WORK CAPACITY AND FATIGUE IN DRAUGHT RUMINANT ANIMALS

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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November 1993

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ABSTRACT

The aim of this study was to define the work capacity of ruminant animals and to investigate the various factors which could limit or enhance this capacity.

A series of three experimental studies was conducted comparing work capacity and physiological and metabolic responses to work in untrained and trained animals.

The first experiment involved the comparison of four untrained and four trained swamp buffalo which were walked on a treadmill at 0.69 m/sec for a maximum period of three hours, while pulling four different draught loads equivalent to either 0, 5, 8 or 11% of their live weight.

In the second experiment, three Indonesian breeds of cattle (six of each), either untrained or trained, were compared. The breeds (Ongole,Bali and Madura) were worked in pairs, each pair pulling a draught load equivalent to 12% of the combined live weight of the pair, while walking around a dirt track at approximately 0.69 m/sec for a maximum period of three hours.

The third experiment involved the comparison of six untrained and six trained merino wethers which were walked on a treadmill at speeds of either 0.67, 1.04 or 1.38 m/sec for a maximum period of three hours while pulling a draught load equivalent to 11% of their respective live weight.

In all experiments, changes in body temperature, respiration rate and pulse rate were monitored as well as changes in the concentration of selected blood metabolites in the circulation. The uptake/output of the blood metabolites were also measured in wethers used in the third experiment.

The work capacity of each species was found to increase significantly after only a short period of training; the advantage of training being more clearly demonstrated at higher levels of work. Improvements in the cardiovascular system and the oxidative capacity of these animals were responsible for this enhanced work capacity.

Hyperthermia appeared to be the major factor causing the onset of fatigue in the working ruminants studied. The oxygen saturation of blood was reduced and the likely depletion of

the glycogen reserves of the animals in hyperthermia might also have had a role in the onset of fatigue.

The accumulation of lactate in the blood occurred in animals subjected to heavy work loads. This was more noticeable in the untrained animals and in the Bali and Madura breeds than in the Ongole. However, no acidosis occurred but a condition of mild respiratory alkalosis was observed.

In sheep it was found that the hind-limb muscles did not always produce lactate but in fact took up the metabolite, presumably to use as a fuel. It is likely that this would also have occurred in cattle and buffalo.

It was suggested from evidence presented in this study that under normal working conditions, it would be unlikely that the work capacity of draught animals would be limited by lactic acidosis; rather, hyperthermia would most probably be the major problem.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AmT	Ambient temperature
ARC	Agricultural Research Council
AT	Anaerobic threshold
ATP	Adenosine triphosphate
AV	Arteriovenous
BB	Black bulb
CF	Crude fibre
CNS	Central nervous system
CO	Cardiac output
CO_2	Carbon dioxide
Cr	Creatine
DM	Dry matter
EE	Energy expenditure
FFA	Free fatty acids
G-1-P	Glucose-1-phosphate
H^+	Hydrogen ion
H_2PO_4	Diprotonated inorganic phoshate
IMP	Inosine monophosphate
LDH	Lactate dehydrogenase
MAFF	Ministry of Agriculture, Fisheries and Food
Mm	Maintenence energy requirement
N	Nitrogen
NAD^+	Nicotinamide-adenine dinucleotide
NADH	Nicotinamide-adenine dinucleotide, reduced
$NaHCO_3$	Sodium bicarbonate
NH_3	Ammonia
O_2	Oxygen
O_2SAT	Oxygen saturation
OBLA	Onset of blood lactate accumulation
PCO_2	Partial pressure of carbon dioxide
Pcr	Phosphocreatine
PCV	Packed cell volume
PFK	Phosphofructokinase
Pi	Inorganic phosphate
pO_2	Partial pressure of oxygen
PR	Pulse rate
PVC	Polyvinyl chloride
RR	Respiration rate
RT	Rectal temperature

SR	Sarcplasmic reticulum
ST	Skin temperature
T-tubules	Transverse-tubules
TCA	Tricarboxcylic acid
TCO_2	Total carbon dioxide
VE	Ventilation

Kartiarso, Martin D and Teleni E (1987) Nutritional study using a treadmill at James Cook University. *DAP Project Bulletin* **3**, 11–15

Kartiarso, Martin D and Teleni E (1989) The pattern of utilization of body reserves by working cattle and buffalo. *DAP Project Bulletin* **8**, 7

Komarudin-Ma'sum, Teleni E, Martin DG and Affhandy L (1991) A comparison of the work capacity of Ongole (*Bos indicus*), Bali (*Bos sondicus*) and Madura cows. I. Untrained. *Draught Animal Bulletin* **2**, 7–15

Komarudin-Ma'sum, Teleni E, Martin DG and Affhandy L (1991) A comparison of the work capacity of Ongole (*Bos indicus*), Bali (*Bos sondicus*) and Madura cows. II. Trained. *Draught Animal Bulletin* **2**, 16–22

Martin DG and Teleni E (1988) Blood flow, blood pH and CO_2 output across the hind-limb muscles of working ruminants. *Proc Nut Soc Aust* **13**, 105

Martin D and Teleni E (1989) Fatigue in buffaloes on different work loads. *DAP Project Bulletin* **8**, 2–6

Martin D, Kartiarso and Teleni E (1989) Muscle blood flow in draught animals. *DAP Project Bulletin* **9**, 17–18

Teleni E and Martin DG (1991) Concentrations of some blood metabolites in trained and untrained cows. *Proc Nutr Soc Aust* **16**, 25

Teleni E, Bakrie B, Martin D, Boniface AN and Murray RM (1988) Physiologicl responses and changes in some blood metabolites in working ruminants. *Proc VI World Conference on Animal Production*, Helsinki. p467

Teleni E, Komarudin-Ma'sum, Martin DG and Bakrie B (1991) Concentration of blood metabolites in working Ongole, Bali and Madura cows. *Draught Animal Bulletin* **2**, 27–33

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GENERAL INTRODUCTION

There are over 400 million draught animals in the world todayBarwell & Ayres 1982) playing a significant role in food production for the ever-increasing world population. The use of these domesticated animals in agriculture dates back to 14th century BC.

Today, approximately two billion people depend on draught animal power for the cultivation of their land and for transportation. Nearly 50% of the cultivated areas of the world are prepared using draught animals Ramaswamy 1985). In addition, it has also been estimated that these animals haul over 25 million carts.

Draught animals include cattle, buffalo, horses, mules, donkeys, camels, yaks and elephants. However, this study is focussed on cattle and buffalo as they are the main species involved in land preparation for cropping. These animals provide not only draught power for ploughing, but also manure, transport, meat, milk and rental income for the farmer.

On smallholder farms, tractors are considered unsuitable (both in economic and practical terms) especially on hillsides, waterlogged fields and narrow terraces. More than 100 million farms in developing countries are less then two hectares. On these farms, family labour is readily available and draught animals are major assets to the farmers (Ramaswamy 1985).

Prolonged work, particularly in hot and humid conditions, can adversely affect the health and longevity of the animals. Live weight loss, physical weakness and increased susceptibility to disease and even death from exhaustion can occur (Anon 1972). In a given area, the amount of work which an animal is required to undertake in land cultivation would be relatively fixed. It might be expected therefore, that the degree of work-related stress which an animal would suffer would vary with its body size, i.e. the larger the body size of the animal, the less the stress it would suffer and *vice versa*.

This thesis is part of an on-going research program of the *Nutritional Physiology and Metabolism Unit*, on exercise physiology and its various applications. The experiments described were undertaken to define the work capacity of ruminant animals and to investigate the various factors which could limit or enhance this capacity.

REVIEW OF THE LITERATURE

2.1 Skeletal Muscle: Structure and Contraction

Skeletal muscle fibres consist mainly of protein filaments which are grouped into bundles called myofibrils. The myofibrils are surrounded by extracellular fluid (cytoplasm) and are enclosed by an excitable membrane (sarcolemma). The cytoplasm contains mitochondria, internal membrane systems of the sarcoplasmic reticulum (SR), the transverse tubules (T-tubule), glycogen, adenosine triphosphate (ATP), phosphocreatine (Pcr) and glycolytic enzymes. Individual and groups of fibres (figure 2.1) are surrounded by connective tissues. Muscles are supplied by a vast network of blood capillaries and nerve fibres.

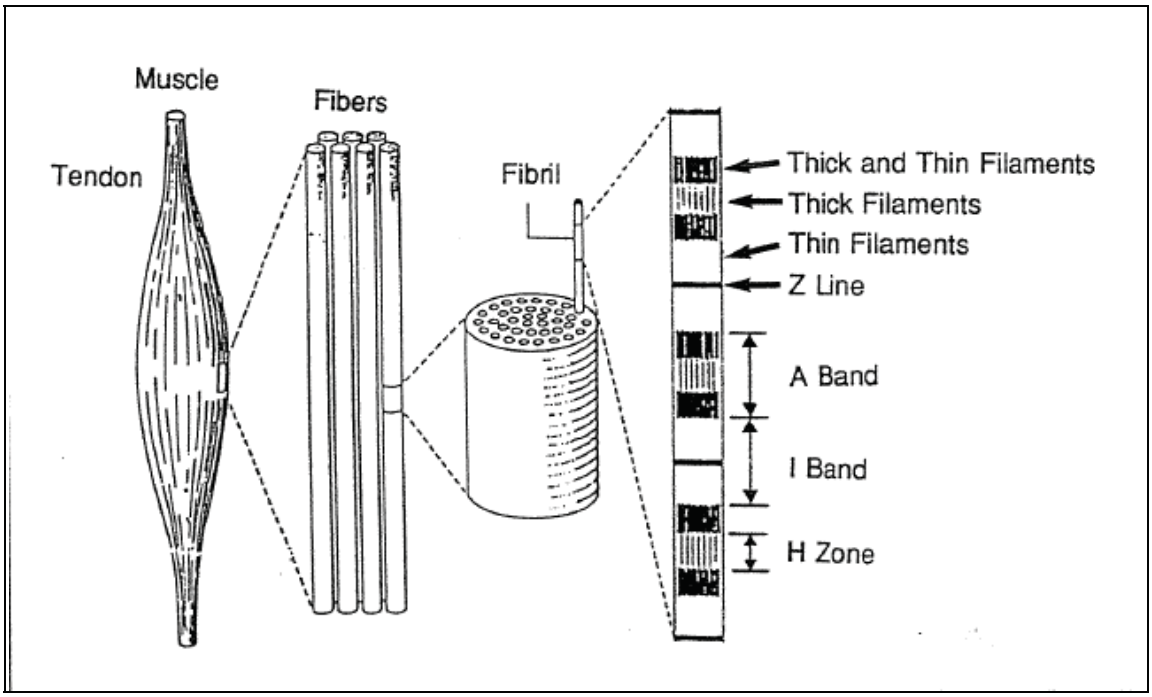


Figure 2.1 Diagram of muscle structure showing the arrangements of fibres in a striated muscle. The cross-striations on the myofibrils can be seen with light microscopy. (from Keynes & Aidley 1991).

Myofibrils consist of thick and thin protein filaments which slide past each other to cause muscle contraction. This sliding filament theory was independently formulated by Huxley and Hanson (1954) who used phase-contrast microscopy of myofibrils from glycerol extracted muscles and by Huxley and Niedergerke (1954) who used interference microscopy of living muscle fibres.

The thick filaments consist of an ATPase myosin which enzymatically hydrolyses

ATP to form adenosine diphosphate (ADP) and inorganic phosphate. This reaction operates only in the presence of Mg^{2+}. The thin filaments contain actin, tropomyosin and troponin. Actin binds to myosin, forming actomyosin which increases the *ATPase* activity by 200-fold (Adelstein & Eisenberg 1980).

Myosin contains mobile sections (S_1 and S_2 subunits) called cross-bridges. These cross-bridges attach to the actin which cause the filaments to slide past each other when the S_1 subunit head rotates (figure 2.2).

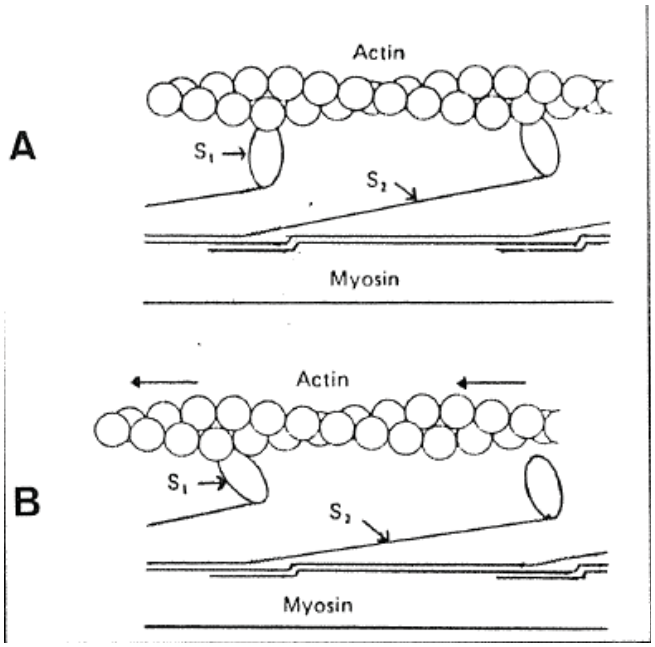


Figure 2.2 Sliding is probably brought about, by a rotation of the S_1 subunits about their points of attachment to the thin filament (actin). In (A) the left-hand cross-bridge has just attached, whereas the S_1 subunit of the right-hand one has nearly completed its rotation. In (B) the situation is shown a short time later: the S_1 subunit of the left-hand cross-bridge has rotated, so pulling the thin filament to the left, and the right-hand cross-bridge is now detached. **(from Huxley 1976)**

The energy for one cycle of cross-bridges is provided by one mole of ATP.

Cross-bridge formation and hence sliding of the filaments can only take place through the process of *excitation-contraction coupling* after the filaments receive nerve impulses.
The action potential causes a depolarisation of the T-tubules (invaginations of the cell surface membrane) which causes the SR to release Ca^{2+}. The SR are membrane-bound sacs between the myofibrils which store Ca^{2+} against a concentration gradient by an ATP-dependent Ca^{2+} pump. The process by which the T-tubules and the SR interact is still unclear, however, it is thought that they are connected via a foot protein which undergoes a conformational change to open the Ca^{2+} channel in the SR membrane (figure 2.3).

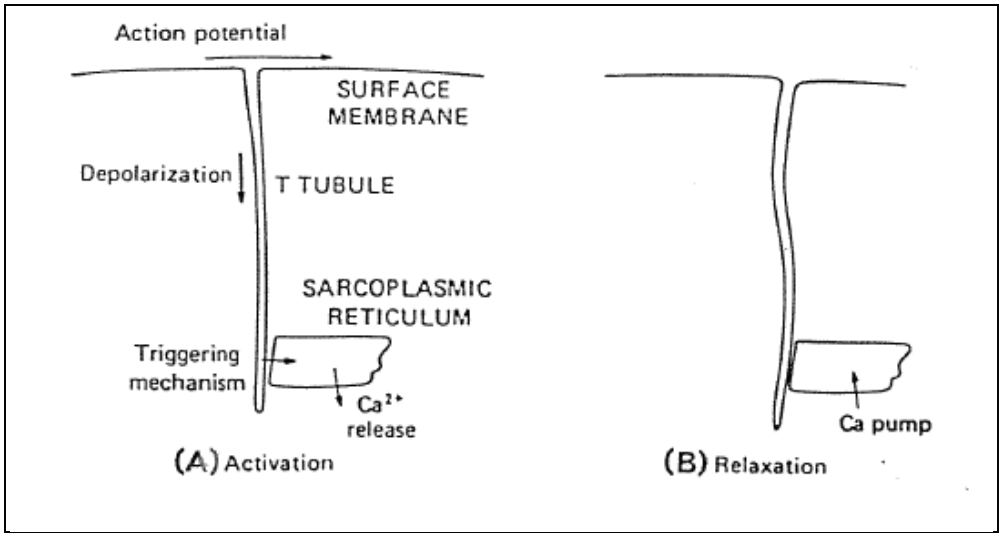


Figure 2.3 Schematic representation of the coupling process in skeletal muscle. During activation calcium ions are released from the sarcoplasmic reticulum (A). They are then pumped back into the sarcoplasmic reticulum, so causing relaxation (B).

In humans, Ca^{2+} are stored in the SR at a high concentration, but at a low concentration, e.g. 50 nM, in the cytoplasm at rest. The Ca^{2+} released into the cytoplasm after excitation is at a concentration of 1000–5000 nM (Allen et al. 1992). Calcium ions bind to one of the three subunits of Troponin (*viz* TnC, TnT or TnI) causing a conformational change which is transmitted to the tropomyosin and then to actin. This change exposes the active site of the actin which binds to the mobile section of myosin (cross-bridge formation). The energy released when ATP splits enables the two filaments to slide past each other, producing muscle contraction.

When muscle is relaxed, the Ca^{2+} is pumped back into the SR by the ATP-dependent Ca^{2+} pump. The Ca^{2+} is bound by a protein (calsequestrin) inside the SR. The troponin and tropomyosin inhibit the interaction of actin and myosin in the absence of Ca^{2+}.

The release of Ca^{2+} from the SR is pH-dependent. For example, Nakamaru and Schwartz (1972) showed that Ca^{2+} was released at pH 6.45–7.7 (extracellular) but not at pH 6.1–6.3.

The reduction in concentration of ATP, after muscle contraction, stimulates glycolysis, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. The relative contribution of these to the regeneration of ATP depends on the fibre composition of the muscle.

2.1.1 Muscle fibre types

Skeletal muscle is composed of two types of fibres, *viz* the slow twitch (type I) and the fast twitch (type II). The fibres are classified using a system by Brooke and Kaiser (1970) according to their speed of contraction and their dependence on anaerobic and aerobic metabolism for supply of ATP (see table 2.1). This classification is based on the staining of myosin-ATPase after acid or alkaline pre-incubations. This is consistent with the different techniques developed by Fitzsimon and Hoh (1981: electrophoretic studies) and Snow et al. (1981: immunological methods).

Type I contains a type of myosin ATPase that is different from other fibres (Brooke & Kaiser 1970). The fibres have a high rate of aerobic energy production (high endurance capacity) due to their high content of mitochondrial and oxidative enzymes. They also have a high myoglobin content and an extensive capillary network to facilitate oxygen diffusion.

Type II fibres also have a high rate of energy production but are limited in endurance capacity. The fibres are specialised in anaerobic production of energy, although some do have aerobic capacity. Type II contains three types of myosin-ATPase. These are types IIA, IIB and IIC fibres. Type IIA is fast-twitch but is also highly aerobic. Type IIB is anaerobic as described above and IIC is a rare fibre, the precise function of which is unknown.

Table 2.1 Human skeletal muscle fibre types and their properties **(from Saltin et al. 1977)**

	TYPE I (slow-twitch)	TYPE IIA (fast-twitch oxidative)	TYPE IIB (slow-twitch glycolytic)
ATPase activity after pre-incubation at pH 10.3	-	+++	+++
ATPase activity after pre-incubation at pH 10.3 and pH 4.6 – 4.8	-	-	+++
Speed of contraction	Slow	Fast	Fast
Glycolytic capacity	Low	Moderate	High
Oxidative capacity	High	Moderate	Low
Glycogen store	Moderate – High	Moderate – High	Moderate – High
Triacylglycerol store	High	Moderate	Low
Capillary supply	Good	Moderate	Poor

2.1.2 Energetics of contraction

Adenosine triphosphate is the universal energy currency in the body. It provides energy for muscle contraction, the active transport of molecules and ions and is used in the synthesis of biomolecules from simple precursors. Muscles convert chemical energy into mechanical energy by the hydrolysis of ATP. The amount of ATP in muscle is very limited (approximately 6 mmol/kg muscle; McKinen 1983) and would provide enough energy for only 1–2 seconds of muscle contraction. For sustained exercise, ATP must be regenerated to provide a continuous supply of energy to the muscle.

Regeneration of ATP is achieved by two distinct processes:–

✎ *Anaerobic Phosphorylation*
This involves adenosine diphosphate (ADP), Pcr and anaerobic glycolysis.

- *Phosphocreatine reaction*

$$\text{Pcr} + \text{ADP} \xrightarrow[\text{kinase}]{\text{creatine}} \text{Cr} + \text{ATP}$$

- *Myokinase reaction*

$$2\text{ADP} \xrightarrow[\text{kinase}]{\text{myoadenylate}} \text{ATP} + \text{AMP (adenosine monophosphate)}$$

- *Glycolysis*

$$\text{G} + 3\text{ADP} + 3\text{Pi} \rightarrow 2\ \text{Lactate} + 3\text{ATP}$$

✎ *Aerobic Phosphorylation*
This involves the reduction of substrates and their oxidation via the TCA cycle and respiratory chain (see figure 2.4).

Although anaerobic metabolism provide pathways leading to a more rapid yield of ATP, the pathways are less efficient than aerobic phosphorylation which is a much slower process due to limitations in oxygen transport. The amounts of ATP yielded by the anaerobic and aerobic pathways are shown in table 2.2.

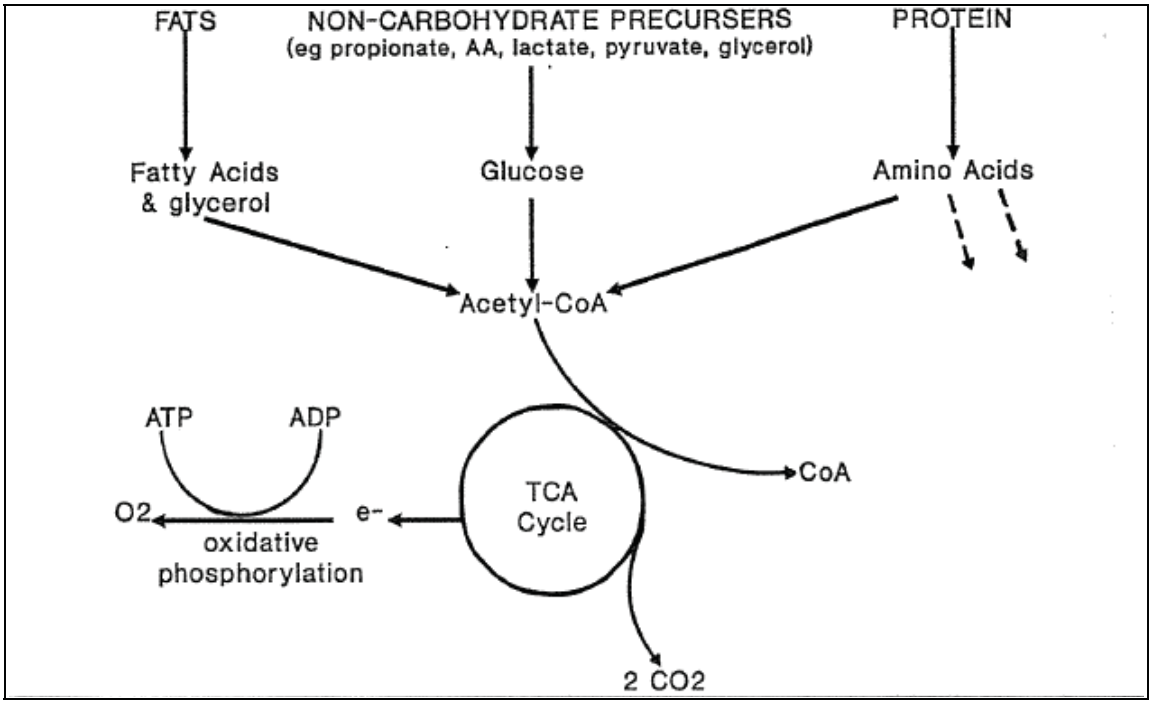


Figure 2.4 Diagram showing substrates for ATP production via the TCA cycle and respiratory chain.

Table 2.2 Yields of adenosine triphosphate (ATP) from anaerobic and aerobic pathways **(from McKinen 1983; Bayly 1989)**

Source	ATP Yield (moles)
Anaerobic Phosphorylation	
Phosphocreatine reaction	1
Myokinase reaction	1
Anaerobic glycolysis	
• Intramuscular glycogen	3
• Blood glucose	2
Aerobic Phosphorylation	
Glucose (intramuscular glycogen)	39
Fat (palmitic acid)	129*

* assuming complete oxidation

The immediate source of energy for muscle is intramuscular ATP. To replenish the depleted supply of ATP in the short term, degradation of Pcr and ADP and anaerobic glycolysis occur very rapidly. Indeed the degradation of Pcr and the process of glycogenolysis occur concomitantly from the onset of exercise (Bonen et al. 1989). These changes were demonstrated by Brzezinska (1987) in a study where dogs were exercised at moderate intensity until exhaustion (71 minutes).

The amount of ATP in muscle progressively decreased towards exhaustion while those of

ADP and AMP increased (figure 2.5). The changes in concentrations of Pcr and creatine in muscle showed opposite trends during exercise; most of the degradation of Pcr occurring in the first 30 minutes of exercise (see figure 2.6). It was also shown that the concentration of muscle glycogen decreased and that of glucose increased until the last 10 minutes of exercise when it decreased (figure 2.7). Pyruvate and lactate progressively increased during exercise (figure 2.8).

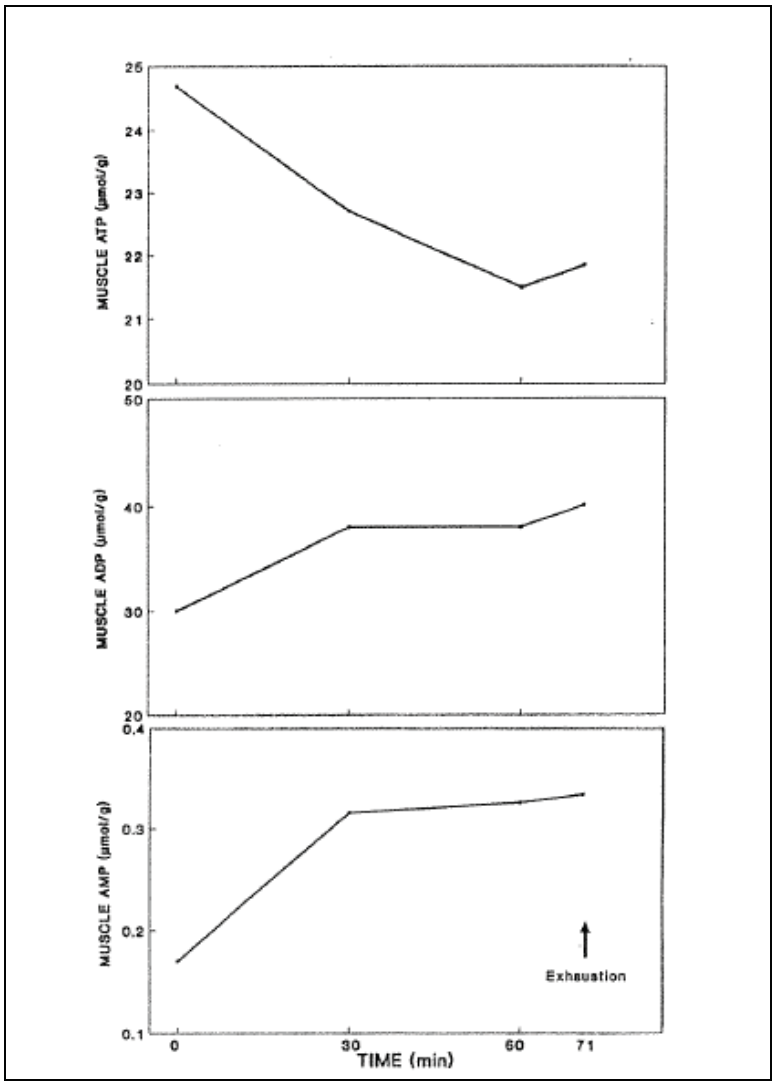


Figure 2.5 Muscle concentrations of ATP, ADP, AMP during exercise in dogs **(adapted from Brzezinska 1987).**

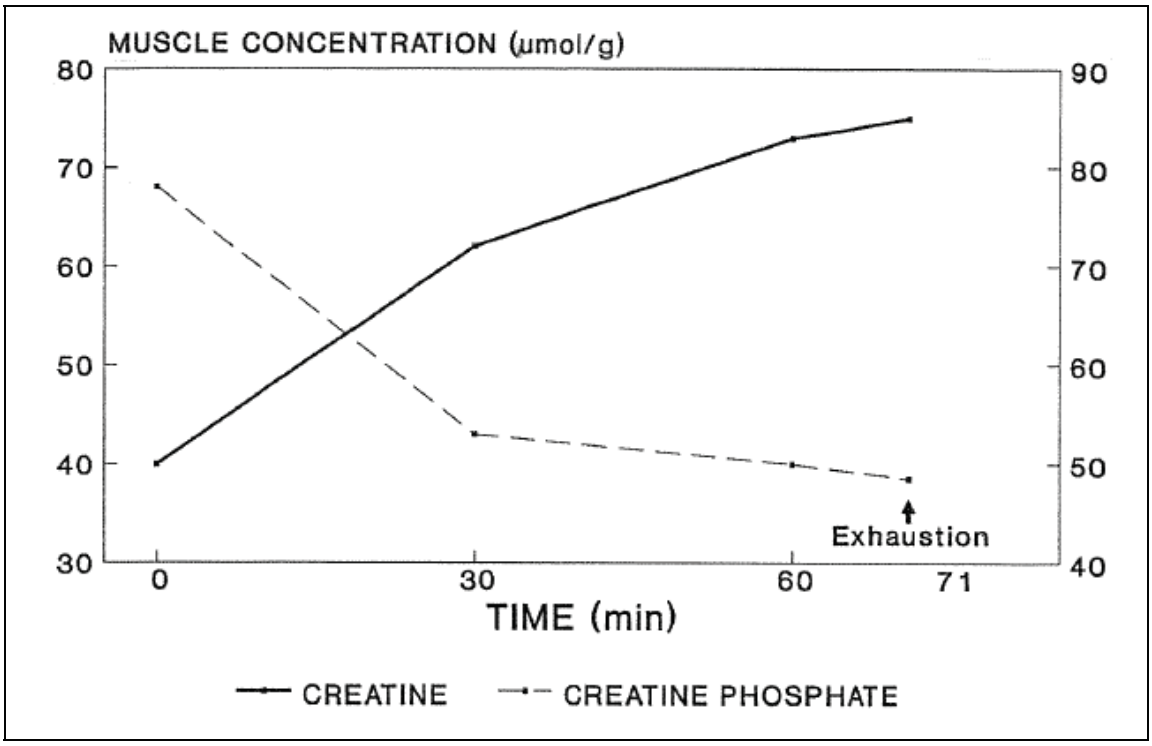


Figure 2.6 Muscle concentrations of creatine phosphate and creatine during exercise in dogs. (adapted from Brzezinska 1987)

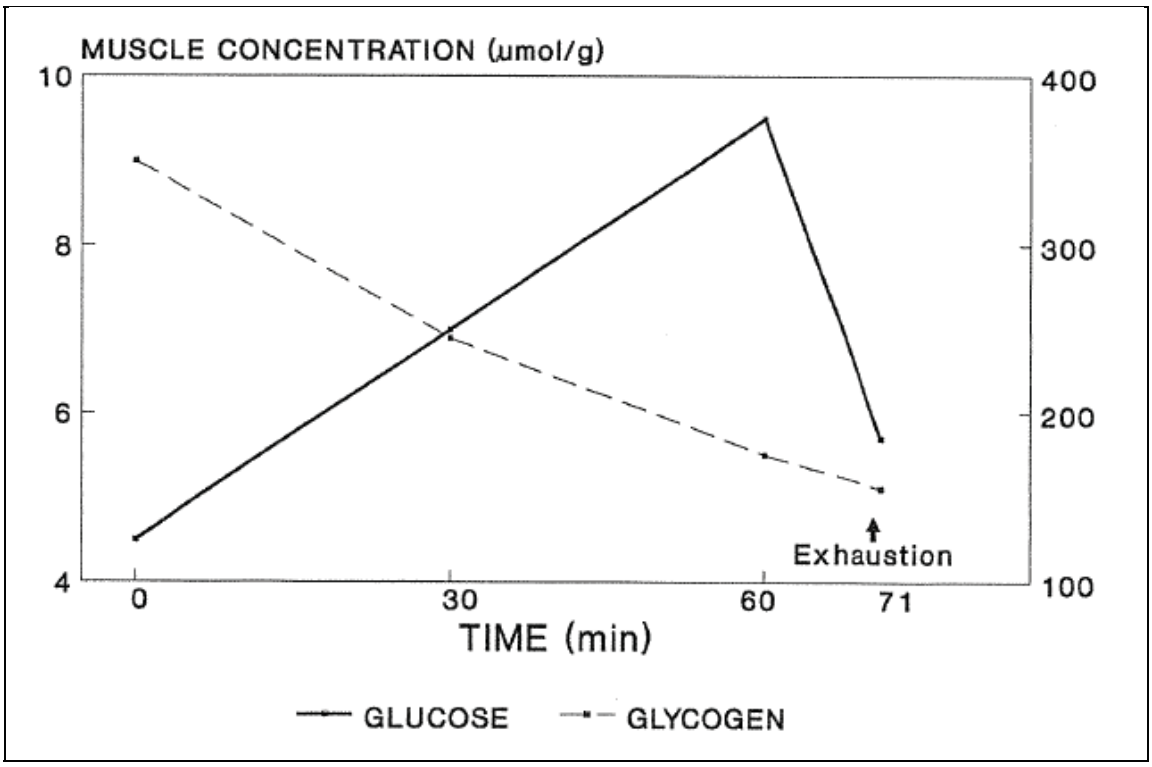


Figure 2.7 Muscle concentrations of glycogen and glucose during exercise in dogs. (adapted from Brzezinska 1987)

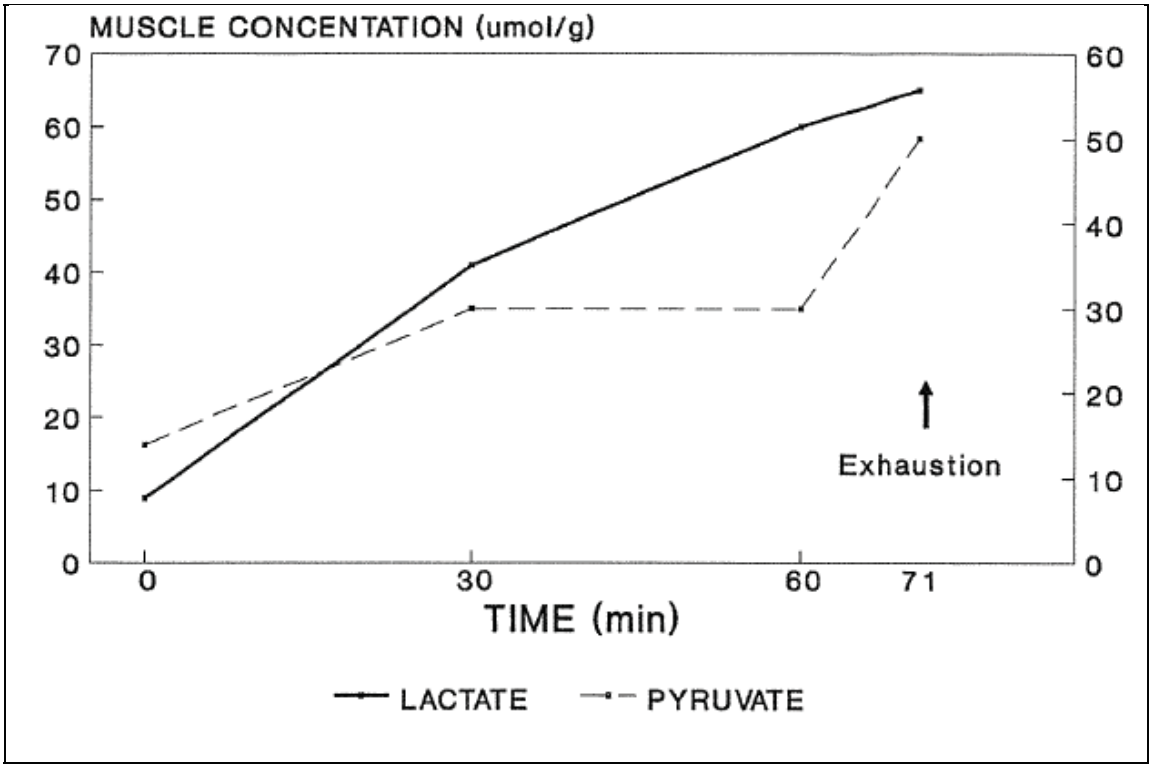


Figure 2.8 Muscle concentrations of pyruvate and lactate during exercise in dogs. (adapted from Brzezinska 1987)

The major sources of energy for muscle contraction in exercise are glucose and free fatty acids (FFA) (Bjorntorp 1991); the FFA becoming increasingly important as exercise is prolonged (Astrand 1967; Pethick 1984). These two substrates are derived from intramuscular and extramuscular stores of glycogen and triglycerides respectively.

