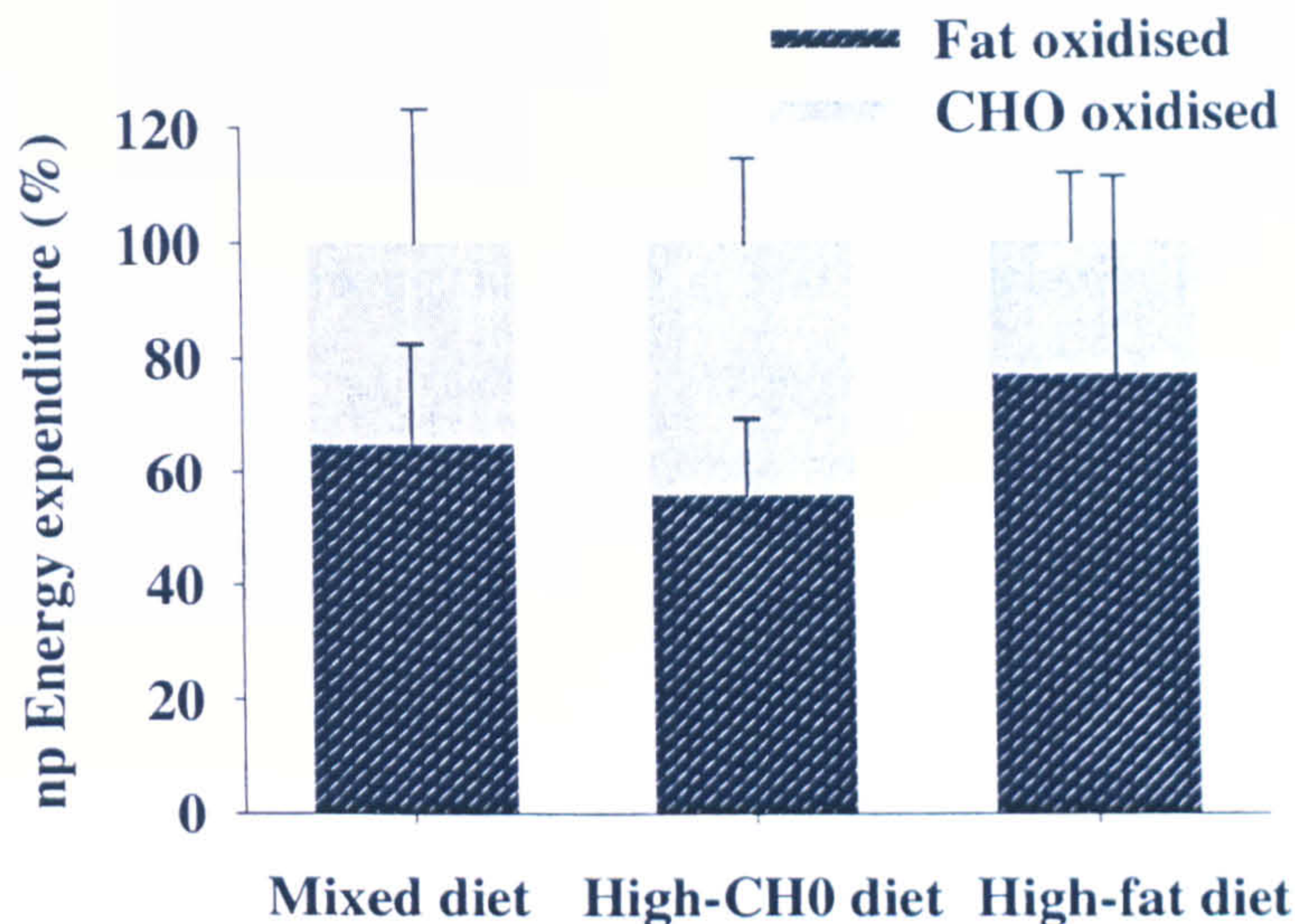


Figure 7.3. Percentage of non-protein energy expenditure linked to total carbohydrate and lipid oxidation over the 450-min protocol. Values are percentage (SD); $n = 8$ for all 3 meals. There were no significant differences between the three diets of the contribution of fat oxidation or CHO oxidation to non-protein energy expenditure.

Table 7.3. Substrate oxidation during the 450-min protocol.

	Mixed diet	High-CHO diet	High-fat diet
CHO oxidation, g/min	0.66 ± 0.11	0.88 ± 0.13	$0.45 \pm 0.09^{* \psi}$
Fat oxidation, g/min	0.55 ± 0.08	$0.49 \pm 0.06^{\phi}$	$0.68 \pm 0.10^{*}$
Energy expenditure, kJ/min	30.9 ± 1.9	32.2 ± 3.8	32.4 ± 5.4

Values are mean \pm SD for 8 subjects for all 3 meals. * significant differences between the high-fat diet and the high-CHO diet (* $P < 0.05$). ϕ significant differences between the high-CHO and the mixed diet ($\phi P < 0.05$). ψ significant differences between the high-fat and the mixed diet ($\psi P < 0.05$).

7.3.3 Blood glucose and plasma insulin concentrations: Prior to exercise and during the lunch break after ingestion of the large CHO intakes, blood glucose and plasma insulin concentrations were higher on both the mixed and high-CHO diets compared to the fat ($P < 0.05$; Table 7.4; statistics in Fig. 7.4.). For all trials the blood glucose concentration

showed a gradual decrease in the subsequent 225 min of exercise prior to lunch. The snack at 90 min did not produce any change in either glucose or insulin, in any trial (Fig. 4). A surge in blood glucose was evident following lunch (270 min) before a gradual decline during the final 180 min of exercise (Fig. 7.4). Again, there was no change in either glucose or insulin following the snack at 360 min (statistics in Fig. 7.4).

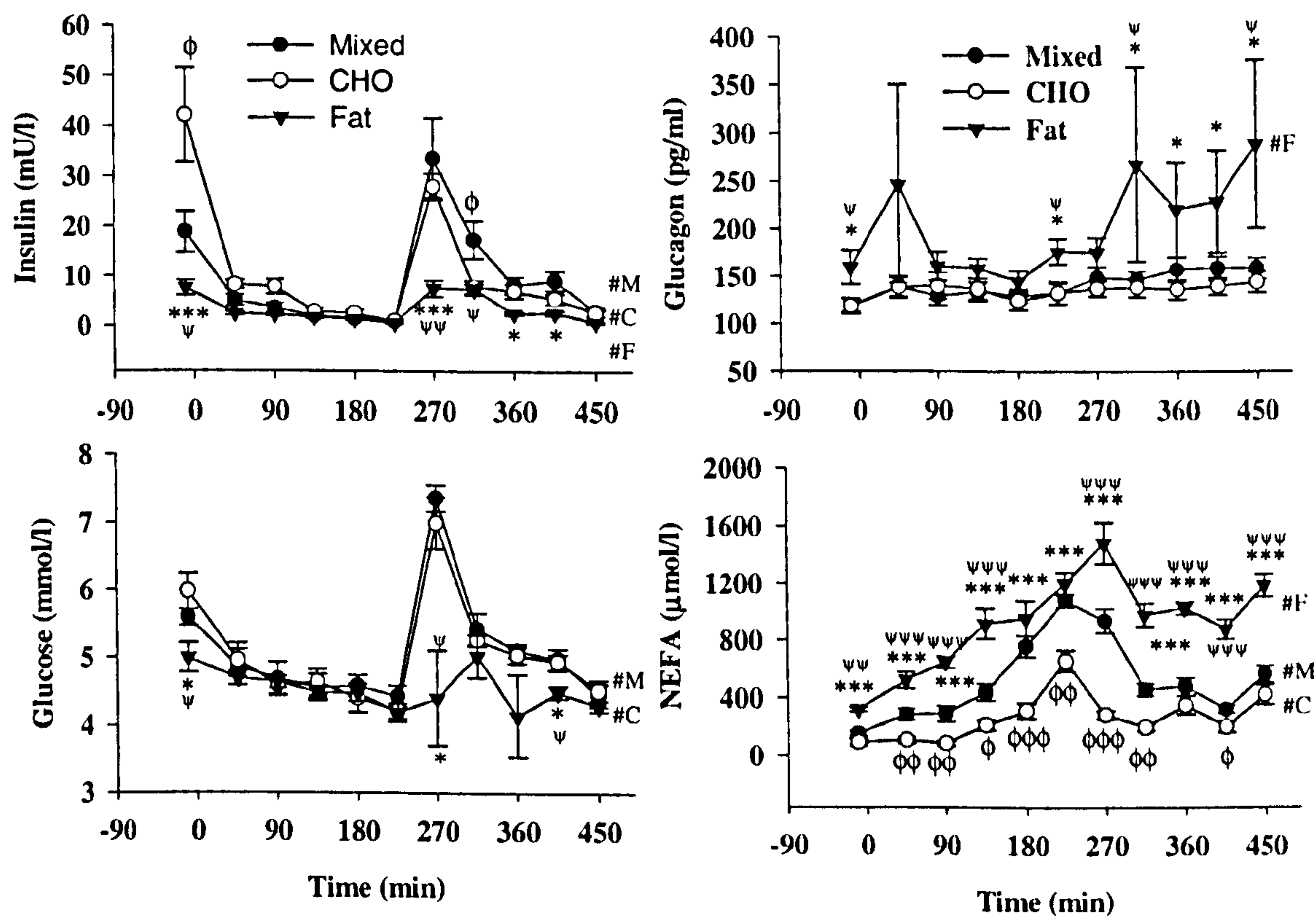


Figure 7.4. Plasma insulin, glucose, glucagon and NEFA concentrations during the 450-min exercise protocol after the 3 different diets. Values are means (SD); n = 8 for all 3 meals. * significant differences between the high-fat diet and the high-CHO diet (* $P < 0.05$, ** $P < 0.01$, * $P < 0.001$). φ significant differences between the high-CHO and the mixed diet (φ $P < 0.05$, φφ $P < 0.01$, φφφ $P < 0.001$). ψ significant differences between the high-fat and the mixed diet (ψ $P < 0.05$, ψψ $P < 0.01$, ψψψ $P < 0.001$). #F, #M, #C, significant change over time in the high-fat, mixed and high-CHO diets, respectively ($P < 0.05$).**

7.3.4 Plasma metabolite concentration: Plasma NEFA concentrations (Fig. 7.4.) were significantly greater both before exercise and during exercise with the high-fat diet than the high-CHO trials (statistics in Fig. 7.4.). Similarly, the NEFA concentrations were higher in the mixed diet than the high-CHO on the majority of the time points except at rest, 360 min and 450 min (Fig. 7.4.). The higher NEFA concentrations in both the high-fat and mixed diets are reflected in the higher area under the curves ($P < 0.05$; Table 7.4). Plasma TAG concentrations were lower at rest and during the first 90 min of exercise in the high-CHO diet compared with the high-fat and mixed diets. After this time point there were no significant difference in TAG concentrations among the diets. When comparing the area under the curves among the three diets (Table 7.4), there were trends for lower plasma TAG in the high-CHO trial ($P = 0.058$), although the differences did not reach statistical significance, probably as a result of the considerable between-subject variability (Fig. 7.5.). The areas under the curve showed higher concentration of 3-OHB in both the mixed and the high-fat diets compared to that of the high-CHO diet (Table 7.4). Apart from at rest and at 45 min, these higher concentrations of 3-OHB are reflected throughout the 450 min of exercise (statistics in Fig. 7.5.). Similarly, the areas under the curve showed higher concentrations of plasma glycerol in the high-fat diet compared with the high-CHO diet ($P < 0.05$), in spite of the relatively large between-subject variability (Table 7.4). The higher concentrations of glycerol in the high-fat diet were evident at 90 min, 135 min, 180 min, 315 min, 405 min and 450 min (Fig. 7.5.).

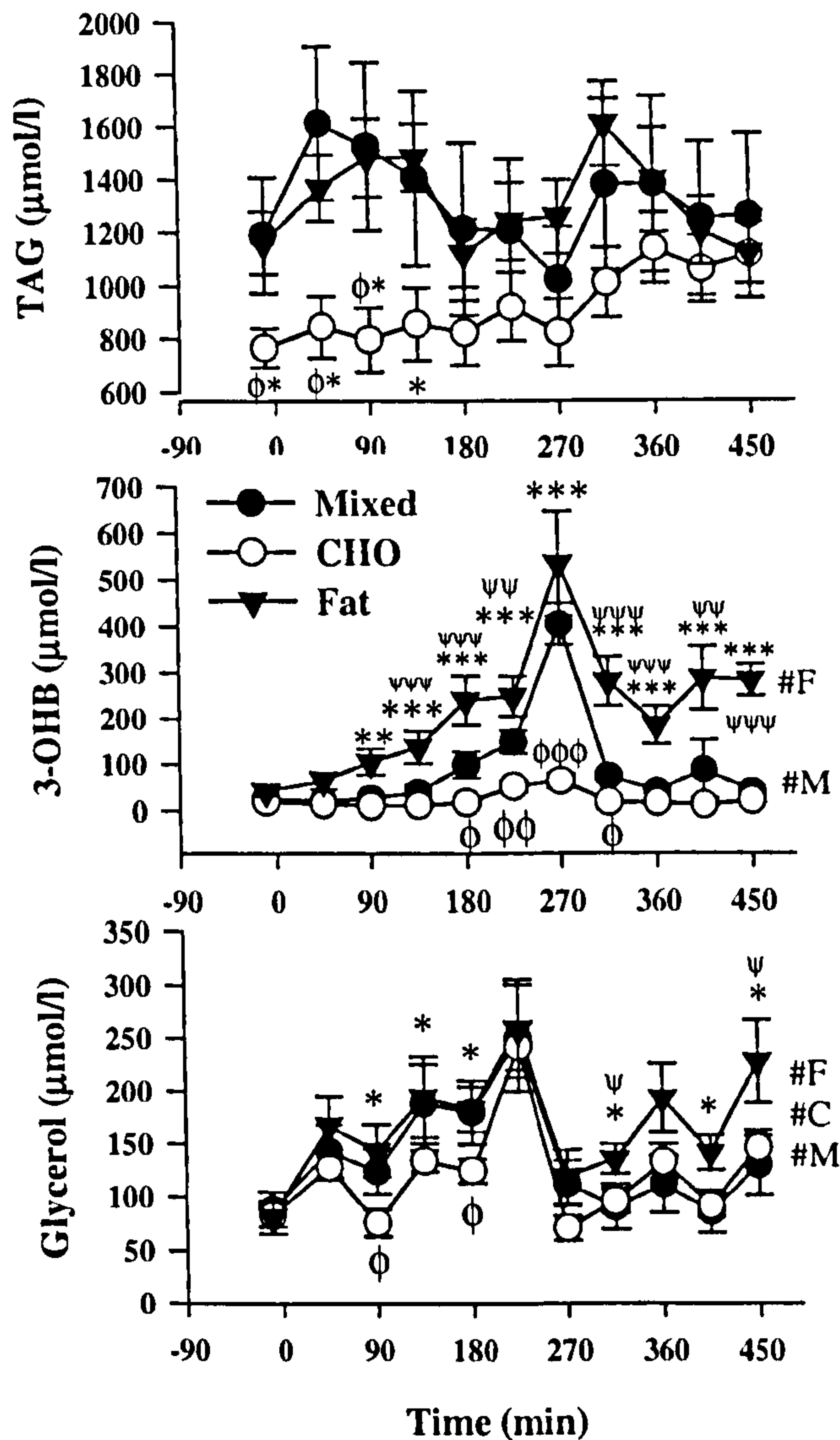


Figure 7.5. Triacylglycerol (TAG), 3-hydroxybutyrate (3-OHB), and glycerol concentrations during the 450-min exercise protocol after the 3 different diets. Values are means (SD); $n = 8$ for all 3 meals. * significant differences between the high-fat diet and the high-CHO diet (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). ϕ significant differences between the high-CHO and the mixed diet (ϕ $P < 0.05$, ϕϕ $P < 0.01$, ϕϕϕ $P < 0.001$). ψ significant differences between the high-fat and the mixed diet (ψ $P < 0.05$). #F, #M, #C, significant change over time in the high-fat, mixed and high-CHO diets, respectively ($P < 0.05$).