The proportional contributions of the various energy-yielding substrates, and of anaerobic and aerobic metabolism, to the generation of ATP during exercise depend on the concentration of FFA in plasma, training status and the size of glycogen stores in muscle, the oxidative capacity of muscle and the intensity and duration of the exercise (Issekutz et al. 1966; Bayly 1989; Bjorntorp 1991).

During rest, muscle metabolism in dogs and humans is entirely aerobic in which plasma FFA is a major oxidative substrate (Layzer 1990). Ruminants, however, utilise glucose and acetate (Bell et al. 1985; Pethick 1984) as major energy-yielding substrates (see table 2.3).

Table 2.3 Metabolite concentration in the blood and the maximum contribution to the oxidation of skeletal muscle in the hind limb of the fed sheep and humans **(from Pethick 1984)**

Metabolite	Sheep[b]		Human[c]	
	Arterial concentration (mM)	Contribution to oxidation (%)	Arterial concentration (mM)	Contribution to oxidation (%)
Acetate	1.20	30–40		ND[a]
Ketone bodies	0.35	15	0.17[d] 0.17	
Long chain fatty acids	0.06		0.6–0.7	2–10 70–110
Glucose[e]	2.95–3.81	5 30–57	4.2–4.8	10–30

[a] not determined
[b] Pethick and Lindsay (1982) and Bird et al. (1981)
[c] Jansson (1980), Ahlborg et al. (1974) and Dagenais et al. (1976)
[d] Ballard (1972)
[e] Contribution to oxidation has been corrected for lactate release or uptake

During exercise in the three species mentioned, both glycogen and plasma FFA are utilised. Pethick (1984) in his study of sheep, confirmed the increasing importance of FFA as a fuel for muscle in sustained exercise. His sheep were walked on a treadmill at 5 km/h for 2 h. He also showed that the use of glucose by muscle was rapid but sustainable over the 2-h period, and that the other fuels (*viz* acetate and ketone bodies) were used at similar rates during rest and exercise. Data showing the percentage contributions of metabolites to the hind-limb muscle of sheep during exercise are presented in table 2.4.

Table 2.4 The maximum contribution of circulating and endogenous metabolites to oxidation in skeletal muscle during sustained exercise **(from Pethick 1984)**

Metabolite	Maximum contribution to oxidation (%)		
	Duration of exercise		
	15 min	50 min	120 min
Acetate Ketone bodies Long-chain fatty acids	8	8	8
Glucose[a]	2	4	4
Endogenous substrates in muscle[b]	15	26	40
	21	25	29
	54	37	19

[a] Corrected for lactate uptake or output
[b] Computed by difference

Blood glucose concentration must remain constant so as to adequately supply the central nervous system (CNS) and brain. This homeostasis is maintained by liver glycogen and,

indirectly, by skeletal muscle glycogen which provides lactate, pyruvate and alanine as precursors for gluconeogenesis during exercise. The increasing utilisation of FFA (see table 2.3) as energy-yielding substrates during prolonged exercise, contributes to the maintenance of a constant concentration of blood glucose.

The increased concentration of FFA in plasma has an inhibitory effect on the uptake of glucose by muscle and on the depletion of glycogen stores (Rennie & Holloszy 1977). This effect is referred to as glycogen-sparing. Randle et al. (1964) first suggested that an increase in concentration of plasma FFA would increase the TCA cycle activity, leading to increases in concentrations of both acetyl-CoA and citrate. The acetyl CoA inhibits *pyruvate dehydrogenase* (E.C.1.2.4.1) and the citrate inhibits *phosphofructokinase* (E.C. 2.7.1.11) (PFK). The resultant reduction in the rate of glycolysis and an increase in glucose-6-phosphate further inhibits *glucose-hexokinase* (E.C.2.7.1.1) and the absorption of glucose into the muscle cell. This regulation of glycolysis by PFK was suggested by Randle et al. (1964) who demonstrated it in *in vitro* studies. However, it has not been demonstrated *in vivo* (Jenkins et al. 1989; Johnson et al. 1989).

The results of the study by Piatti et al. (1991) support the hypothesis proposed by Randle et al. (1964). They showed that FFA inhibited the uptake of glucose by muscle in the absence or presence of insulin. Whether the glucose-FFA cycle actually operates during exercise is somewhat controversial. In a study of humans, increasing the concentration of FFA in plasma during the first 30 minutes of exercise, decreased carbohydrate utilisation (Costill et al. 1977; Hargreaves et al. 1988). Increasing the concentration of FFA later in exercise, when FFA concentrations are normally higher, had no effect (Ravussin et al. 1986). Hargreaves et al. (1991) reported that humans infused intravenously with intralipid* and heparin, to increase the concentration of plasma FFA, showed an inhibition of uptake of glucose by muscle at rest, during exercise and during recovery. They hypothesised that the observed effect involved glucose transport rather than the glucose-FFA cycle. The mechanism by which FFA interfere with glucose metabolism remains unclear.

Glycolysis

Aerobic and anaerobic glycolysis are important pathways that are regulated at two rate-limiting steps involving the enzymes: *phosphorylase* (E.C.2.4.1.1) and *PFK*.

In aerobic glycolysis, glycogen is metabolised to pyruvate which enters the mitochondria where it is completely oxidised to carbon dioxide (CO_2) and water. In anaerobic glycolysis,

* Intralipid = 20% triglyceride emulsion

however, pyruvate is converted to lactate by *lactate dehydrogenase* (LDH) (E.C.1.1.1.27).

Phosphorylase degrades glycogen to glucose-1-phosphate (G-1-P). This enzyme has an active form called *phosphorylase a* and an inactive form, *phosphorylase b*. It is inhibited by high concentrations of ATP and glucose-6-phosphate and is activated by high concentrations of AMP, adrenaline and also calcium ions.

$$\text{Glycogen (n residues)} + \text{Pi} \xrightleftharpoons{\textit{phosphorylase}} \text{G-1-P} + \text{glycogen (n-1 residues)}$$

The other rate-limiting step in glycolysis involves *PFK*. The enzyme is inhibited by high concentrations of ATP and citrate as well as low pH (Asmussen 1979; Hermansen 1981). It is activated by inorganic phosphate and AMP. Hormonal regulation appears to play a minor role in glycolysis (Gollnick et al. 1970; Harris et al. 1971).

$$\text{Fructose-6-Phosphate} \xrightarrow{\textit{PFK}} \text{Fructose 1,6-diphosphate} + \text{ADP} + \text{H}^+ + \text{ATP}$$

2.2 Fatigue

The endurance capacity of an animal is the period for which it can sustain work until it is exhausted. Exhaustion occurs when the work rate of the animal starts to decline. This can be due largely to the onset of muscular fatigue which is defined as a failure by muscle to maintain a required or expected force (Edwards 1981) because of its reduced capacity to regenerate ATP (Sahlin 1992).

Although the processes leading to the onset of fatigue have not been totally elucidated, the condition has been classified as being either central or peripheral (see Bigland-Ritchie 1981; Gandevia 1992; Sahlin 1992).

2.2.1 Central fatigue

Central fatigue is closely associated with the function of the CNS and it is related to the mood or motivation to work. Ammonia (NH_3) is a potent neurotoxin and it can cause fatigue by impairing CNS function (Mutch & Barister 1983). Furthermore, variations in the concentration ratio of aromatic amino acids (AA):branched chain AA, which can occur during prolonged exercise, can affect the concentrations of neurotransmitters in the brain and hence the central drive and mood to work (Blomstrand et al. 1988).

2.2.2 Peripheral fatigue

Peripheral fatigue involves the transmission (neuromuscular junction, muscle membrane and endoplasmic reticulum) and contractile mechanisms of muscle filaments. It is thought that the following three general processes contribute to peripheral fatigue:

- *Neural fatigue*
  This occurs when there are depletion of neurotransmitter substances [e.g. acetylcholine, 5-hydroxytryptamine, noradrenaline, dopamine, adrenaline, glycine, gamma-aminobutyric acid, glutamic acid, aspartic acid, leucine, encephalin and substance P] and changes in membrane potential. Electrolyte balance and Na^+-K^+ pumps are responsible for these membrane potentials.

- *Intramuscular metabolite accumulation*
  This refers specifically to the accumulation of lactic acid in muscle from anaerobic glycolysis.

- *Intramuscular fuel depletion*
  There is an exponential decrease in work rate with reduction in intramuscular fuel (glycogen and triglycerides).

2.2.3 Factors involved in fatigue

Ammonia

Ammonia production during exercise occurs either through the purine nucleotide cycle (Lowenstein 1972) or through catabolism of AA from muscle protein (Graham et al. 1991).

Purine nucleotide cycle
Ammonia is produced in the purine nucleotide cycle when AMP is catabolised to inosine monophosphate (IMP). The reaction is catalysed by the enzyme *AMP deaminase* (E.C.3.5.4.6) and partially buffers H^+ from lactate production (Sahlin et al. 1978). The activity of this enzyme is greater in fast-twitch then in slow-twitch muscle fibres (Bockman & McKenzie 1983).

$$\text{AMP} \xrightarrow{\text{AMP deaminase}} \text{IMP} + NH_3$$

Adenosine monophosphate deaminase is inhibited by guanosine triphosphate (GTP). Adenosine monophosphate and ADP have been found to be potent stimulators of the

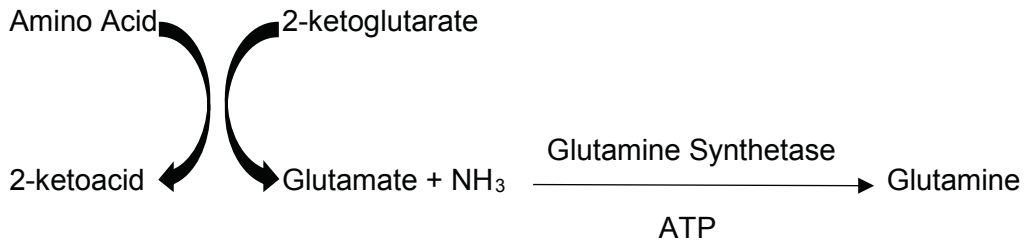
enzyme (Wheeler & Lowenstein 1979). High concentrations of IMP have been observed in fatigued muscle and is closely related to increased and decreased concentrations of muscle lactate and Pcr respectively (Sahlin et al. 1989). During low intensity exercise, Sahlin and Broberg (1990) found no measurable increase in muscle concentration of IMP or NH_3. At high intensity exercise, however, both IMP and NH_3 accumulated in working muscles. The authors and Sewell et al. (1992) found that these increases were stoichiometrically matched with the decrease is total adenine nucleotides (ATP+ADP+AMP). After prolonged exercise, low intramuscular glycogen concentrations were found to be associated with AMP deamination (Broberg & Sahlin 1989) and that the formations of lactic acid and AMP were inversely related. The nature of the relationships may indicate that the production of NH_3 occurs at the deterioration of energy supply. The formation of IMP from AMP probably eliminates the AMP concentration which stimulates glycolysis, thereby preventing further acidosis. It has been shown that pH has a direct effect on *AMP deaminase* activity which increases as the pH in muscle approaches 6.5 to 6.1 (Wheeler & Lowenstein 1979). However, the degradation of AMP has been reported to have occurred, in the absence of lactate accumulation and acidosis, in iodoacetate-poisoned muscle (Sahlin et al. 1981; Dudley & Terjung 1985) and in muscles of *myophosphorylase-* (E.C.2.4.1.1) (Coakley et al. 1989) and *PFK*-deficient patients (Bertocci et al. 1991). This demonstrates that the degradation of AMP may be dependent on the availability of other energy-yielding substrates. Results of kinetic studies by Ronca-Testoni et al. (1970) and Wheeler and Lowenstein (1979) are consistent with this suggestion.

Protein degradation

Ammonia may be produced by the catabolism of AA from muscle protein (Broberg & Sahlin 1989). Graham et al. (1991) observed that during moderate exercise in humans, there was a substantial and progressive release of NH_3 from leg muscle. They also observed an increased release of AA, including indispensable AA. Whether the increased release of AA from muscle is a result of an increased rate of muscle protein degradation, or a decreased rate of protein synthesis is unclear.

Increased concentration of plasma FFA can reduce the amount of NH_3 released from muscles (Graham et al. 1991). It may also increase the clearance rate, possibly by the liver, of circulating AA (Graham et al. 1991).

The NH_3 produced from the deamination of AA may be used glutamine production in skeletal muscle. Glutamate and NH_3 combine in the presence of ATP and the enzyme, *glutamine synthetase* (E.C.6.3.1.2) to form glutamine.



The rate of this reaction has been observed to increase significantlly at high concentrations of NH_3 in muscles of rats, dogs and monkeys (Hill et al. 1972). The rate of glutamine synthesis can also be stimulated by metabolic acidosis (Ruderman & Berger 1974) in which the excretion of excess H^+ in the urine is facilitated by NH_3 from the AA (Bergman & Heitman 1978).

Alkalosis

Alkalosis may be associated with a delay in the onset of fatigue. Metabolic alkalosis is characterised by an increased concentration of bicarbonate (HCO_3^-) in blood while respiratory alkalosis is characterised by reduced partial pressure of carbon dioxide (pCO_2) (Spriet et al. 1986)

In alkalosis, there is an increased concentration of plasma lactate due to an increase in the rates of release of lactate from working muscles (Mainwood et al. 1972; McCartney et al. 1983; Spriet et al. 1986).

Results of studies on the question of whether fatigue is delayed by alkalosis are inconsistent. For example, Spriet et al. (1986) reported a 15 to 20% reduction in the rate of lactate accumulation in muscles of exercised rat, although no increase in the endurance capacity of the animals was observed. Other studies showed that an increase in intracellular pH of muscles prior to exercise, may lead to increased rates of anaerobic glycolysis (Heisler 1975). Costill et al. (1984) suggested that alkalosis could delay the onset of fatigue by increasing the rate of FFA oxidation. It was also demonstrated that in humans, ingestion of $NaHCO_3$ could delay the onset of fatigue (Jones et al. 1977; Sutton et al. 1981). The increased endurance observed was associated with the maintenance of glycolysis and an increased rate of release of lactate and H^+ from muscles. Studies where no improvement in work endurance of animals was observed (e.g. Spriet et al. 1986) may have used animals in which

the *excitation-contraction coupling* mechanism (see section 2.1) was already operating at a maximum.

There appears to be general agreement that alkalosis would cause an increase in concentration of plasma lactate due to an increased rate of release of lactate from working muscle. Whether this would cause a delay in the onset of fatigue is yet uncertain. Intensity and duration of exercise are likely to be important considerations.

$H_2PO_4^-$

The diprotonated form of Pi, $H_2PO_4^-$, is of interest in fatigue due to changes in the two variables H^+ and Pi, which have separate and combined effects on fatigue (Cooke et al. 1988). The $H_2PO_4^-$ was found to be responsible for the reduction in the strength of muscle contraction (Nosek et al. 1987). Cooke et al. (1988) showed that at pH 7 half of the Pi in muscle were in the diprotonated form and at pH 6, all were in the diprotonated form. The negative relationship between the concentrations of both $H_2PO_4^-$ and H^+ in human muscles (tibialis anterior and adductor pollices) and the strength of contraction of the tissue during aerobic and anaerobic exercise, was further demonstrated in the study of Weiner et al. (1990). It appears that the reason for such a relationship is the inhibition of *ATPAse* activity by H_2PO_4 and H^+. Studies using muscles of frog (Miller et al. 1988) and rabbit (Nosek et al. 1987) showed that $H_2PO_4^-$ directly inhibits the contractile mechanism of muscle by inhibiting cross-bridge formation. This probably occurs through competition from Pi for the catalytic binding site of ATPase in activated fibres (Webb et al. 1986).

Potassium and sodium

The movement of ions across the cell membrane is of major importance in the transportation of substrates, regulation of secretion of hormones and in the control of muscle contraction. The maintenance of the ionic gradient (Na^+ – K^+ balance) between extra- and intracellular fluid uses ATP. Estimates of the amount of energy expended in the maintenance of this ionic gradient are quite variable (see table 2.5).

Table 2.5 The contribution of Na^+, K^+–*ATPase* to muscle energy expenditure *in vitro* as determined by O_2 consumption and by microcalorimetry*

Muscle	% Contribution	Reference
Rat soleus	6*	Chinet et al. 1977
Mouse soleus	7*	Biron et al. 1979
Rat diaphragm	12	Ismail-Beigi & Endelman 1971
Sheep intercostal	18	McBride 1986
Mouse soleus	19	Gregg & Milligan 1980
Hamster diaphragm	23	Horwitz & Eaton 1977
Sheep intercostal	23	Early et al. 1988
Lamb intercostal	31	McBride & Early 1987
Rat pectoralis	34	Guernsey & Stevens 1977
Sheep sternomandibularis	37	Gregg & Milligan 1982a
Cattle sternomandibularis	41	Gregg & Milligan 1982b
Rat diaphragm	41–51	Asano et al. 1976

Exercise causes a change in the Na^+ and K^+ concentrations in cells. The major change involves the efflux of K^+ from muscle cells causing an accumulation of the ion extracellularly (Medbo & Sejersted 1990; Dorup & Clausen 1993). An increase in extracellular K^+ concentration would stimulate heart and respiration rates, and would also cause vasodilation within the muscle. These would result in an increase in blood flow (Lindinger & Sjogaard 1991).

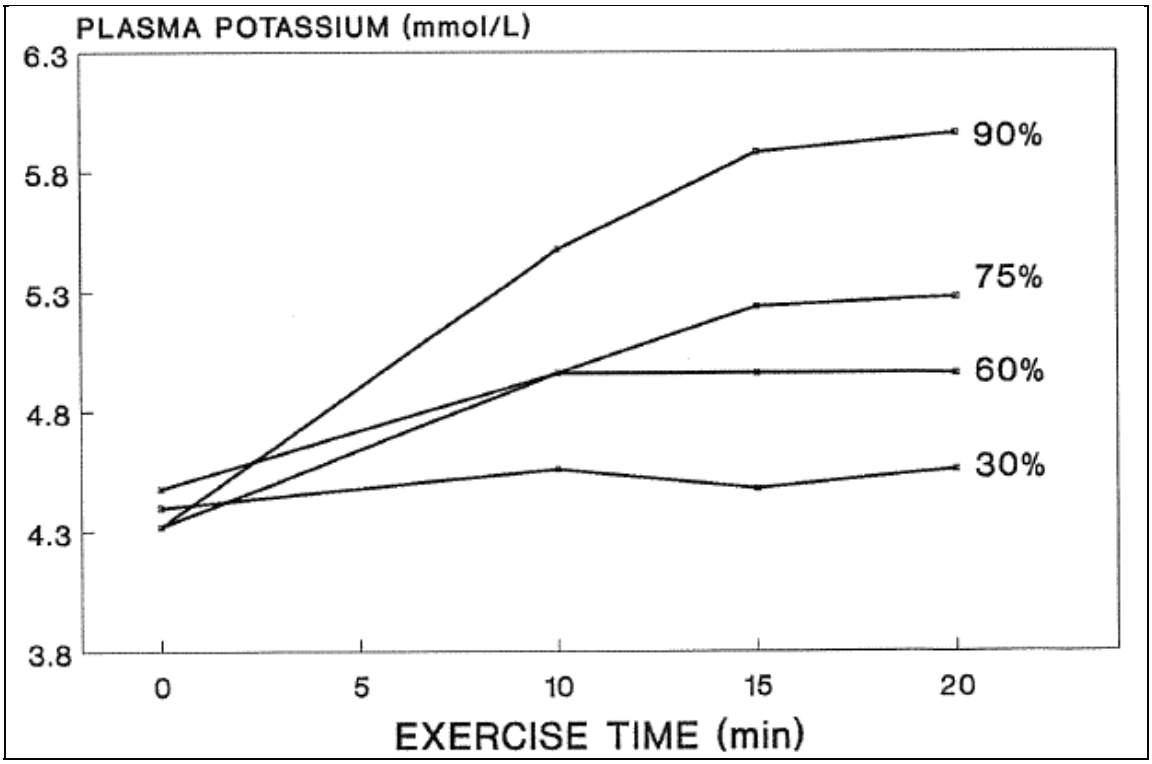


Figure 2.9 Mean increases in plasma K^+ concentrations in humans (arm) with increasing

exercise intensity (% V O_2 max) and duration of exercise. [Neilsen et al. (1984), cycling or swimming]

The increase in extracellular K^+ concentration has also been identified as a factor causing fatigue (Medbo & Sejersted 1990; Lindinger & Sjogaard 1991). Some published data on changes in concentration of K^+ in plasma during exercise are presented in figure 2.9.

There are three main causes of the increase in extracellular K^+ concentration during exercise:–

- A decrease in plasma volume (haemoconcentration) due to a shift of water out of the plasma (Sjogaard & Saltin 1982; Lindinger & Heigenhauser 1988);
- the small amount of K^+ released from erythrocytes, and possibly from haemolysis (Reinhart et al. 1983); and
- a release of K^+ from contracting muscles due to the activity of ATP-sensitive K^+ channels.

A reduction in muscle pH reduces the activity of the ATP-dependent K^+ channels (Davies et al. 1992) by causing H^+ to bind to the site of ATP activity. This competitive binding causes a 15–fold reduction in K^+ channel activity thus opening the K^+ channels and releasing K^+ (Davies et al. 1992).

Kolb (1990) identified four K^+ channels:–

- Inwardly rectified K^+ channel – activated by hyperpolarisation.
- Delayed rectifier K^+ channel – responsible for repolarising phase of an action potential.
- Calcium activated K^+ channel – activated by Ca^{2+} influx into cytoplasm.
- ATP sensitive K^+ channel – activated by local decreases in the concentration of ATP and results in outward transport of K^+ from the cell.

When muscle contracts each action potential causes the efflux of Na^+ into and K^+ out of the cell. Repeated contractions would cause a deficiency of Na^+ and an accumulation of K^+ in the T-tubules (see figure 2.3). The Na^+ – K^+ pump does not have the capacity to transport K^+ back into the cell (Clausen et al. 1987) during exercise, hence the K^+ accumulation. The accumulation of K^+ extracellularly and the decreased Na^+ concentration would cause a depolarisation of the cell membrane (Lindinger & Sjogaard 1991) resulting in a decreased amplitude of the action potential (Sandercock et al. 1985). The reduced action potential would then reduce the rate of release of Ca^{2+} from the SR (see figure 2.3), therefore reducing the force of muscle contraction.

Failure of excitation–contraction coupling

Calcium ions are an important intracellular link in the *excitation-contraction coupling* that produces muscle contraction. It is thought that the release of Ca^{2+} plays a major role in the failure of this mechanism at fatigue. The failure of this mechanism may be caused by the failure of either the surface membrane potential, the T-tubular action potential, the release of Ca^{2+} from the SR, or the SR Ca^{2+} pump to pump Ca^{2+} back into the SR. Eberstein and Sandow (1963) were the first to suggest the importance of the failure of *excitation-contraction coupling* in fatigue. They showed that fatigue could be overcome by the addition of caffeine (a drug which acts directly on the SR Ca^{2+} channels causing Ca^{2+} to be released). They suggested therefore that the contractile mechanism can still operate if sufficient Ca^{2+} were supplied and that fatigue occurs when the release of Ca^{2+} from the SR is inadequate. Their results are difficult to interpret, however, as caffeine also increases the sensitivity of the contractile protein to Ca^{2+} (Wendt & Stephenson 1983).

The decline in the concentration of ATP in muscle during exercise is thought to be linked closely to the failure in the release of Ca^{2+}, as ATP is required for the opening of the Ca^{2+} channel in the SR (Smith et al. 1985). In addition, a reduction in the concentration of ATP also causes a class of K^+ channels to open (Noma 1983) thereby stabilising the membrane potential which may reduce the action potential and the rate of release of Ca^{2+} from the SR.

It has also been hypothesised (Allen et al. 1992) that as the concentration of ATP decreases, the amount of Ca^{2+} pumped into the SR will gradually decrease resulting in a decreased release of Ca^{2+} from the SR. The results of studies by Lee et al. (1991) who observed a rise in the concentration of extracellular Ca^{2+} in muscle towards the end of fatigue, are consistent with this hypothesis. However, Gonzalez-Serratos et al. (1978) using the electron microprobe analysis technique, found no change in the Ca^{2+} content of the SR.

There is a reduced sensitivity of the contractile proteins (actin and myosin) to Ca^{2+} during fatigue. Godt and Nosek (1989) found that at the onset of fatigue there was a 1.7–fold reduction in the sensitivity of contractile proteins to Ca^{2+}. The maximum Ca^{2+}-activated contraction force of muscle was reduced by 30%. It was suggested (Blanchard & Solaro 1984) that this might be due to the competition by H^+ for the Ca^{2+} binding sites in troponin. Such competition is likely to intensify under acidotic conditions in muscle when the increased H^+ concentration may inhibit the interaction of actin and myosin. However, it has also been found that, at Ca^{2+} saturation, the tension between the contractile proteins can decline. Work by Cooke and Pate (1985) and Godt and Nosek (1989) showed that the intramuscular accumulation of Pi, from the breakdown of Pcr and ATP, has a negative effect on the production of force in cross-bridge formation. The cross-bridge formation is also inhibited by

decreasing pH values. For example, a drop in pH value in muscle from 7 to 6.6 was shown to cause a reduction in the number of cross-bridges and the force they produced (Lannergren & Westerblad 1991).

The release of Ca^{2+} from SR is dependent on pH. Nakamaru and Schwartz (1972) demonstrated that at pH values of 6.45 to 7.7 but not 6.1 to 6.3, Ca^{2+} would be released from the SR. The binding of Ca^{2+} to the SR at the lower pH values is probably due to the fact that the optimum pH for *ATPase* activity is 7.0 to 7.5 (Battle et al. 1993).

Protein

The breakdown of muscle protein can occur during exercise, but the resulting AA are not considered as major energy-yielding substrates (Graham et al. 1991). However, Dohm et al. (1980) suggested that such a loss of protein may be a factor which contributes to fatigue in exercise. Protein catabolism may be influenced by the availability of energy-yielding substrates and Henderson et al. (1985) observed large increases in the oxidation of AA such as leucine, in the early period of exercise.

Glycogen depletion

The rate of glycolysis in skeletal muscle can increase by 100–fold during exercise (Newsholme & Start 1973). Such a rate could lead to a rapid depletion of muscle glycogen and subsequently, fatigue. For example, in humans at rest, muscles contain 6.9 g glycogen/100 g muscle, and when fatigued this value is reduced to 1.7 g/100 g (Ahlborg et al. 1967).

Although glycogen is present at the same concentration in all fibre types at rest, different fibre types utilise glycogen at different rates during exercise. At fatigue, fast-twitch fibres contain less glycogen than slow-twitch fibres (Saltin 1981). Saltin (1981) demonstrated that the concentration of glucose in plasma remained fairly constant during exercise. However, 10 minutes before the onset of fatigue, the concentration decreased significantly, and the uptake of glucose was greatest by muscles low in glycogen.

Adrenaline stimulates the breakdown of glycogen by stimulating the activation of *phosphorylase b* (Podolin et al. 1991). Temperature increases also have been found to accelerate the rate of glycolysis (Kozlowski et al. 1985).

Acidosis and hyperthermia

Acidosis is characterised by a reduction in pH, and in muscle, this is largely due to the accumulation of lactic acid. It is considered to be a major cause of fatigue during exercise (see section 2.3). Also important to the onset of fatigue during exercise is hyperthermia which is thought to cause fatigue by a variety of mechanisms (see section 2.4).

2.3 Lactic Acid

At physiological pH, lactic acid ($C_3H_6O_3$) dissociates into a proton (H^+) and an anion ($C_3H_5O_3^-$). Between the production of lactic acid in muscle and its removal, the dissociated forms exist and can exert different influences. Increased concentration of H^+ is more critical than lactate or undissociated lactic acid. The lactate ion has no known inhibitory effects on muscle contraction (Saltin 1990) but H^+ as well as lactic acid can produce acidosis (Metzger & Fitts 1987; Allen et al. 1989) when present in large enough concentrations. The concentration of lactic acid and its effect on acid-base balance of the body, depends on its rate of production, removal and diffusion from muscle cells to blood.

2.3.1 Production of lactic acid

The major source of lactic acid production is anaerobic glycolysis. In the final stage of the reaction, pyruvate is converted to lactic acid by the enzyme *lactate dehydrogenase (LDH)*.

$$\text{Glucose} + 3\text{ADP} + 3\text{Pi} \longrightarrow 2\ \text{Lactic acid} + 3\text{ATP}$$

$$\text{Final Reaction:}\quad \text{Pyruvate} + \text{NADH} + \text{H}^+ \underset{\text{LDH}}{\rightleftharpoons} \text{Lactic acid} + \text{NAD}^+$$

The production of ATP by this pathway (compared with the aerobic pathway) is very fast but it is less efficient (see section 2.1.2).

Hill and Lupton (1923) first proposed the theory of "oxygen debt" from the observation that at low-intensity exercise, the concentration of lactic acid in plasma increased slowly. At greater intensity, it increased rapidly. They proposed that at high work rates, the supply of oxygen (O_2) to muscle was inadequate, thus increasing the rate of anaerobic glycolysis. Jobis and Stainsby (1968) suggested, however, that the output of lactic acid by muscle was not due to an inadequate supply of O_2. They used the fluorescence of mitochondrial reduced nicotinamide adenine dinucleotide (NADH) as an indication of mitochondrial oxidation/ reduction state and found that, the transient production and release of lactic acid by muscle, during several different contractions, were not related to a lack of O_2 in the mitochondria. Connett et al. (1984) using dog muscle also reported that the values for the partical pressure of O_2 (pO_2) in muscle cells remained above those considered to be adequate for repetitive muscle contractions. Jobis and Stainsby (1968) and Connett et al. (1984) suggested that a

general lack of supply of O_2 to muscle was not the likely cause of the increased output of lactic acid by this tissue during exercise.

Contracting muscle appears to be the major site of lactic acid production and accumulation during exercise, whereafter it is released into the blood stream (Fletcher & Hopkins 1907). The small intestine has been identified as a site for lactic acid production from the catabolism of dietary glucose. This has been shown in rats, rabbits and dogs (Hildman et al. 1980; Davis et al. 1984; Smadja et al. 1988). The liver is also capable of producing lactic acid and releasing lactate into the circulation (Davis et al. 1985; Wasserman et al. 1987). The fact that gluconeogenesis also occurs in the liver, may explain the segregation of its functions into different metabolic zones (*viz* periportal and perivenous). Epidermal cells of the skin are also capable of taking up glucose and releasing lactate (Johnson & Fusaro 1972).

Increased concentrations of arterial adrenaline and lactate are positively correlated (r=0.97) over a wide range of concentrations (Gregg et al. 1989). This increase in adrenaline has been found to raise blood lactate by increasing production as well as decreasing clearance.

Increased concentrations of adrenaline have been found to increase the concentration of lactate in blood of dogs by causing a reduced uptake by, or an increased lactate output from tissues other than contracting skeletal muscle (Stainsby et al. 1985). This finding was supported by that of Ahlborg (1985) who reported that adrenaline also stimulated release of lactate from inactive (arm) muscle during cycling in humans. Spriet et al. (1988) however, reported that adrenaline stimulated glycogenolysis in contracting skeletal muscle.

Insulin is another hormone found to increase the production of lactic acid (Taylor et al. 1991).

The energy-yielding substrates used during exercise depend on the speed and intensity at which the exercise is performed. These determine the proportional contributions of aerobic and anaerobic metabolism to ATP generation (see figures 2.10 and 2.11).

Gollnick and Hermansen (1973) attempted in their study, to identify the intensity and duration of work at which the production of lactic acid would be increased. During light to moderately heavy work (up to 50% of maximum O_2 uptake) lactic acid concentrations in plasma were unchanged or even decreased. With moderate to heavy work (50–85% of maximum O_2 uptake) there was a rapid increase in lactic acid concentration which stabilised or even decreased, if exercise was continued past 10 minutes. During very heavy exercise (≈90% of maximum O_2 uptake) there was a continuous increase in lactic acid concentration, and fatigue resulted. From these observations, they concluded that, work rate associated with 50–60% maximum O_2 uptake, is probably the critical level above which lactic acid production

would be accelerated.

The activity of *LDH* has been found to increase during prolonged exercise (Karlsson 1971) but not change during short work periods less than 30 minutes (Gollnick et al. 1967).

2.3.2 Lactic acid transport

While a few lactic acid molecules are able to diffuse across cell membranes, the majority are transported in conjunction with Na^+ or H^+ by a lactate transport system. There is evidence that these transporters exist in erythrocytes, brush border cells of the small intestine, kidney and muscle (sarcolemma) (Hildman et al. 1980; Storelli et al. 1980; Smadja et al. 1988; Juel 1991).

The exchange of lactic acid between muscle cells and other tissues is affected by lactic acid concentration and proton gradients, and it is not completely due to the rate of glycolysis (Stainsby & Brooks 1990). The lactate carrier transports lactate and H^+ in a ratio of 1:1 and is responsible for 50 to 90% of total flux (Juel 1991). The movement of lactate is also dependent on blood flow and capillarisation (Saltin 1990), with equilibrium between muscle and blood appearing to take 5–10 minutes (Gollnick & Hermansen 1973). Hermansen (1971) showed that a blood-brain barrier exists to protect the brain from acidosis. He observed that, although lactate concentrations in plasma increased from 1.2 to 14.6 mM during exercise in humans, there was no change in the concentration of lactic acid in the cerebrospinal fluid. This is in agreement with the findings of Harris et al. (1962).

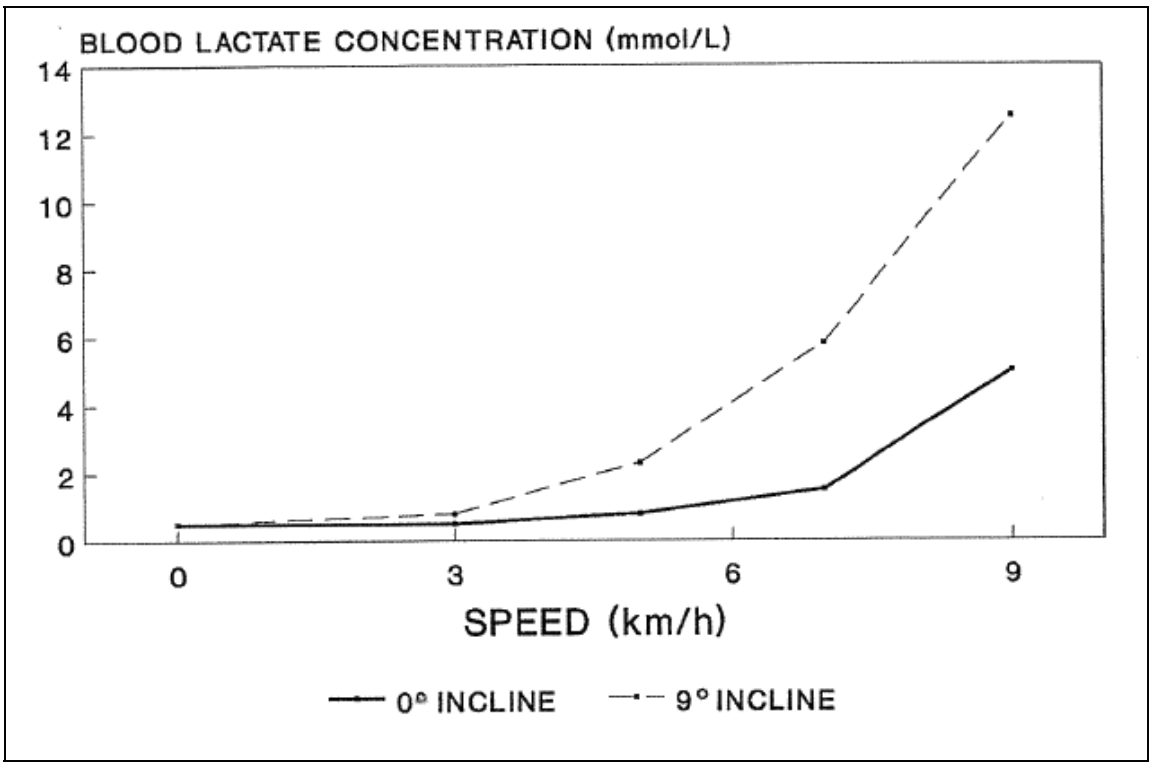


Figure 2.10 The effect of exercise intensity on the mean concentration of lactate in arterial blood of five sheep exercised for 25 minutes.

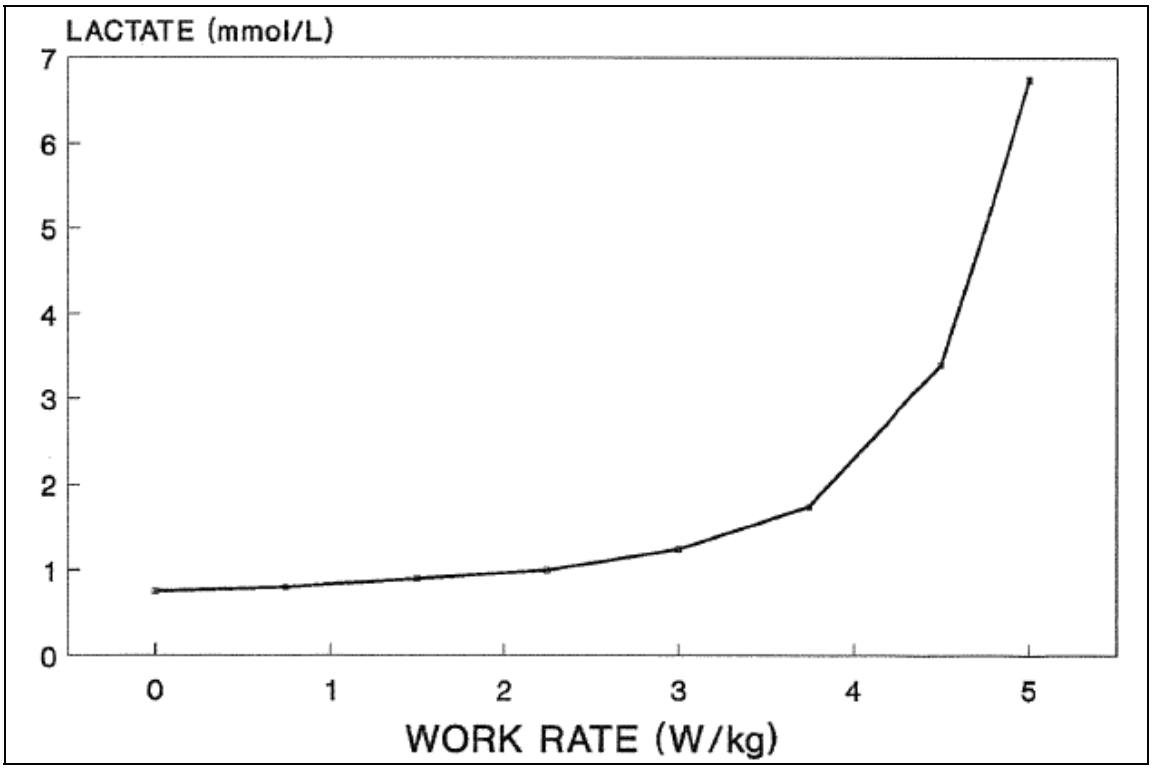


Figure 2.11 The effect of work rate (W) on the concentration of lactate in venous blood. **(from Oyono-Enguelle et al. 1990)**

2.3.3 Removal of lactic acid

Skeletal muscle, liver, gut and the heart are able to remove lactate from the blood during exercise (Himwich et al. 1930; Gertz et al. 1988). Sweat and urine have been found to eliminate lactate to some extent (Krebs 1964).

Skeletal muscle, probably due to its large mass and high perfusion, is a major site for the uptake and oxidation of lactic acid (Stanley et al. 1986). Stanley et al. (1986) using isotope tracers, estimated that approximately half the amount of lactic acid formed during sustained submaximal exercise in humans was removed by oxidation in contracting skeletal muscle. Results of studies using dogs (Issekutz et al. 1976), rats (Donovan & Brooks 1983) and humans (Stanley et al. 1986) indicate, that the proportion of lactate removed and oxidised by muscle depends on O_2 consumption at sustained submaximal exercise. There is also evidence which indicate that, at increased concentrations of plasma lactate, the uptake of the metabolite by muscle is also increased (Gladden 1989).

Liver is another important site for the uptake of lactate (Ahlborg et al. 1974; Pethick et al. 1991) which is used in this organ as a precursor in gluconeogenesis and glycogen synthesis. Other tissues, which can take up lactate from the circulation, include those of the gut and heart. In sheep, for example, the gut can be an increasingly important sink for lactate when the plasma concentration of the metabolite is high and the pathways for its uptake and metabolism by the liver are saturated (see Pethick et al. 1991). The heart also is capable of taking up lactic acid. However, the uptake and production of the metabolite by this organ may occur simultaneously (Gertz et al. 1988).

2.3.4 "Lactate shuttle"

An hypothesis based on an extension of the "Cori Cycle" (Cori 1931) and "Glucose Paradox" concepts (Newguard et al. 1983; Foster 1984) (that lactic acid transported to the liver for gluconeogenesis is not from blood-borne glucose from the diet) was suggested by Brooks (1985). According to this hypothesis, the formation and distribution of fuel in the form of lactate provides a central intermediate in metabolism in different tissues and different cells of the same tissue. Lactic acid, formed in muscle cells with high rates of glycogenolysis and glycolysis, provides energy for adjacent cells and those of other tissues in the body as well as being a major gluconeogenic precursor. Results of studies which are consistent with this hypothesis have been reported by a number of workers (e.g. Ahlborg et al. 1974; Donovan & Brooks 1983).

2.3.5 Acidosis

Acidosis has a wide range of effects and can indirectly or directly (due to H^+) cause fatigue (see section 2.2.3). The pH values of blood at which fatigue occurs in different species are shown in table 2.5. The reduction in pH values during fatigue in the different species ranges from 0.22 to 0.73. In order to avoid the destruction of acid-labile cell components, the production of lactic acid must be regulated. Hill (1955) observed that the formation of lactic acid ceased when pH was 6.3.

It is suggested from evidence presented in a number of studies (e.g. Trivedi & Danforth 1966; Hermansen 1981; Hultman et al. 1981) that the reduction in intracellular pH inhibits the activity of the enzymes: *PFK* and *phosphorylase* which are the two key rate-limiting enzymes in glycolysis. *In vitro* studies (Danforth 1965; Ui 1966) showed that the activity of these two enzymes were almost completely inhibited at pH 6.4.

Table 2.6 The pH values of blood associated with the onset of fatigue in different species

pH				
Rest	Fatigue	Change	Species	Reference
6.98	6.46	–0.52	human	Bergstrom (1962)
7.40	7.18	–0.22	human	Bergstrom (1962)
7.06	6.33	–0.73	rats	Metzger & Fitts (1987)
7.22	6.94	–0.28	rats	Metzger & Fitts (1987)
–	6.41		human	Hermansen & Osnes (1972)
–	6.60	–	human	Sahlin et al. (1976)
7	6.45		human	Hermansen & Osnes (1972)
7	6.60	–	human	Lannergren & Westerblad (1989)
–	–	–0.50	frogs	Renaud et al. (1986)
–	–	–0.40	frogs	Renaud et al. (1986)
		–0.44		
		–0.38		

Regulation

The regulation of H^+ is a key ventilatory stimulus in humans, with the respiratory elimination of carbonic acid being essential for the maintenance of the acid-base balance. The ventilatory mechanism is important in the control and neutralisation of the acidifying effects of metabolic byproducts of muscle metabolism (CO_2 and H^+).

During exercise, the non-bicarbonate buffering systems operate initially, and is related to the hydrolysis of Pcr and formation of inorganic PO_4 (formation of $HPO_4/H_2PO_4^-$). Once this small non-bicarbonate source is consumed, the HCO_3^- takes over as the buffering system which could account for 92% of proton buffering (Beaver et al. 1986). The HCO_3^- and lactate appear to change reciprocally and stoichiometrically (Pethick et al. 1991; Stringer et al. 1992).

2.3.6 Anaerobic threshold (AT)

The term AT describes that point above which lactate concentration in blood begins to increase rapidly. This is due to a greater contribution of anaerobic metabolism to energy production (Wasserman et al. 1973). Many terms have been presented over the years to describe this rapid accumulation of lactate in blood during exercise; *viz*, metabolic acidosis threshold, aerobic-anaerobic threshold, onset of blood lactate accumulation (OBLA), onset of plasma lactate accumulation, lactate turning point, lactate threshold, excess lactate and individual anaerobic threshold.

The term, OBLA, seems to be a more appropriate expression than AT to describe lactate accumulation in blood.

The intensity of exercise has been described as treadmill running velocity, cycle ergometer power outputs or O_2 uptake.

Kinderman et al. (1979) reported that in humans, a concentration of blood lactate of 4 mM could be used as a predictor of AT. However, this concept was not supported by results of other studies, e.g. on lactate threshold (Oyono-Enguelle et al. 1990) on individual anaerobic threshold (Stegman et al. 1981) and on OBLA (Sjodin and Jacobs 1981).

It has been suggested that ventilatory threshold may be an indicator of AT. Some measures which have been used for assessing this are maximum O_2 consumption, ventilation (V_E), respiratory exchange ratio and the V_E:V_{O2}max ratio. This ventilatory response is related to the need to buffer lactic acid production. The V-slope analysis was presented by Beaver et al. (1986a). It is a plot of CO_2 output and of oxygen uptake. The AT is determined by the cross-over point of the regression lines of the two slopes (see figure 2.12). Beaver et al. (1986b) found a high correlation between excess CO_2 (non-metabolic) and lactate. Although a relationship between these appears to exist (Koike et al. 1990) the underlying mechanism(s) for the relationship is not known.

2.4 Hyperthermia

Hyperthermia or heat stress is a rise in body temperature. It is considered to be a cause of fatigue if the thermoregulatory mechanisms of the body are not able to maintain the body temperature below intolerable limits. Hyperthermia causes an increase in rectal temperature (RT) as well as pulse rate (PR) and respiration rate (RR) (MacDougall et al. 1974; Oyono-Enguelle et al. 1993).

The RT does not necessarily reflect accurately the actual core temperature of the body, since

the rate of equilibration of temperature throughout the body is slow. Hodgson et al. (1993) found that the RT of horses continued to rise for a period after work ceased. The change in RT appears to lag behind that of muscle and blood. Lindholm and Saltin (1974) observed a difference of approximately 2☜C in rectal and muscle temperatures of horses after exercise.

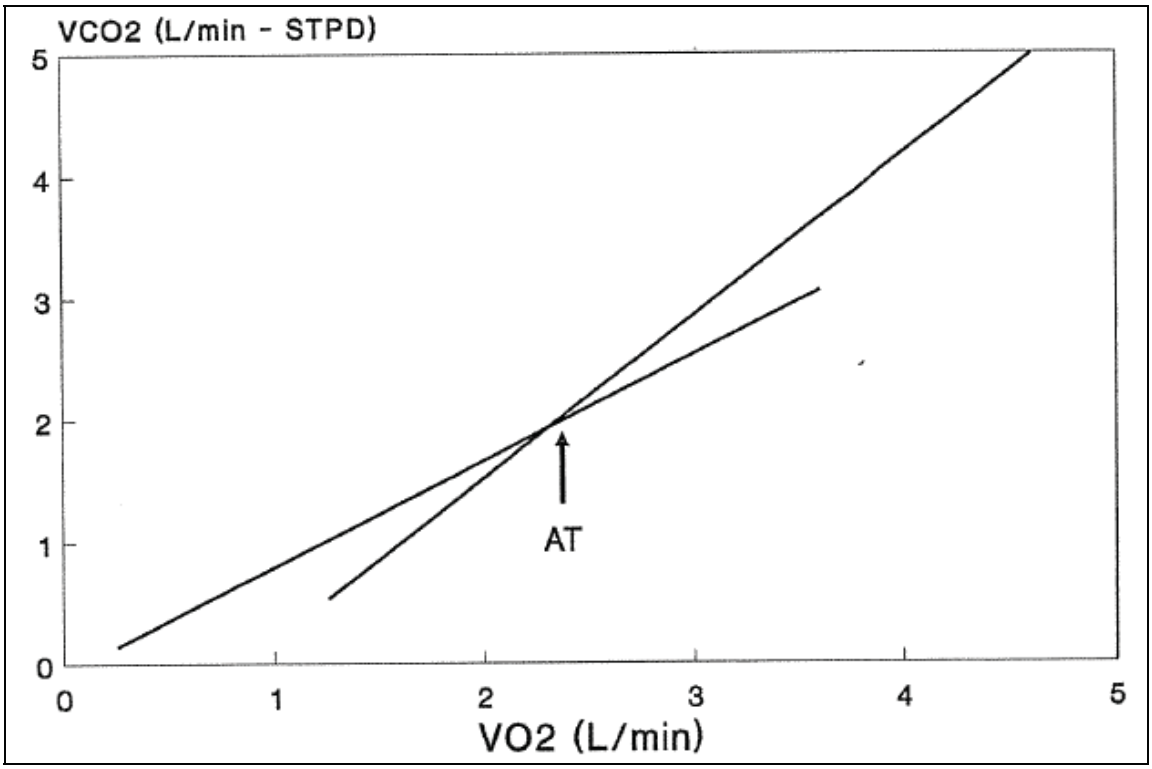


Figure 2.12 Regression lines showing anaerobic threshold from the inflection point of the V-slope, CO_2 production (VCO_2) versus O_2 uptake (VO_2 **(from Beaver et al. 1986a)**

Hyperthermia can be of metabolic and environmental origin. It is therefore easy to see why a draught animal performing work for long periods in a hot environment (e.g. tropics), could become heat-stressed.

2.4.1 Causes of hyperthermia

Environmental

Solar radiation and ambient temperature (AmT) are greatest in the tropics and the absorption of heat by working animals depends on the time of day and season in which they work. The horizontal nature of the bodies of cattle and sheep compared to humans, gives these animals a greater exposure to solar radiation on a given sunny day. Coat or skin colour can also affect absorption of heat. For example, buffalo with black skin and sparse hair coverage will tend to absorb more solar radiation than will cattle or sheep. The terrain in which an animal works also has some effect on the absorption of heat from the environment. A bare ground reflects about 25% of total solar radiation falling upon it (Lee 1950) hence the increased potential to absorb heat during the ploughing of bare fields.

Humidity and wind velocity affect the rate at which evaporative cooling occurs. The greatest rise in body temperature has been recorded in animals exposed to hot and humid conditions, than to any other conditions, e.g. hot and dry, humid and cold. Upadhyay and Madan (1985a) found that cattle performed less work in hot and dry and hot and humid periods compared to winter. Upadhyay and Madan (1985a) subjected cattle to three hours of work, pulling loaded carts in ambient temperatures and relative humidities respectively of 39.85☜C and 17.64% (hot/dry), 33.95☜C and 70.75% (hot/humid) and 19.03☜C and 55.29% (cold/dry). During the hot/humid season, the cattle were fatigued after two hours of work but for both the hot/dry and cold/dry seasons, the animals completed three hours of work. The walking speed of the animals ranged from 3.0 to 4.5 km/h.

Metabolic

Contracting muscle during exercise produces heat as a byproduct of metabolism. Some of this energy is used by the contractile proteins and the rest is liberated as heat energy. The intensity of exercise is closely related to increases in body temperature. An increase in RT of 2.5☜C above the resting normal value has been proposed as intolerable to cattle (Upadhyay & Madan 1985b). These authors found that cattle were unable to work when their RT increased by more than 2.5☜C above resting value.
Metabolic heat production is also influenced by the energy-yielding substrates used. The oxidation of glucose, for example, releases less heat than does the oxidation of FFA (Kruk et al. 1987). These authors artificially increased the concentrations of glucose and FFA in the blood of exercising dogs. When glucose was infused intravenously into these animals, the exercise-induced increases in muscle and rectal temperatures, and O_2 consumption were lower than corresponding values recorded in dogs infused intravenously with FFA only. It is clear from this that the relative contributions of glucose and FFA to muscle metabolism will

influence the amount of O_2 which is required for a given amount of work. It has been estimated that 11% more energy per unit of O_2 consumed is yielded when glucose is oxidised than when FFA are (DiPrampero 1981).

2.4.2 Control of temperature regulation

The heat regulatory centre in the body is the hypothalamus. It regulates RR and depth, cutaneous blood flow and sweating and is closely associated with the endocrine system. The pre-optic and anterior hypothalamus control the responses to heat by heat sensitive neurons. Schmid and Pierau (1993) observed that in slices of pre-optic and anterior hypothalamus, 48% of the cells were warm-sensitive, 47% temperature-insensitive and 5% cold-sensitive. These neurons are sensitive to blood temperature as well as to nerve impulses from extra-hypothalamic sources such as the skin, spinal cord and lower brain stem. Warming of the anterior hypothalamus has been found to stimulate thermoregulatory responses (Hammel 1968).

2.4.3 Cooling mechanisms (heat dissipation)

Cooling mechanisms vary between species. Humans perspire and dogs pant to dissipate body heat. Cattle, sheep and buffalo use a combination of sweating and panting. Cattle rely heavily on sweating (MacFarlane 1968) but they also pant. Sheep rely mainly on panting (Baker & Hayward 1968) but do also sweat. Buffalo, however, rely mainly on panting and wallowing. Buffalo have one-sixth the density of sweat glands found in cattle and are sparsely covered with hair (Bunyavejchewin et al. 1985). The species are therefore less able than cattle to reduce their body heat by evaporative cooling. Farmers keep these animals from being heat-stressed by allowing them to wallow before and after work, by smearing them with mud or by splashing water on them.

Panting

Panting is an evaporative cooling mechanism involving the respiratory tract and nasal cavity. Bovids possess a group of blood vessels at the base of the skull called the carotid rete (a cranial retia mirabilia) which acts as a heat exchanger. These arterial networks are also found in cats and pigs (Simoens et al. 1987). A less complex version is found in dogs (Baker & Chapman 1977). These structures are absent in horses (Simoens et al. 1987) and humans (DuBoulay et al. 1975; McFarland et al. 1979). The carotid rete [see figure 2.13(A)] is a compact vascular network that branches along the carotid artery before reaching the Circle of Willis [cerebral arterial circle; see figure 2.13(B)].

Warm arterial blood from the heart passes through the carotid rete, and while in the carotid

rete, is cooled by counter-current heat exchange by cool venous blood returning from cutaneous and mucosal areas such as the horns, the skin of the head and the nose and mouth. The veins in the horns of goats have been reported to be cooled by ambient air (Taylor 1966), while the oral and nasal mucosal veins are cooled by panting (Baker & Hayward 1968). There is some evidence in sheep (Ohale & Ghosal 1982) that a similar thermoregulatory system is associated with ocular arteries (rete mirabile opthalmicum).

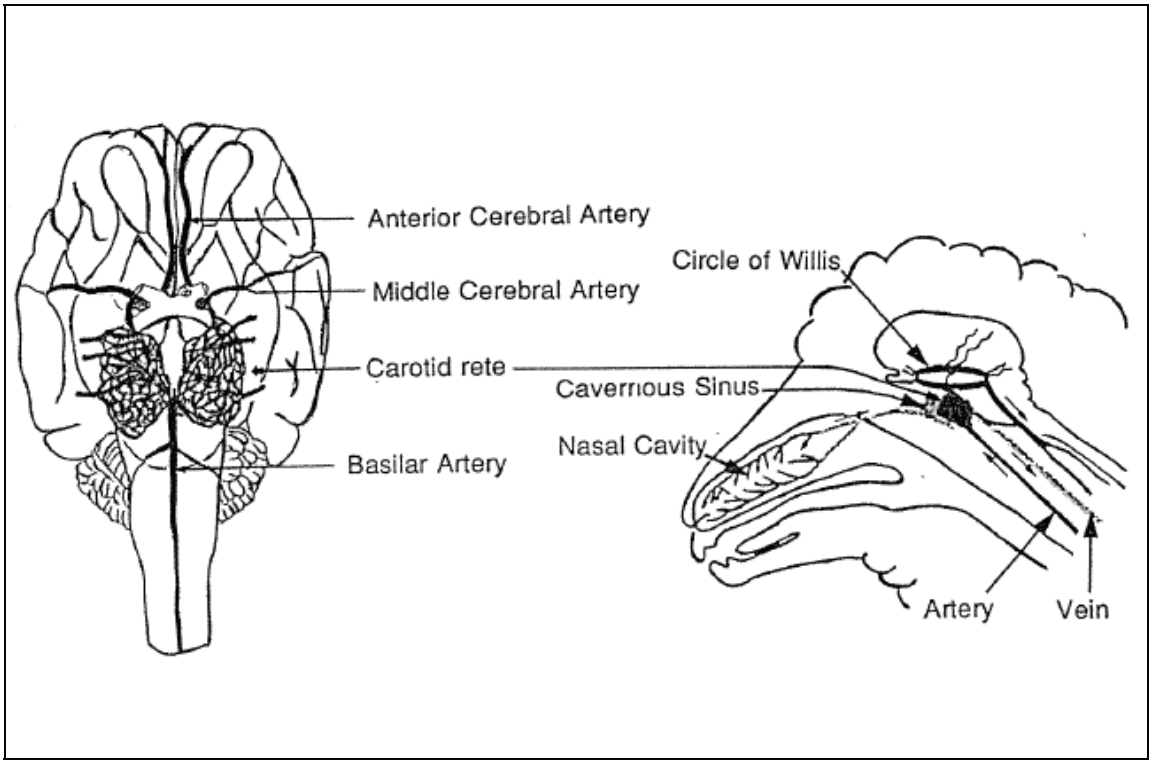


Figure 2.13 Diagrams showing (A) the carotid rete and (B) Circle of Willis, cavenous sinus and nasal cavity illustrating the direction of venous and arterial bloodflow. (A: from Baker & Hayward 1968; B: Bligh 1973)

It is thought that there is a critical temperature which affects brain function and causes work to cease (fatigue). Evidence suggests that the control of brain temperature to below 40.5☜C is crucial for continuing exercise. Chesy et al. (1985) reported that in cattle exercised for 30 minutes, the brain temperatures stabilised at 40.3–40.5☜C despite increasing body temperatures. When exercise was prolonged, however, and the core body temperature increased slightly above 42☜C, the temperature of the hypothalamus started to rise above 40.5☜C. At this point, panting changed from closed- to open-mouthed and the animals could not continue to work beyond 5 to 10 minutes. This suggests that 40.5☜C is the highest temperature that the brain can tolerate although body tissues can tolerate much higher temperatures. The critical brain temperature in humans was found to be 39.5–41☜C (Brinnel et al. 1987). In cattle and sheep, 40.5☜C was also the hypothalamic temperature at which panting changed from closed- to open-mouthed in experiments by Findlay and Ingram (1961)

and Ingram and Whitlow (1962).

Baker and Hayward (1968) demonstrated that sheep are capable of maintaining brain temperature when the environmental temperature was raised from 20–25☜C to 45–50☜C. Although the temperature of the central arterial blood increased from 39 to 40.1☜C, that of the cerebral arterial blood increased from 38.5 to 39.1☜C. It is clear from this that the carotid rete does not allow the blood temperature of the brain to increase by as much as the body temperature does.

Sheep tend to be much more heat-tolerant and are able to maintain their temperature homeostasis. They do not exhibit neurological signs of hyperthermia until their RT are quite high compared to other species (see table 2.7 below).

Table 2.7 Critical rectal temperature (☜C) and behavioural changes in different animals

Animal	Rapid respiration	Panting	Agitation	Staggers	Gasping
Mouse	–	41.1	40.6	41.7	–
Rat	39.4	40.6	40.6	41.7	–
Rabbit	39.4	40.0	41.7	–	41.7
Pig	38.9	39.2	41.1	–	41.1
Cat	38.9	39.4	41.7	–	41.7
Dog	37.5	37.8	40.6	42.8	41.7
Sheep	39.4	41.1	42.2	43.3	42.8

Heat can be dissipated from the buccal cavity and tongue by evaporative cooling and increased salivation is associated with this. Hodgson et al. (1993) demonstrated that, in horses working at 40, 65 and 90% V_{O2}max, the respiratory heat loss was 30, 19 and 23% of the heat produced by exercise. Heat loss through the buccal cavity and tongue can be twice the amount of heat lost through the nose in dogs (Taylor 1977) which are open-mouthed panters. Sheep on the other hand, pant through their noses and only open their mouths when core temperatures are raised by more than 1☜C.

In mild heat-stress, panting is usually closed-mouthed, rapid and shallow. During severe hyperthermia, panting changes to open-mouthed and becomes deeper and slower. Hales and Findlay (1968) found that in cattle, when deep body temperature increased from 38.5 to 42☜C over 2–3 hours of work, two phases of respiration occurred. In the first, RR increased 10–fold and respiratory volume increased 5–fold, showing rapid and shallow panting. In the second phase, breathing was deeper and slower. During this time, RR was 7–fold the resting value and the respiratory volume increased 8–fold. This second phase is associated with respiratory alkalosis when CO_2 elimination is excessive (Lee 1950).

Sweating

Sweating is a form of evaporative heat loss from the surface of the skin. The disadvantage of heat loss through sweating compared to panting is the loss of water and electrolytes.

Sweating capacity varies with species. Horses have twice the capacity of humans (Hodgson et al. 1993) and buffalo have a very low capacity. Sweating rate increases with work intensity (Hodgson et al. 1993) and is stimulated by increases in body temperature. For example, Hodgson et al. (1993) found in horses that the onset of sweating occurred when the temperature of the blood (carotid artery) reached 38.5☜C at three different work intensities. Heavy sweating in humans was suggested to contribute to a reduction in total K^+, thus increasing the risk of heat-stroke (Knochel et al. 1972). However, a more recent study by Costill (1986) found no evidence to suggest that K^+ was deficient in muscle or blood of humans during heavy sweating in prolonged exercise.

2.4.4 Hyperthermia as a cause of fatigue

Hyperthermia may contribute to fatigue. It is positively correlated with rises in blood lactate, increases in the rate of glycolysis and blood flow to the skin. Neilson et al. (1990) reported that there were no limitations to the blood flow to muscles, or to the availability and uptake of substrates by muscles in heat-stressed humans. The authors suggested that fatigue may have been caused by a negative effect on the CNS, thereby altering brain function and motivation. Bell and Hales (1985) on the other hand, reported reductions in blood flow to muscles of exercising sheep which were heat-stressed.

2.4.5 Lactic acid and heat stress

It has been reported by several authors that the concentration of lactate in blood increased with increasing body temperatures in humans (MacDougall et al. 1974; Oyono-Enguelle et al. 1993), rats (Zurovski et al. 1991), dogs (Kozlowski et al. 1985) and goats (Fiestcorn et al. 1982)— see figure 2.14.

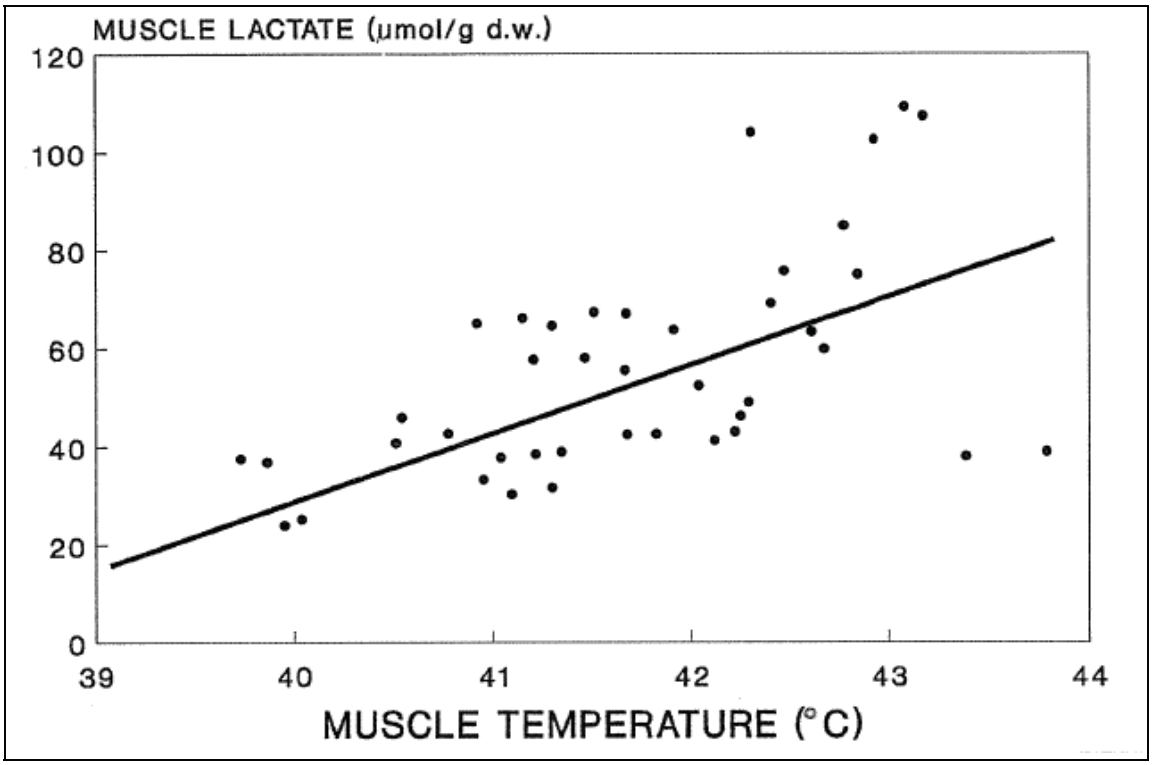


Figure 2.14 Regression of muscle lactate (LA) content on muscle temperature in dogs. **(from Kozlowski et al. 1985)**

Zurovski et al. (1991) found in exercised rats, that there was a temperature threshold of 41–41.5☜C at which there was an accelerated increase in the concentration of lactate in blood. A threshold of 41☜C in cattle was also reported by Hales et al. (1967). Such increases in lactate concentration during hyperthermia may be due to a combination of several factors. For example, the rate of anaerobic glycolysis may accelerate during hyperthermia (Edwards et al. 1972; Kozlowski et al. 1985). It is also possible that the rate of removal of lactic acid from the circulation by tissues might be reduced during hyperthermia, although Oyono-Enguelle et al. (1993) suggested that this might not be the case in moderate exercise.