7.3.4 *Plasma hormone concentrations*: The high-fat diet resulted in a significantly higher area under the curve for glucagon concentration when compared with the high-CHO diet, although a large within-subject variability was evident (Table 7.4).

Table 7.4. Areas under the curve for metabolite and hormonal data.

	Mixed diet	High-CHO diet	High-fat diet
<u>Metabolites</u>			
NEFA ($\mu\text{mol/l}$)	540 \pm 86	265 \pm 57 ^ϕ	935 \pm 137** ^ψ
Glycerol ($\mu\text{mol/l}$)	147 \pm 40	123 \pm 24	182 \pm 49*
TAG ($\mu\text{mol/l}$)	1316 \pm 789	920 \pm 340	1328 \pm 327
3-OHB ($\mu\text{mol/l}$)	99 \pm 4	125 \pm 6 ^ϕ	234 \pm 100*
Lactate (mmol/l)	0.6 \pm 0.1	0.8 \pm 0.1	0.5 \pm 0.1
Glucose (mmol/l)	5.1 \pm 0.3	5.0 \pm 0.3	4.6 \pm 0.2* ^ψ
<u>Hormones</u>			
GH (ng/ml)	2.3 \pm 1.4	2.9 \pm 2.5	3.3 \pm 1.9
Glucagon (pg/ml)	141 \pm 27	135 \pm 28	200 \pm 60*
Insulin (mU/l)	9.1 \pm 4.5	9.2 \pm 1.9	3.1 \pm 1.0*
Cortisol (nmol/l)	360 \pm 54	330 \pm 65	342 \pm 66
Noradrenaline (nmol/l)	3.7 \pm 1.2	3.8 \pm 1.8	3.5 \pm 1.4
Adrenaline (nmol/l)	0.5 \pm 0.6	0.5 \pm 0.2	1.2 \pm 1.7

Values are mean \pm SD for 8 subjects for all 3 meals. Areas under the curve were divided by time baseline to represent average value over the respective 450 min protocol. NEFA, non-esterified fatty acids; TAG, triacylglycerol; 3-OHB, 3-hydroxybutyrate; GH, growth hormone. * significant differences between the high-fat diet and the high-CHO diet (* $P < 0.05$, ** $P < 0.01$). ^ϕ significant differences between the high-CHO and the mixed diet (^ϕ $P < 0.05$). ^ψ significant differences between the high-fat and the mixed diet (^ψ $P < 0.05$).

The increases in glucagon concentrations were most apparent prior to exercise, at 225 min and during the last 180 min of exercise (statistics in Fig. 7.4.). Adrenaline, growth

hormone and cortisol showed significant changes over time (statistics in Fig. 7.6.), with no differences between the different diets (Table 7.3; Fig 7.6). This interaction was most pronounced at the sample just prior to lunch at 225 min where a surge in the hormones was evident in a marked stress response (statistics in Fig. 7.6). Plasma cortisol concentrations remained similar throughout the exercise protocol, exhibiting a normal diurnal variation in concentrations with, as mentioned, a significant surge on all diets prior to the rest for lunch (Fig. 7.6).

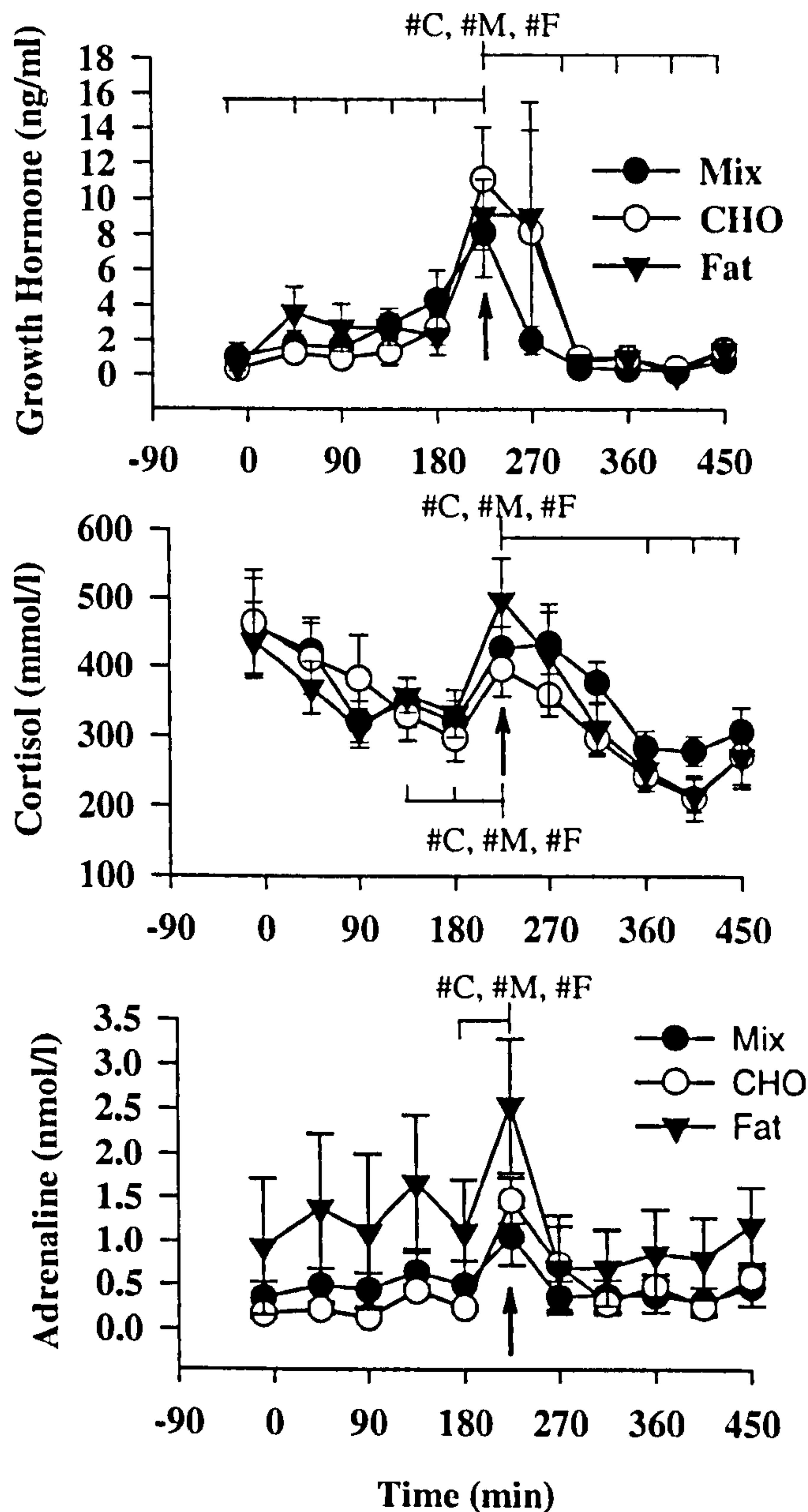


Figure 7.6. Growth hormone, cortisol and adrenaline concentrations during the 450-min exercise protocol after the 3 different diets. Values are means (SD); $n = 8$ for all 3 meals. Repeated measures ANOVA for all hormones showed significant effects of time ($P < 0.05$) but not meal type. #F, #M, #C, significant change over time, at the 225 min time point, in the high-fat, mixed and high-CHO diets, respectively ($P < 0.05$). The arrows denotes surge in the stress hormones prior to the 45-min rest for lunch.

7.3.5 Heart rate and RPE: Heart rate and RPE increased significantly above pre-exercise values for all trials. Both the heart rate and RPE values were significantly higher during the high intensity walking compared with walking at low intensity. Despite the change in heart rate and RPE in accordance with the exercise intensities, there were no significant differences observed among the three trials at any point (data not shown).

7.3.6 Subjective measurements: Although there were significant effects of time ($P < 0.001$) on ratings of hunger, fullness and satiety over the protocol, there was no significant effect of meal type among the three conditions. Likewise, although there was a gradual increase in ratings of fatigue throughout the exercise, differences were not significant (Fig. 7.7). Furthermore, there were no differences in ratings of thirst, nausea, strength of appetite, or desire to eat (data not shown) between the experimental trials.

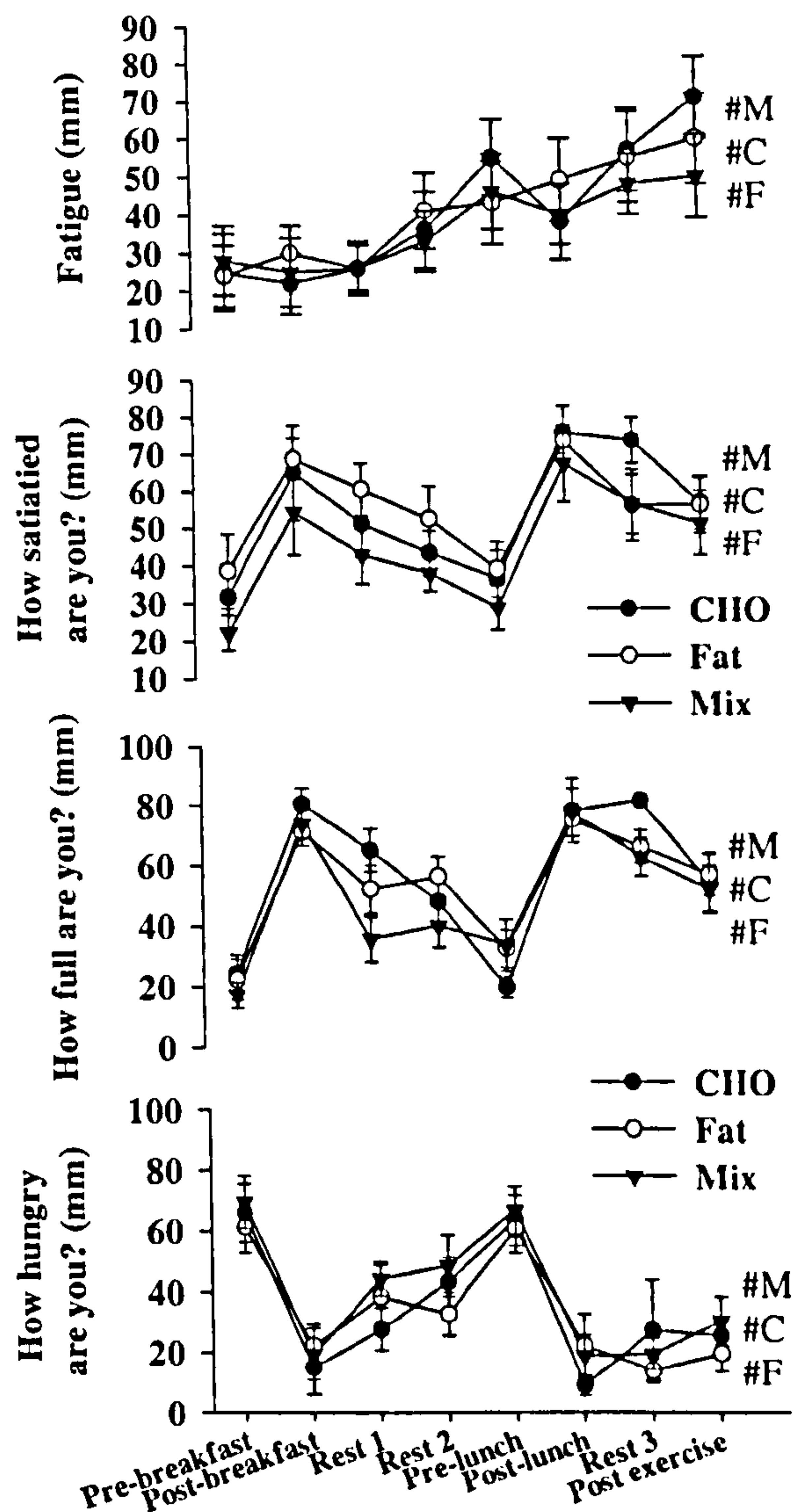


Figure 7.7. Fatigue, satiety, fullness and hunger at intervals during the prolonged exercise protocol. Values are means (SD); $n = 8$ for all 3 meals. Repeated measures ANOVA for fatigue, satiety, fullness and hunger showed significant effects of time but no effects of meal type. #F, #M, #C, significant change over time in the high-fat diet, mixed and high-CHO diet, respectively ($P < 0.05$).

7.4 DISCUSSION

The present study has yielded two important findings. Firstly, the metabolic responses were, to an extent, susceptible to dietary manipulations. After each diet, although the total fat and CHO oxidation corresponded to the amount of each substrate administered, the main source of energy in all trials was fat oxidation. However, the dietary manipulation did significantly alter the metabolic and hormonal milieu. Secondly, the absence of any change in heart rate, RPE or subjective ratings of fatigue between the dietary manipulations during prolonged exercise is an important, rather than uninteresting, observation. This suggests that dietary composition will not adversely affect physiological and subjective factors over one day. However, the high-fat diet resulted in a negative CHO balance over the exercise period. In accordance with previous studies which have involved higher intensity exercise (Costill et al., 1988; Starling et al., 1997), high-fat diets might not be so good for further exercise even at low to moderate intensities. Decreases in the glycogen stores (Costill et al., 1988) and/or muscle triacylglycerol concentrations (Starling et al., 1997), especially if continued over a few days of walking, would be detrimental to the ability to sustain the activity.