2.4.6 Blood flow and heat stress

Hyperthermia during exercise presents a "conflict of interests" to the cardiovascular system which is required to provide an increased rate of blood flow, to the skin for increased rate of heat dissipation, and to muscles for increased rate of transportation of O_2 and substrates to and byproducts of metabolism from the tissue.

During exercise, blood flow to muscles increases and that to other regions in the body, e.g. the splanchnic bed, decreases (Bell et al. 1983). In sheep, such increases could account for half the cardiac output (CO) (Bell et al. 1983). The blood flow to the myocardium and respiratory muscles also increases in proportion to the intensity of exercise. The increase in

CO is largely due to the increase in PR. The latter is stimulated by an increase in body temperature in cattle (Whittow 1965), dogs (Hales & Dampney 1975) and in humans (Rowell 1974). Other factors which may stimulate PR include increases in extracellular K^+ (Lindinger & Sjogaard 1991) and sympathoadrenal activity (Galbo 1985). Immediately prior to exhaustion, the CO of an exercising animal has been known to decrease. The reduction tends to coincide with the point at which the maximum PR is reached or about to be reached (MacDougall et al. 1974).

In humans, the blood flow to the skin is increased greatly during hyperthermia. This is due to the great reliance by humans on evaporative cooling to reduce body temperature. It is not uncommon therefore, that during hyperthermia, an increased proportion of the blood is redirected from skeletal muscle, kidneys and liver (Rowell 1983).

In sheep and dogs, blood flow is increased to the respiratory passage, nose, buccal cavity and tongue and to a lesser extent to the skin, unlike the situation in humans (Hales 1983). Bell et al. (1983) found that the total blood flow to the skin of sheep actually decreases in hyperthermia. There were increases in blood flow, however, to specific regions such as the ears, fore limbs, hind limbs and kidneys (Bell & Hales 1985; see figure 2.15).

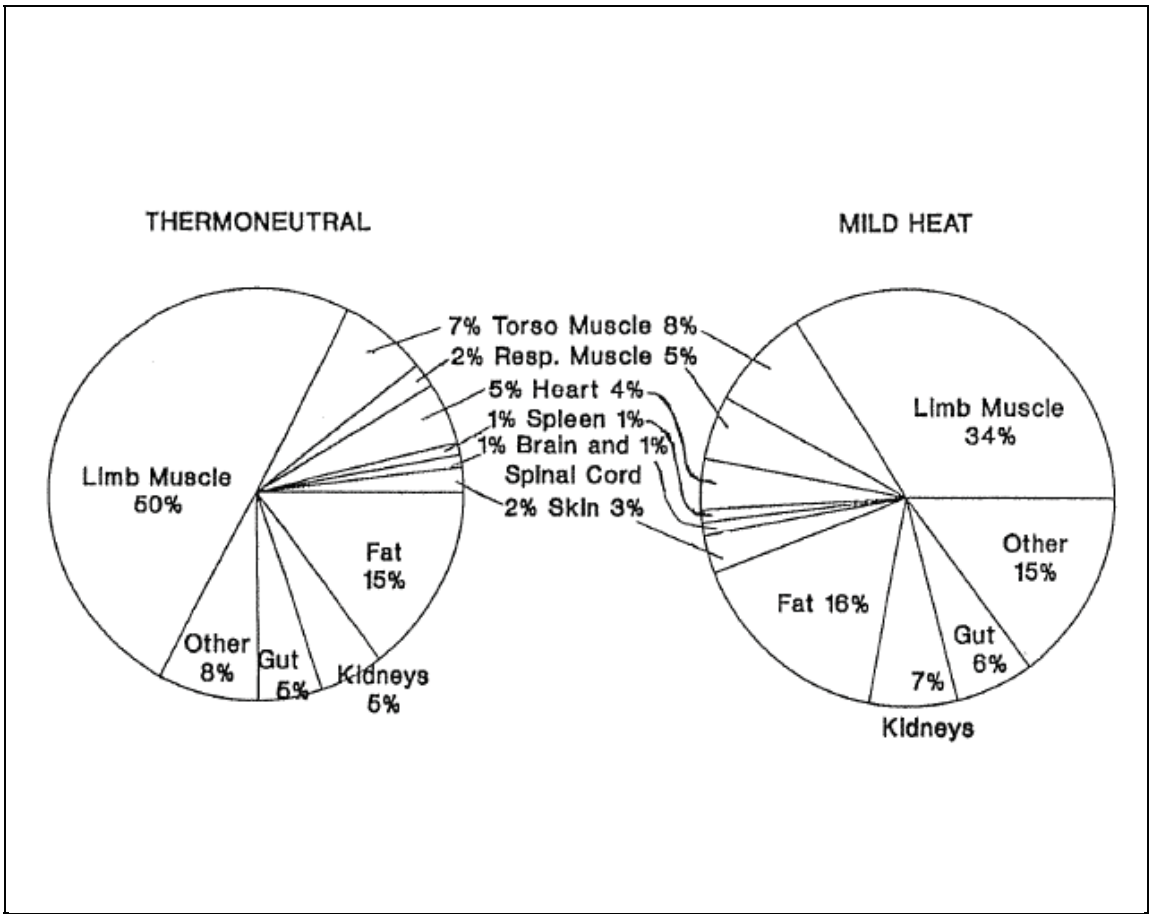


Figure 2.15 Data on the distribution of blood to various tissues in sheep during exercise and mild heat stress. (adapted from Bell & Hales 1985)

2.5 Training

Repeated endurance exercise or training improves the coordination of nervous, hormonal and cellular regulations. The effects of training include:

- an increased work capacity
- a greater rate of utilisation of fat as a fuel
- a lower rate of use of glycogen
- a decrease in the concentration of lactate in blood and muscle
- an increased metabolic clearance rate of lactate.

2.5.1 Cardiovascular system

Training induces a gradual buildup of the capacity to transport O_2 efficiently to muscles. The circulatory system directs blood flow to muscles more effectively due to increased capillarisation (Rosler et al. 1985; Bjorntorp 1991). The hypertrophy of the heart causes an increase in stroke volume (Sutton 1992). Henriksson (1977) reported an increase of 8% in blood flow to muscles in trained humans. Myoglobin content (Yakovlev 1976) and the red O_2-consuming muscle fibres are also increased after training. Overall, the result of training is the

improved transportation and extraction by muscle of available O_2 (Rennie et al. 1974; H enriksson 1977; Rosler et al. 1985; Sutton 1992; figure 2.16).

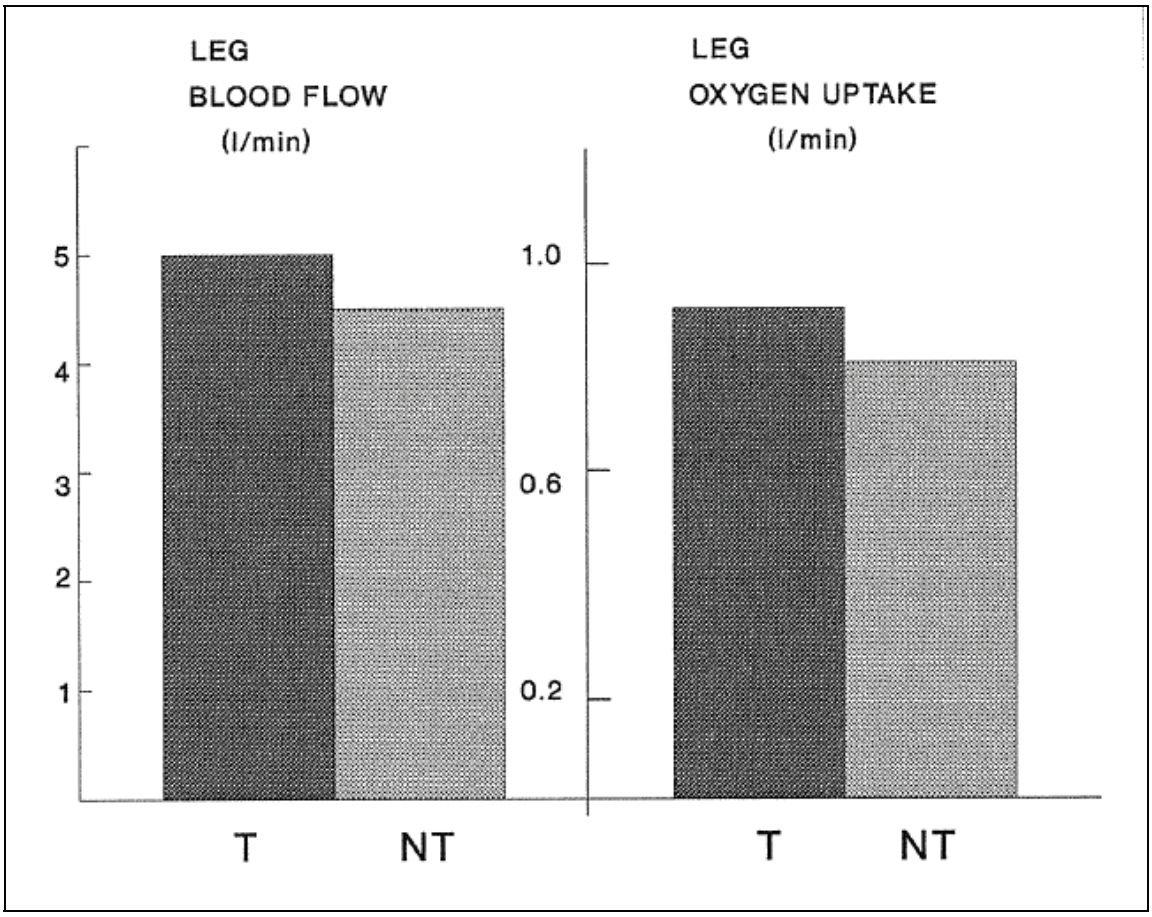


Figure 2.16 Blood flow and oxygen uptake in the trained (T) and non-trained (NT) leg muscle of humans **(adapted from Henriksson 1977)**

2.5.2 Fat utilisation

Training increases the capacity of skeletal muscle to use fat as an energy source (Henriksson 1977; Abernethy et al. 1990; Bjorntorp 1991). This increased capacity results in the sparing of glycogen and in improved work capacity.

At the start of exercise, the adrenalin which is secreted causes a rapid increase in the arterial concentration of FFA by stimulating the hormone-sensitive *lipase* (E.C.3.1.1.3). In addition, growth hormone (GH) is also secreted. It has a slower, but a more prolonged effect on lipolysis (Winder et al. 1979; Crampes et al. 1986).

In trained animals, the fat cells have an increased sensitivity to hormonal stimulation (Bjorntorp 1991). This results in a pattern of mobilisation of FFA that more closely match their

pattern of utilisation in trained animals. The patterns of mobilisation and utilisation of FFA in untrained animals are not as closely matched. Training also increases the deposits of triglycerides directly adjacent to the mitochondria (Hoppeler et al. 1973).

Enhanced fat metabolism, relative to carbohydrate metabolism in trained subjects (see figure 2.17) is due to the increased oxidative capacity of trained muscles (Henriksson 1977). The increased activity of the oxidative enzymes *pyruvate dehydrogenase*, *carnitine acyltransferase* (E.C.2.3.1.7), *succinate dehydrogenase* (E.C.1.3.99.1) and *malate dehydrogenase* (E.C.1.1.1.37) after training (Shantz et al. 1986) gives rise to a more favourable condition for acetyl-CoA units, derived from α-oxidation of fatty acids, to enter the TCA cycle (Gollnick & Saltin 1982; Gollnick et al. 1985). In addition, the activity of lipid-oxidising enzymes, including those involved in the α-oxidation of fatty acids, is increased after training (Bjorntorp 1991). The utlisation of fat is also enhanced after training due to the increased transportation of ADP into the mitochondria, thereby inhibiting *PFK* (Mansour 1965). *Lipoprotein lipase* (E.C.3.1.1.34) activity, responsible for the increased uptake of very low density lipids is also enhanced after training (Bjorntrop 1991).

2.5.3 Glycogen sparing

In trained animals, during exercise, there is a slower rate of depletion of glycogen stores (see figure 2.17) and a slower rate of oxidation of plasma glucose (Coggan et al. 1990). This glycogen-sparing effect is thought to be due to the sensitivity of the enzyme *PFK* to citrate. The enhanced rate of FFA metabolism results in an increase in the concentration of citrate (Rennie et al. 1976) which passes through the mitochondrial membrane and inhibits the activity of *PFK* (Newsholme & Start 1973).

Stores of glycogen in both the fast- and slow-twitch muscle fibres have been found to be larger in trained animals (Abernethy et al. 1990). The activity of *glycogen synthase* (E.C.2.4.1.11) in these animals is also increased (Taylor et al. 1972). Additionally, it appears in the exercising and trained animal, that the volume of blood flow to the splanchnic bed is increased concommitently with a major partitioning of CO to muscles and skin. This allows the liver of the trained animal to maintain the rates of gluconeogenesis, using particularly, the lactate released from muscle as a major substrate (Donovan & Brooks 1983). It appears also, that glucose homeostasis is under better regulatory control in the trained animal. This is reflected by the relatively stable concentration of blood glucose in this animal during exercise (Donovan & Brooks 1983). Reports by Ploug et al. (1990) also indicated that training increases insulin-stimulated and contraction-stimulated glucose transportation in all muscle fibre types, thus enhancing glucose homeostasis.

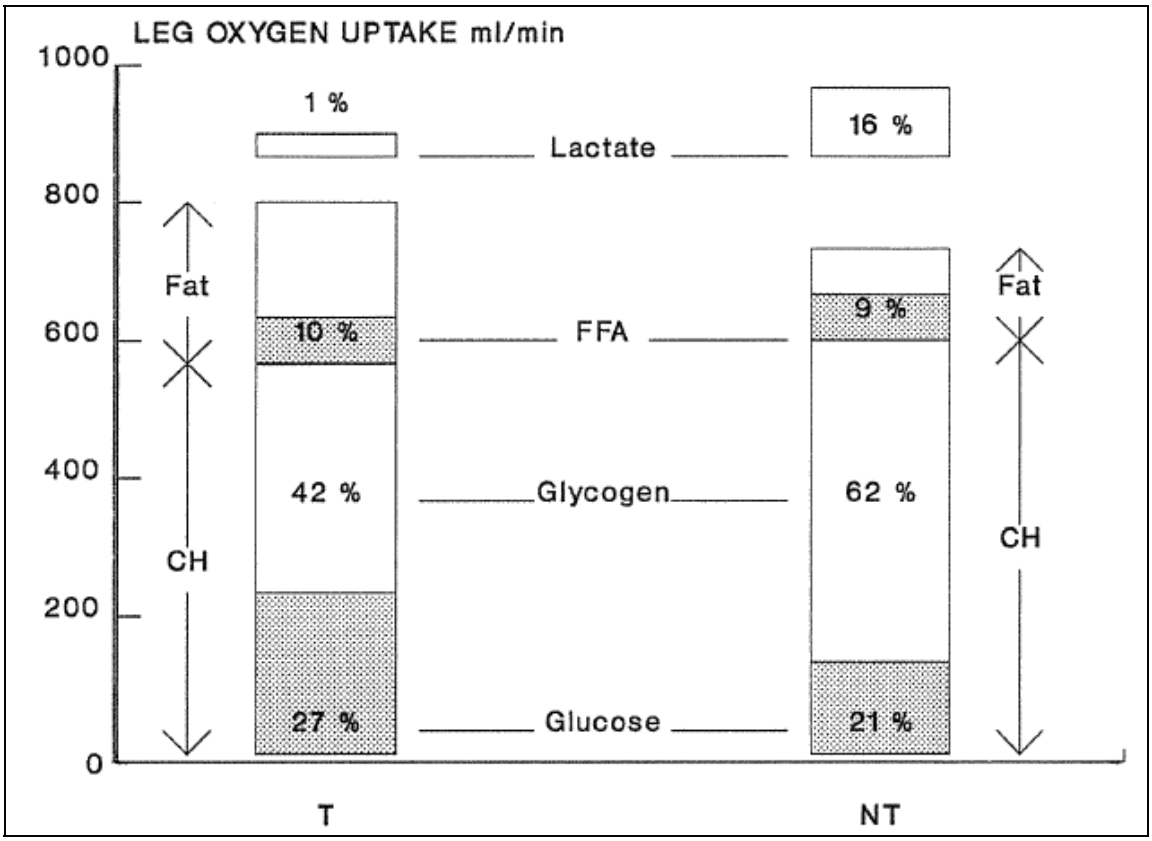


Figure 2.17 Contribution of carbohydrate (CH) and fat oxidation to the total leg oxygen uptake in the trained (T) and the non-trained (NT) leg during the post-training metabolic study. (adapted from Henriksson 1977)

A significant reduction in the activity of enzymes involved in glycogen degradation, anaerobic glycolysis and gluconeogenesis was reported for trained rats by Green et al. (1983). There was a reduction in the activity of *phosphorylase a*, *LDH* and *Fructose–2,6–Diphosphatase* (E.C.3.1.3.11) by 44, 39 and 75% respectively. In other studies (Taylor et al. 1972; Gollnick et al. 1973) however, the opposite seems to be the case.

2.5.4 Lactate and training

At a given work rate, the concentrations of lactate in muscle and in blood are lower in the trained than in the untrained animal (Henriksson 1977; Donovan & Brooks 1983; MacRae et al. 1992). The lower values in the trained animal are probably due to a reduced rate of lactic acid production (MacRae et al. 1992). MacRae et al. (1992) reported a 13% reduction in the rate of lactic acid production after training in humans. They proposed that this was due to a reduced output of adrenaline which occurs after training (Winder et al. 1979). Donovan and Brooks (1983) on the other hand, did not observe a reduction in the rate of production of lactic acid in trained rats subjected to different work loads.

The rate of lactate clearance during exercise appears to be increased by training (see

Donovan & Brooks 1983; MacRae et al. 1992). The major use of the lactate cleared from the circulation is probably as a gluconeogenic substrate (Donovan & Brooks 1983).

Training has been shown (Donovan & Brooks 1983) to reduce the concentration threshold at which lactate clearance occurs. Evidence supporting this was presented by Pilegaard et al. (1993). They observed that in rats trained intensively on a treadmill, there was an increased capacity (60%) in the muscle membrane transport system, which is partially responsible for the flux of lactate between muscle and blood. The change in capacity was found to be due to an increase in the number of lactate-transport proteins and also to a higher affinity of lactate for the transport proteins.

From the results of a number of studies (e.g. Donovan & Brooks 1983; Oyono-Enguelle et al. 1990; MacRae et al. 1992) it would appear that the increased rate of lactate clearance, rather than the reduced rate of glycolysis (MacRae et al. 1992) has the greater influence on the concentration of lactate in muscle and blood.

2.5.5 Fibre types and size

Although it is still uncertain as to whether fibre types can actually be changed by training, size, particularly of fast-twitch muscle fibres, increases after strength and endurance training (Tesch & Karlsson 1985). However, no differences have been found in the size of slow-twitch fibres.

Tesch and Karlsson (1985) observed differences in the percentage of slow- and fast-twitch fibres in different muscle groups in athletes who had been trained in different sports. Runners, for example, had a greater percentage of slow-twitch fibres in the *vastus lateralis* while the kayakers had a higher percentage of slow-twitch fibres in the deltoids.

Hypertrophy in muscle after training, is due to hypertrophy of the fast-twitch rather than the slow-twitch fibres (Tesch & Karlsson 1985). Myofibrillar and SR proteins, myoglobin (Yakovlev 1976) and mitochondrial proteins (Holloszy 1967) have been found to increase due to training, indicating that the increase in these are responsible for the hypertrophy.

There is some evidence (Andersen & Henriksson 1977; Jansson & Kaijser 1977) to support the theory that fast-twitch fibres can be converted to slow-twitch fibres. It is thought (Jansson et al. 1978) that the type IIC fibres found in endurance athletes might represent an intermediate stage in the transformation of fast-twitch to slow-twitch fibres.

2.5.6 Other effects

Training causes an increased capacity of skeletal muscle to form alanine and pyruvate. It also increases mitochondrial and cytoplasmic *alanine aminotransferase* activity by 80 and 50% respectively (Molé et al. 1973). Leucine oxidation also is enhanced by training (Henderson et al. 1985).

Training also enhances ligament and tendon strength (Tipton et al. 1971).

2.6 Conclusion

It can be concluded from this review that there are a range of factors that can, either directly or indirectly cause the onset of fatigue during exercise. The acid-base balance, hyperthermia and the depletion of substrates appear to be the main factors. There are areas of information in this review that need clarification, due either to a lack of published data, or to conflicting reports from different authors.

An important area requiring investigation, is the work loads and/or physiological states under which lactic acid accumulation would cause acidosis and fatigue in ruminants. Alkalosis, on the other hand, may occur and so far there are conflicting reports on whether the condition would delay the onset of fatigue and enhance work capacity.

Hyperthermia is probably a major cause of fatigue in exercising animals and would be of particular concern in the tropics. It appears that it may cause the onset of fatigue in animals by reducing blood O_2 saturation (O_2SAT) and by facilitating the depletion of glycogen stores.

The utilisation of substrates for ATP production in the ruminant muscle, at different work rates and physiological states (untrained and trained) also needs investigation, especially with regards to the potential of lactic acid (lactate shuttle) and protein as sources of energy.

SECTION 3A

A COMPARISON OF WORK CAPACITY OF UNTRAINED AND TRAINED BUFFALO (*BUBALUS BUBALIS*)

3A.1 Introduction

Fatigue is a complex phenomenon which limits work capacity of an animal. There are several factors which may contribute to fatigue.

The need for ATP by an animal increases with increasing work loads. Normally the aerobic dissimilation of glycogen is able to meet such a demand by the contracting muscle but, depending on the intensity and the duration of work, anaerobic dissimilation may become the dominant pathway of ATP production. Under such a situation the accumulation of lactic acid from anaerobic glycolysis can be large and may cause acidosis which has been suggested (Metzger & Fitts 1987; Allen et al. 1989) to be one of the factors contributing to the onset of fatigue. On the other hand, it has also been suggested (Ahlborg et al. 1967; Henriksson 1977; Bjorntorp 1991) that the rapid depletion of glycogen stores and the slow rate of FFA oxidation, both contribute to the slow rate of ATP production and the onset of fatigue. Another important factor suggested (Edwards et al. 1972; Kozlowski et al. 1985) to contribute to the onset of fatigue is the increased rate of metabolic heat production. Upadhyay and Madan (1987) suggested that a rise in RT of 2.5°C or greater, above normal resting values, cannot be tolerated by the working animal.

Swamp buffalo (*Bubalus bubalis*) are particularly prone to heat stress due to their inability to dissipate heat rapidly. This is mainly due to the low number of sweat glands that the animal possesses. This problem is exacerbated by their dark skin colour, and sparse covering of hair.

It is therefore reasonable to assume that in the context of working buffalo, heat stress may be a major factor contributing to the onset of fatigue. However, it has been shown that there is a positive relationship between increased heat production and accumulation of lactic acid (MacDougall et al. 1974; Zurovski et al. 1991; Oyono-Enguelle et al. 1993) suggesting a possible interaction between these two factors. It would be interesting therefore to see the role of each in the onset of fatigue in such an animal.

Physical training has been shown to increase the capacity of humans to undergo endurance exercise (Sutton 1992; Rennie et al. 1974). This increased capacity is partly due to a reduced rate of lactic acid production, but mainly due to the increased rate of lactic acid clearance by the muscle cells (Donovan & Brooks 1983; MacRae et al. 1992). Additionally there is an improved cardiovascular capacity. The increased oxidative capacity of muscles achieved by training allows the tissues to utilise FFA at an increased rate; thus sparing glycogen and delaying the onset of fatigue.

From the above and the review of the literature (see section 2) it may be hypothesised that the accumulation of lactic acid in working muscles will induce a condition of acidosis, causing fatigue and limiting work capacity.

The aim of this study was to test the hypothesis using untrained and trained buffalo.

3A.2 Materials and Methods

3A.2.1 Location

This study was conducted at the Department of Biomedical and Tropical Veterinary Sciences, in accordance with the *Guidelines for Housing and Care of Laboratory Animals* issued by the Experimentation Ethics Review Committee of James Cook University, Townsville.

3A.2.2 Experimental animals

Animal management and feeding

The animals used in this study were non-pregnant female swamp buffalo, four years old and with a mean live weight of 363 ± 31 kg. They were housed individually in covered cement floor pens (4.3 m x 3.4 m) which were cleaned daily.

The animals were fed 7 kg of hammermilled rice straw (*Oriza sativa*) plus 500 g cottonseed meal/hd/d. The composition of the rice straw and cottonseed meal are shown in table 3A.1. Feeding was carried out daily at 1600 h and clean drinking water was available to each animal at all times. Animals were inspected daily for signs of ill-health.

Table 3A.1 The composition of rice straw and total dietary nitrogen content

Rice Straw Composition (%)	
Nitrogen	0.8
Neutral detergent fibre	70
Organic matter	81
Total Dietary Nitrogen	1.15

(Source: Teleni et al. 1991)

Training

Training consisted of a 25 d (5 d/wk for 5 wks) period, during which the animals were walked on a treadmill for 2 h/d at 0.69 m/sec, each pulling a draught load of 20 kg (equivalent to approximately 6% of the live weight of the animal). The buffalo were introduced to the work routine on the treadmill gradually, prior to the 25 d training period.

Catheters

One day before the measurement period, chronic indwelling polyethylene catheters, (1 mm ID x 1.5 mm OD: Dural Plastics and Engineering, Dural, NSW, Australia) were inserted into the left and right external jugular veins of each animal to facilitate blood sampling and determination of PR. The catheters were filled with 0.9% sterile physiological saline (Baxter, Viaflex, Baxter Health Care Pty Ltd, Toongabbie, NSW, Australia) containing heparin at 125 IU/mL (CSL Limited: Parkville, Victoria, Australia) and an antibiotic at 2 g/L (Penbritin: Beecham Veterinary Products, Dandenong, Victoria, Australia). A plastic tube (5 mm diameter) was placed over the catheter as a protective sleeve. A foam collar (30 cm wide) was wrapped around the neck of each buffalo to protect the catheters.

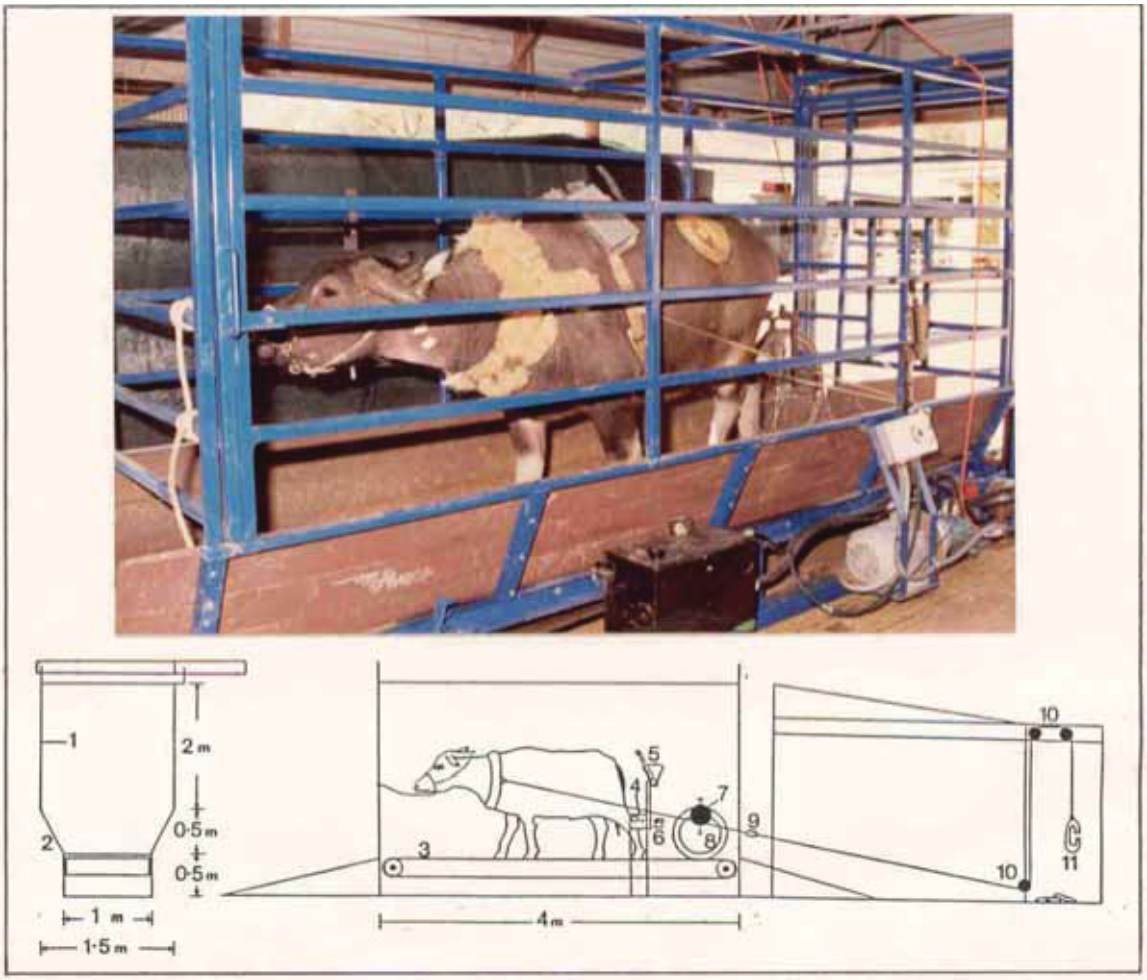
3A.2.3 Equipment

Treadmill

The treadmill used was a modified horse walker manufactured from steel to our specifications by Dinkum Studs, Peak Crossing, Australia (photograph 3A.1). It was housed in a partially open-sided shed which allowed free air movement through it. The load was suspended outside the treadmill from a rope via a series of pulleys to the harness (as shown in photograph 3A.1).

Harness (collar)

A collar was fitted around the neck of the buffalo to facilitate the attachment of the draught load via traces (see photograph 3A.1). The collar was made from a car tyre (non-treaded area) the inside of which was lined with sheep skin (see photograph 3A.1) to prevent chafing of the neck. The collar was locked into position around the neck by a car seat belt lock (see photograph 3A.1).



Photograph 3A.1 Buffalo walking on the treadmill. A diagrammatic representation showing the components of the treadmill 1. steel frame; 2. wooden angled sides; 3. rubber belt; 4. on/off switch and timer; 5. speed control lever; 6. speedometer; 7. odometer; 8. bicycle wheel; 9. load cell; 10. pulleys; 11. load.

Load

The load used was a 25 L plastic drum, the weight of which could be varied to requirement by varying the amount of water in it. Ship chain links were used as additional weights when required (see photograph 3A.1). The weights were suspended through an arrangement of pulleys as illustrated in photograph 3A.1.

Draught force measurement

A load cell (XTRAN SIW−4.55 KN: Applied Measurement Australia Pty Ltd, Victoria) was attached at a point posterior to the junction of the trace (see photograph 3A.1). An ergometer, odometer and elapsed working time unit were used in the measurement of draught force, distance travelled and working time (Tropag Consultants, Centre for Tropical Veterinary Medicine, University of Edinburgh, Scotland). The odometer was connected to a bicycle wheel (66 cm diameter) which was mounted to run on the belt of the treadmill (see photograph 3A.1).

3A.2.4 Experimental design

Two physiological states, untrained and trained, were compared using four buffalo in a completely randomised Latin square design (4 work loads x 4 periods) in which measurements were taken on two animals at a time over a four-day period. Measurements were repeated after the animals were trained. The buffalo were subjected to work by each pulling a draught load equivalent to 0, 5, 8 or 11% of its live weight for 3 h/d while walking at a speed of 0.69 m/sec on a treadmill.

During the *Pre-work* period, blood samples were taken while the animals were in their pens. They were then walked slowly to the treadmill to begin the working period. When work ceased they were walked back to their pens for the *Recovery* period. No feed or water was given during the measurement period. Time schedule for the *Pre-work, Work* and *Recovery* periods are shown in figure 3A.1. Start times were alternated between animals each day.

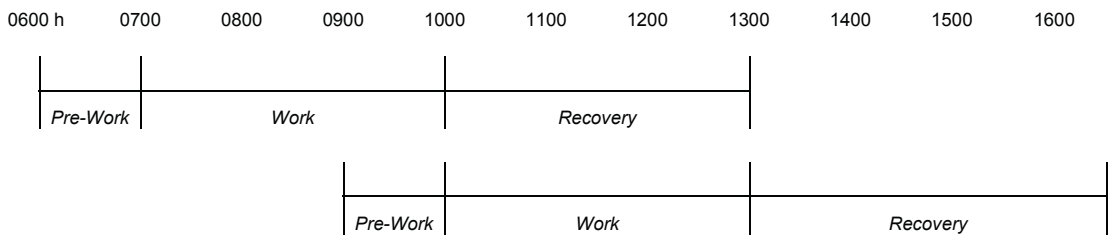


Figure 3A.1 The daily time schedule for the *Pre-work, Work* and *Recovery* periods for each pair of animals during the four-day measurement period

3A.2.5 Experimental procedure

All animals were made fully accustomed to the experimental procedure, well before the time for sampling and measurement arrived.

Samples and blood sampling

During the blood sampling period, a polyvinyl chloride (PVC) extension tube (1 mm ID x 2 mm OD: Dural Plastics and Engineering) with a 3-way-stop-cock on the end was connected to the catheter. This enabled samples of blood to be taken easily while the buffalo was on the treadmill. The PVC extension tube was removed at the end of each day.

Blood samples (10 mL) for analysis of packed cell volume (PCV) and relevant metabolites were taken half hourly, one hour before work started (*Pre-work* period), during work (3 h) and at hourly intervals for 3 h immediately after work ceased (*Recovery* period). Blood samples (2 mL) for bloodgas analysis were taken hourly.

The 10 mL blood samples were taken, using a 10 mL syringe, via the jugular venous catheter and transferred into 10 mL tubes containing lithium heparin. The tubes were inverted gently, two to three times, to ensure thorough mixing of the heparin and blood and then stored in an ice bath until centrifugation. Blood samples were centrifuged (Clements Centrifuge: RCF ~3500 rpm = 3000 *g*; 24 cm radius) for 10 minutes at 5000 *g*, after which plasma was collected into 5 mL plastic vials and stored at -20°C.

The 2 mL blood samples for bloodgas analysis were taken anaerobically using a 2.5 mL syringe containing approximately 5 μL of heparin (500 IU/mL) as an anticoagulant. Any air bubbles in the syringe were immediately expelled, the syringe capped and placed in an ice bath until analysed for blood gases.