7.4.1 Substrate oxidation and balances: In resting conditions, there is a clear hierarchy in the maintenance of macronutrient balances, with CHO and protein having the highest priority (Abbott et al., 1988; Schrauwen et al., 2000). Fat oxidation, on the other hand, is only marginally influenced by fat intake during resting conditions. Prolonged exercise will generally lead to a negative energy balance due to difficulties in matching sufficient energy intake to the high-energy turnover as a consequence of the exercise. Since fat oxidation is determined mainly by the difference between energy expenditure and CHO and protein oxidation, fat balance is strongly correlated with energy balance (Schrauwen et al., 2000). The relationship between fat oxidation and energy balance becomes apparent when the substrate balances are considered, i.e. the amounts of substrates ingested minus the amounts oxidized (Fig. 7.2). Although there were no differences in the negative energy balance among the three trials, the fat balance was more negative on the CHO diet than in the mixed and fat trials. This highlights that despite the negative fat

balance in all trials, the CHO intake can, to a certain extent, decrease the amount of fat oxidized. The failure of the dietary CHO to promote CHO oxidation to the extent shown in resting studies (Bobbioni-Harsch et al., 1997; Whitley et al., 1997) is most probably both a consequence of the large negative energy balance (Schrauwen et al., 2000) and a result of the hormonal/metabolic state which favours fat oxidation at low to moderate intensities (Horowitz and Klein, 2000; Jeukendrup and Jentjens, 2000). These results suggest that high amounts of dietary CHO during prolonged walking decrease the contribution of fat oxidation, but only to a limited extent.

The high-fat diet led to an increased fat oxidation which reduced the magnitude of the negative fat balance, but produced a greater negative CHO balance. This may be important, as the high-fat diet was low in CHO, suggesting some use of muscle and or liver glycogen stores. Previous studies have suggested that glycogen stores are important in determining the rate at which fat oxidation is adapted to fat intake (Schrauwen et al., 1997a; Schrauwen et al., 1997b; Schrauwen et al., 1998). Because fat oxidation does not adapt rapidly to the increased fat intake with a high-fat diet, subjects will be in a negative CHO balance. This means that high-fat diets, in the present study, will lead to a reduction in glycogen stores, and an increase in fat oxidation (Flatt, 1987). This questions the benefit of high-fat strategies during prolonged exercise, which may entail high-energy deficits in relation to the high-fat strategy. For example, high-fat diets may actually promote glycogen utilisation as opposed to glycogen sparing, potentially leading to early fatigue.

Previous exercise studies in which subjects were fed isoenergetic diets (Whitley et al., 1998) or intralipid and heparin infusion (Ravussin et al., 1986) have shown that, despite marked alterations in substrate availability in plasma, the pattern of substrate oxidation during exercise is remarkably resistant to alteration by dietary means. These contrast with the results of the present study where the relative rate of fat and CHO oxidation varied with the different diets. The disparity of results is most likely explained by the differing diets and the differing intensities of activity used, which would affect the substrate oxidative response (Coyle, 2000). For example, in a number of the studies

isoenergetic diets were used which have entailed a lower fat intake (expressed at percentage of total energy intake) and higher exercise intensities (Whitley et al., 1998; Burke et al., 2000; Carey et al., 2001), both of which would decrease the substrate oxidative response (Coyle, 2000).

There was an enhanced fat oxidation during the high-fat trial. Recent studies have suggested that the increase in fat oxidation following a high-fat diet can be accounted for by both adipose derived fatty acids oxidation and from triacylglycerol -derived fatty acid oxidation (VLDL and/or intramuscular TAG) (Sidossis et al., 1995; Turcotte et al., 1995; Schrauwen et al., 2000). Nevertheless, the relative contribution of plasma triacylglycerols to energy production during exercise remains unclear (Horowitz and Klein, 2000). Because fat ingestion acutely increases plasma triacylglycerol, as demonstrated in the present study, quantifying the contribution of this energy source during exercise will resolve whether fat ingestion can contribute substantially to energy metabolism during exercise.

The greater increase in fat utilisation observed on the high-fat condition is not, apparently, without its limitations. The oxygen requirement for the oxidation of fat can be up to 16% greater than that required to produce the same amount of ATP from the oxidation of CHO. One litre of oxygen can oxidize glycogen and produce approximately 6.5 moles of ATP compared with 5.6 moles when palmitate is oxidized (Astrand and Rodahl, 1986). Consequently, a change toward fat oxidation should produce a higher cardiovascular stress (Newsholme, 1981; Sherman and Leenders, 1995). However, in previous studies in which the plasma NEFA has been elevated acutely by either a high fat meal or by Intralipid-heparin infusion, no effect on oxygen uptake or HR during exercise has been reported (Hargreaves et al., 1991; Vukovich et al., 1993; Pitsiladis et al., 1999). In agreement, the present study showed no differences in $\dot{V}O_2$, HR or RPE between the three trials.

7.4.2 Metabolic and hormonal responses: The present study confirms the well-established observation that both meals pre-exercise and during exercise, can profoundly

affect the pattern of substrate availability in the plasma. In the present study a high-CHO meal (438 g CHO and 35 g fat) which included breakfast, snacks and lunch resulted in a significant suppression of plasma NEFA, 3-OHB and glycerol during the 450-min exercise protocol compared with an enhanced plasma NEFA, 3-OHB and glycerol concentration after an isoenergetic high fat meal (63 g CHO and 196 g fat) and isoenergetic mixed meal (302 g CHO and 84 g fat). This result is similar to previous work by Coyle et al. (1985) and Montain et al. (1991) who reported a suppression of plasma NEFA concentrations during submaximal exercise in endurance trained cyclist after ingestion of CHO loads several hours before exercise. These observations highlight the potential for manipulating fatty acid supply by dietary means.

It was notable, however, that there was a marked stress response as shown by elevations in the concentrations of adrenaline, cortisol and growth hormone just prior to the lunch break. This increase occurred in all three dietary conditions (Fig. 6) and the point coincided with the longest period without ingestion of food whilst still walking. This suggests that snacks, in combination with the breakfast and lunch may confer some protection against a marked increase in stress hormones. Apart from the spike just prior to lunch, plasma cortisol showed an actual decline throughout the prolonged exercise protocol, probably as a result of increased clearance (Smoack et al., 1991) and the circadian variation (Waterhouse et al., 1997).

7.4.3 Subjective measurements: The effects of dietary manipulation during very prolonged exercise on hunger responses have not been quantified previously. Short-term studies which have sought to measure satiating efficiency have produced equivocal results. Indirect evidence from controlled weight loss studies have suggested diets high in CHO suppress appetite and subsequent energy intake (Duncan et al., 1983; Tremblay et al., 1989; Heini et al., 1998). The suggested mechanism(s) for this suppression of appetite was that of insulin which exerts this anorexic effect (Heini et al., 1998). In the present study, a significant decrease in circulating insulin levels in the high-fat trial was evident suggesting that, during prolonged exercise, insulin plays no role in modulating any of the subjective ratings of hunger, satiety or fullness. However, some other resting studies

appear to have demonstrated that fat has a satiating action equivalent to CHO (Van Stratum et al., 1978; Foltin et al., 1990; Rolls et al., 1991). In the present study, despite the differing rates of digestion and absorption of CHO and fat, we did not detect any differences in ratings of appetite during the protocol. Further research into prolonged exercise and appetite is clearly warranted.

The results from this study clearly show that the differing dietary manipulations resulted in a similar energy deficit. The similar negative energy balance suggests that a wide range of dietary patterns may be acceptable for those trying to lose weight by incorporating moderate intensity exercise into their routine. Prolonged walking may be considered a useful adjunct in a weight loss programme.

In summary, the availability of fatty acids, and of other substrates, and the pattern of substrate oxidation and balance, during prolonged walking are altered by dietary means. The main source of energy in all trials was predominantly fat oxidation, although diet influenced the degree of total CHO and fat oxidation. These results emphasize that the close relationship between fat and CHO metabolism following isoenergetic meals can be somewhat displaced, most probably due to the prolonged low to moderate intensity of the exercise and subsequent negative energy balance. In accordance with previous studies which have involved more high intensity exercise (Costill et al., 1988; Starling et al., 1997), high-fat diets might not be so good for further exercise even at low to moderate intensities. Decreases in the glycogen stores (Costill et al., 1988) and/or muscle triacylglycerol concentrations (Starling et al., 1997), especially if continued over a few days of walking, would be detrimental to the ability to sustain the activity.

Therefore, the most important consideration may be the potential effects of energy intake per se on recreational participants; this particular area was the focus of the final study.

CHAPTER 8

PHYSIOLOGICAL, METABOLIC AND PERFORMANCE IMPLICATIONS OF A PROLONGED HILL WALK: EFFECTS OF DIFFERENT ENERGY INTAKES

8.1 INTRODUCTION

Several kinds of prolonged exercise such as cycling, hill walking, mountaineering and military manoeuvres induce a negative energy balance due to smaller energy intake than expenditure. The influence of fasting on metabolic changes and physical performance has been studied extensively (Dohn et al., 1986; Loy et al., 1986; Gleeson et al., 1988) and it appears that short-term fasting reduces physical performance and modifies the metabolic response to exercise. Furthermore, there is increasing evidence that a negative energy balance may have several adverse effects on health e.g., on the immune system (Chandra, 1990) as well as on sex hormones and bone mineralisation (Drinkwater, 1983; Calloway, 1987; Santora, 1987).

Studies conducted on military personnel have indicated, despite sustaining energy deficits of between 3 to 7 MJ/day, no obvious or reported ill-effects on participants (Forbes-Ewan et al., 1989; Hoyt et al., 1991; Jones et al., 1993). Performance measures were not made during these studies.

Guezennec et al. (1994) investigated physical performance and metabolic changes induced by combining prolonged exercise with three different energy intakes. Twenty-seven male soldiers were randomly assigned to three groups receiving 7.6 MJ/day (low intake), 13.4 MJ/day (medium intake), or 17.6 MJ/day (high intake). The soldiers took part in a 5-day combat course of heavy and continuous physical activity (energy expenditure was estimated to exceed 21 MJ/day), with less than 4 h of sleep per solar day. Maximal oxygen uptake ($\dot{V}O_2$ max) and anaerobic performances were measured before and after the combat course. Whilst the soldiers on the medium and high energy intake maintained their measured performances, those on the low energy intake showed a significant decrease in $\dot{V}O_2$ max (8%) and in anaerobic power (14%). The data suggest that only a severe energy deficit leads to a small decrease in performance.

Hill walking is such an activity which is likely to entail both high energy expenditure and large participation from recreational activists. The high overall energy expenditure is due to the prolonged nature of the activity. An important and unique consideration is that hill walking is a recreational activity that attracts a wide range of participants with varying age and fitness levels.

In the initial study (Chapter 5), unremarkable changes in reaction time (cognitive function), mood state and grip strength were evident after a 12-km strenuous hill walk. The data from this study suggest that, despite serious physiological stress, the subjects demonstrated normal motor control during the walk. Furthermore, in the questionnaire-based study (Chapter 4), based on 100 hill walkers, a weak but significant relationship was identified between subjects consuming a low energy intake and an increased incidence of injury. In addition, the results from the questionnaire indicated normal distances covered of 18 – 26 km over 6 – 8 hours in duration. Such distance and duration will likely entail large energy expenditures, and potentially elicit a large deficit in energy. The implications of such an acute energy deficiency on recreational participants remain unclear and have not been investigated in this context.

The results from the previous chapters suggest that the vast amount of hill walkers may be undertaking the activity with relatively low-energy intakes and subsequently sustaining high-energy deficits. Furthermore, Chapter 7 highlighted that despite the ability of fat intake to alter the metabolic responses to exercise, it may be of little benefit to the walker, and may actually impair performance if activity was continued over consecutive days. Therefore, the most important consideration may be the potential effects of energy intake per se on recreational participants.

The purpose of the present study therefore was to determine the effects of two different energy intakes on some relevant responses that are important in the safety of hill walkers, such as the potential thermal stress, impaired psychomotor performance and the ability to maintain glycaemia. It was aimed to extend the previous investigations into a hill walking event over a more prolonged period than considered in the initial study (Chapter 5).

Based on the previous studies (Chapters 4, 5 and 6) it was postulated that, due to the large energy cost of such hill walking events, subjects receiving a low energy intake may be more prone to compromises in performance than their counterparts receiving a high energy intake. Furthermore, as evident in the earlier studies (Chapter 5, 6, 7), it was hypothesised that the low energy intake stimulates metabolism in such a way that

blood glucose concentrations is maintained, mediated via an increased mobilisation of fat.

8.2. METHODS

8.2.1 Subjects

Sixteen male subjects participated in this study. All subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. The study was approved by the Liverpool John Moores University Human Ethics Committee (further details in section 3.9.1). The physical characteristics of the subjects are shown in Table 8.1. The majority of the subjects were active and experienced hill walkers. Experiments were conducted over three weeks in January.

Percentage of body fat (%fat) was estimated as described in section 3.1.3. Peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) was established by using a continuous incremental treadmill running test to exhaustion, as described in section 3.2. All subjects were studied during January, with a minimum of 2 days between each condition. Subjects completed a 2-day dietary and physical activity diary recorded before each of the two trials, and were asked to keep their diet and activity the same before each test day. In this way, variations in diet and exercise before the two trials were minimized.

Table 8.1. Physical characteristics of the subjects

	Mean \pm SD	Range
Age, years	24 \pm 3	19 - 29
Height, m	1.79 \pm 0.08	1.70 - 1.86
Body mass, kg	76.3 \pm 11.8	62 - 94
BMI, kg/m ²	24.6 \pm 2.3	18 - 27
Body fat, %	16.2 \pm 5.3	5.4 - 26
$\dot{V}O_{2\text{ peak}}$, (ml/kg/min)	51.8 \pm 6.5	43 - 64

BMI, body mass index; Mean values \pm SD, based on 16 subjects.

8.2.2. Protocol and Procedures

In a balanced design, subjects completed a 21-km hill walk under two different dietary conditions. The course varied in elevation from 100 m to 902 m above sea level and consisted of a range of gradients and terrain typical of a mountainous hill walk. A cottage was used as a temporary field laboratory and for living accommodation, and was located at the start and end of the hill walk. Subjects woke each morning between 05:00 and 05:30 hours and completed the preliminary experiments prior to the hill walk, shown in Figure 1. Self-paced walking began each day between 07:00–08:00 hours. Subjects were instructed to record their nude body mass each morning before consuming any food or beverages and after voiding, with calibrated balance scales accurate to 0.1 kg, and immediately upon completion of the walk. Following the initial weighing, and preliminary tests, the participants inserted a rectal temperature probe to a depth of 10 cm beyond the anal sphincter. The rectal probe was connected to a data logger (Squirrel meter 1000, Grant Instruments, Cambridge, UK) which recorded data every 6 min. During the walk a rest period of approximately 1 – 3 min was allowed every time environmental temperature measurements were made, and a 15-min stop at approximately half-way through the course. All subjects carried a lightweight water-proof backpack that contained spare clothing, food and water supply for the walk, and the data logger described above. The loaded pack weighed approximately 9.5 kg, which is consistent with a hill walking scenario.

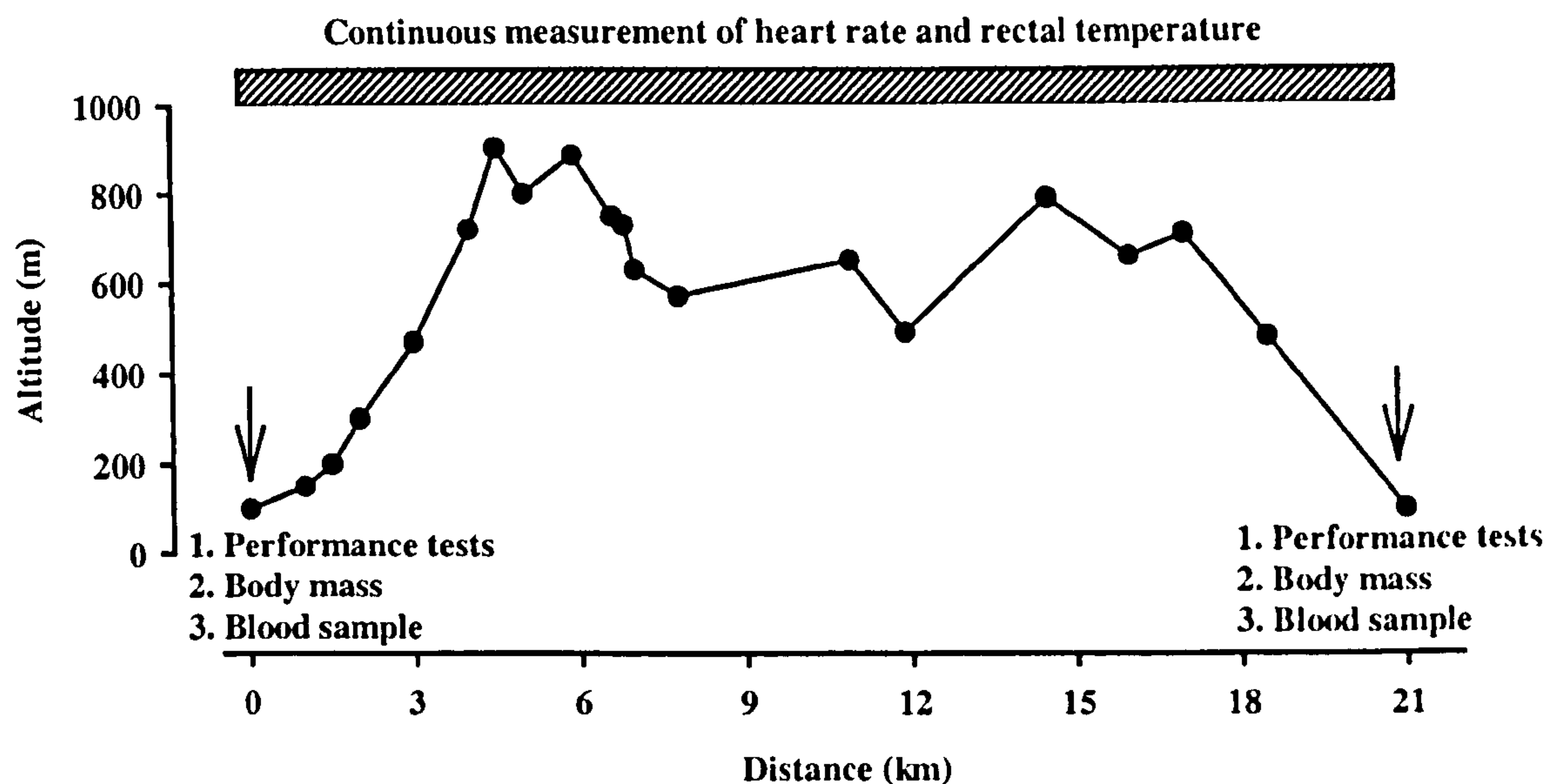


Fig 8.1. Hill profile and summary of experimental design.

8.2.3. Diet

Subjects were asked to fast from 20:00 hours and then consume a standardized breakfast of 2.5 MJ. For the duration of the hill walk, subjects were then given either a high or low energy intake diet in a balanced design. The diets were divided into three equal amounts, and subjects were encouraged to consume one amount by 7 km, one by 14 km and the final amount by 21 km.

The constituents for both the diets were chosen from a range of snacks which included commercially available products such as biscuits, chocolate bars, flapjacks and cheese sandwiches. In both conditions subjects were instructed, and monitored by the same investigator, to consume approximately 400 ml/h of water. The total energy intake on the high-energy intake diet was 12.5 MJ, compared with 2.5 MJ on the low-energy diet. Both diets had similar macronutrient distribution, as given in Table 8.2. The low-energy intake was based on the results from the previous chapters (Chapters 4 and 5), which recorded similar intakes used by participants.

Table 8.2. Total composition of test meals*

	High-energy Intake	Low-energy Intake
Fat (g)	135	26
% Saturated	41	39
%Energy	39	38
CHO (g)	401	74
% Simple	54	56
% Energy	51	52
Protein (g)	74	15
% Energy	10	10
Total		
Energy, kJ	12679	2589

Both diets had similar proportions of simple sugars relative to total carbohydrate, and similar saturated: unsaturated fatty acid values.

8.2.4. Measurements and Analysis:

8.2.4.1 Temperature and heart rate: The measurements of rectal temperature and heart rate are as described in section 3.7.1. Due to technical problems during the walks, where the data logging system malfunctioned, the rectal temperature data are based on 14 subjects.

8.2.4.2 Environmental measurements: Measurements of the environmental conditions (wind speed, dry and wet bulb temperatures), and calculation of the wind chill index are given in section 3.7.2.

8.2.4.3 Psychomotor and performance measurements: In the morning, prior to walking and immediately upon completion of the hill walk, subjects completed a battery of psychomotor and performance tests which included choice reaction time (cognitive processing time), Profile of Mood State (POMS), grip and leg strength (muscular power), flexibility, dynamic and static balance and kinaesthetic differentiation tests. All these psychomotor and performance tests were carried out as described in section 3.8 and 3.9.

The subjects were fully familiarised with the use of the equipment, and each test was performed three times in a balanced fashion. The reliability and test-retest reproducibility of these tests have been recently described (Rinne et al., 2001).

8.2.4.4 Blood and urine sampling and analysis: Blood samples were taken and stored as described in section 3.4.1. A portion (20 μ l) of blood was used immediately for the measurement of haemoglobin and haematocrit in order to calculate changes in plasma volume. These methods and calculations are given in section 3.4.2. The concentrations of the metabolites plasma NEFA and TAG, glycerol, 3-OHB and glucose were measured as described in sections 3.5.1-3.5.3. Equally, the concentrations of the hormones insulin, cortisol and growth hormone were measured as described in section 3.5.4.

An index of dehydration was determined in triplicate using urine osmolality, as described in section 3.6.1. For the urine osmolality, a 5-ml sample was produced after the first void of the day. Another 5-ml urine sample was then collected immediately upon completion of the walk.

8.2.4.5 Statistical analysis: Variables are presented as means \pm standard deviation (SD). Data were initially tested for normality, before being analysed by a two-way repeated-measures analysis of variance (ANOVA). The ANOVA results were corrected by the Huynh-Feldt ϵ -adjusted degrees of freedom when the violation to sphericity was minimal (>0.75) and the Greenhouse-Geisser correction used when sphericity was violated (<0.75), and significant condition and condition-time interactions were identified (Field, 2000). Post hoc tests (Honestly Significantly Different) were performed to isolate any significant differences. Student's paired t-tests ascertained between-condition differences when a variable was measured once. For data which were not normally distributed, the Kruskal-Wallis test followed by the Wilcoxon matched-pair signed rank test where appropriate was used for analysis. Statistical significance was set at $P \leq 0.05$ for all statistical tests.

8.3. RESULTS

8.3.1. Exercise duration and diet compliance: All subjects completed the 21-km hill walk. The mean (range) duration for the hill-walk was 7 h 28 min (5 h 51 min - 10 h 56 min). There were no differences between the different energy conditions and time to complete the walk. The differences in the time to complete the walk were due mainly to variations in weather conditions and terrain. Ten subjects experienced very sustained wet and windy weather during both of their walking conditions. During a number of these adverse conditions wind speeds of up to 60 mph (100 km/h) were recorded.

During the high-energy intake, three of the subjects had difficulties in consuming all of the food. The amount of unconsumed food was less than 1.4 MJ per person. Since this amount was less than 10% of the required intake, data for these subjects were included in subsequent analysis. All subjects consumed all the food on the low-energy diet.

8.3.2. Thermoregulatory, heart rate and environmental data: The mean (range) of the recorded environmental data for air velocity, air temperature and wind chill index was 3.7 (0.9 - 15.8) m/s, 8.8 (1.9 - 14.1) °C and 638 (258 - 1365) kcal/m²/h, respectively. These figures highlight the variability in the weather conditions over the period of testing. The surface conditions on the walks tended to vary with the weather. Little snow or ice was encountered due to the wet and relatively mild conditions.

The profile of the rectal temperature measurements is given in Fig 8.2. Although there was a clear trend of a lowered rectal temperature during the low-energy intake conditions, this did not reach statistical significance at any time point. However, when comparing the ten subjects who experienced sustained wet and windy conditions on both dietary conditions, a significant difference between the high- and low-energy intake conditions ($P < 0.05$) was evident during each measurement point during the last 9 km of the walk. A number of the subjects in each of the low- and high-energy intake conditions exhibited marked decreases in rectal temperature (Fig 8.2). One subject's rectal temperature fell below 35°C; after warming and monitoring, his rectal temperature rapidly increased to normal levels and he sustained no ill effects. It was noteworthy that the subjects completed their walks in sustained wet and windy weather and, whilst they were adequately dressed for the conditions, they were all very lean individuals with an estimated percentage body fat less

then 8%.

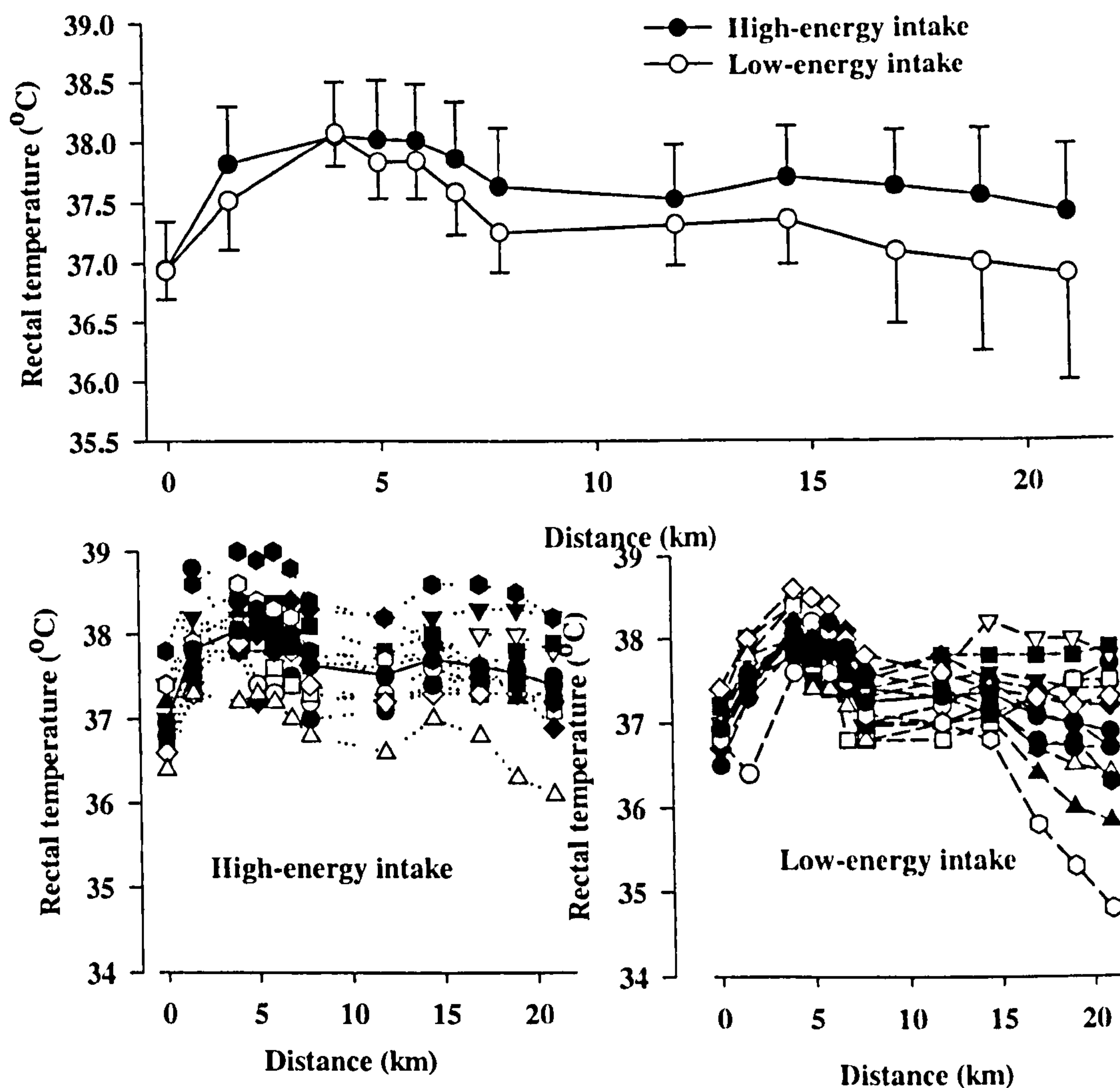


Figure 8.2. Mean (top) and individual rectal temperature results (below) during the hill walk. Values are Mean \pm SD; n = 14.

The heart rate profile is given in Fig 8.3. No between group differences were evident in the heart rate responses. The mean heart rate for the duration of the walk was 132 ± 19 (b/min) and 135 ± 21 (b/min) in the high and low-energy intakes, respectively.