After each blood sample was taken, the catheter was flushed with sterile physiological saline and filled with heparinised saline as described in section 3A.2.2.

Measurements

Environmental
Environmental parameters were measured at hourly intervals.

Ambient temperature (AmT)

Ambient temperature was recorded using a mercury thermometer (range: 0–50°C; increments: 1°C; Laboro, England). The thermometer was suspended close to the sampling area and at the same level as the treadmill.

Black bulb (BB)

The BB is a net radiometer used to estimate heat absorption by the animal from the environment. This was constructed using a black copper ball (24 cm diameter) containing a thermistor and located beside the AmT thermometer. The temperature of the BB was read using the Sensortek unit (previously described).

Relative humidity

Wet and dry bulb thermometer readings were obtained from the weather station at James Cook University. Percent relative humidity was calculated from these readings.

Physiological

Physiological parameters were recorded at hourly intervals. During the *Work* period the treadmill was stopped for approximately 3–5 mins to allow physiological measurements to be taken.

Rectal temperature

A thermocouple rectal probe was used in conjunction with a Sensortek digital readout unit (Model BAT–12, Sensortek Inc, USA). This measurement was taken by placing the probe against the wall of the rectum, approximately 10 cm anterior to the anal sphincter. The temperature value was recorded only when the digital readout had stabilised.

Skin temperature (ST)

A thermocouple skin probe and the Sensortek unit (as described above) were used to measure ST. The probe was placed on the rump in approximately the same position for each measurement.

Respiration rate

Respiration rate was determined by the number of flank movements counted in a 15-second interval. This value was multiplied by 4 to give breaths per minute.

Pulse rate

An air bubble was created within the stream of heparinised saline in the jugular catheter. This catheter had been inserted close enough to the heart so as the pulsing of the bubble could be clearly seen. The pulsing of the bubble was counted by eye in a 15-second interval and then multiplied by 4 to give heart beats per minute.

Work and draught force

The distance travelled registered by the odometer (see section 3A.2.3) was recorded hourly. Distance travelled was calculated as described in section 3A.2.8.

3A.2.6 Fatigue assessment

Each animal was assessed for signs of fatigue using a fatigue score card (see table 3A.2) adapted from that of Upadhyay and Madan (1985c; see table 3A.3). Assessment was undertaken each time by the same person. An animal was considered fatigued when a score of 20 was reached.

Table 3A.2 Score card showing fatigue assessment parameters

Physiological Changes		
Pulse Rate—	each 10 beats/min above resting value	= 1 point
Respiration Rate—	each 15 breaths/min above resting value	= 1 point
Rectal Temperature—	each 0.5°C above resting value	= 1 point
Distress Symptoms		
Leg incoordination and movement inhibition		= 0–5 points
Mouth frothing		= 0–5 points
Total accumulated points when work should cease		= 20 points

Table 3A.3 Fatigue score card for draught animals

Table 3A.3 Fatigue score card for draught animals (Upadhyay & Madan 1985).

	Score scale					Total
	1	2	3	4	5	
Respiration rate (breaths/min)	+Ro+15	Ro+30	Ro+45	Ro+60	Ro+75	5
Heart rate (beats/min)	+Ho+10	Ho+20	Ho+30	Ho+40	Ho+50	5
Rectal temperature (°C)	+To+0.5	To+1.0	To+1.5	To+2.0	To+2.5	5
Frothing	First appearance	Dribbling of saliva starting	Continuous dribbling	Appearance of froth on upper lip	Full mouth frothing	5
Leg uncoordination	Strides uneven	Occasional dragging of feet	Movements of legs uncoordinated and frequent dragging of feet	No coordination in fore and hind legs	Unable to move because of uncoordination	5
Excitement	Composed	Disturbed	Nostrils dilated and bad temperament	Movement of eye wall prominent with excitement	Furious and trying to stop	5
Inhibition of progressive movement	Brisk	Free movement	Slow walking	Very slow	Stop walking	5
Tongue protrusion	Mouth closed	Occasional opening of mouth	Frequent appearance of tongue	Continuous protrusion of tongue	Tongue fully out	5

+Ro,Ho,To – represent initial respiration rate, heart rate and rectal temperature, respectively

3A.2.7 Laboratory analyses

Bloodgas

The 2 mL blood samples were analysed for pH, total carbon dioxide (TCO_2), O_2SAT, bicarbonate (HCO_3) and pCO_2 using a 1312 Bloodgas Analyser (Allied Instrumentation Laboratory, Ma, USA).

Haemoglobin

Haemoglobin concentration was determined using a T660 Coulter Counter (Coulter, Australia) on the residual sample from the bloodgas analysis.

Packed cell volume

The PCV was determined in microhaematocrit tubes (in duplicate), containing well mixed blood. These were centrifuged at 10 000 rpm for 10 minutes (Heraeus-Christ microhaematocrit centrifuge, Biofuge A, West Germany) and then read using a Hawksley Micro-haematocrit reader (Hawksley & Sons Ltd, England).

Glucose

Plasma glucose was determined using the Technicon colorimetric method, number SE40036FJ4 as modified from the procedure of Gochman and Schmitz (1972) using a Technicon Auto-Analyser II [Technicon (Ireland) Ltd, Swords Co, Dublin]. This glucose method combines the specificity of glucose oxidase (EC1.1.3.4: Boehringer Mannheim GmBH, West Germany) with a peroxidase indicator reaction. In the presence of peroxidase, 3–methyl–2–benzothiazolinone hydrazone oxidatively couples with N,N–dimethylaniline to form a stable, intensely coloured, water soluble indamine dye.

Urea

Plasma urea was determined using the Technicon colorimetric method, number SE40001FD4 which is a modification of the procedure of Marsh et al. (1965). In a relatively weak acid solution, diacetyl monoxime is hydrolysed to diacetyl which, in turn, reacts directly with urea in the presence of acidic ferric ions. The presence of thiosemicarbazide intensifies the colour of the reaction.

Free fatty acids

Plasma FFA were determined in duplicate, using the microtitration technique of Dole (1956) as modified by Kelly (1965). The FFA were extracted using a mixture of isopropyl alcohol, heptane and sulphuric acid, then titrated against tetrabutyl ammonium hydroxide in methanol using phenol red as the indicator.

Lactate

The concentration of lactate was determined in deproteinised plasma by the method of Hohorst (1965). The enzyme, *LDH* (E.C.1.1.1.2.7: Boehringer Mannheim, West Germany) was used to convert lactate to pyruvate and the production of NADH measured colorimetrically using an Abbott Bichromatic Analyser 100 (Abbott Diagnostics, Dallas, USA).

3A.2.8 Calculations

Energy Expenditure (EE)
Energy expenditure was calculated using the equation from Lawrence (1985) where,

EE used for work = energy for walking + energy for pulling loads (see equation 1 below).

EE =AFM + W/C……**(1)**

where:
A = energy used to move 1 kg of body weight 1 m horizontally (J)
F = distance travelled (km)
M = live weight (kg)
W = work done whilst pulling loads (kJ)
C = efficiency of doing mechanical work
and A = 2.09 ± 0.062.....Lawrence (1985)
C = 0.389 ± 0.010.....Lawrence (1985)
F = distance calculated from odometer readings

where:

$$1 \text{ distance count} = \frac{3.142 \times 26 \times 25.4 \times 256}{360} \quad \text{……..(2)}$$

W = calculated from equations (3) and (4) below.

W = DF x Distance……..**(3)**

where:
DF = draught force

DF (kg) =AF (kg) x cosα……..**(4)**

see Figure 3A.2 below where:

AF = angle force (kg)

α = angle of pull

Maintenance energy requirements were calculated using equation

Mm (MJ/d) = 8.3 + 0.09 LW (kg)…….MAFF (1984) **(5)**

where:

Mm = maintenance energy requirement

LW = live weight

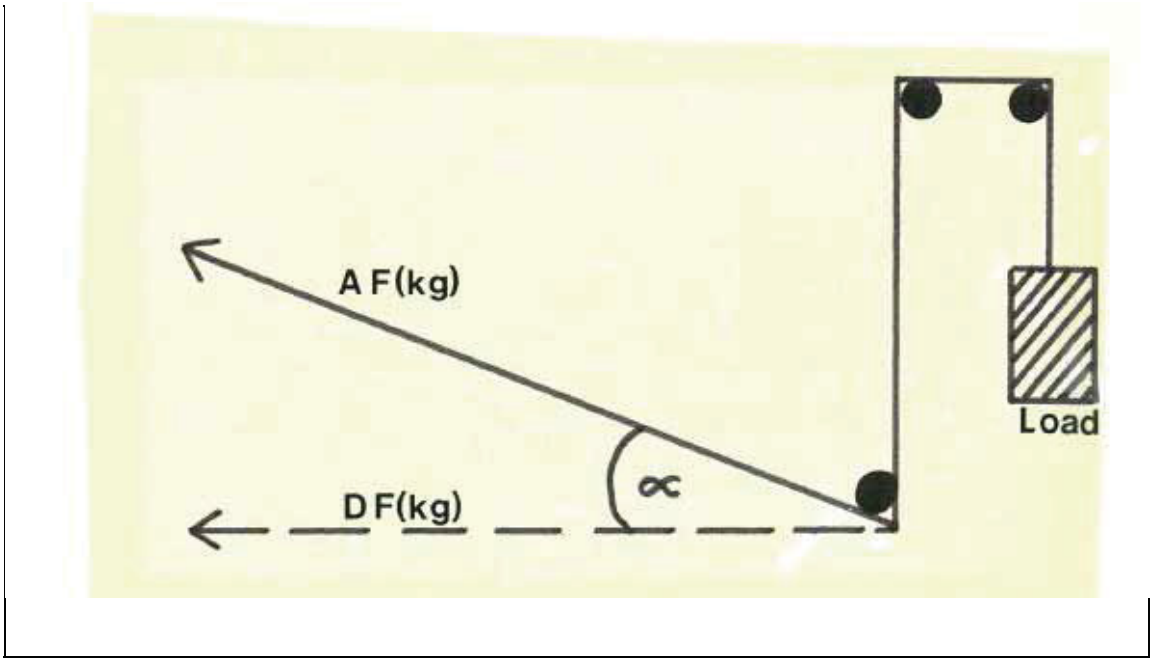


Figure 3A.2 Relationship between angle force (AF), draught force (DF), angle of pull (α) and load.

3A.2.9 Statistical analysis

Data were computed and subjected to analysis of variance using the Statistix 3.0 program (NH Analytical Software, USA).

3A.3 Results

Not all animals across the different work loads and physiological states were able to complete the 3 h work schedule. Therefore these results are a comparison for 1.5 h during the *Work* period.

3A.3.1 Environmental conditions

The mean AmT during the experimental period was 26.1°C (minimum: 10.5°C and maximum: 33.8°C). Relative humidity averaged 62% and the BB averaged 26.5°C (minimum: 10.5°C and maximum: 39.3°C).

3A.3.2 Work capacity and energy expenditure

The mean duration of work, distance travelled and EE are presented in table 3A.4.

Duration of work

The maximum working time allowed was 3 h. None of the untrained buffalo were able to complete 3 h while pulling load 4, but 1 of the trained animals were able to complete 3 h on the same load. For load 1, all animals were able to complete the 3 h work schedule. Duration of work was not significantly affected by physiological state (table 3A.5). The respective increase in duration of work by the trained animals compared with the untrained animals were 3, 17 and 20% for loads 2, 3, and 4 respectively (see table 3A.4). It should be noted that these percentage increases do not take into account the fact that some of the animals which completed the 3 h work schedule would have been able to continue working for a longer time.

Table 3A.4 Mean duration of work, distanced travelled, energy expenditure (EE) and EE:Maintenance energy requirement (Mm) ratio of untrained (U) and trained (T) buffalo pulling four different loads
Values are means ± standard errors (n=4)

	Duration of work (mins)		Distanced travelled (km)		Energy expenditure[2]	
Load[1]	U	T	U	T	MJ/d	EE/Mm
1	180 ± 0	180 ± 0	8.78 ± 0.00	8.78 ± 0.00	94.37 ± 0.47	2.30 ± 0.003
2	168 ± 4	174 ± 3	8.17 ± 0.18	8.42 ± 0.18	97.50 ± 0.96	2.38 ± 0.003
3		150 ± 6	6.22 ± 0.06	7.32 ± 0.30	99.42 ± 0.98	2.43 ± 0.003
	128 ± 11					
4	107 ± 8		5.29 ± 0.39	6.22 ± 0.46	101.34 ± 1.01	2.47 ± 0.003
		128 ± 10				

[1] Loads 1, 2, 3 and 4 = 0, 5, 8 and 11% of live weight, respectively
[2] n=8

Distance travelled

Physiological state had no significant effect on distance travelled (table 3A.5). Corresponding to the duration of work the percentage increases in distance walked by the trained animals were 0, 3, 18 and 19% compared to the untrained animals for loads 1, 2, 3 and 4 respectively.

Energy expenditure

The data for EE in table 3A.4 were calculated from pooled data from eight measurements (including untrained and trained animals) and indicates the rate of EE for the different loads. The EE increased from load 1 to 4 by approximately 7%. In relation to Mm the increase in EE ranged from 2.3 for load 1 to 2.47 for load 4 (see table 3A.4).

Fatigue assessment

Fatigue scores recorded during work are summarised in table 3A.6.

Training had no significant effect (P=0.5259) on fatigue scores although there was a trend for fatigue scores to be lower for trained buffalo (see figure 3A.3). There was a significant difference (P=0.0281) between loads for fatigue scores. The scores for untrained buffalo on

loads 3 and 4 were similar to that for trained buffalo on load 4 (see figure 3A.3). Data have been pooled for physiological states and are presented in table 3A.6

Table 3A.5 Means of pooled data on duration of work, distance travelled, energy expenditure (EE) and EE:Maintenance energy requirement (Mm) ratio of buffalo for physiological states and work loads
Values are means ± standard errors (SE) (n=16)

	Treatments					
	Physiological states		Work loads[1]			
	Untrained	Trained	1	2	3	4
Duration of work (min)	146	158	180	170	139	117
± SE	2	2	0	2	5	4
Significance	P=0.3426		P=0.0001			
Distanced travelled (km)	7.1	7.7	8.8	8.3	6.8	5.7
± SE	0.1	0.1	0.0	0.1	0.2	0.2
Significance	P=0.3425		P=0.0001			

[1] n=8
1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively

Table 3A.6 Mean fatigue scores for untrained and trained buffalo pulling four different loads
Values are means ± standard errors (n=4)

Load	Untrained	Trained	Pooled
1	5.25 ± 1.80	4.75 ± 1.03	5.00 ± 1.42
2	9.50 ± 1.50	6.75 ± 2.25	8.13 ± 1.88
3	16.38 ± 1.38	9.25 ± 1.55	12.82 ± 1.47
4	16.50 ± 2.27[1]	17.25 ± 1.33	16.88 ± 1.80
			P(0.0281)

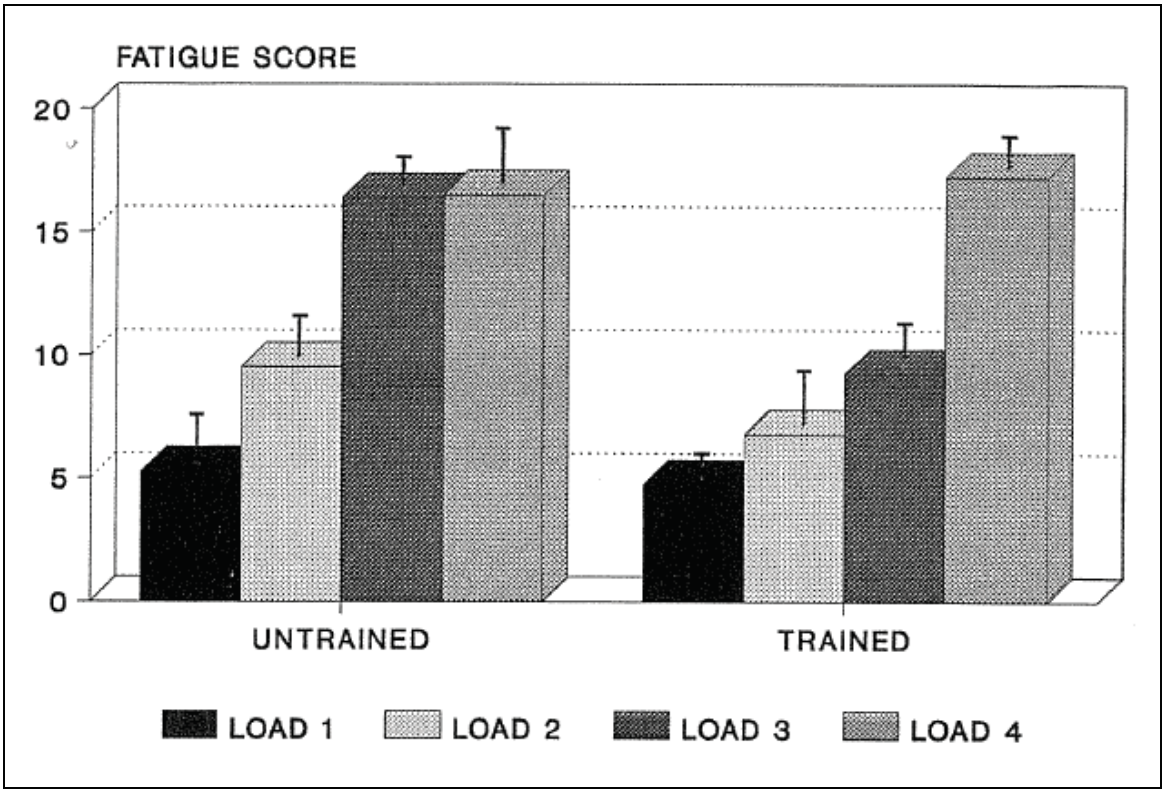


Figure 3A.3 Mean fatigue score (0-20) for loads 1 to 4 for untrained and trained buffalo Loads 1, 2, 3 and 4 are equivalent to 0, 5, 8 and 11% of live weight respectively. Vertical lines above bars represent standard errors.

The fatigue scores increased with increasing work load (see table 3A.6). The percentage increase between loads 1 and 2 and 2 and 3 were 60 and 75% respectively but the increase in fatigue score between loads 3 and 4 was only 21%.

3A.3.3 Physiological Variables

Skin temperature

Data on ST of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.4.

Pooled data, comparing the differences in mean ST between physiological states, work loads and between work status are presented in table 3A.7.

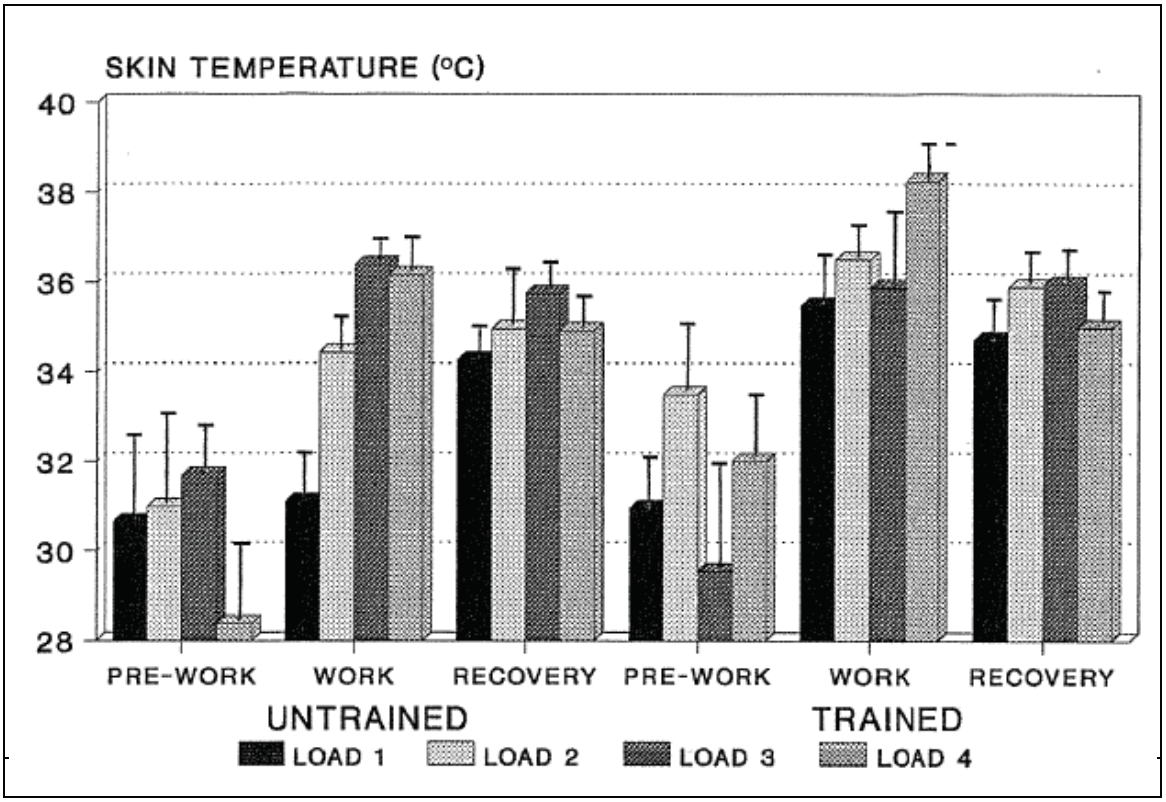


Figure 3A.4 Mean skin temperature of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively Vertical lines above bars represent standard errors.

Table 3A.7 Means of pooled data on skin temperature (ST) of buffalo or physiological states, work loads and work status
Values are means ± standard errors (SE).

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
ST (°C)	34.3	35.0	33.6	34.8	35.1	34.9	31.0	36.0	35.2
± SE	0.31	0.31	1.1	1.2	1.3	1.0	0.9	0.6	0.3
Significance	P=0.2834		P=0.3919				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

Skin temperature was not significantly affected by either physiological state or work load (table 3A.7) although there was a trend of increasing ST with increasing work load (figure 3A.4). Work status significantly affected ST (table 3A.7). Skin temperature increased by 5°C during *Work* and decreased only slightly during *Recovery* (figure 3A.5).

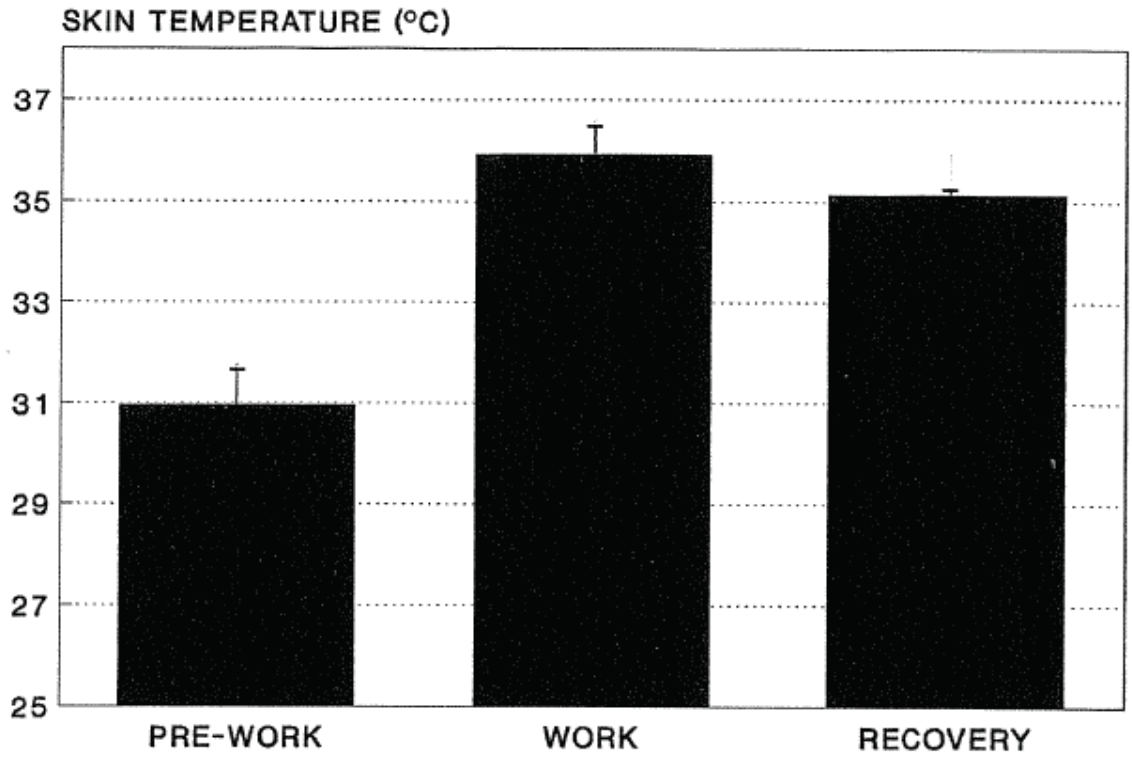


Figure 3A.5 Means of pooled data on skin temperature of buffalo during the *Pre-work, Work* and *Recovery* periods Vertical lines above bars represent standard errors.

The effect of work on ST is clearly illustrated in figure 3A.5 where data were pooled across physiological states and work loads for the three work states.

The changes in the pattern of ST over time across work status are presented in figures 3A.9 to 3A.16.

Rectal temperature

Data on RT of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.6.
Pooled data, comparing the differences in mean RT between physiological states, work loads and between work status are presented in table 3A.8.

Rectal temperature was not significantly affected by either physiological state or work load (table 3A.8) although there was a trend of increasing RT with increasing load (see figure 3A.6). Work status significantly affected RT (table 3A.8).

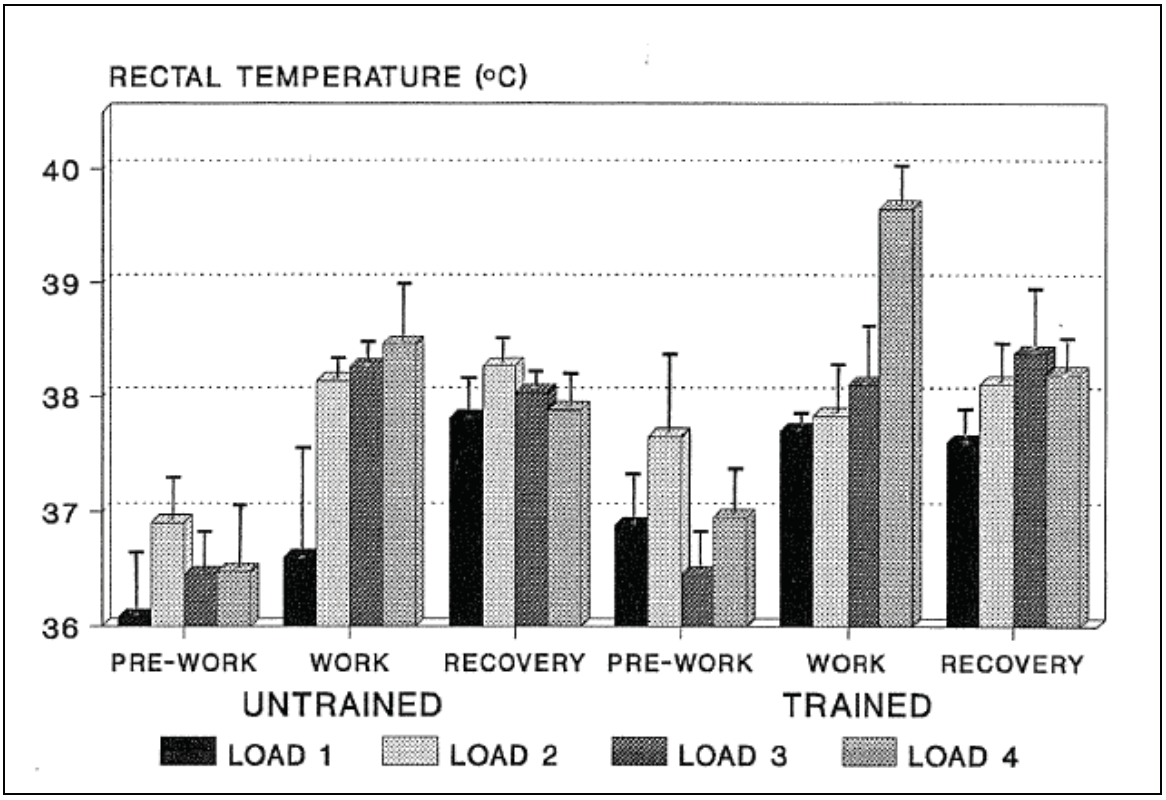


Figure 3A.6 Mean rectal temperature of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively Vertical lines above bars represent standard errors.

Rectal temperature increased by an average of 1.6°C during 1.5 h work but only decreased slightly (0.3°C) during recovery (see table 3A.8).

Table 3A.8 Means of pooled data on rectal temperature (RT) of buffalo or physiological states, work loads and work status
Values are means ± standard errors (SE).

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
ST (°C)	37.8	38.0	37.4	38.0	37.9	38.2	36.7	38.3	38.0
± SE	0.1	0.1	0.5	0.4	0.3	0.4	0.3	0.3	0.1
Significance	P=0.3467		P=0.0957				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

The changes in the pattern of RT over time across work status are presented in figures 3A.9 to 3A.16.

Respiration rate

Data on RR of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.7.

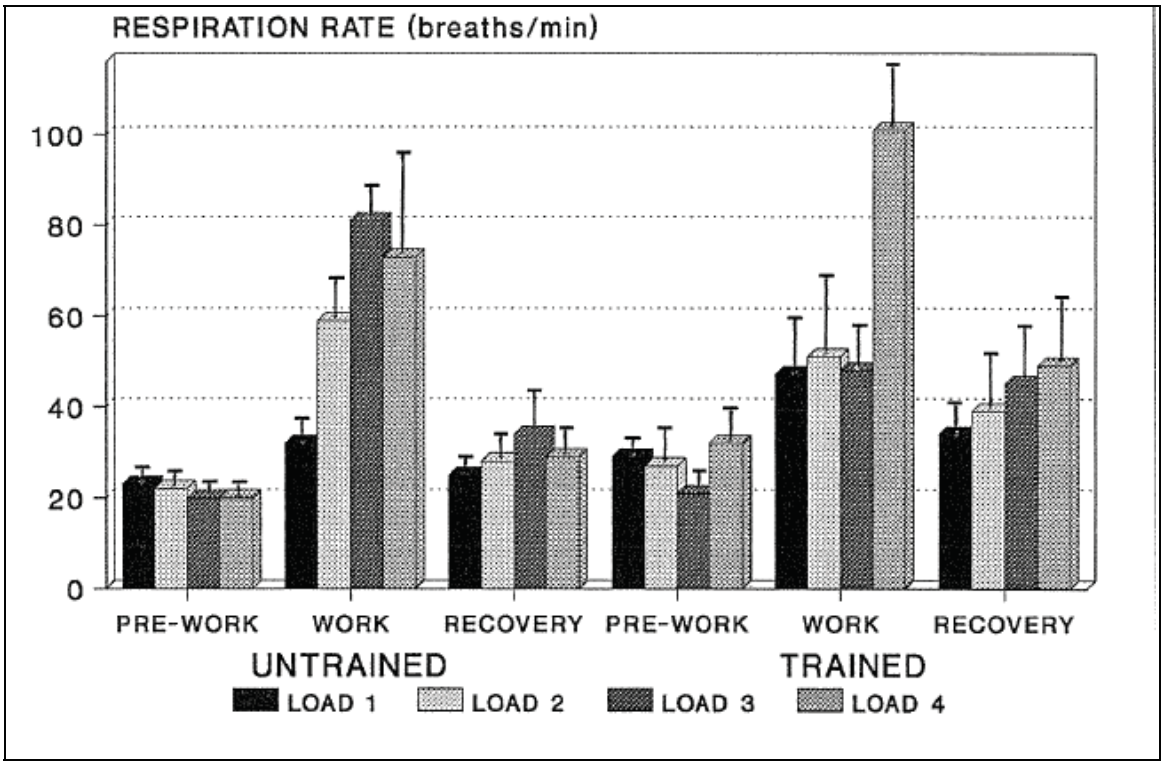


Figure 3A.7 Mean respiration rate of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the differences in mean RR between physiological states and work loads and between work status are presented in table 3A.9.

Respiration rate was not significantly affected by physiological states (table 3A.9). The difference between work loads approached significance (P=0.0631) with a trend of increasing RR with increasing work load (table 3A.9). Work status significantly affected RR (table 3A.9).

Respiration rate increased by 138% to a mean value of 68 breaths/min after 1.5 h of work. Subsequent to work, RR decreased but remained above *Pre-work* values (figure 3A.7).

Table 3A.9 Means of pooled data on respiration rate (RR) of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE).

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
RR (breaths/min)	37	45	30	35	44	53	24	68	34
± SE	2	3	5	8	6	9	2	7	2
Significance	P=0.1868		P=0.0631				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

The changes in the pattern of RR over time across work status are presented in figures 3A.9–3A.16.

Pulse Rate

Data on PR of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.8.

Pooled data, comparing the differences in mean PR between physiological states and work loads and between work status are presented in table 3A.10.

Table 3A.10 Means of pooled data on pulse rate (PR) of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE).

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
PR (beats/min)	54	48	44	47	52	60	38	76	45
± SE	2	1	3	4	4	7	1	10	1
Significance	P=0.2880		P=0.1420				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

Pulse rate was not significantly affected by either physiological state or work loads (table 3A.10) although there was a trend of increasing PR with increasing work load (see

figure 3A.8). There is also a trend for PR to be lower for trained as compared to untrained buffalo (figure 3A.8). Work status significantly affected PR (table 3A.10).

Pulse rate increased by 100% to a mean value of 76 beats/min. Subsequent to work PR decreased but remains above *Pre-work* (see figure 3A.8).

The changes in the pattern of PR over time across work status are presented in figures 3A.9–3A.16.