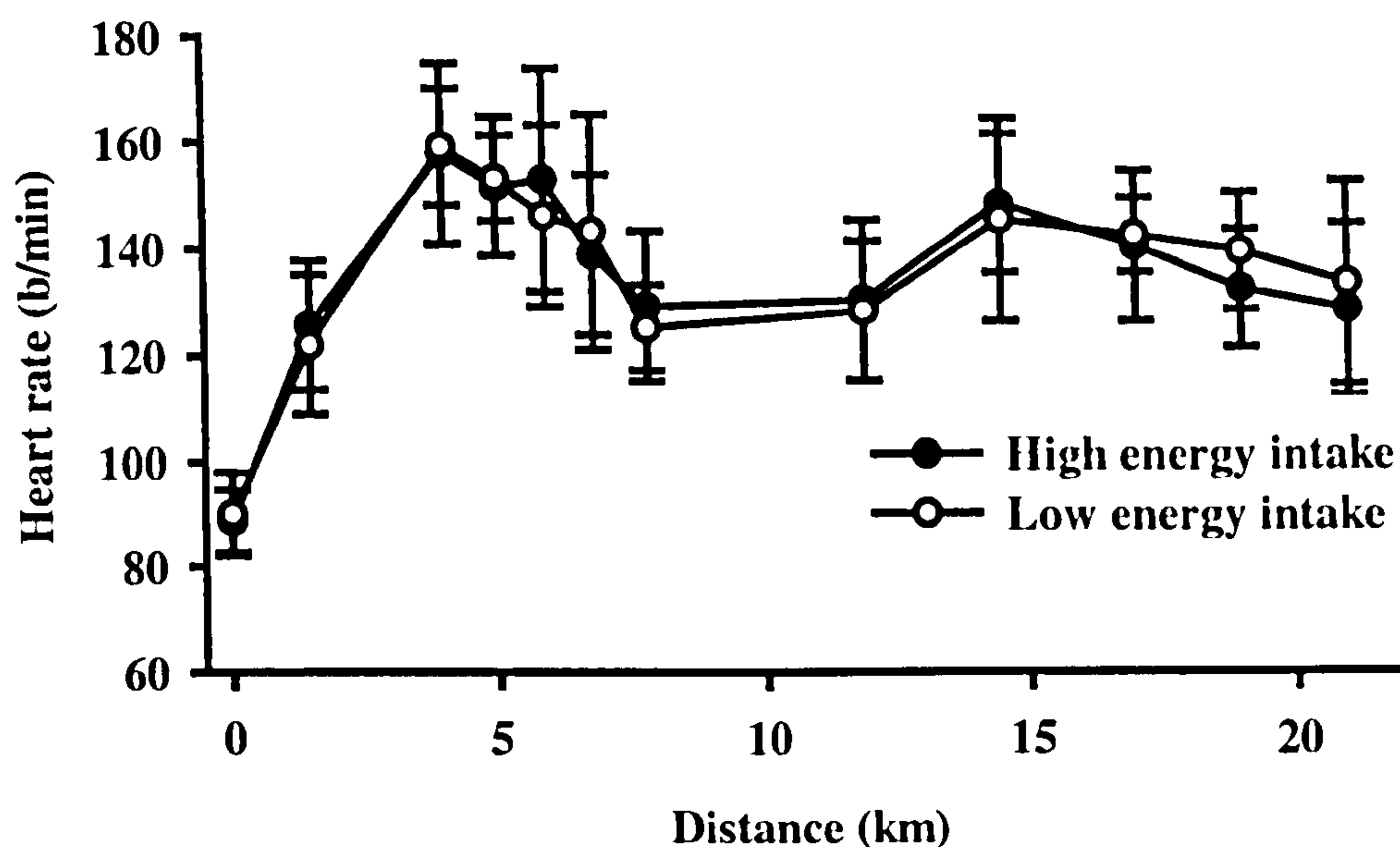


Figure 8.3. Heart rate results during the hill walk. Values are Mean \pm SD; n = 15.

8.3.3 Performance and psychomotor responses:

Subjective responses: Table 8.3 gives the recorded POMS. Although there was a trend of increased ratings of fatigue in both groups, only the increased ratings of fatigue in the low-energy intake reached significance ($P < 0.05$). These increases in fatigue were also apparent in the subjective ratings of fatigue, using a visual analogue scale. Again, the increased ratings were more noticeable in the low-energy intake condition (Fig 8.4). Both dietary groups showed a marked increase in rating of vigour upon completion of the walk ($P < 0.001$; table 8.3).

Subjective ratings of hunger, fullness and nausea are illustrated in Fig 8.4. As may have been anticipated, ratings of hunger were lower and ratings of fullness were significantly higher on the high-energy intake when compared with the low ($P < 0.05$). There were no differences in subjective ratings of nausea, indicating that subjects in the main found the high-energy intake acceptable.

Psychomotor responses: Table 8.4 gives the results from the measured psychomotor performance tests. The only statistical differences between the different energy intakes were evident in the reaction time and balance tests. The high-energy intake group showed

a decreased ($P < 0.05$) reaction time in both 1- and 2-finger reaction time, whereas no change was displayed in the low-energy intake, or in 4- and 8-finger reaction time tests in either group. In other words, reaction time performance was worse on the low-energy intake condition when compared with the high-energy intake. The high-energy intake group displayed no change in the balance tests. Conversely, in the low-energy intake condition, a deterioration of both forward ($P < 0.01$) and static balance ($P < 0.05$) was evident when compared with pre-walk values.

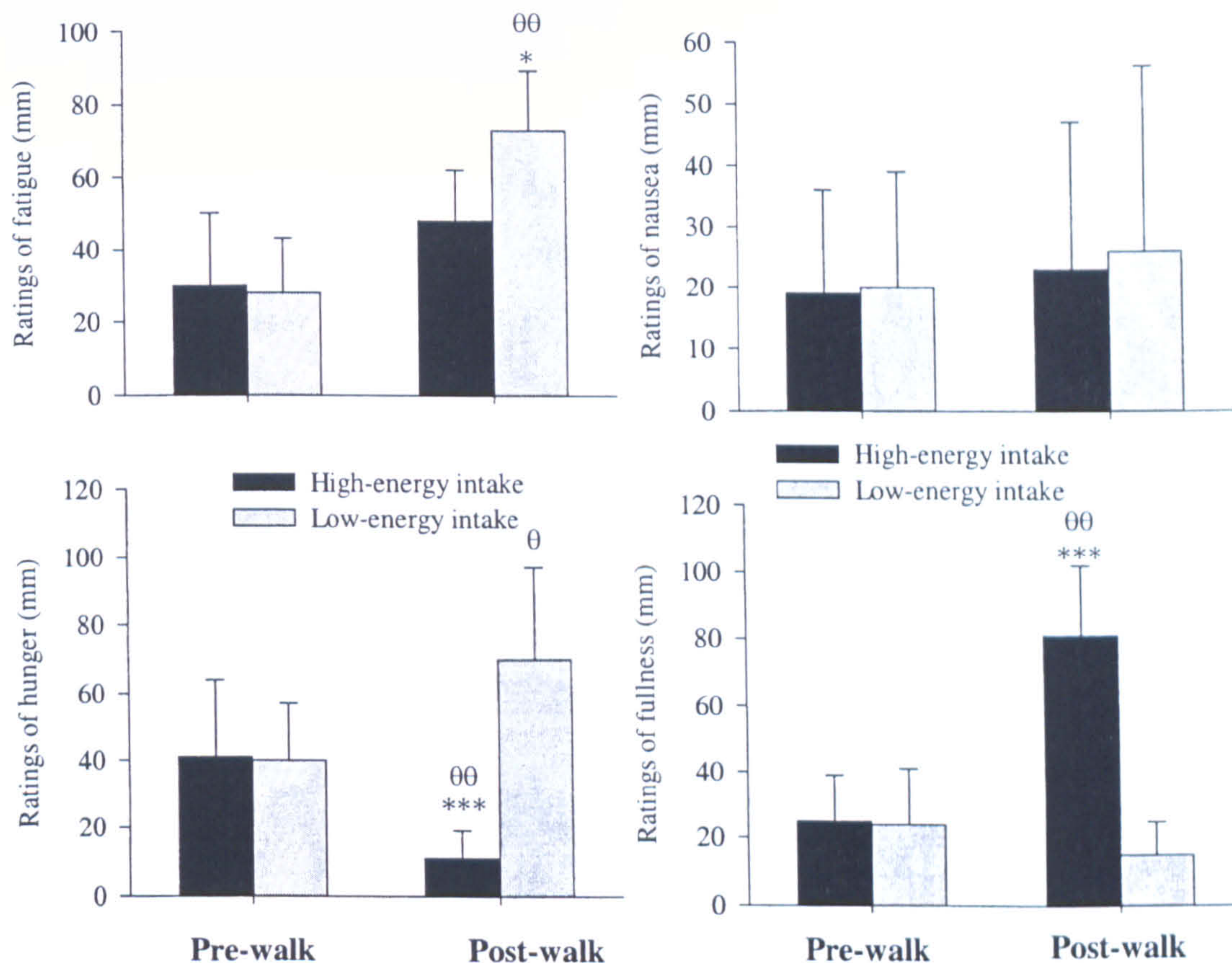


Figure 8.4. Subjective fatigue and appetite responses prior and immediately following the hill walk. * denotes significant between group difference ($* P < 0.05$; $*** P < 0.001$). θ denotes significant change from pre-walk values ($\theta P < 0.05$; $\theta\theta P < 0.01$). Values are mean \pm SD, $n = 16$.

Table 8.3. Profile of mood states prior and immediately following the hill walk.
Mean values \pm SD, based on 16 subjects.

	High-energy intake		Low-energy intake	
	Pre	Post	Pre	Post
Mood state (t-score)				
Tension	32.6 \pm 2.6	31.4 \pm 1.0	32.1 \pm 2.0	31.3 \pm 0.7
Depression	37.4 \pm 1.1	38.1 \pm 2.3	37.7 \pm 1.5	38.1 \pm 1.6
Anger	37.3 \pm 1.0	38.0 \pm 2.0	37.8 \pm 1.8	37.5 \pm 1.1
Vigour	5.3 \pm 6.2	35.0 \pm 6.8***	4.3 \pm 5.2	33.0 \pm 6.8***
Fatigue	37.4 \pm 2.4	44.4 \pm 9.2	38.6 \pm 3.7	48.1 \pm 4.4**
Confusion	31.5 \pm 2.1	32.0 \pm 3.8	31.1 \pm 1.9	31.3 \pm 2.2

Symbol * denotes significant change from pre-walk values (** $P < 0.01$; *** $P < 0.001$).

Table 8.4. Psychomotor and performance results prior and immediately following the walk. Mean values \pm SD, based on 16 subjects.

	High-energy intake		Low-energy intake	
	Pre	Post	Pre	Post
Reaction time (ms)				
1-finger	493 \pm 36	402 \pm 34*	483 \pm 40	473 \pm 54
2-finger	457 \pm 28	417 \pm 25*	456 \pm 39	440 \pm 80
4-finger	555 \pm 93	553 \pm 105	545 \pm 108	537 \pm 77
8-finger	691 \pm 128	667 \pm 117	660 \pm 97	676 \pm 128
Balance (s)				
Forwards	4.5 \pm 0.8	4.0 \pm 0.8	4.3 \pm 0.7	6.9 \pm 1.6**
Backwards	4.8 \pm 1.0	4.7 \pm 0.7	4.9 \pm 0.9	4.6 \pm 0.9
Static	4.2 \pm 3.1	5.4 \pm 4.7	4.2 \pm 2.4	6.2 \pm 2.1*
Kinaesthetic differentiation (cm)				
Jump to 50 cm	3.1 \pm 1.3	2.1 \pm 1.5	2.6 \pm 0.9	2.2 \pm 1.0
Jump to 75 cm	2.5 \pm 1.2	2.4 \pm 1.2	2.2 \pm 1.5	2.7 \pm 1.4
Jump to 100 cm	2.6 \pm 1.3	3.2 \pm 3.0	2.4 \pm 1.5	3.0 \pm 2.0
Performance				
Flexibility (cm)	22 \pm 8.4	23 \pm 8.7	21 \pm 9.6	22 \pm 9.3
Grip strength (kg/m ²)	39 \pm 8.7	39 \pm 7.6	39 \pm 6.2	38 \pm 7.9
Max leg strength (kg)	136 \pm 37	156 \pm 44	141 \pm 30	148 \pm 38
Max jump (cm)	209 \pm 21	196 \pm 32	207 \pm 19	199 \pm 37

Symbol * denotes significant change from pre-walk values (* $P < 0.05$; ** $P < 0.01$).

8.3.4 Subjective observations: During the low-energy intake condition, nine of the sixteen subjects showed marked changes in behaviour during the walk. These changes were especially evident after the first 10 km. The majority of these subjects showed marked signs of withdrawal from voluntary conversation, slowing down in pace and, in four cases, aggressive and negative behaviour. These symptoms are generally

considered as some of the early signs and symptoms of exposure and of hypoglycaemia. Incidentally, these signs and symptoms were present predominately in combination with adverse, wet and windy weather conditions. Furthermore, four of the subjects on the low-energy intakes sustained minor injuries during the walk. These injuries were muscular based in three subjects, who still managed to complete the walk. One subject had to be evacuated from the mountain early due to potential tendon damage in the knee resulting from a slip during a down-hill section at approximately 8 km into the walk. This subject still walked 13 km and data have been included in the final analysis.

8.3.5 Blood and urine constituents: There were no significant changes in plasma volume during the walk. Consequently, circulating concentrations have not been corrected for haemo-concentration. Figures 8.5 and 8.6 give the concentrations of the blood constituents measured prior and immediately following the walks. The general changes in blood metabolism were an increased mobilisation of fat and a greater hormonal 'stress' response, during the low-energy intake condition. During the low energy intake, insulin concentrations were suppressed ($P < 0.001$) immediately following the walk compared with pre-walk values, whereas on the high energy intake, the insulin concentrations were significantly elevated upon completion of the walk. The NEFA, 3-OHB and glycerol concentrations were all significantly higher, immediately following the walk, on the low-energy condition when compared with the high-energy intake trial. Likewise, the low-energy intake trial showed significantly higher concentrations of growth hormone and cortisol when compared with activity under the high-energy intake condition. During the high-energy intake, the plasma TAG concentrations were significantly elevated from both pre-walk values and from that of the low-energy values. Whilst the blood glucose concentrations were significantly lowered immediately following the walk, during the low-energy intake condition, no subjects became hypoglycaemic (blood glucose concentration < 3 mmol/l) although it cannot be ruled out that there was no hypoglycaemia during the walk. Urine osmolality decreased from the morning concentrations (535 ± 132 and 523 ± 142 ; mosmol/kg) to that collected upon completion of the walk (188 ± 102 and $\pm 191 \pm 81$; mosmol/kg; $P < 0.001$) in the high- and low-energy intake group, respectively.

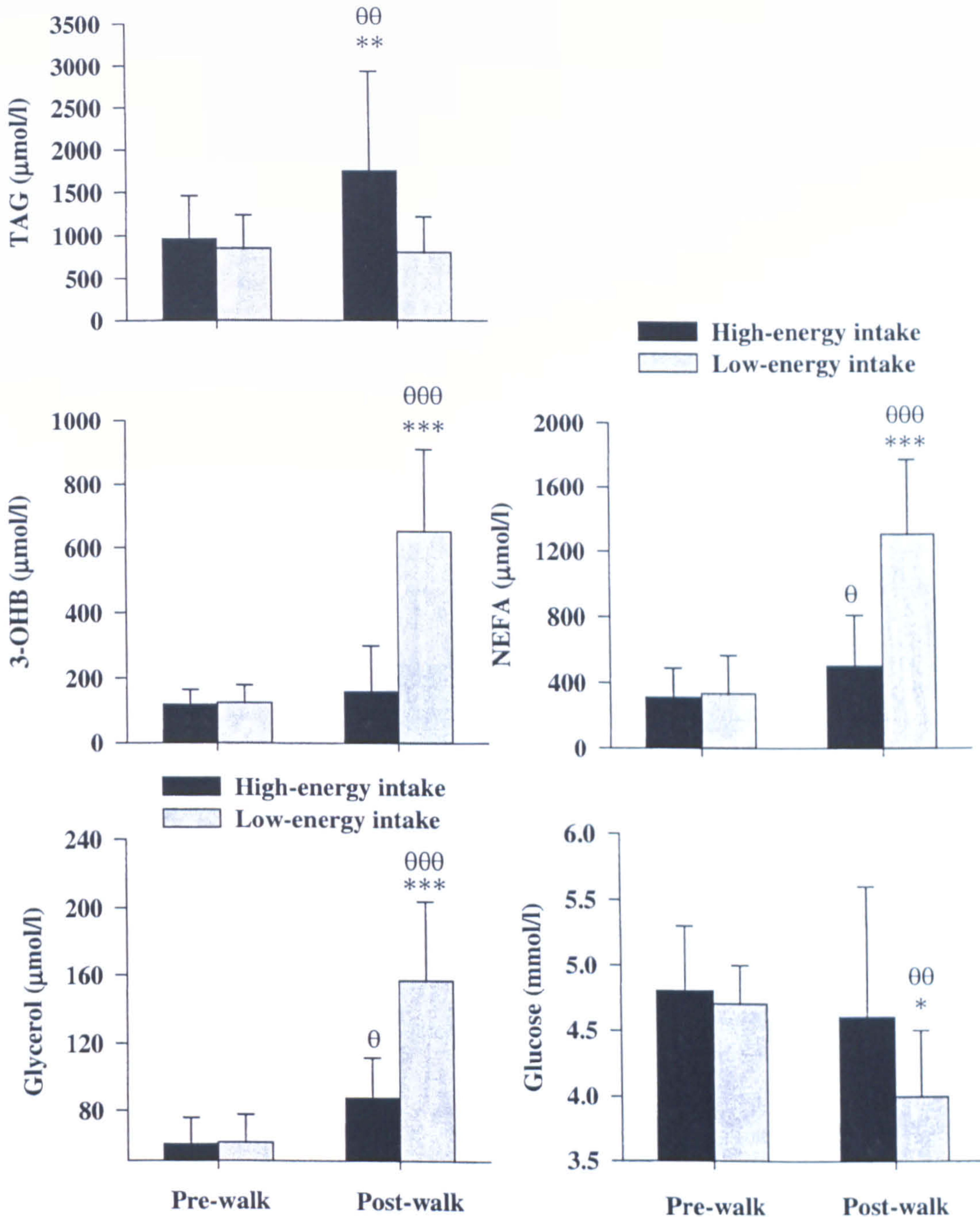


Figure 8.5. Changes in blood metabolites prior, and immediately following completion of the walk. * denotes significant between group difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). θ denotes significant change from pre-walk values (θ $P < 0.05$; $\theta\theta$ $P < 0.01$; $\theta\theta\theta$ $P < 0.001$). Values are mean \pm SD, $n = 16$.

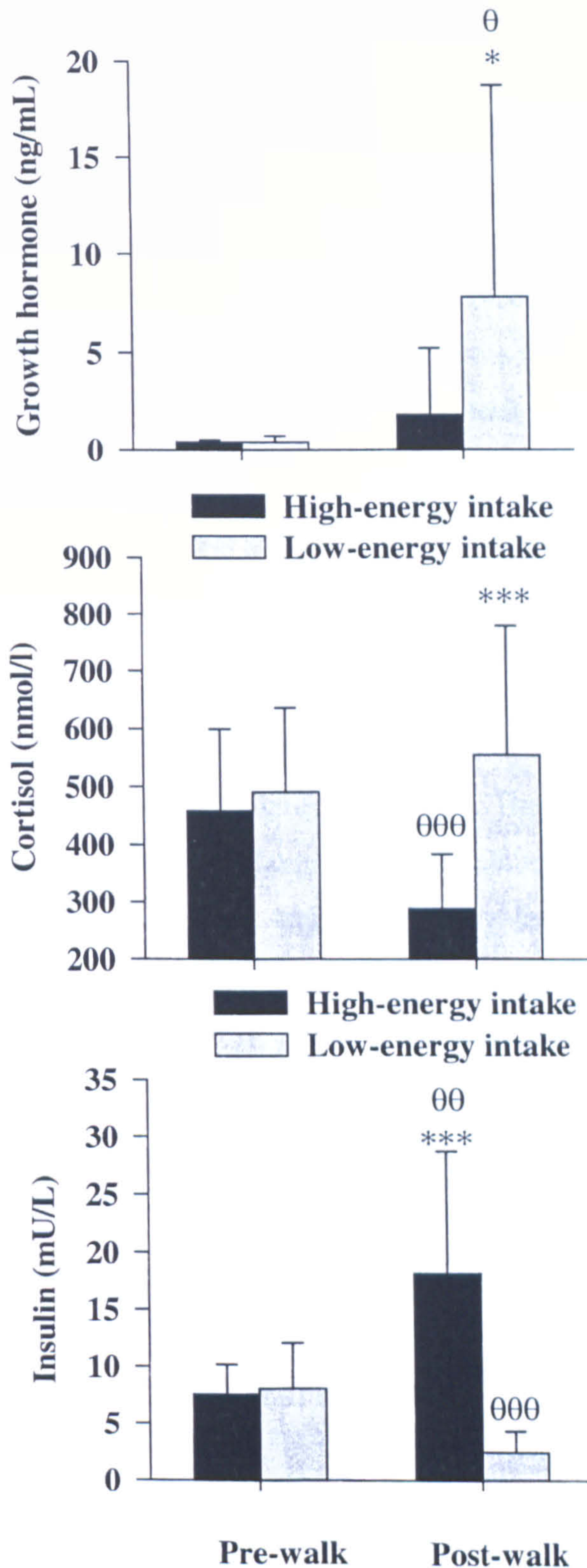


Figure 8.6. Changes in plasma insulin, cortisol and growth hormone prior and immediately following completion of the walk. * denotes significant between group difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). θ denotes significant change from pre-walk values (θ $P < 0.05$; $\theta\theta$ $P < 0.01$; $\theta\theta\theta$ $P < 0.001$). Values are mean \pm SD, $n = 16$.

8.4. DISCUSSION

The present study has yielded a number of important findings. Firstly, during the low-energy intake, mean blood glucose concentration levelled off at the low-mid range of normoglycaemia whereas, on the high-energy intake, they were significantly elevated compared with the low-energy intake. The maintained blood glucose levels were most probably mediated via an increased mobilisation of fat in the low-energy group whereas in the high-energy intake, fat mobilisation was suppressed and CHO utilisation was promoted. Secondly, the demanding nature of the walks was reflected in some impairment in the measured performance tests. Generally, the low-energy intake group showed a slower 1- and 2-finger reaction time (perception task indicator) and an impaired ability to balance, when compared with the high-energy intake group. Finally, the data on temperature regulation suggest that, subjects receiving a low-energy intake may be somewhat compromised in their ability to maintain their body temperature when compared with their counterparts consuming a high-energy intake. The mechanism(s) by which a low-energy intake may compromise thermoregulatory ability are unclear. In addition, despite adequate insulation, the data suggest that very lean subjects may be more susceptible to the dangers of hypothermia.

8.4.1. Levels of energy expenditure

Previous data obtained in Chapter 6 over 10 days of hill walking showed that hill walks of similar intensity induce an energy requirement estimated to be between 18.5 - 25.5 MJ. In the initial study (Chapter 5), subjects covered the exact 8 km of the walk used in the present study, before descending. Since the present study extended the walk of the previous study by a further 8 km of strenuous walking, it is reasonable to estimate the energy expenditure to exceed 20 MJ for the walking period.

8.4.2. Effect on psychomotor and physical performance

The main result of the present study was that the high-energy intake group had an improvement in 1 and 2-finger reaction time (perception task indicator) whereas there was no change in the low-intake group. Choice reaction time (4 and 8-finger) was unchanged in both groups. The influence of different energy intake on cognitive performance has been previously described in relation to CHO ingestion during prolonged exercise. Previous studies have investigated the effects of CHO-electrolyte feeding on cognitive performance

following prolonged exercise lasting more than 1 h, and results have been quite diverse.

For example, Reilly and Lewis (1985) have shown that CHO ingestion improves cognitive performance in a 120-min cycling task at 60% of maximal oxygen uptake, whereas Ivy et al. (1983) failed to observe any effect of CHO ingestion on reaction time performance after 150-min at 40 % $\dot{V}O_2$ max. In a recent study, Collardeau et al. (2001) showed that CHO-electrolyte ingestion during a 100-min run resulted in an improvement in choice reaction time, when compared to a placebo group. Single reaction time, a perception task indicator, was unchanged in both conditions (Collardeau et al., 2001).

One unanticipated result in the present study is that there was no change in choice reaction time (cognitive processing), kinaesthetic differentiation or physical performance (maximal leg and grip strength, maximal jump and flexibility) after the 21-km hill walk, when a decrement might have been expected, especially in the low-energy intake group. There could be several explanations for the stability of these variables. Firstly, although a negative effect of exercise on cognitive performance is often suggested in the literature (Collardeau et al., 2001), through the action of some physiological mediators that are influenced by exercise (Etnier et al., 1997), experimental results have not confirmed this hypothesis, and it is difficult to prove the influence of these mediators on cognitive and physical function during exercise (Struder et al., 1996). One further possibility may be that the fatigue induced by a 21-km hill walk is not great enough to produce a decrease in mental performance or in the other unchanged physical performance tests. As pointed out by Tomporowski and Ellis (1986), the effect of fatigue could be modified by a positive effect of the exercise-induced increase in arousal, or incentive variables. In support of this exercise-induced increase in arousal was the 6- to 7-fold increase in ratings of vigour, assessed using the Profile of Mood State questionnaire (Table 8.3). Thus, even during exhaustive exercise, subjects may be able to compensate for the negative effect on cognitive performance.

Whilst the impairment in the low-energy intake compared with the high-energy intake was moderate, this impairment may well be an influencing factor in susceptibility to both fatigue and injury in the mountainous environment. A relevant observation was that four of the subjects on the low-energy intakes sustained minor injuries during the walk. Whilst it is somewhat speculative to identify the exact cause of these injuries since they occurred in severe weather conditions, no injuries were noticed in the high-energy intake group over

these weather conditions. There is some evidence that low muscle glycogen levels, as may be anticipated during the low-energy intake, are associated with increased injury risk in alpine skiing, especially in recreational skiers (Ericksson et al., 1977). The explanation is that glycogen depletion of the fast twitch fibres will limit the ability to develop a high muscle tension in a short period of time (needed to correct false turns or inadequate timing) (Coyle, 1992). Physical inability to correct movements in time will lead to increased injury risks (Coyle, 1992). This relationship between low muscle glycogen levels and a physical inability to correct moves could well be mediated through balance impairment. In the present study, this balance impairment was evident only in the low-energy intake condition. Although a failure to provide adequate fuel to sustain the activity may be one factor influencing the susceptibility to injury, the potential mechanisms by which this may occur are unclear but may also be centrally mediated.

8.4.3. Thermoregulatory responses

The observations of the present study regarding the thermal stresses involved in a hill walking event, with different energy intakes, have provided some novel results. Although not statistically significant, there was a marked trend for a lowered rectal temperature during the low-energy intake condition. The reasons for this lowered temperature are not clear. All experiments were conducted in a balanced design which prevented any single effect of weather alone. Furthermore, subjects were measured in pairs, each under a different dietary intake. Whilst the subjects on the high-energy intake may have some advantage from the thermogenic effect of a greater food intake, which may reflect a small increase in whole body metabolism, this would probably be marginal due to the exercise hyperthermia and the relatively small amount of food consumed. One possibility may be that the subjects on the high-intake had to stop more frequently to consume food. At these time points, for logistical and safety purposes, the subjects on the low-intake also stopped. During these stops the subjects on the low-intake were very stationary whilst the subjects consuming the high-energy intake were more active inasmuch as they had to remove their rucksacks, locate and consume their food, replace rucksack, then move on. Since heat loss is likely to occur rapidly at this point, this potential to lose heat may be one contributory factor involved in the apparently lowered rectal temperature in subjects consuming the low-energy intake. Further work into this area may have important implications for the development of hypothermia in the mountainous environment.

The initial physiological responses to cold exposure to maintain core temperature in the cold are peripheral vasoconstriction to reduce heat loss and shivering to generate heat. Although shivering was not quantified directly, pronounced shivering was noted in six of the subjects receiving the low-energy intake and in two receiving the high-energy intake. Once peripheral vasoconstriction is maximised, core temperature can only be maintained by an increased heat production, i.e., shivering, which is thought to be the major contributor to the cold-induced increase in heat production (Doubt, 1991). The lowered temperature may be one factor underlying the subjective observations, predominantly in the low-energy intake group.