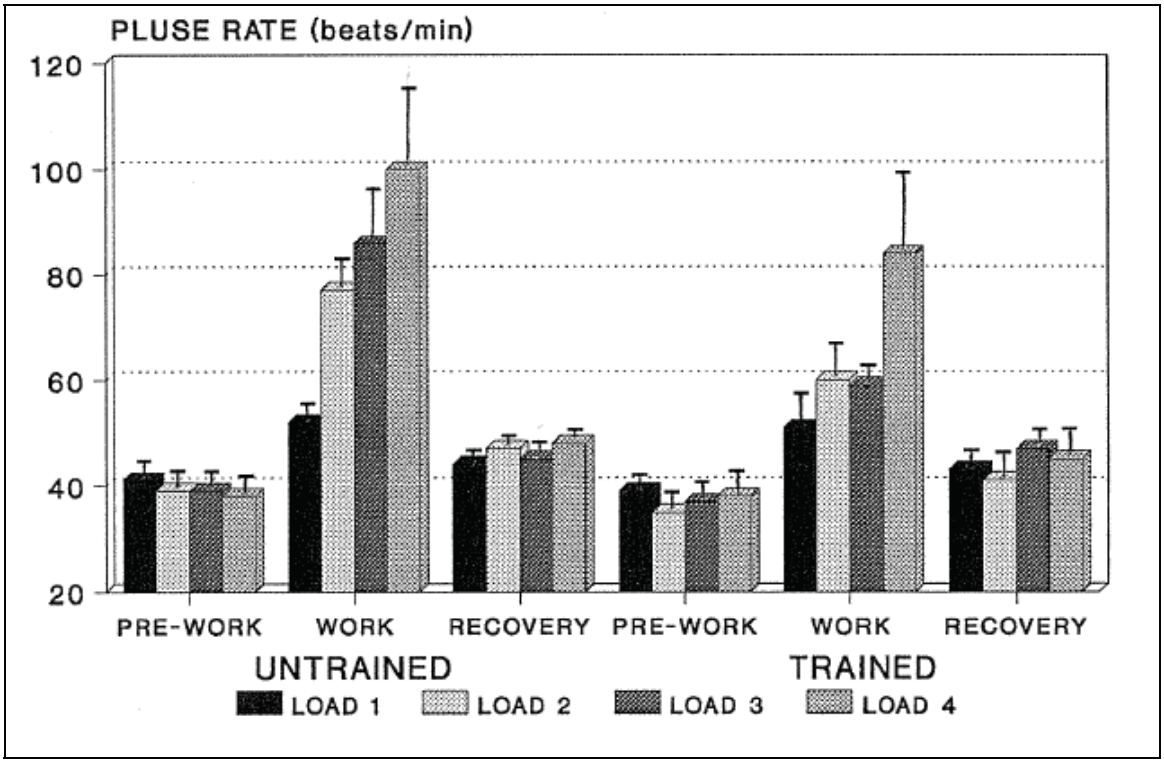


Figure 3A.8 Mean pulse rate of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

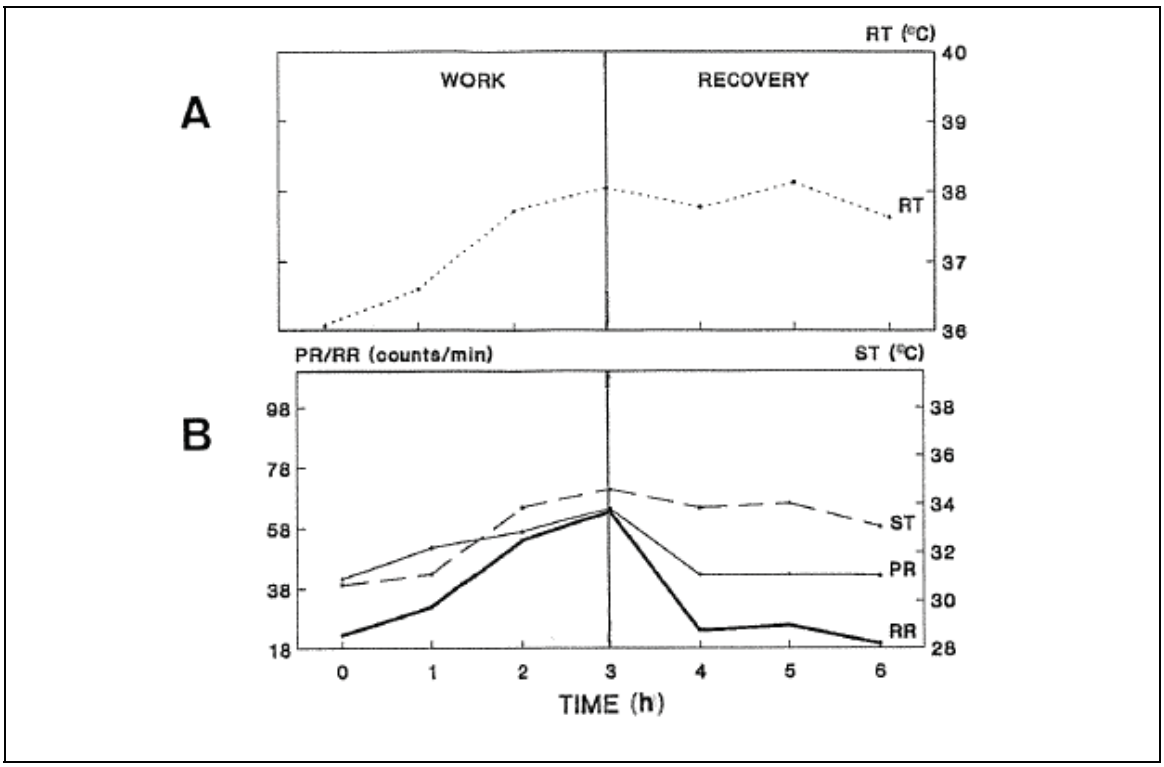


Figure 3A.9 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of untrained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 0% of live weight.

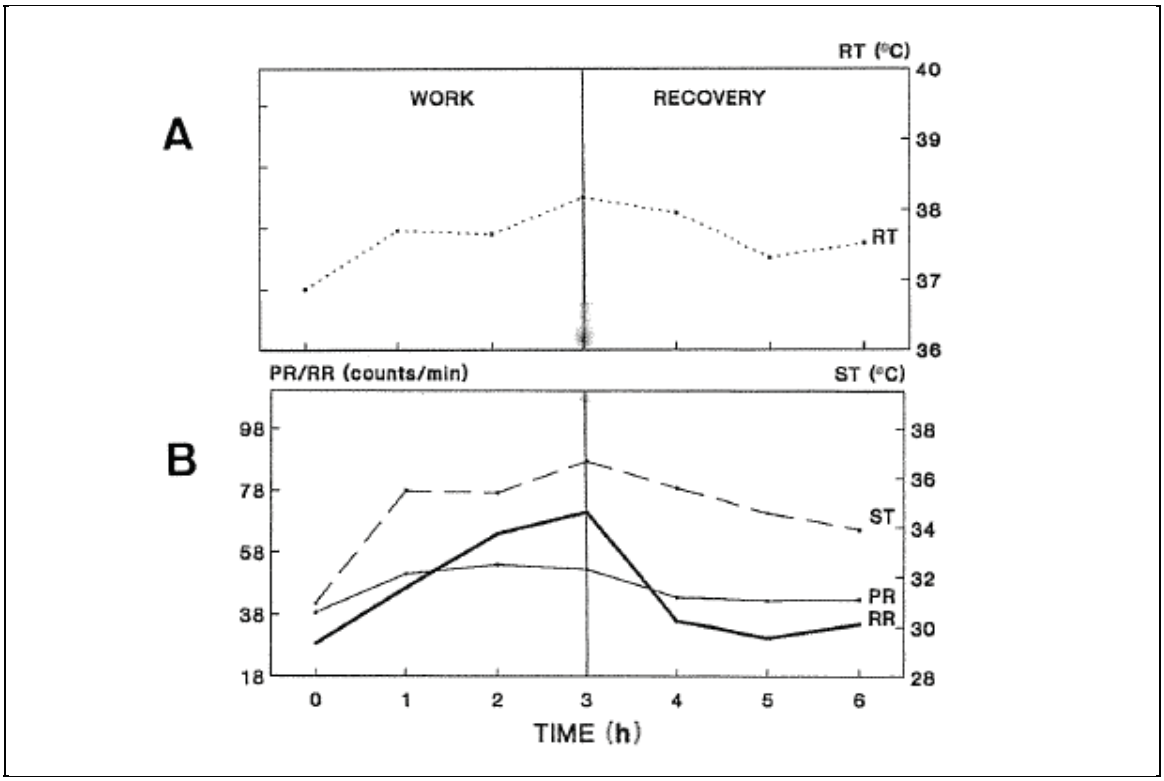


Figure 3A.10 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 0% of live weight.

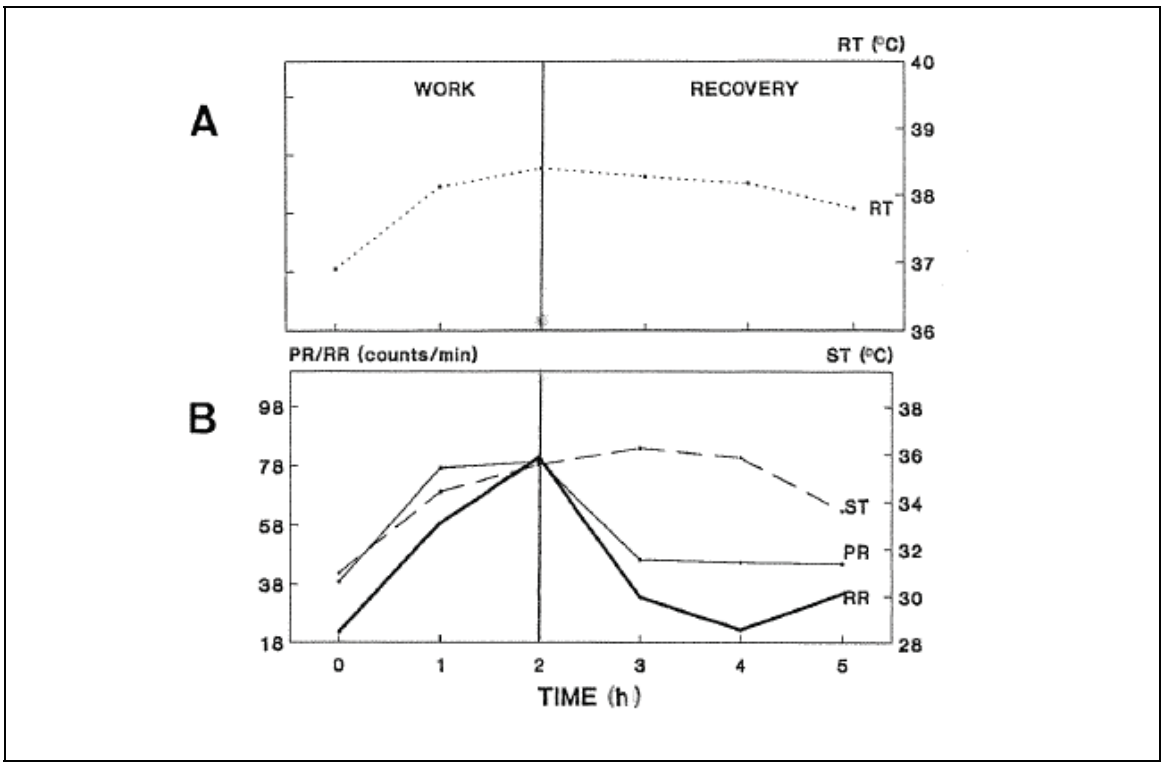


Figure 3A.11 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of untrained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 5% of live weight.

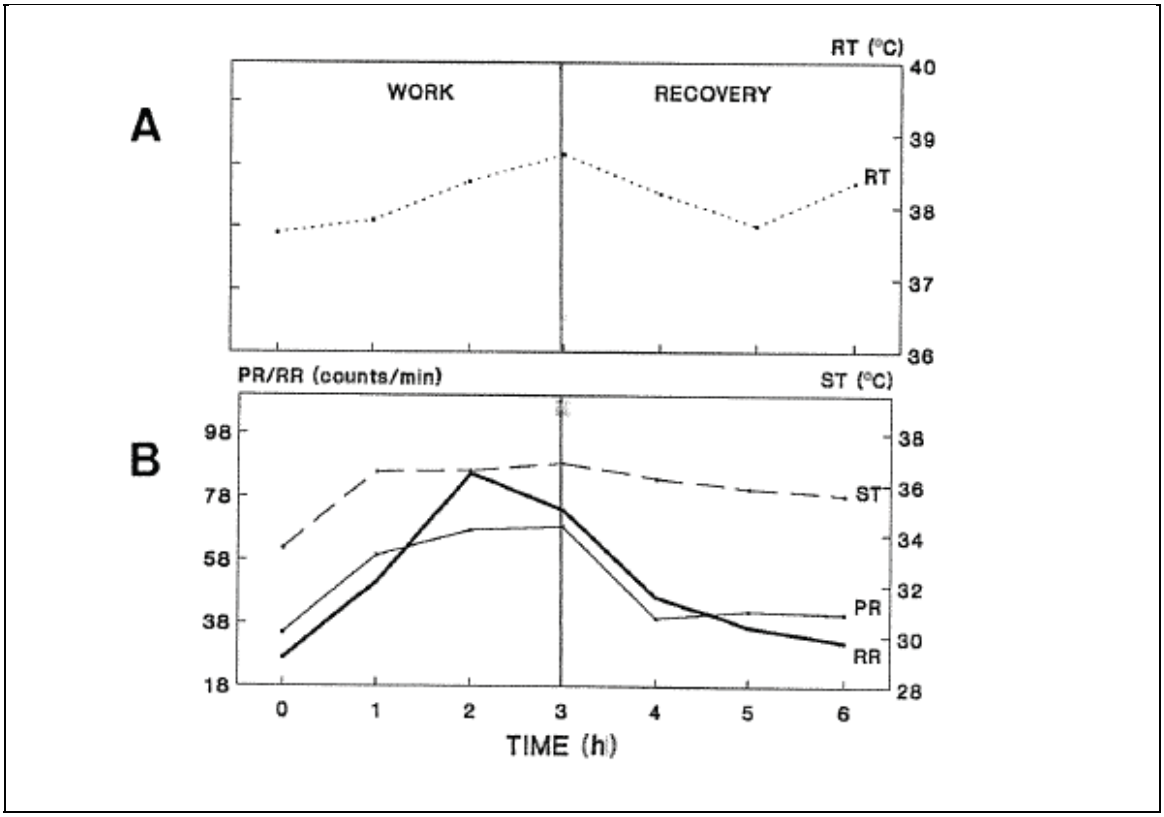


Figure 3A.12 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 5% of live weight.

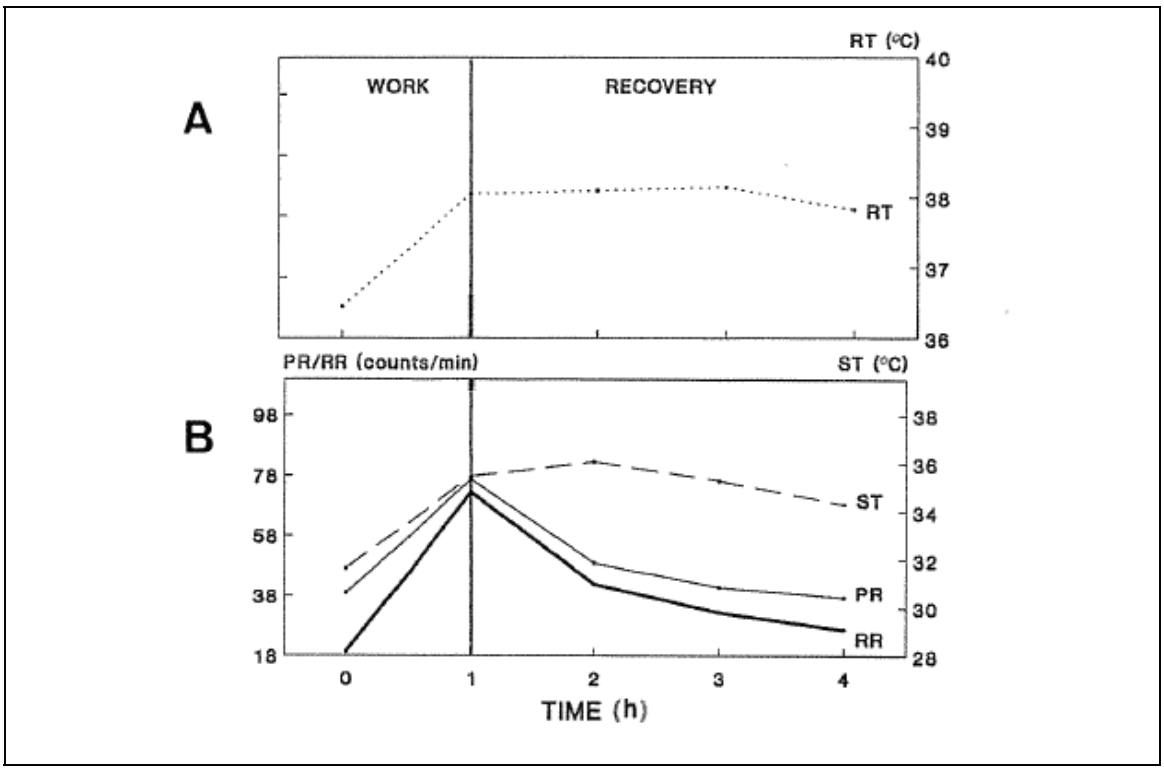


Figure 3A.13 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of untrained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 8% of live weight.

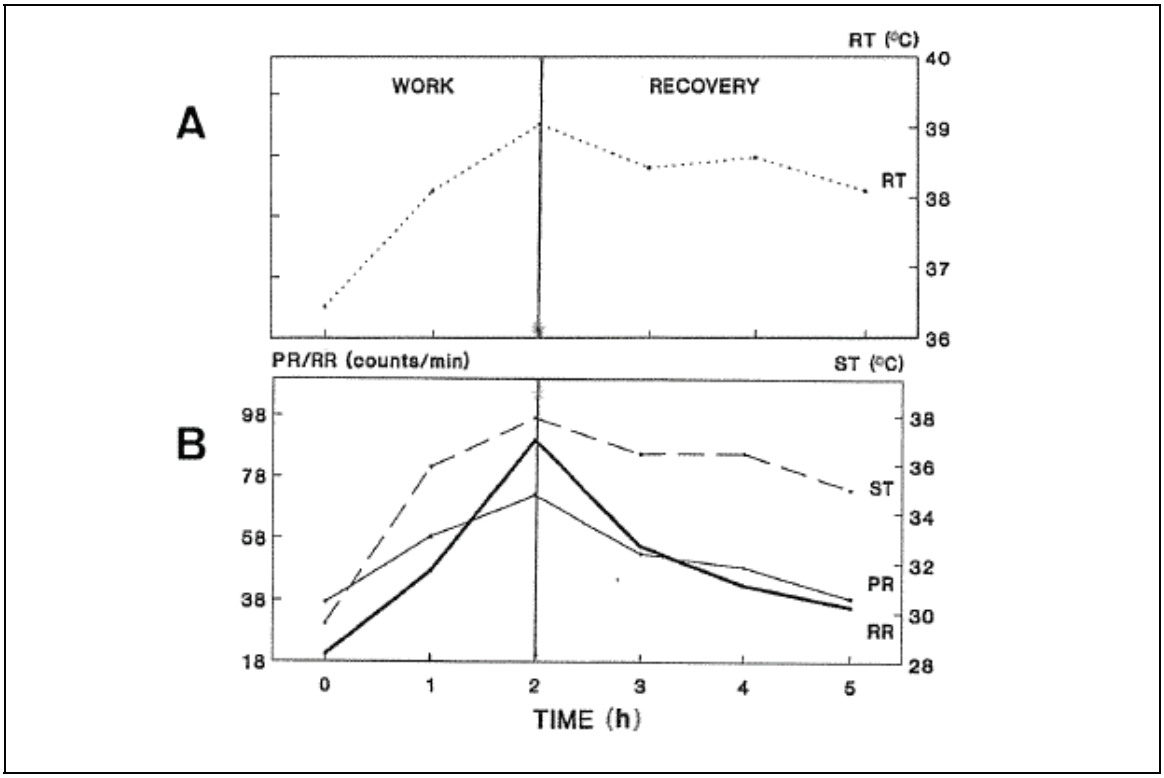


Figure 3A.14 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 8% of live weight.

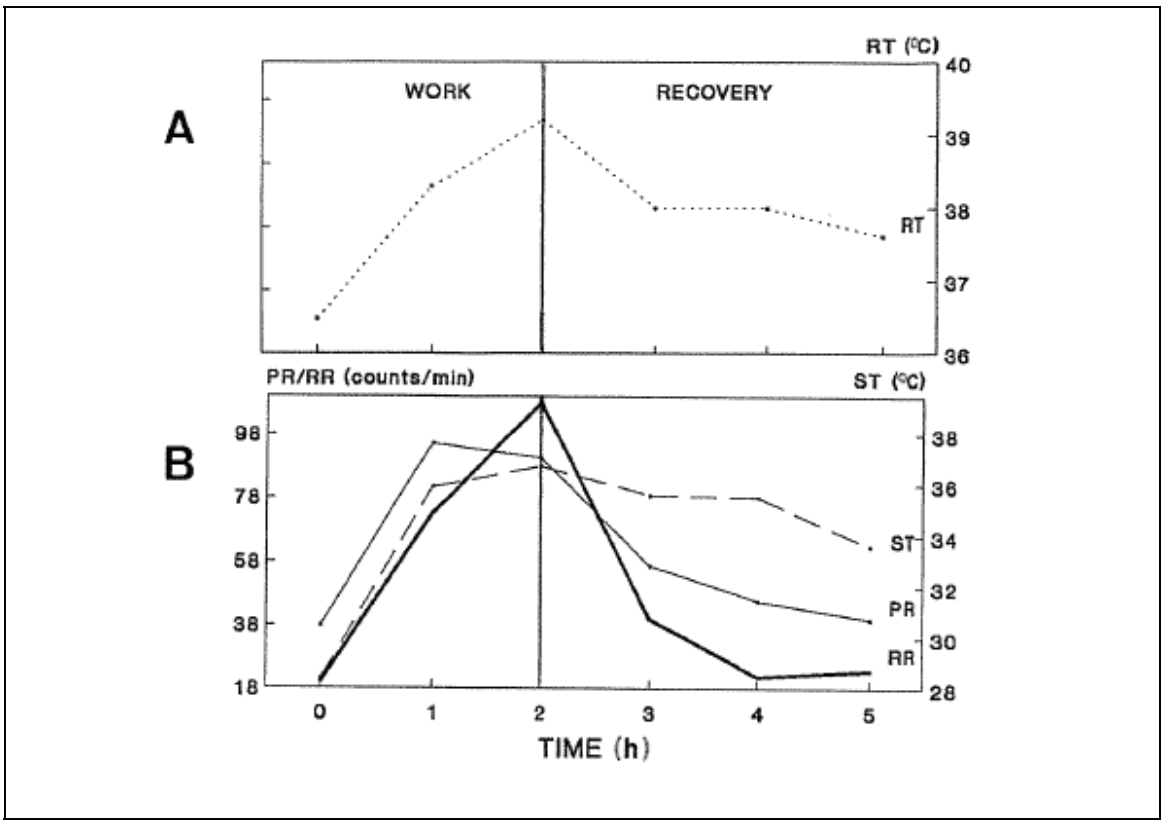


Figure 3A.15 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of untrained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 11% of live weight.

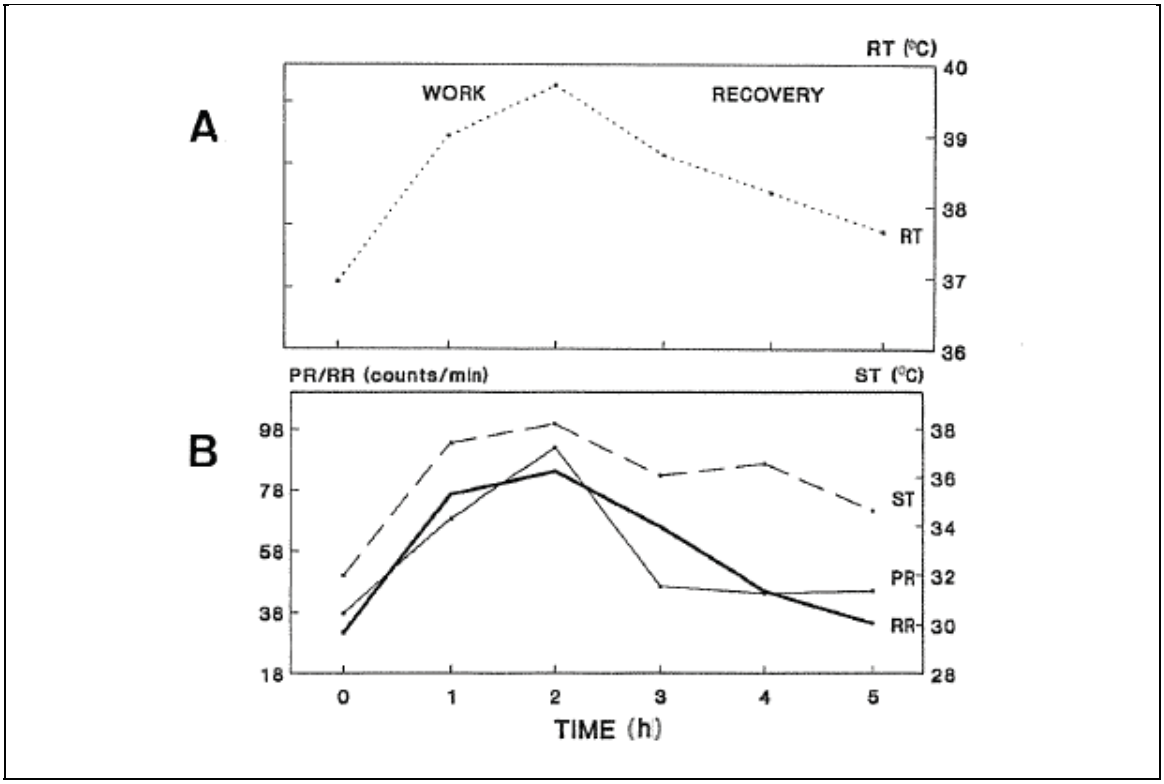


Figure 3A.16 Changes in (A) rectal temperature (RT), (B) skin temperature (ST), pulse rate (PR) and respiration rate (RR) of trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 11% of live weight.

Blood Parameters

Packed cell volume
Data on PCV of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.17.

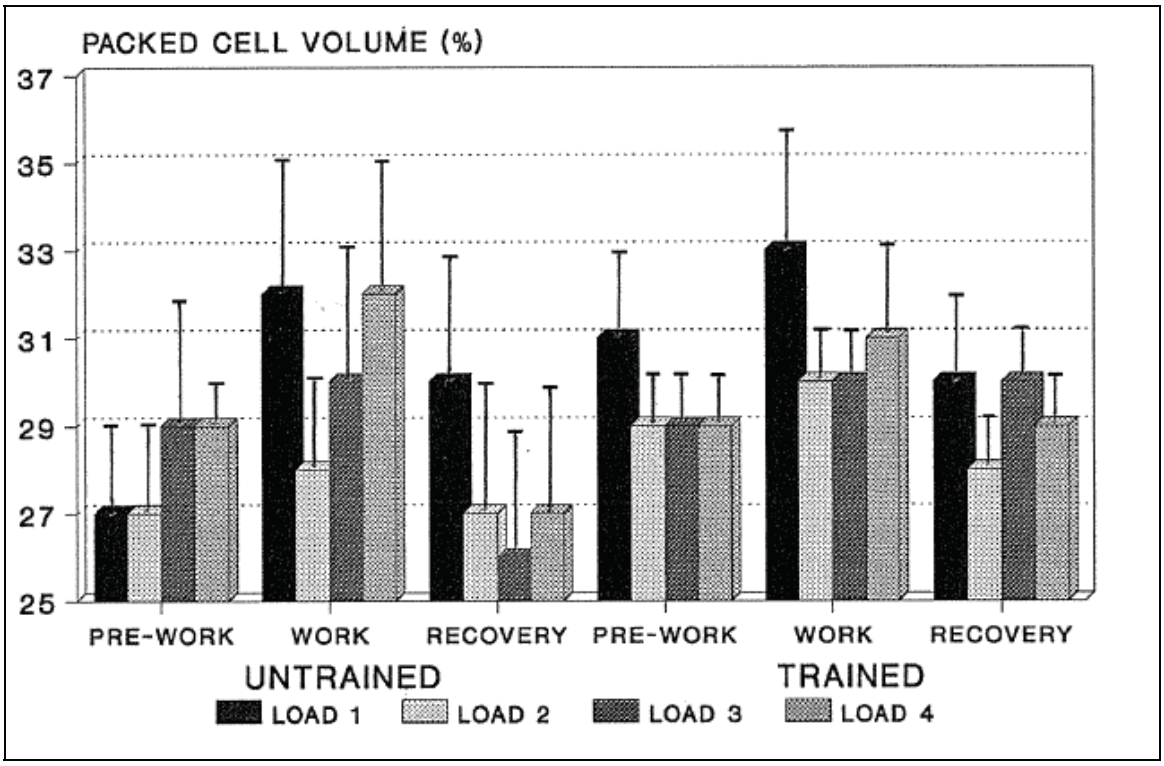


Figure 3A.17 Mean packed cell volume of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the differences in mean PCV between physiological states, work loads and between work status are presented in table 3A.11.

Packed cell volume was not significantly affected by work load (table 3A.11). There were significant differences in PCV between both physiological states and work status.

Table 3A.11 Means of pooled data on packed cell volume (PCV) of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
PCV (%)	28	30	30	28	28	29	29	31	28
± SE	1	1	2	2	2	2	1	1	1
Significance	P=0.0076		P=0.0723				P=0.0063		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

The effects of work and physiological state are clearly illustrated in figure 3A.18 where data were pooled across work loads. The mean PCV increased during the *Work* period by 11 and 7% respectively in both untrained and trained buffalo (see figure 3A.18). Subsequent to work, PCV in the untrained animals decreased to below *Pre-work* values during the *Recovery* period, while corresponding values in the trained animals remained equal to *Pre-work* values. The differences between physiological states were highlighted during *Pre-work* and *Recovery* periods when PCV was higher in trained than untrained buffalo (figure 3A.18).

The changes in the pattern of PCV over time across work status are presented in figures 3A.21–3A.24.

Haemoglobin

Data on haemoglobin concentrations in blood of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.19.

Pooled data, comparing the difference in mean haemoglobin concentrations between physiological states, work loads and between work status are presented in table 3A.12.

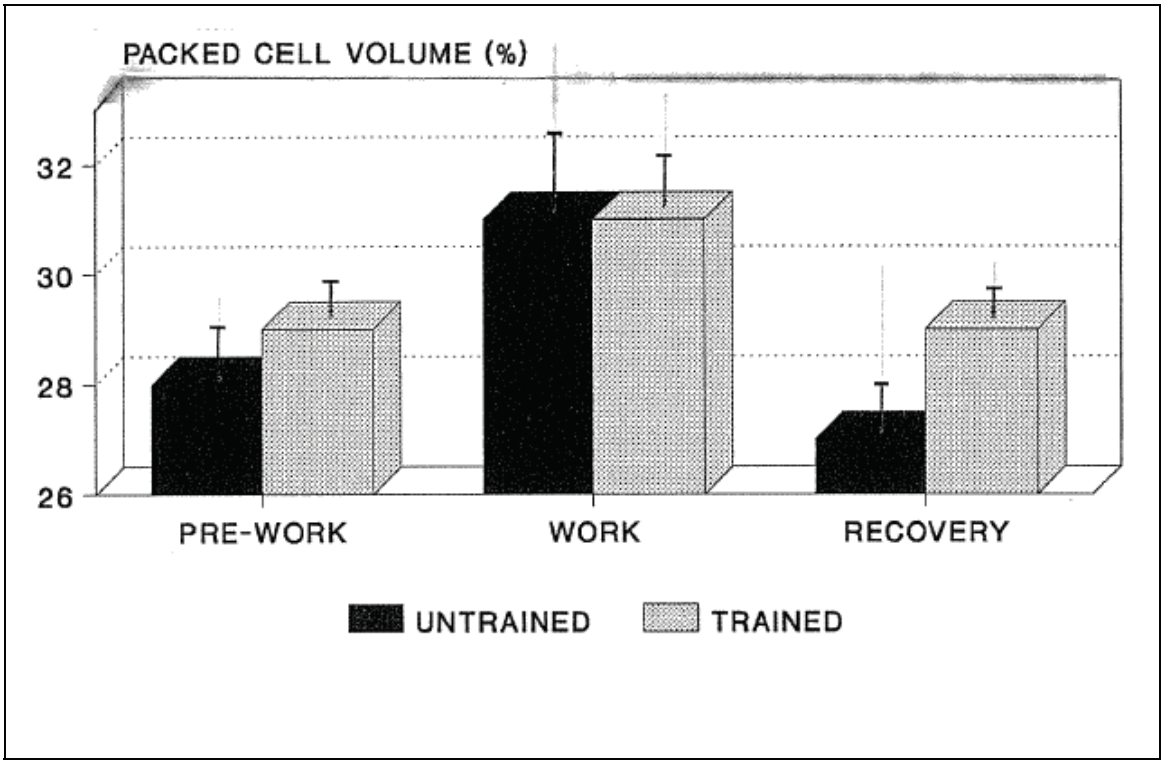


Figure 3A.18 Means of pooled data on packed cell volume of buffalo during the *Pre-work, Work* and *Recovery* periods. Vertical lines **above bars represent standard errors.**

Table 3A.12 Means of pooled data on haemoglobin concentration of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
Haemoglobin (g/L)	102.20	110.66	108.46	104.27	106.26	106.72	108.25	109.12	104.92
± SE	0.18	0.13	0.80	0.59	0.59	0.75	0.35	0.36	0.20
Significance	P=0.0000		P=0.6075				P=0.2271		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

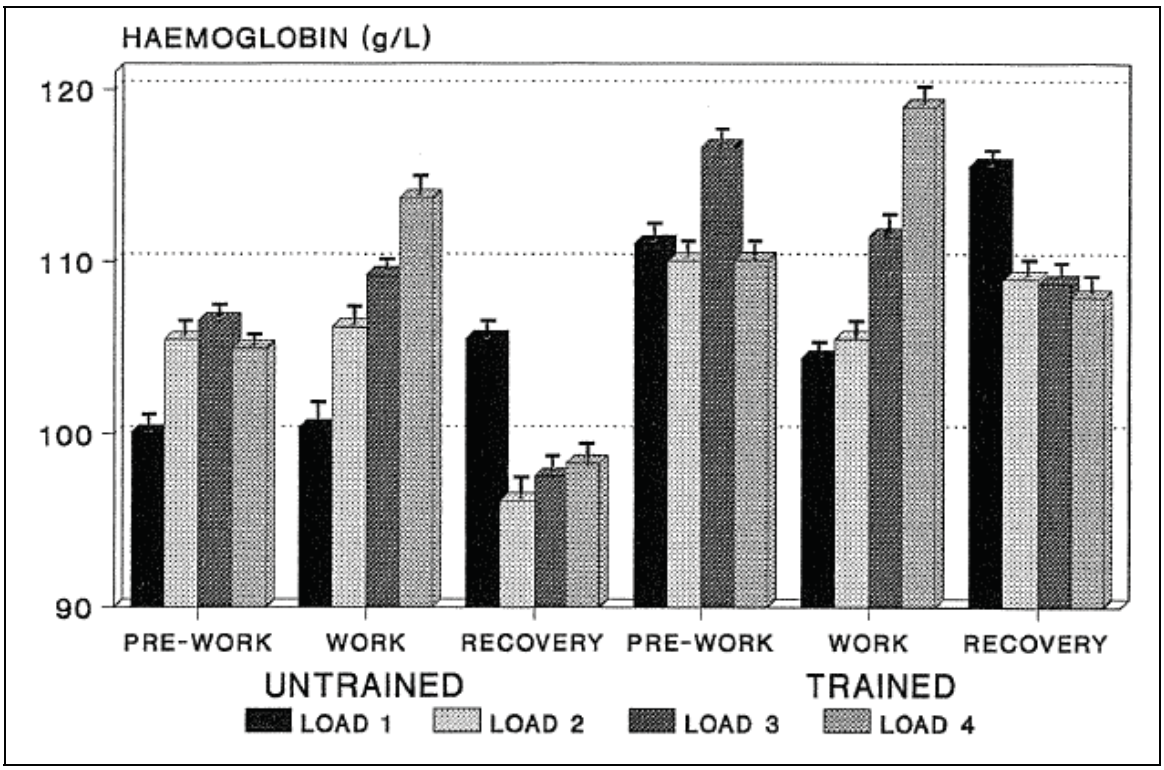


Figure 3A.19 Mean concentrations of haemoglobin in blood of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Haemoglobin concentration was not significantly affected by either work load or work status (table 3A.12). Physiological state significantly affected haemoglobin concentration (table 3A.12). There was a trend in untrained buffalo of increased haemoglobin concentration during the *Work* period while corresponding values in the trained animals decreased except for load 4 (see figure 3A.19).