An interesting observation was that one subject during the high-energy intake trial and two subjects in the low-intake condition exhibited marked decreases in rectal temperature (Fig 8.2). In one of these subjects, on the low-energy intake condition, rectal temperature fell below 35°C. It was noteworthy that all these subjects completed their walks in sustained wet and windy weather and, whilst they were adequately dressed for the conditions, they were all very lean individuals with an estimated percentage body fat less than 8%. However, there was no evident statistical relationship between percentage body fat and the lowered post-walk rectal temperatures, probably due to the confounding influence of insulation, exercise and environmental weather conditions. Whilst the importance of the insulation of fat has been confirmed by several research groups (Pugh and Edholm, 1955; Hayward and Keatinge, 1981; Collins, 1983), the majority of these experiments have been conducted in relation to water immersion studies and did not consider the effects of clothing and exercise. The data from the present study suggest that, even when clothing is adequate, the very lean may still be at a greater risk of hypothermia than their fatter counterparts.

8.4.4. Effect on blood metabolism

During the low-energy intake, mean blood glucose concentration levelled off at the low-mid range of normoglycaemia. This maintenance of blood glucose levels became possible due to a series of metabolic adaptations gearing to meet the requirements of glycolytic tissues. Insulin secretion is known to decrease during short and prolonged fasting (Cahill, 1970), as well as during exercise (Pruett, 1970; Galbo and Gollnick, 1984; Burstein et al., 1991). In the present study, insulin responded rapidly to the combined stimuli of low-energy intake and exercise, declining to over 50% of its initial concentration, thus enabling

lipolytic and proteolytic processes to be initiated as part of gluconeogenesis. This lowered insulin was also reflected in a marked fat mobilisation, characterised by a 2- to 5-fold increase in NEFA, 3-OHB and glycerol concentrations. Conversely, during the high-energy intake the reverse was evident; insulin levels were increased, which would be expected to lead to a decrease in adipose tissue lipolysis as reflected in the suppressed NEFA, 3-OHB and glycerol concentrations, compared with the low-energy intake.

Despite the high-energy expenditures, and the differing energy intakes, blood glucose concentrations were well maintained above hypoglycaemic levels in both groups at the time the blood samples were taken. During the low-energy intake, mean blood glucose concentrations levelled off at the low-mid range of normoglycaemia whereas, on the high-energy intake, they were significantly elevated compared with the low-energy intake. During the low-energy intake conditions, the maintained blood glucose concentrations were probably mediated via the marked fat mobilisation. Enhanced fat mobilisation should make it easier to maintain blood glucose by decreasing carbohydrate oxidation and promoting gluconeogenesis (Ahlborg et al., 1974; Marniemi et al., 1984). Conversely, during the high-energy intake conditions, the large amount of CHO intake (401 g) consumed during the walking period would be expected to decrease the amount of energy derived from fat oxidation and increase proportionally the amount of energy derived from blood glucose (Ahlborg and Felig, 1976; Krzentowski et al., 1984).

In summary, the data suggest that subjects consuming a low-energy intake may become compromised in their ability to operate safely in the mountainous environment. Whilst the impairment in the low-energy intake compared with the high-energy intake was somewhat moderate, this impairment may well be an influencing factor in susceptibility to both fatigue and injury whilst pursuing outdoor recreational activity.

CHAPTER 9

GENERAL DISCUSSION

9.1 SYNOPSIS OF FINDINGS

The studies described in the present thesis were designed to provide an in-depth physiological, metabolic and ergonomic insight into the prolonged activity of hill walking. There have been few systematic investigations of the stresses associated with this popular recreational pursuit.

In Chapter 4 the characteristic activities of hill walking were identified by means of a questionnaire-based study. The results, based upon 100 walkers, indicated typical distance covered of 18 – 26 km over 6 – 8 hours in duration and an ascent over 600 m. Significant negative relationships were found to exist between the incidence of injuries in walkers and the energy intake during the walk. Despite the activity lasting a full day, quantification of typical energy intakes during such hill walking events showed that, on average, they were not higher than normal reference energy intakes for the associated age groups. The major conclusions from the questionnaire-based study were firstly that there is a high prevalence of lower limb injuries sustained predominately during downhill walking, nearing the end of the walk. Secondly, the typical energy intakes during a walk are probably inadequate to balance the high-energy turnover of such prolonged activity.

A practical implication from the questionnaire-based study is that the use of walking poles may confer some additional protection against lower limb injuries during downhill walking (Jacobson et al., 1997; Schwameder et al., 1999) and may increase efficiency during up-hill sections (Knight et al., 1998). Furthermore, walkers should take more foods with higher energy content to help increase energy intake and provide a measure of protection if the walk becomes unexpectedly prolonged.

The low-energy intakes highlighted in the questionnaire-based study were further evident in an initial field study (Chapter 5) where subjects, despite eating *ad libitum*, consumed a similar amount of energy (~5 MJ) as that quantified in Chapter 4. Specifically, in Chapter 5, subjects completed a self-paced 12-km strenuous hill walk which resulted in dehydration and an average loss of 2 kg in body mass. Despite the high energetic cost of the walk, dehydration, and serious physiological stress, the subjects demonstrated little change in psychomotor control during and after the walk.

Furthermore, in spite of the difference between energy intake and expenditure, blood glucose and triacylglycerol concentrations were maintained. The major source of energy was enhanced fat oxidation, probably from adipose tissue lipolysis.

In previous work, Pugh (1966) proposed that maintaining a $\dot{V}O_2$ of 2-2.5 l/min or 50-60% $\dot{V}O_{2 \max}$ would offset heat loss and combat the debilitating effects of the cold, wet and windy environment. Even though these observations were based on only three subjects, recent work by Weller et al. (1997a) supported Pugh's postulate. Both experiments were conducted in an environmental chamber, subjects exercised on a cycle ergometer and treadmill, respectively. Pugh (1967) and Weller et al. (1997a) showed that when exercise metabolism is reduced, the increase in shivering may be insufficient to prevent a decrease in deep body temperature. The results from Chapter 5 highlighted the variability in $\dot{V}O_2$ in response to the hill walk which is likely to depend on such factors as terrain, gradient, weather conditions, backpack weight, exercise intensity, preceding diet and thermal stress. It was only during the high intensity part of the walk that subjects reached this cut-off point. This 'cut-off' point was clearly variable with individuals. Since the hill walkers in this study walked at their own pace, it could be cautiously concluded that hill walkers do not consistently operate at, or above this 'cut-off' level.

It was noteworthy that when the subjects stopped for lunch and measurements mid-walk for approximately 30 min, the exercise hyperthermia was cancelled out by the decreased heat production and increased heat loss through conduction and radiation. The core temperature continued to fall after subjects began walking following lunch. This temperature 'after-drop' has been reported in a number of studies employing cold water immersion (Golden and Hervey, 1977; Noakes, 2000), but to my knowledge, has not been reported in circumstances such as the present. Even though rectal temperature did not drop below 36°C, this after-drop may describe the reason why hill walkers slip into the first stages of hypothermia after stopping for a rest. The suggested mechanisms for the after-drop are subject to controversy (Golden and Hervey, 1977; Collins et al., 1982; Lloyd, 1986; Webb, 1986).

The main finding from the study described in Chapter 5 was that, even over 1-day of hill walking subjects were operating at a marked negative energy balance. The

imbalance was also reflected in a ~200-g carbohydrate deficit. Furthermore, when subjects stopped for ~30 min for lunch, a marked decrease in rectal temperature was observed. After a rest break, hill walkers may be particularly susceptible to fatigue, injury and possibly hypothermia.

In Chapter 6 the findings of the initial field study (Chapter 5) were extended over 10 consecutive days of hill walking. It was evident from the literature that no previous studies have considered the effect of age on the potential stress of such activities. It was aimed therefore to quantify some relevant responses that are important in the safety of hill walkers, such as the likelihood of dehydration, impaired performance, and the ability to maintain glycaemia and also the possible effect that age may have on these responses.

The results from Chapter 6 showed that participants maintained energy balance via an increased energy intake. Despite the very high energy expenditure (EE) of ~21.6 MJ/day, and physiological stress, body mass was relatively well maintained in both groups. Comparable to the recorded levels of energy expenditure, Dressendorfer et al. (1982) reported energy intake values of 20.2 MJ/day in marathon runners during a 20-day 500-km road race. Also, one of the highest energy intake levels of 20-25 MJ/day reported in Maine lumbermen (Wood and Mansfield, 1904) is comparable to the present study. Indeed, only the measured EEs of 25.4 MJ/day over 22 days in the Tour de France (Westerterp et al., 1986), 15.1 - 34.9 MJ/day in elite cross-country skiers during intensive training (Sjodin et al., 1994) and of 25.7-32.5 MJ/day during an Arctic expedition (Stroud et al., 1997) reached higher values than those of the present study.

In light of the high-energy expenditure values and subsequent physical activity levels in both age groups, subjects were close to the limits of body mass maintenance (Westerterp et al., 2001). The important and novel consideration in the present study is that the participants were monitored during recreational activity, and not with elite performers in extreme situations.

The main difference between the monitored 1-day walk and the 10-day walk was an enhanced fat mobilisation, reflected in lowered plasma insulin and high plasma

NEFA, glycerol and 3-OHB concentrations during the 10 days of walking. Blood glucose levels were maintained in both groups, probably mediated via the marked fat mobilisation. Enhanced fat mobilisation should make it easier to maintain blood glucose by decreasing carbohydrate oxidation and promoting gluconeogenesis (Ahlborg et al., 1974; Marniemi et al., 1984). In agreement with the initial study, described in Chapter 5, TAG concentrations remained unchanged after the first day of walking. However, during the consecutive days of walking, the TAG concentrations were significantly lowered when sampled upon completion of the walks, coinciding with the increased concentrations of circulating NEFA, 3-OHB and glycerol.

As observed in Chapter 5, subjects demonstrated little change in psychomotor control as a consequence of a 1-day hill walk. However, over the 10-day experiment this impairment became apparent in both the young and older age groups. The impairment was more noticeable in the older subjects who also became progressively dehydrated during the 10 days. Taking the observations collectively, due to the marked dehydration and impairment of psychomotor performance, the older age walkers may be more susceptible to fatigue and injury, and in adverse weather conditions the risk of hypothermia in the mountainous environment must be considerable.

The next scope of the work described in this thesis was an investigation of the influence of diet in conditions resembling the physiological stress of a hill walk. In the laboratory-based study described in Chapter 7 the potential benefits of macronutrient manipulation was considered over 7 h 30 min of simulated walking. The results indicated that the availability of fatty acids, and of other substrates, and the pattern of substrate oxidation and balance, during prolonged walking can be altered by dietary means. The main source of energy in all trials was predominantly fat oxidation, although diet influenced the degree of total CHO and fat oxidation. These results emphasize that the close relationship between fat and CHO metabolism following isoenergetic meals can be somewhat displaced, most probably due to the prolonged low to moderate intensity of the exercise and subsequent negative energy balance. In accordance with previous studies which have involved more high intensity exercise (Costill et al., 1988; Starling et al., 1997), high-fat diets might not be so good for further exercise even at low to moderate intensities. Decreases in the glycogen stores (Costill et al., 1988) and/or muscle triacylglycerol concentrations (Starling et al.,

1997), especially if continued over a few successive days of walking, would be detrimental to the ability to sustain the activity. However, diets with moderate amounts of fat (e.g., 50%) may be acceptable for such activities; this proportion of fat could help increase energy intake via high-energy dense food. The use of more energy dense food may also enable the walker to cut down on the amount of food required to be carried for this purpose.

The results from the previous chapters suggest that a vast amount of hill walkers may be undertaking the activity with relatively low-energy intakes and subsequently sustaining high-energy deficits. Furthermore, Chapter 7 highlighted that despite the ability of fat intake to alter the metabolic responses to exercise, it may be of little benefit to the walker, and may actually impair performance if activity was continued over consecutive days.

Importantly, the consequences of such high acute energy deficiency on recreational participants remain unclear and have not been investigated in this context. This particular area of acute energy deficiency was the focus of Chapter 8. In random order, subjects completed a strenuous 21-km hill walk with either a high (15 MJ) or low dietary energy intake (5 MJ). Generally, consumption of the low-energy intake led to a marked deterioration in performance compared with the high-energy dietary conditions. Adverse subjective observations were noticed in the majority of the conditions when subjects consumed the low-energy dietary conditions. Although not statistically significant, there was a marked trend towards a lower rectal temperature during the low-energy intake condition. Furthermore, during the low-energy intake, mean blood glucose levels levelled off at the low-mid range of normoglycaemia whereas, on the high-energy intake, they were significantly elevated compared with the low-energy intake. The maintenance of blood glucose levels was most probably mediated via an increased mobilisation of fat in the low energy group whereas in the high intake trial, fat mobilisation was suppressed and CHO utilisation was promoted.

It is concluded from the completion of the studies in chapters 4, 5, 6, 7 and 8 that the original aims of this thesis have been realised. A holistic approach was utilised in order to 'gauge' the typical activity patterns and stresses occurring during hill walking activities. The initial questionnaire provided a means to collect epidemiological data

from participants. Further insights into the physiological and metabolic demands were then gained through the use of a more vigorous approach in the laboratory and field-based studies described. Whilst the laboratory-based study provided a well-controlled environment to monitor metabolic responses to prolonged walking, it clearly lacked the in-the-field conditions. Likewise, the field studies provided a means to collect more ecologically valid data but, due to climatic conditions, differences in insulation and preferred pace and nutritional intakes, they lacked the controlled conditions of the laboratory. An overall perspective of hill walking can only be considered from the unification of the epidemiological, laboratory-based and field-based studies described in this thesis. This overall perspective of hill walking and the factors which may influence the overall strain experienced by participants during a hill walk are illustrated in Figure 9.1.

The model depicted in Figure 9.1 illustrates the range of influencing factors which may place an impact on the overall strain experienced by hill walking participants. As shown, the main factors are the characteristics of the participant, the associated demands of the hill walk, the ability to maintain adequate thermal balance and behavioural state. Whilst all these factors are intimately related, they may also be adversely affected during conditions of inadequate fluid and/or food supply.