The effect of physiological state on haemoglobin concentration is clearly illustrated in figure 3A.20 where data were pooled across work loads and work status for the two physiological states.

Overall the haemoglobin concentration was higher (8%) in trained as compared to untrained buffalo (see figure 3A.20).

The changes in the pattern of haemoglobin concentration over time across work status are presented in figures 3A.21–3A.24.

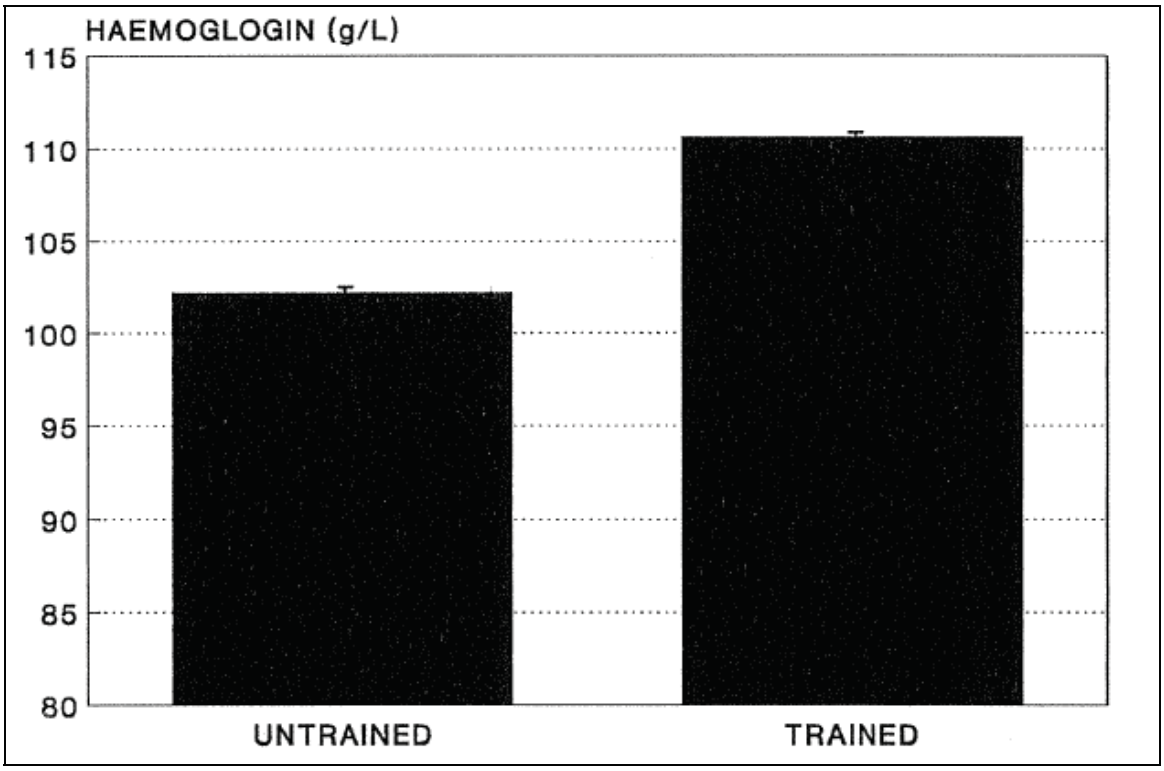


Figure 3A.20 Means of pooled data on concentration of haemoglobin in untrained and trained buffalo. Vertical lines **above bars represent standard errors.**

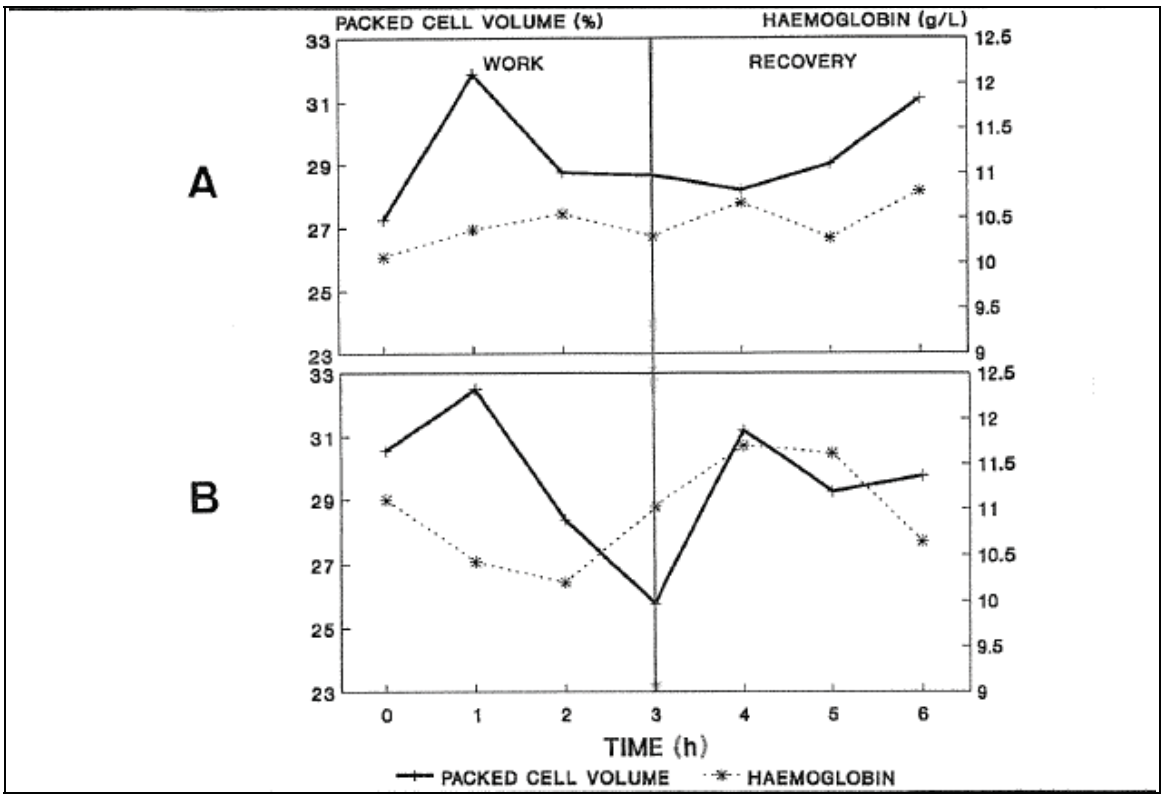


Figure 3A.21 Changes in packed cell volume and haemoglobin concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 0% of live weight.

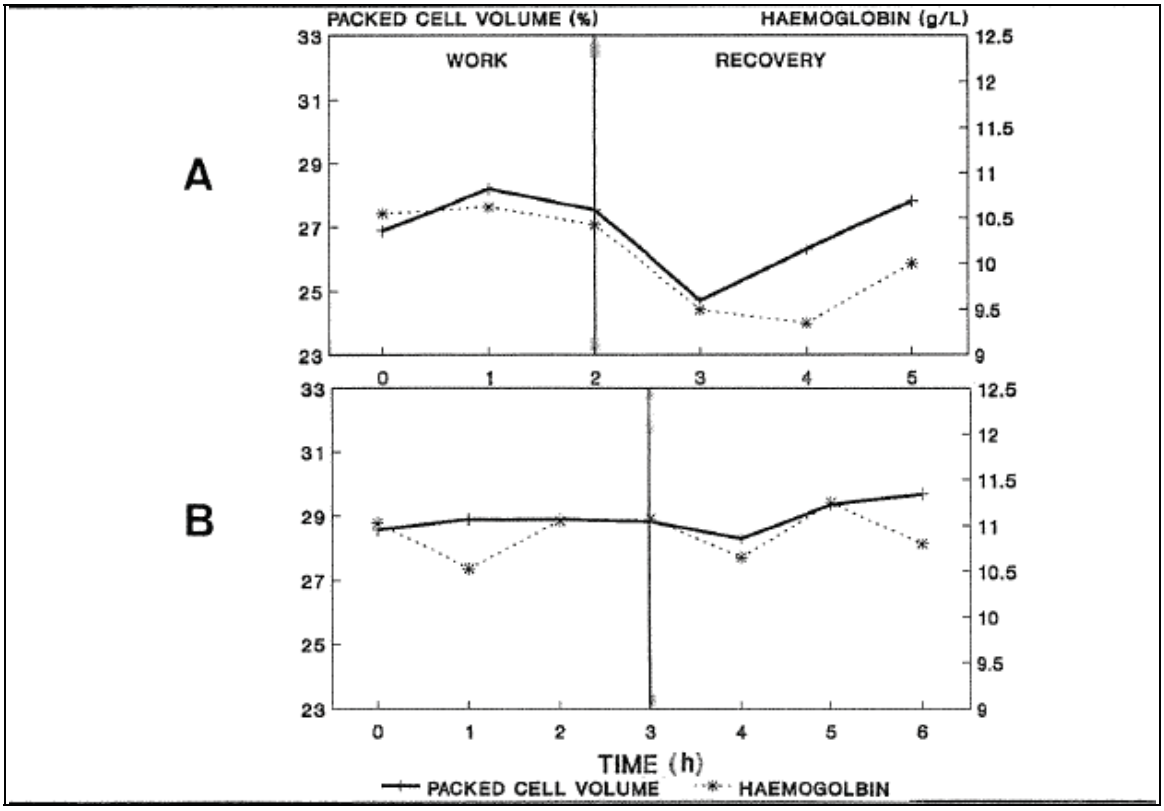


Figure 3A.22 Changes in packed cell volume and haemoglobin concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 5% of live weight.

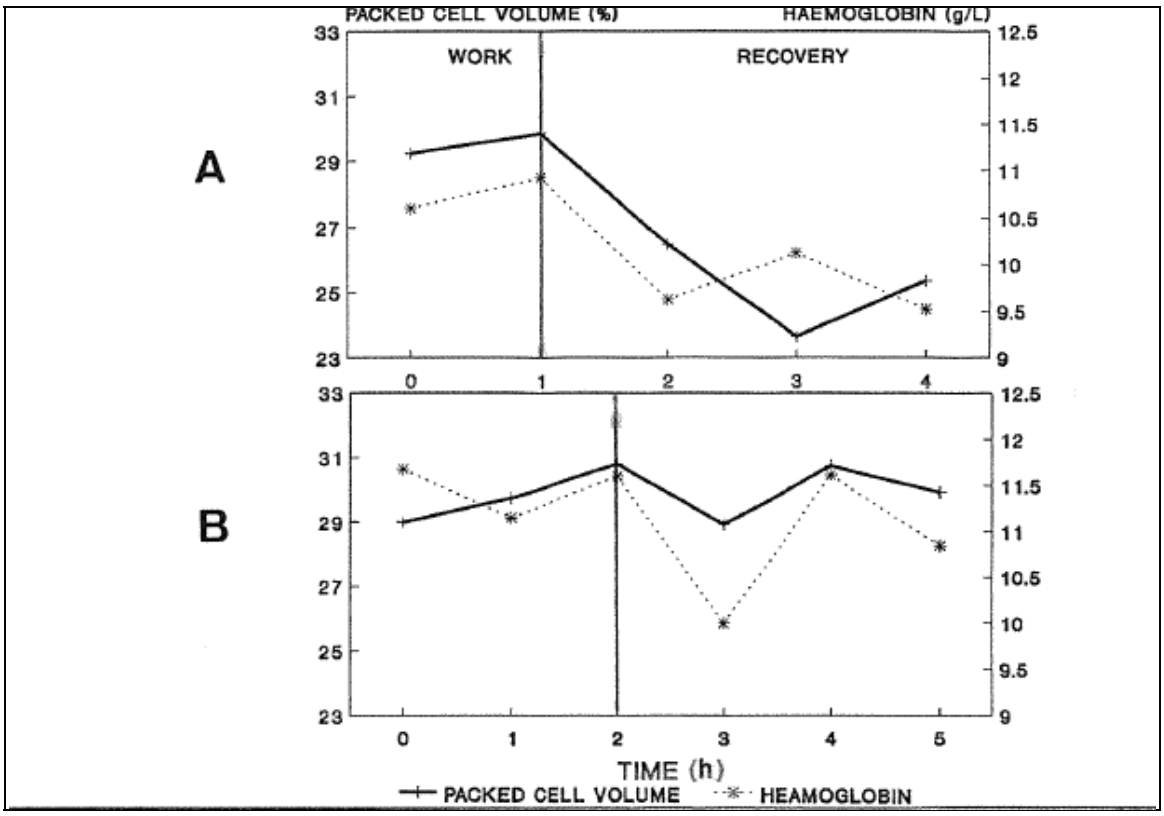
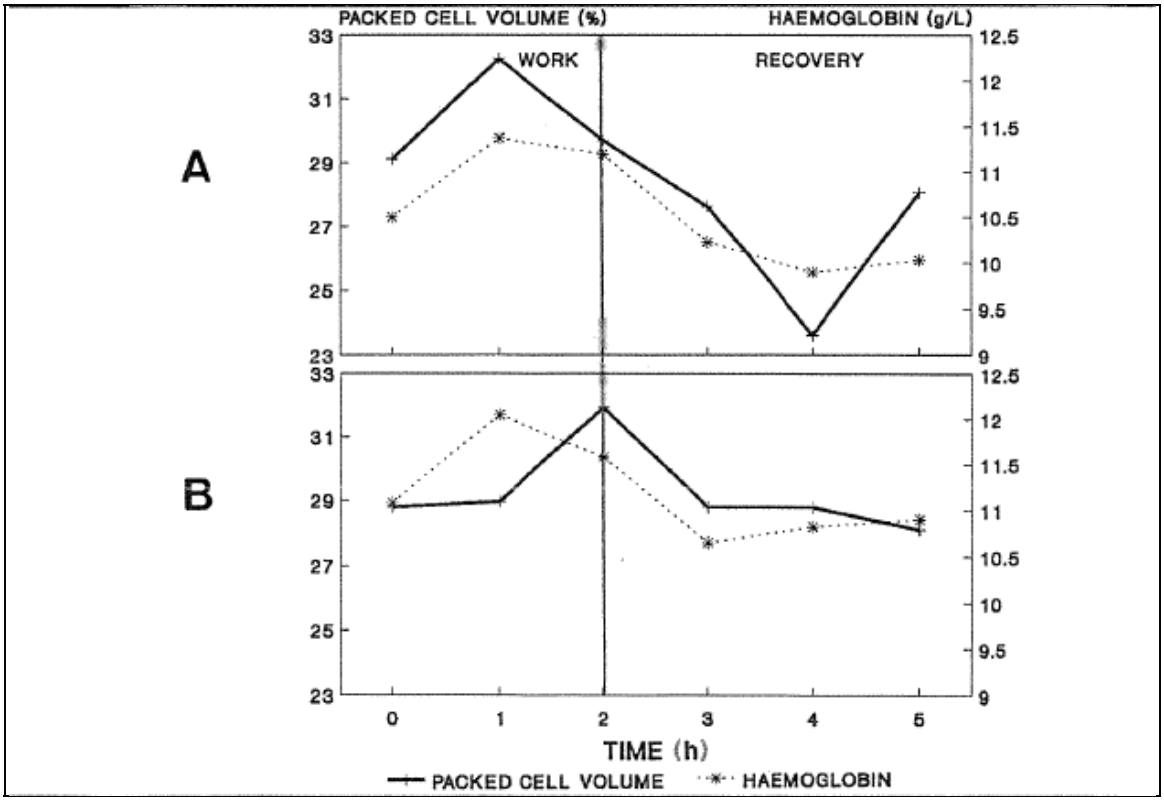


Figure 3A.23 Changes in packed cell volume and haemoglobin concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load



equivalent to 8% of live weight.

Figure 3A.24 Changes in packed cell volume and haemoglobin concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 11% of live weight.

pH

Data on pH of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.25.

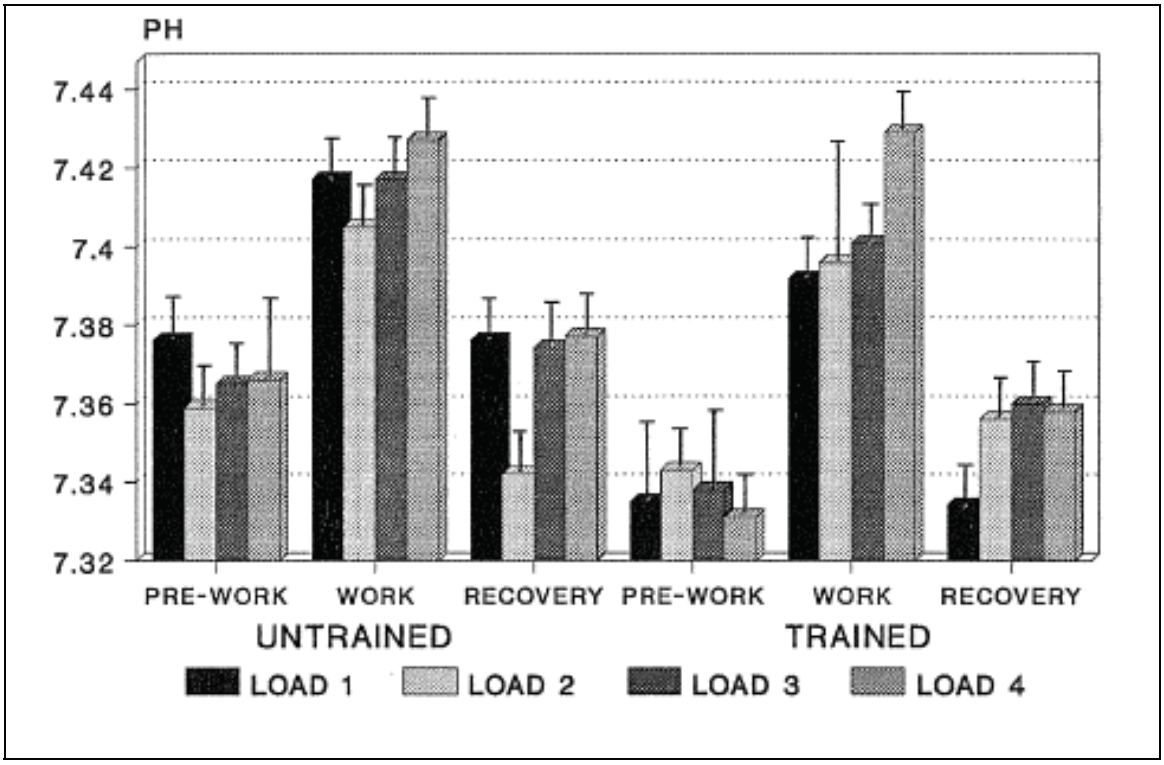


Figure 3A.25 Mean pH of untrained and trained buffalo under different work status (*Pre-work, Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the differences in mean pH between physiological states, work loads and between work status are presented in table 3A.13.

Table 3A.13 Means of pooled data on pH in blood of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
pH	7.38	7.36	7.37	7.36	7.37	7.38	7.35	7.41	7.36
± SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Significance	P=0.0568		P=0.6106				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

The mean pH was not significantly affected by work loads although there was a trend of increasing pH with increasing work load (figure 3A.25). The difference between physiological

states approached significance (P=0.0568). The mean pH was higher in the trained than in the untrained buffalo (table 3A.13). There were significant differences in pH between work status.

Overall pH increased by 0.06 in the *Work* period (table 3A.13) and decreased in the *Recovery* period to remain above *Pre-work* values (figures 3A.26).

The effect of work status on pH is clearly illustrated in figure 3A.26 where data were pooled across physiological states and work loads for the three work status.

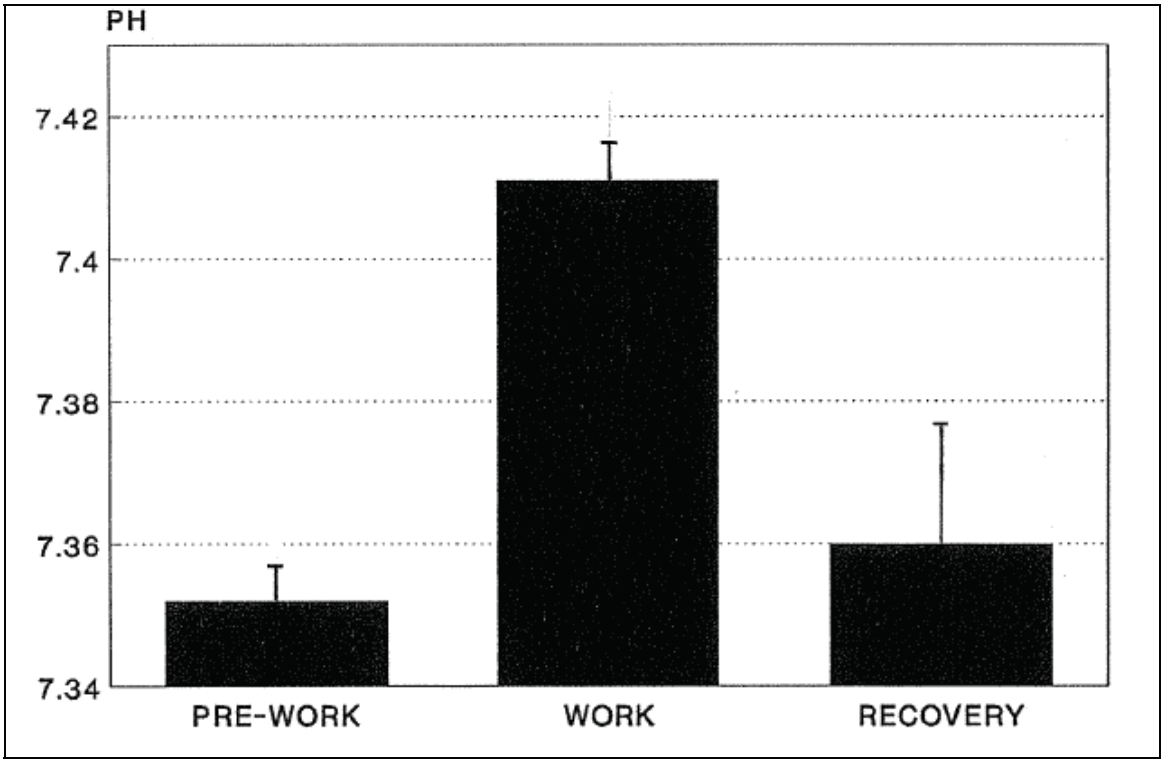


Figure 3A.26 Means of pooled data on pH of buffalo during the *Pre-work, Work* and *Recovery* periods. **Vertical lines above bars represent standard errors.**

The changes in the pattern of pH over time across work status are presented in figures 3A.27–3A.30.

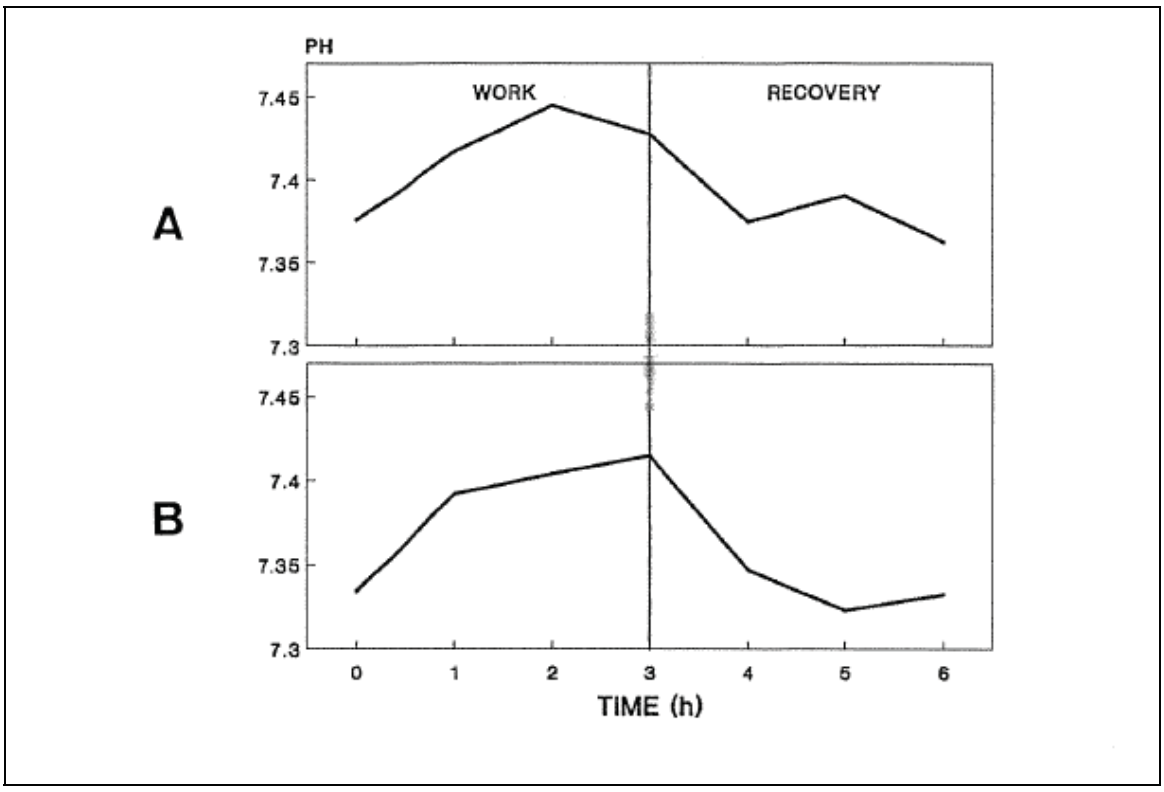


Figure 3A.27 Changes in the pattern of pH of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 0% of live weight.

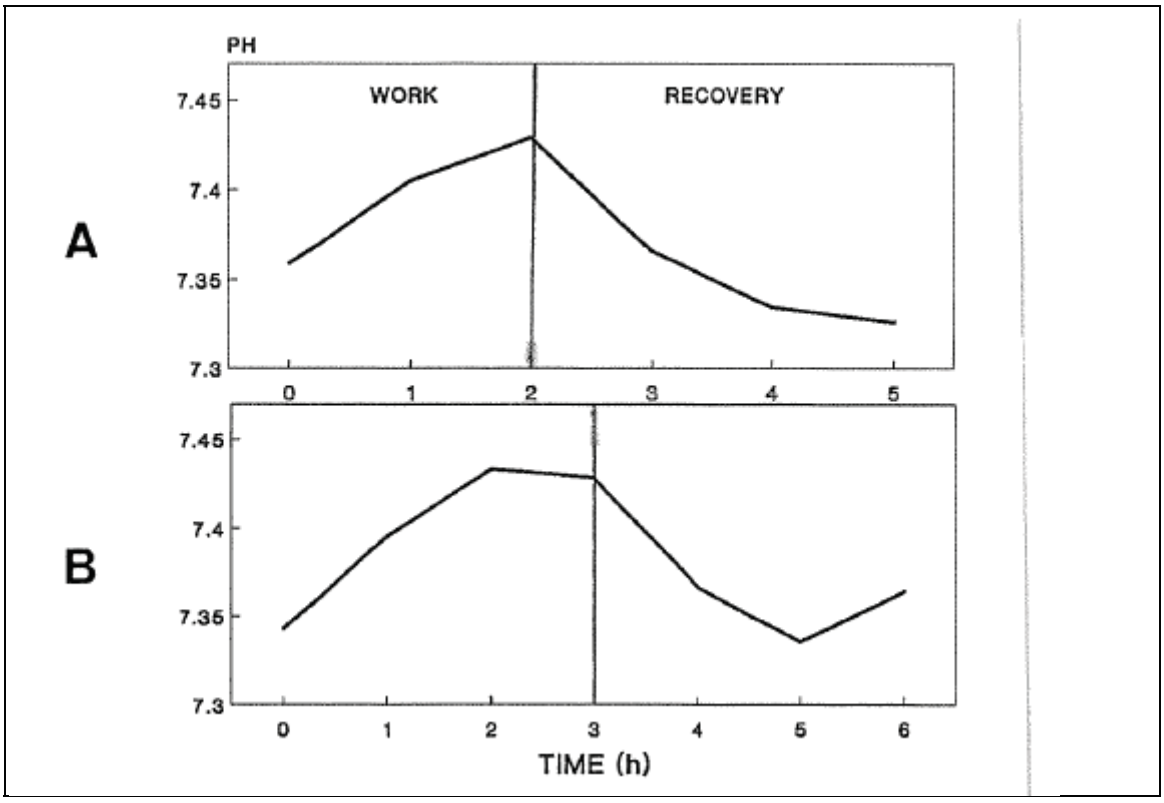


Figure 3A.28 Changes in the pattern of pH of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 5% of live weight.

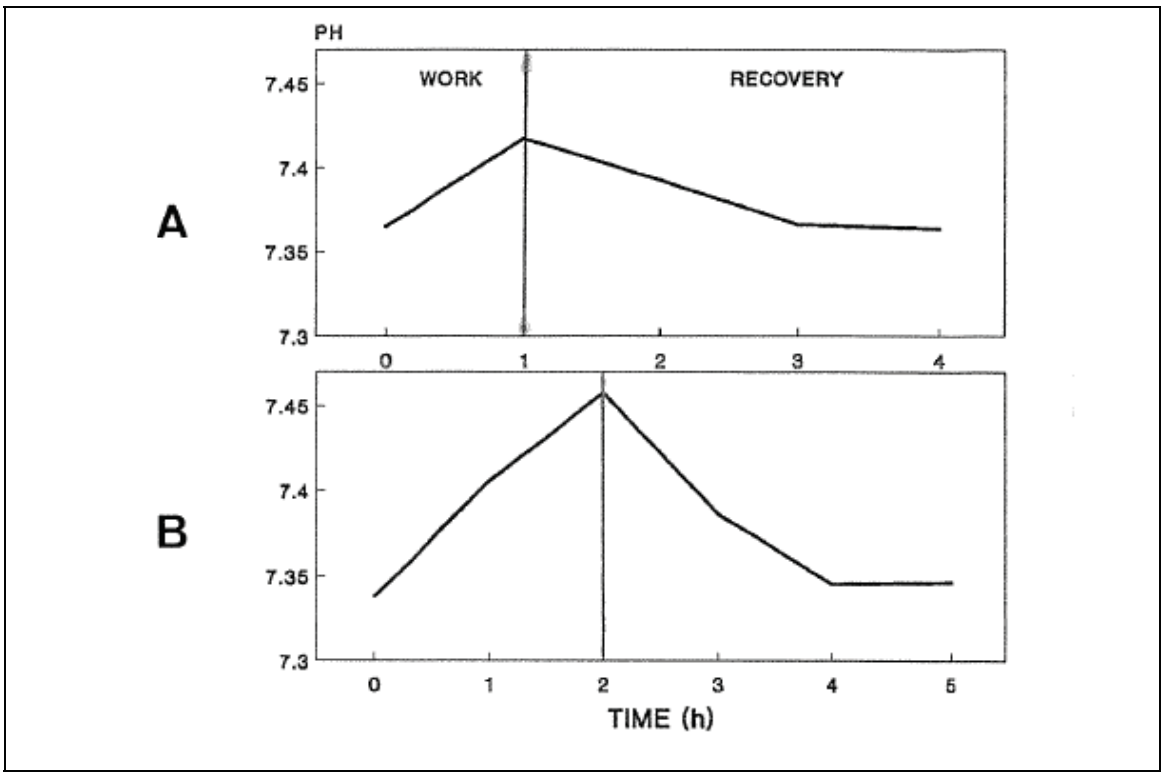


Figure 3A.29 Changes in the pattern of pH of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 8% of live weight.