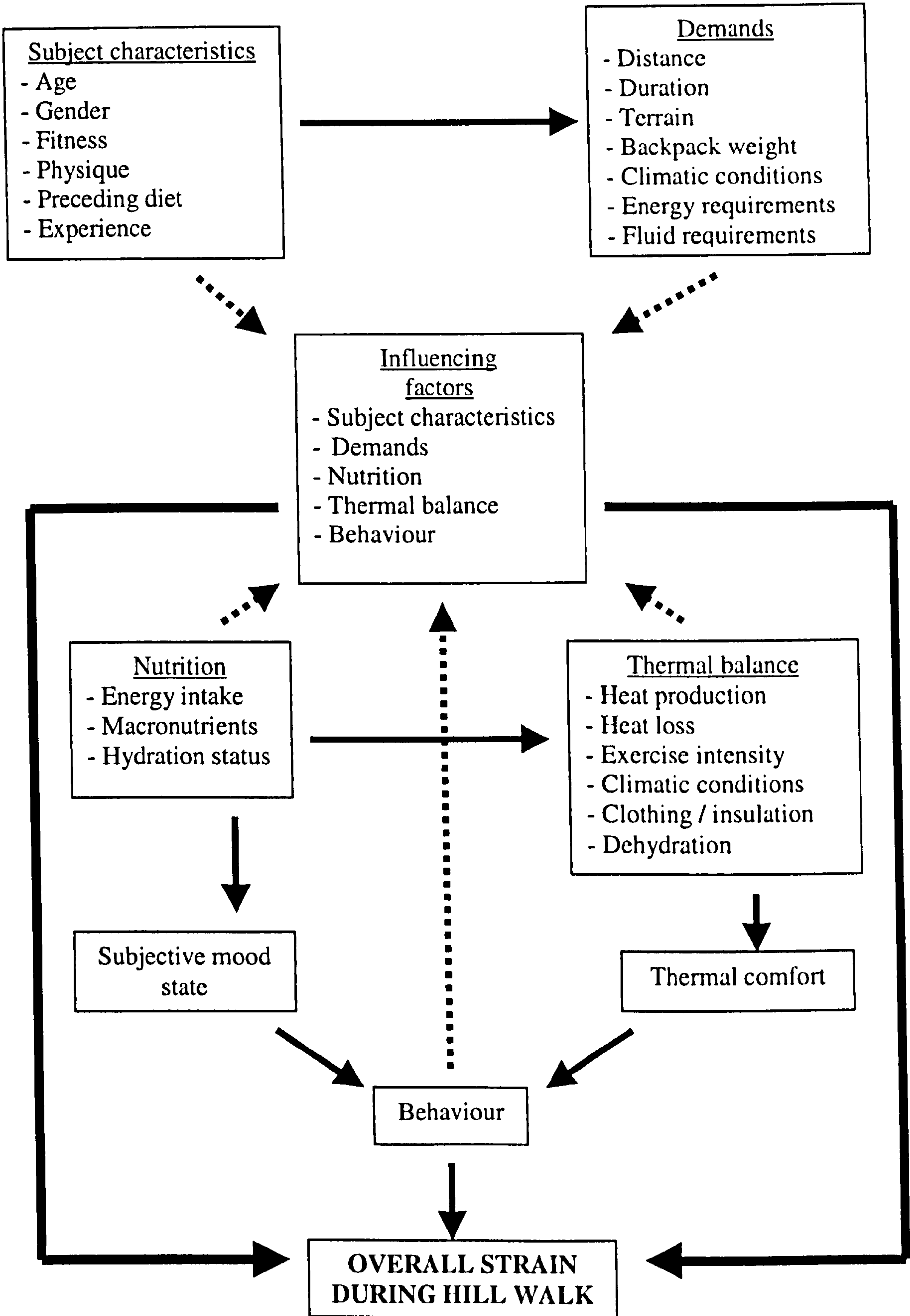


Figure 9.1. Model to illustrate the factors which may influence the overall strain experienced during a hill walk.

In summary, in Chapter 4, despite typical distance covered of 18 – 26 km over 6 – 8 hours in duration, the recorded energy intake during the hill walk was only 3.5 MJ. In Chapter 5 an almost identical energy intake was recorded over a strenuous 12-km hill walk. The findings of this study implied a relative high-energy cost of 14.5 MJ for the recorded hill walk. The difference in energy intake and expenditure was reflected in both a loss in body mass and a carbohydrate imbalance of 200 g.

The results from chapter 6 indicated that, despite problems of maintaining energy balance over one day of walking, hill walkers can rectify and maintain this energy balance over more prolonged periods by means of an increased EI. The energy expenditures recorded over the 10-days of strenuous walking of ~21.6 MJ/day were further comparable to that recorded in the initial study.

One potential way of promoting a high-energy intake could be through the use of energy-dense, high-fat foods. However, the results from Chapter 7 suggest that very high-fat diets might not be so good for further exercise even at low to moderate intensities.

Finally, in Chapter 8, the significance of potential high-energy deficits was considered over a prolonged walk. The results indicated that consumption of a low-energy intake (~5.5 MJ) led to a marked deterioration in performance, lowered blood glucose levels and a trend for a lowered rectal temperature and adverse subjective responses, compared with the high-energy (15 MJ) dietary condition.

9.2 CONCLUSIONS

From the studies described, the main conclusions are that the prolonged activity of hill walking has the potential to impose severe stress simultaneously upon several regulatory systems. High energy expenditures and dehydration are likely to be closely linked to the activity. Failure to provide enough fuel and fluid to sustain the activity may lead to a compromised ability to sustain the activity and an increased likelihood of injury. Older age participants may be particularly prone to both dehydration and an impaired ability to operate in the mountainous environment.

9.3 RECOMMENDATIONS FOR FURTHER WORK

From conducting the research in the present thesis and from reviewing the literature, the researcher proposes the following directions for further work in this area of prolonged outdoor exercise.

It would be of interest to investigate further the topic of hypothermia in the mountainous environment. The vast majority of the literature has focused research attention on hypothermia during water immersion. This is somewhat surprising when considering the popularity of mountainous activities and the danger of hypothermia to participants. In particular, the area of the temperature 'after drop' following a rest deserves further research to determine the extent of the 'after drop' and how this may be off-set by following exercise intensity. Likewise, the apparent ability of high-energy food intake to offset a lowered core temperature needs to be further clarified.

Equally, it would be important to perform similar studies on females. It would also be beneficial to determine whether the menstrual cycle phase in females affects their ability to thermoregulate and hydrate, and influences other metabolic-related responses to prolonged outdoor exercise.

Since all the studies in the present thesis were conducted during quite adverse climatic conditions, an investigation of hill walking during more favourable 'summer' conditions is warranted. It is anticipated that under more favourable climatic conditions greater distances and durations of walks are possible. Greater distances and durations may subsequently elicit higher energy expenditures and levels of dehydration than that recorded during studies described in the present thesis.

Finally, closer work with the rescue services in order to design educational material detailing advice to hill walkers is warranted. The design of such educational material should contribute to the safety of walkers active in the mountainous environment.

It is envisaged that conducting further research in this area will lead to a greater understanding of physiological and metabolic regulation over very prolonged

exercise, often undertaken in adverse climatic conditions, linked with both high energy expenditures and dehydration.

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APPENDIX 1 NOT COPIED
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APPENDIX B

MOOD STATE AND SUBJECTIVE QUESTIONNAIRES

Below is a list of words that describe feelings people have. Please read each one carefully. Then CIRCLE the answer, which describes **HOW YOU FEEL RIGHT NOW**. Make sure you answer every question.

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Panicky	0	1	2	3	4
2. Sad	0	1	2	3	4
3. Lively	0	1	2	3	4
4. Confused	0	1	2	3	4
5. Furious	0	1	2	3	4
6. Worn out	0	1	2	3	4
7. Depressed	0	1	2	3	4
8. Downhearted	0	1	2	3	4
9. Annoyed	0	1	2	3	4
10. Exhausted	0	1	2	3	4
11. Mixed up	0	1	2	3	4
12. Sleepy	0	1	2	3	4
13. Bitter	0	1	2	3	4
14. Unhappy	0	1	2	3	4
15. Anxious	0	1	2	3	4
16. Worried	0	1	2	3	4
17. Energetic	0	1	2	3	4
18. Miserable	0	1	2	3	4
19. Muddled	0	1	2	3	4
20. Nervous	0	1	2	3	4
21. Angry	0	1	2	3	4
22. Active	0	1	2	3	4
23. Tired	0	1	2	3	4
24. Bad tempered	0	1	2	3	4
25. Bushed	0	1	2	3	4
26. Alert	0	1	2	3	4
27. Uncertain	0	1	2	3	4

Assessment of appetite profile

1. How hungry are you?

NOT AT ALL |-----| VERY

2. How full are you?

NOT AT ALL |-----| VERY

3. How satiated are you?

NOT AT ALL |-----| VERY

4. How thirsty are you?

NOT AT ALL |-----| VERY

5. How much could you eat?

NOT MUCH |-----| VERY MUCH

6. How strong is your desire to eat?

VERY WEAK |-----| VERY STRONG

7. How is your appetite?

VERY WEAK |-----| VERY STRONG

8. How nauseous are you?

NOT AT ALL |-----| VERY

9. How fatigued are you?

NOT AT ALL |-----| VERY

APPENDIX C

HILL WALKING QUESTIONNAIRE



Liverpool John Moores University

Characteristic Activities of Hill Walking

This questionnaire will contribute to a research project undertaken within the Research Institute for Sport and Exercise Sciences at the Liverpool John Moores University. The questionnaire asks a range of questions concerning a number of aspects related to a typical days hill walk.

Experimental research will be based upon the results of the questionnaire. The overall aim of the subsequent experiments will be to improve mountain safety. Part of which will be detailed advice to hill walkers on such matters as nutrition and fluid intakes appropriate for a prolonged day in the hills, often in adverse conditions.

There are no correct or incorrect answers to the questionnaire but what is of most value are your general personal experiences as best applied to the questions posed. Complete anonymity of questionnaire data is assured.

Thank-you for your time and co-operation.

**Philip Ainslie
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QUESTIONNAIRE ABOUT THE CHARACTERISTIC ACTIVITIES OF HILL WALKING

Thank you for taking the time to complete this questionnaire. Please tick the box or write in the space provided, where appropriate for each question. It is appreciated that your response to a number of questions may vary according to the weather conditions. In this instance an overall average answer, based on your general experience will be appropriate. Please return the questionnaire using the envelope provided.

Q1. What is your gender? (a) Male (b) Female

Q2. Which age bracket do you fall into (year)?

(a) 19 and under	<input type="checkbox"/>
(b) 20 - 29	<input type="checkbox"/>
(c) 30 - 39	<input type="checkbox"/>
(d) 40 - 49	<input type="checkbox"/>
(e) 50 - 59	<input type="checkbox"/>
(f) Over 60	<input type="checkbox"/>

Q3. What is your current a) body weight b) height.....

Q 4. How long have you been an active hill-walker (year)?

a) 0 – 5	<input type="checkbox"/>
b) 6 – 10	<input type="checkbox"/>
c) 11 –15	<input type="checkbox"/>
d) 16 – 20	<input type="checkbox"/>
e) 21 - 25	<input type="checkbox"/>
f) > 25	<input type="checkbox"/>

Q5. How important would you rate the following factors as a reason for your participation?

	i) Fitness	ii) Health	iii) Wellbeing
(a) Very unimportant	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(b) Unimportant	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(c) Neither important or unimportant	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(d) Important	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(e) Very important	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q6. Do you hill-walk (a) mainly above 600m (b) mainly below 600m

Q7. How often do you hill walk?

(a) Once (or more) a week	<input type="checkbox"/>
(b) Once every 2 weeks	<input type="checkbox"/>
(c) Once (or more) a month	<input type="checkbox"/>
(d) Once every 2 months	<input type="checkbox"/>
(e) Once every 4 months	<input type="checkbox"/>
(f) less often than this (specify).....	

Q8. How many days per year do you spend out on the hills?.....

Q9. On average, what duration are you on the hill for?

(a) Less than 2 hour	<input type="checkbox"/>	(d) >6 - 8 hour	<input type="checkbox"/>
(b) >2 - 4 hour	<input type="checkbox"/>	(e) >8 - 10 hour	<input type="checkbox"/>
(c) >4 - 6 hour	<input type="checkbox"/>	(f) More than 10 hour	<input type="checkbox"/>

Q10. Overall, how would you describe the intensity of your walk?

- (a) Very light
- (b) Light
- (c) Moderate
- (d) Difficult
- (e) Very hard

Q11. What distance do you normally cover during your hill walk?

- a) 0 - 8 km (0 - 4.9 m)
- b) 9 - 17 km (5.0 - 9.9 m)
- c) 18 - 26 km (10.0 - 14.9 m)
- d) 27 - 35 km (15.0 - 19.9 m)
- e) > 35 km (> 20 m)

Q12. On average, what is the weight of your backpack?
(1 stone = 6.4 kg)

- (a) 7 kg and under
- (b) 7 - 14 kg
- (c) 15 - 22 kg
- (d) 23 - 30 kg
- (e) 31 - 38 kg

Q13. How long and how often do you stop for a rest during your hill walk?

- a) Less than 10 min/ 1 hour
- b) Less than 10 min/ 2 hour
- c) 10 - 20 min/ 1 hour
- d) 10 - 20 min/ 2 hour
- e) Other (Please specify).....

Q14. How long do you normally stop for lunch?

- a) 0 - 10 min
- b) 11 - 20 min
- c) 21 - 30 min
- d) 31 - 40 min
- e) >40 min

Q15. Have you ever had any injuries/accidents during a hill-walk?

- a) Yes
- b) No - please go to Q18

Q15a. If yes, how many?.....

Q16. If yes, please give details (i.e., time of year; weather conditions; type of accident; treatment?).
Please continue onto the back of this page if required.

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Q17. If yes to Q16, did it occur -

- (a) near to the beginning of the days walk
- (b) close to the middle of the days walk
- (c) near to the end of the days walk

Q18. On average, how much fluid do you normally drink on a hill walk day? (1 litre = 2 pints)

	i)Pre hill-walk	ii)During-hill walk	iii)Post-hill walk
(a) Less than 0.25 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(b) 0.25 - 0.5 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(c) >0.5 - 1 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(d) >1 - 1.5 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(e) >1.5 - 2 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(f) more than 2 litres	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q19. What constitutes this fluid you drink? e.g. 1 litre of squash, approx. 0.5 litres of coffee, and 0.25 litres of hot black-current juice (please specify type; hot; cold etc).

a) Pre hill-walk.....

b) During hill-walk.....

c) Post hill-walk.....

Q20. Typically, what do you eat on a hill-walking day, including breakfast and evening meals. Please be as detailed as possible, listing, for example, numbers of slices of bread (with or without butter), bowls of cereals, skimmed/full fat milk, numbers of Mars Bars, handfuls of peanuts, pints of beer etc.

Time	Food	Portion Size (large/small) or Number of Items
i) Prior to going on the hill (breakfast) (e.g. bowl of cornflakes with 1 pint of full fat milk; 2 slices of brown bread with flora and strawberry jam).		
ii) During your walk (Please list all snacks e.g. nuts and raisons, 2 Mars bars, apple, cheese and salad sandwich on white bread).		
iii) After your walk (Please detail as above)		

Q21. At what time, relative to your hill walk, do you eat a) breakfast, b) evening meal? (Please mark the scale below with a cross (x), corresponding to the timing of your meal).