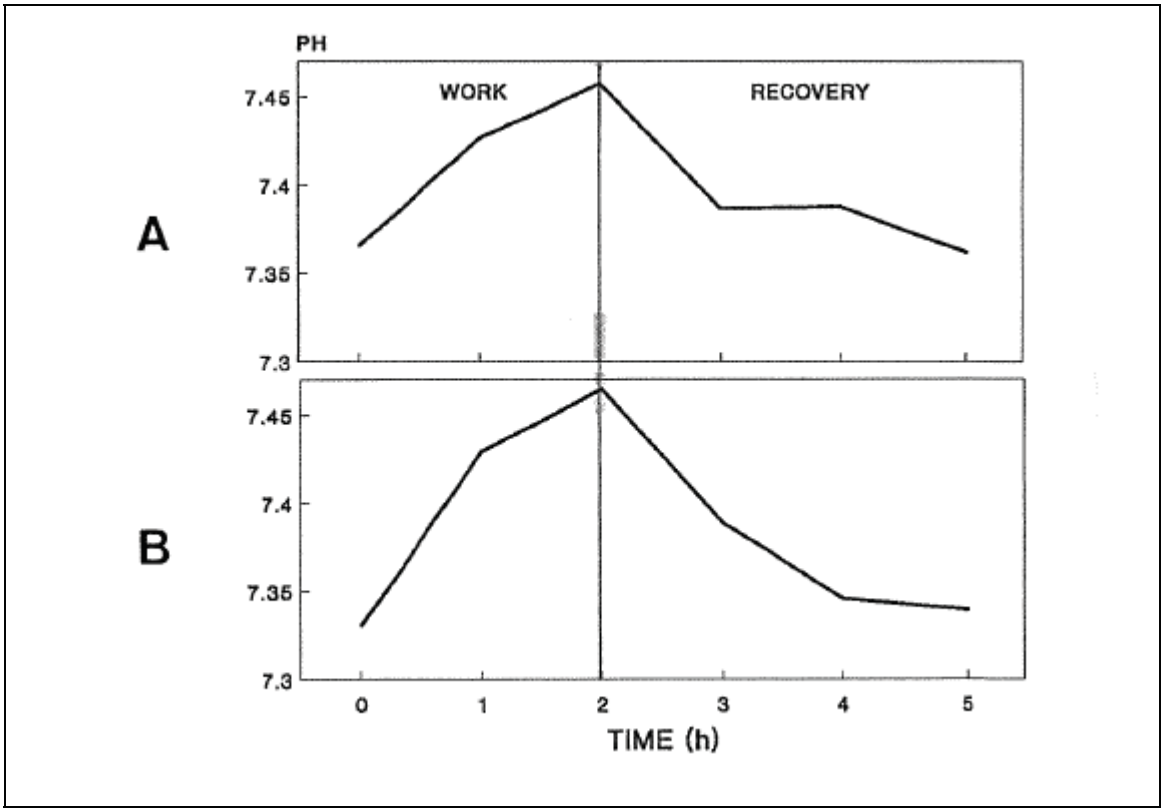


Figure 3A.30 Changes in the pattern of pH of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 11% of live weight.

3A.3.4 Blood Metabolites

Lactate

Data on lactate concentrations in plasma of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.31.

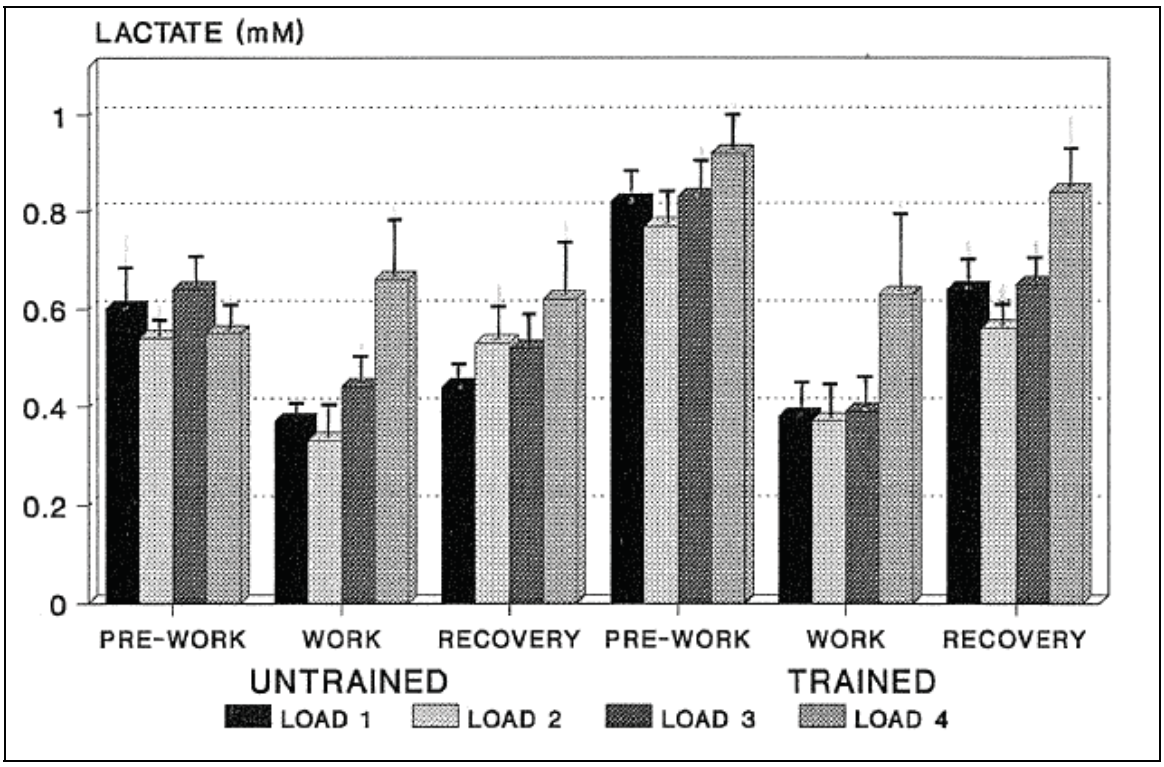


Figure 3A.31 Mean concentrations of lactate in plasma of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean lactate concentrations between physiological states, work loads and between work status are presented in table 3A.14.

All treatments, *viz*, physiological state, work load and work status, significantly affected the concentration of lactate in plasma of buffalo (table 3A.14).

The trained animals had a higher mean lactate concentration in plasma than that in the untrained animals. This difference was mainly due to the differences between physiological states in mean lactate concentrations in the *Pre-work* and *Recovery* periods (figure 3A.31).

Table 3A.14 Means of pooled data on lactate concentration in plasma of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
Lactate (mM)	0.52	0.65	0.54	0.52	0.58	0.71	0.71	0.45	0.60
± SE	0.02	0.02	0.04	0.04	0.05	0.08	0.03	0.04	0.03
Significance	P=0.0007		P=0.0031				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

Overall, there was a reduction in lactate concentration during the *Work* period. The exception was the increase (20%) observed in the untrained animal subjected to load 4 (figure 3A.31). Subsequent to the *Work* period, values of lactate concentration in both the untrained and trained buffalo increased with the exception of untrained animals on load 4 (figure 3A.31).

The differences between work loads was highlighted in the *Work* period (in both the untrained and trained animals) when the lactate concentration was highest in animals on load 4. Values for work loads 1–3 were similar during this period (figure 3A.31).

The changes in the pattern of concentration of lactate in plasma of buffalo over time across work status, on different work loads are presented in figures 3A.37–3A.40. The sharp increase in concentration of plasma lactate in the untrained animals subjected to load 4 is clearly illustrated in figure 3A.40(A).

Glucose

Data on concentrations of glucose in plasma of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.32.

Pooled data, comparing the difference in mean glucose concentrations between physiological states, work loads and between work status are presented in table 3A.15.

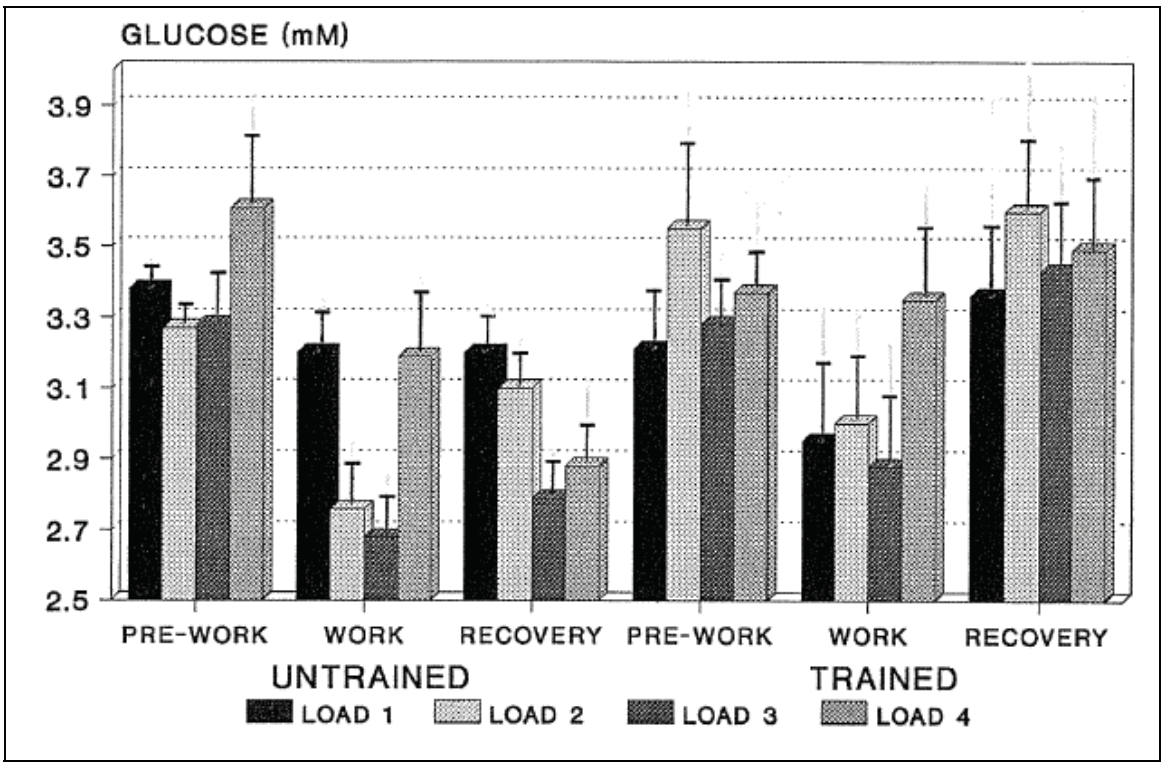


Figure 3A.32 Mean concentrations of glucose in plasma of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Table 3A.15 Means of pooled data on glucose concentration in plasma of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
Glucose (mM)	3.10	3.31	3.22	3.23	3.06	3.30	3.37	3.00	3.23
± SE	0.04	0.05	0.13	0.14	0.13	0.16	0.07	0.08	0.07
Significance	P=0.0008		P=0.0528				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

There were significant differences in plasma glucose concentration between physiological states and between work status (table 3A.15). The difference between work loads approached significance (P=0.0528).

The means of pooled values for glucose concentration in plasma of the trained animals was significantly higher than that of the untrained animals (table 3A.15). The mean concentration of glucose in plasma decreased during the *Work* period by 12 and 9% respectively in both untrained and trained buffalo (figure 3A.33). Subsequent to work, glucose concentrations in the trained animals increased to above *Pre-work* values during the *Recovery* period while corresponding values in the untrained animals remained below *Pre-work* values.This trend is more clearly illustrated in figure 3A.33 where data for different work loads were pooled.

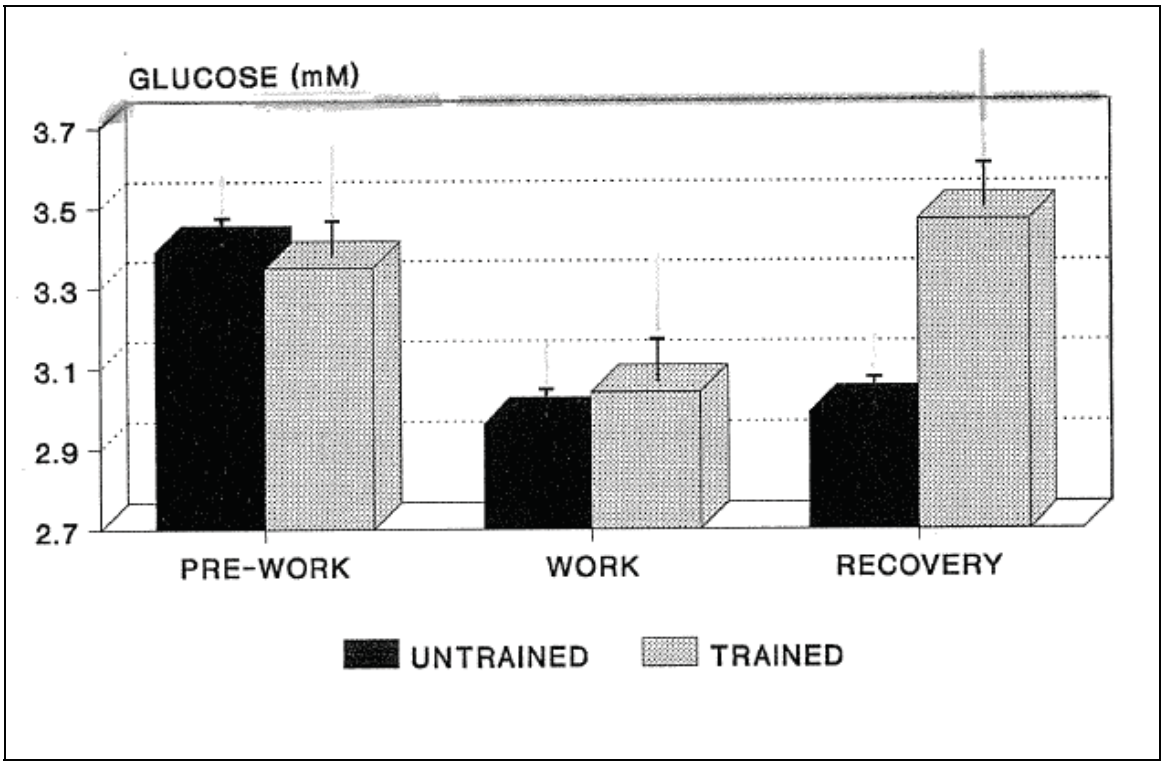


Figure 3A.33 Mean concentrations of glucose in plasma of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

The pattern of changes in glucose concentration over time across work status are presented in figures 3A.37–3A.40.

Urea

Data on concentrations of urea in plasma of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.34.

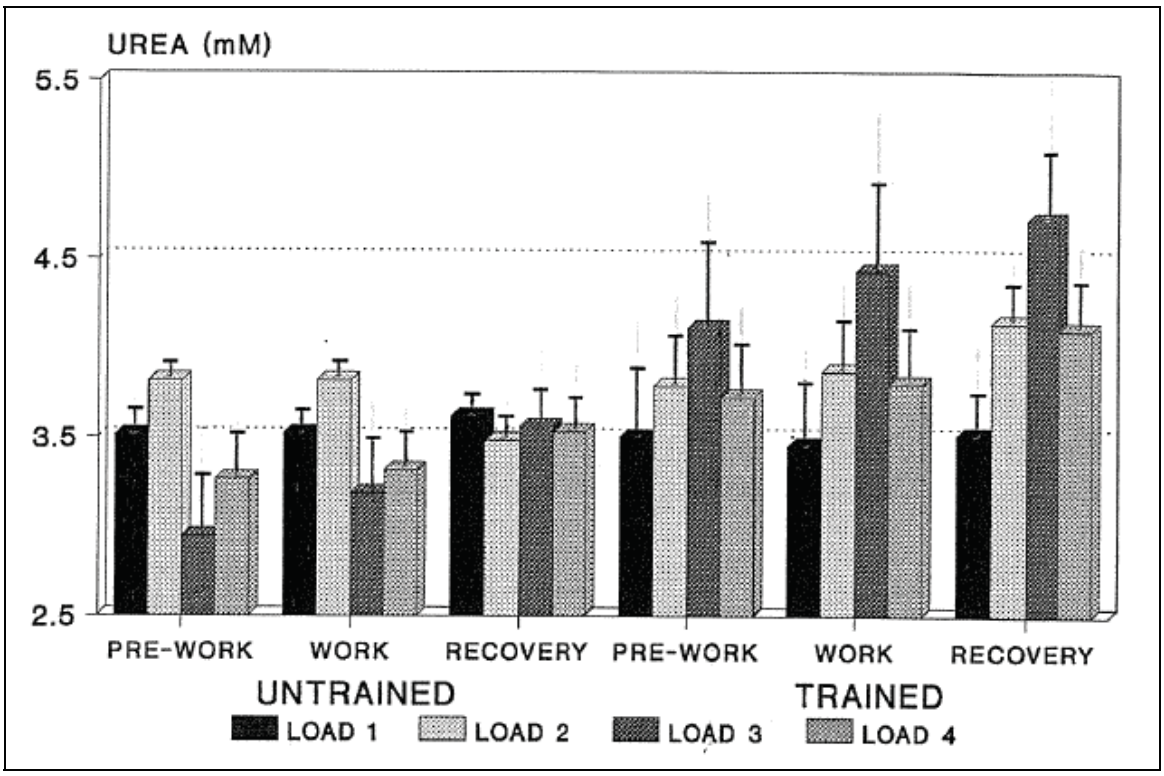


Figure 3A.34 Mean concentrations of urea in plasma of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean urea concentrations between physiological states, work loads and between work status are presented in table 3A.16

Table 3A.16 Means of pooled data on urea concentration in plasma of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
Urea (mM)	3.47	3.86	3.52	3.82	3.85	3.64	3.58	3.67	3.83
± SE	0.05	0.09	0.23	0.17	0.35	0.22	0.14	0.13	0.11
Significance	P=0.0000		P=0.0326				P=0.0645		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

The concentrations of urea in plasma of buffalo were significantly affected by both physiological states and work loads (table 3A.16). The effect of work status approached significance (P=0.0645; table 3A.16).

The concentrations of plasma urea were higher in trained than in untrained animals on loads 2–4 (figure 3A.34). Overall, as work load increased the concentration of plasma urea also increased. The exception was the urea concentration in plasma of animals subjected to load 4 (figure 3A.34).

Except in the case of the untrained animals on load 2, the highest urea concentrations in plasma of untrained and trained animals on each work load were observed during the *Recovery* period (figure 3A.34).

The changes in the pattern of concentration of urea in plasma of buffalo over time across work status are presented in figures 3A.37–3A.40. The general trend is for an increase in urea concentration with time from the *Pre-work* period through *Work* to the *Recovery* period.

Free fatty acids

The values for FFA concentrations in plasma of untrained and trained buffalo under different work loads and work status are presented in figure 3A.35.

Pooled data, comparing the difference in mean FFA concentrations between physiological states, work loads and between work status are presented in table 3A.17.

Table 3A.17 Means of pooled data on free fatty acids (FFA) concentration in plasma of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
FFA (mM)	1.25	1.28	1.08	1.28	1.36	1.34	0.92	1.63	1.25
± SE	0.04	0.05	0.09	0.12	0.11	0.13	0.04	0.08	0.06
Significance	P=0.7625		P=0.0593				P=0.0000		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

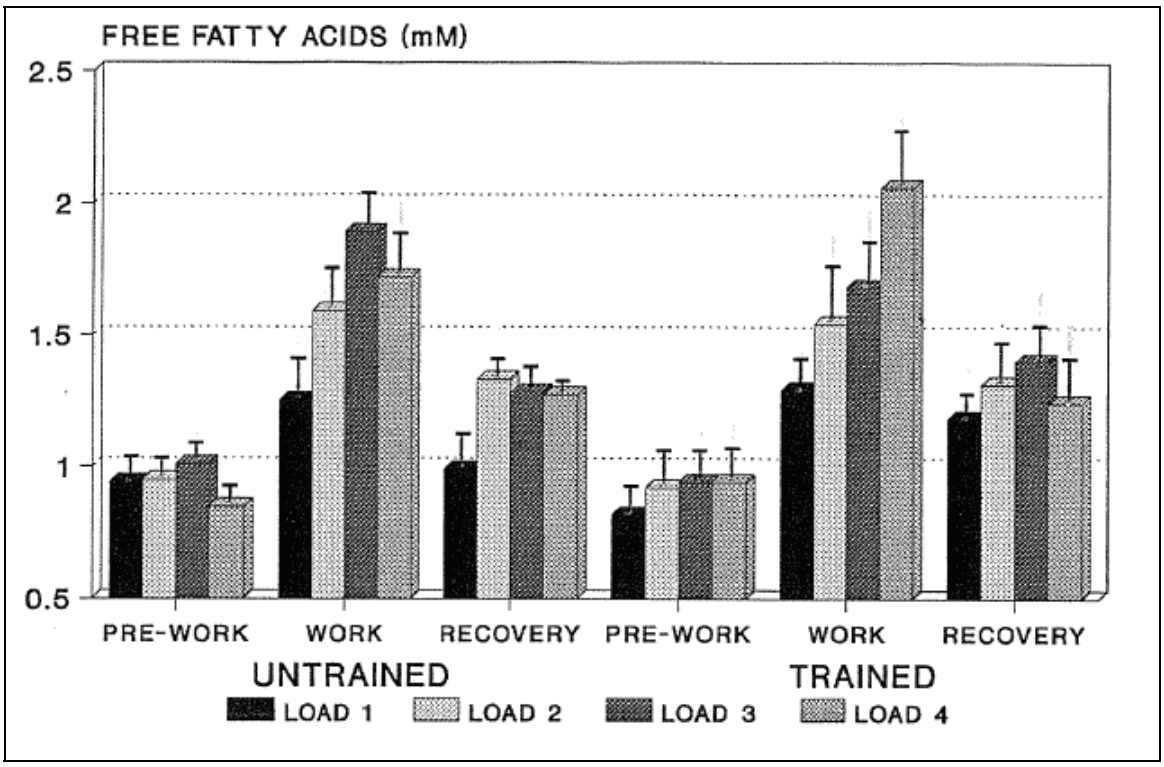


Figure 3A.35 Mean concentrations of free fatty acids in plasma of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

The concentration of plasma FFA was not significantly affected by either physiological state or work load (table 3A.17) although there was a trend of increasing FFA concentration with increasing work load (figure 3A.35). Work status significantly affected the concentration of FFA (table 3A.17).

The percentage increases in FFA concentrations of plasma of untrained buffalo during the *Work* period were 33, 67, 87 and 102% for loads 1, 2, 3 and 4 respectively. The corresponding increases in the trained animals were 57, 67, 79 and 119% respectively.

The effect of work on the concentration of plasma FFA is clearly illustrated in figure 3A.36 where data were pooled across physiological states and work loads for the three work status.

The changes in the pattern of FFA concentration over time across work status are presented in figure 3A.37–3A.40.

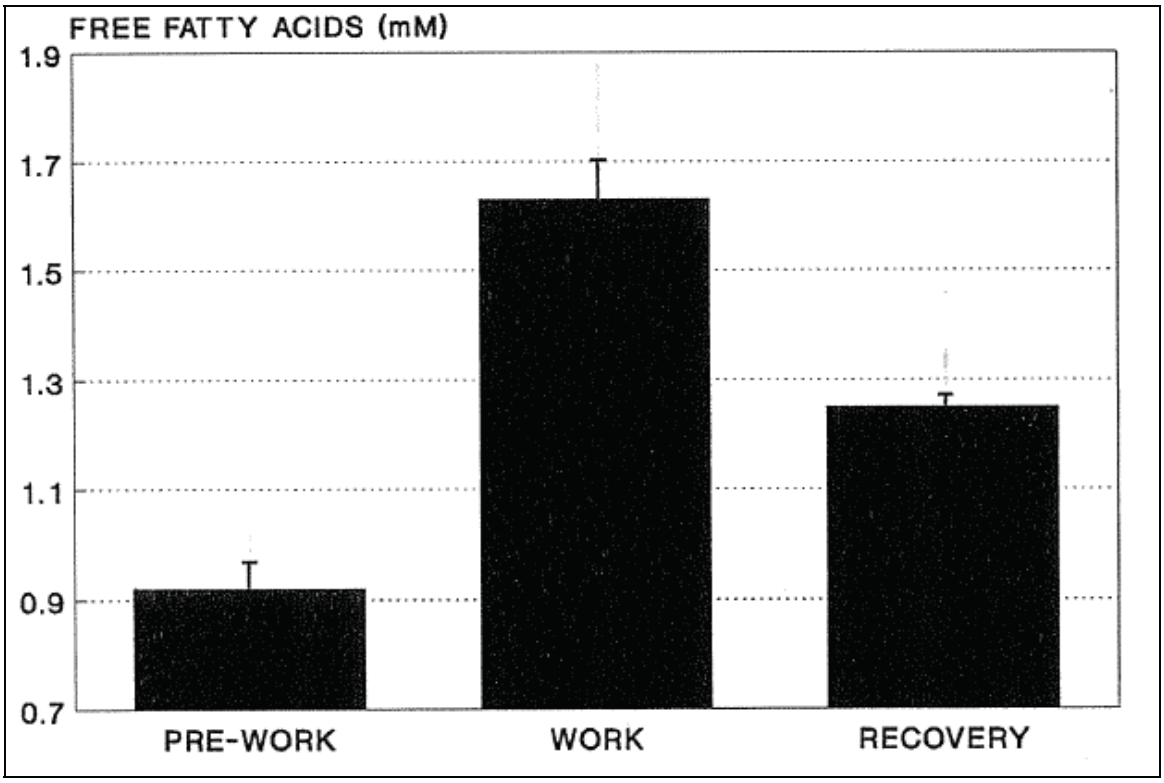


Figure 3A.36 Means of pooled data on FFA concentrations in plasma of buffalo during the *Pre-work*, *Work* and *Recovery* periods. **Vertical lines above bars represent standard errors.**

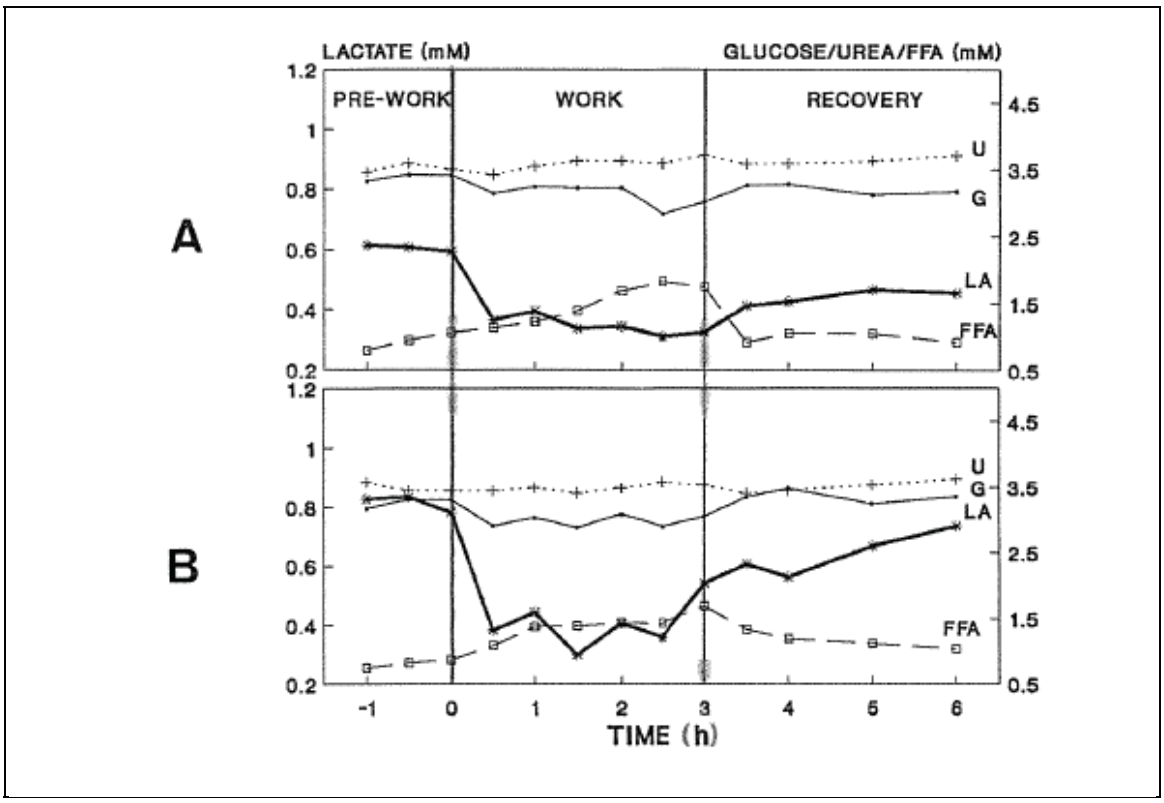


Figure 3A.37 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Pre-work, Work* and *Recovery*) at a load equivalent to 0% of live weight.

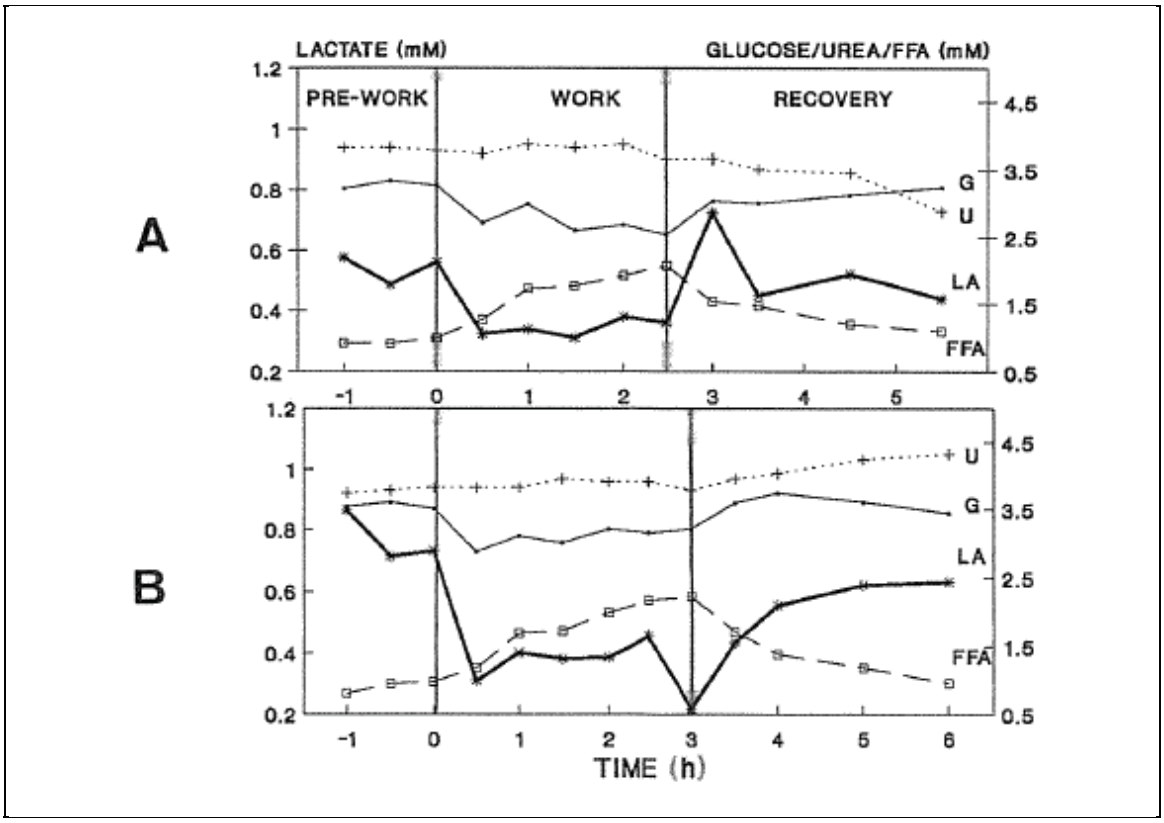


Figure 3A.38 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Pre-work, Work* and *Recovery*) at a load equivalent to 5% of live weight.

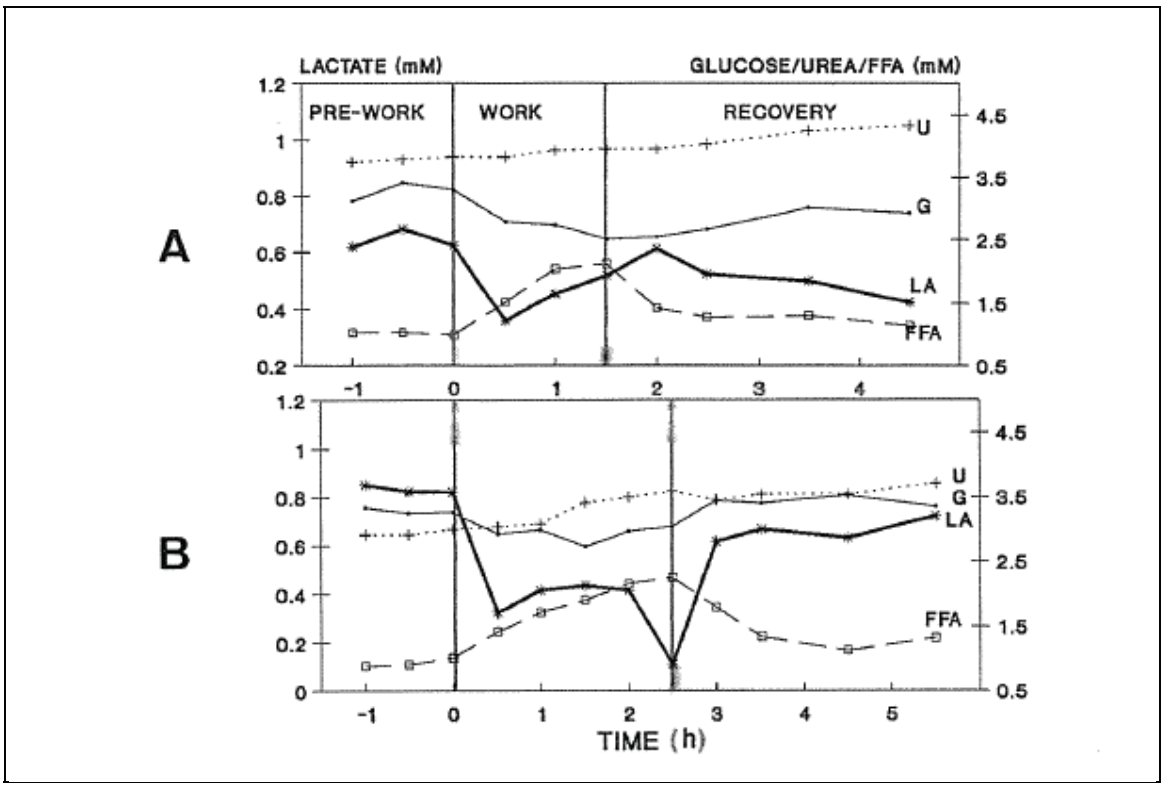


Figure 3A.39 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Pre-work, Work* and *Recovery*) at a load equivalent to 8% of live weight.

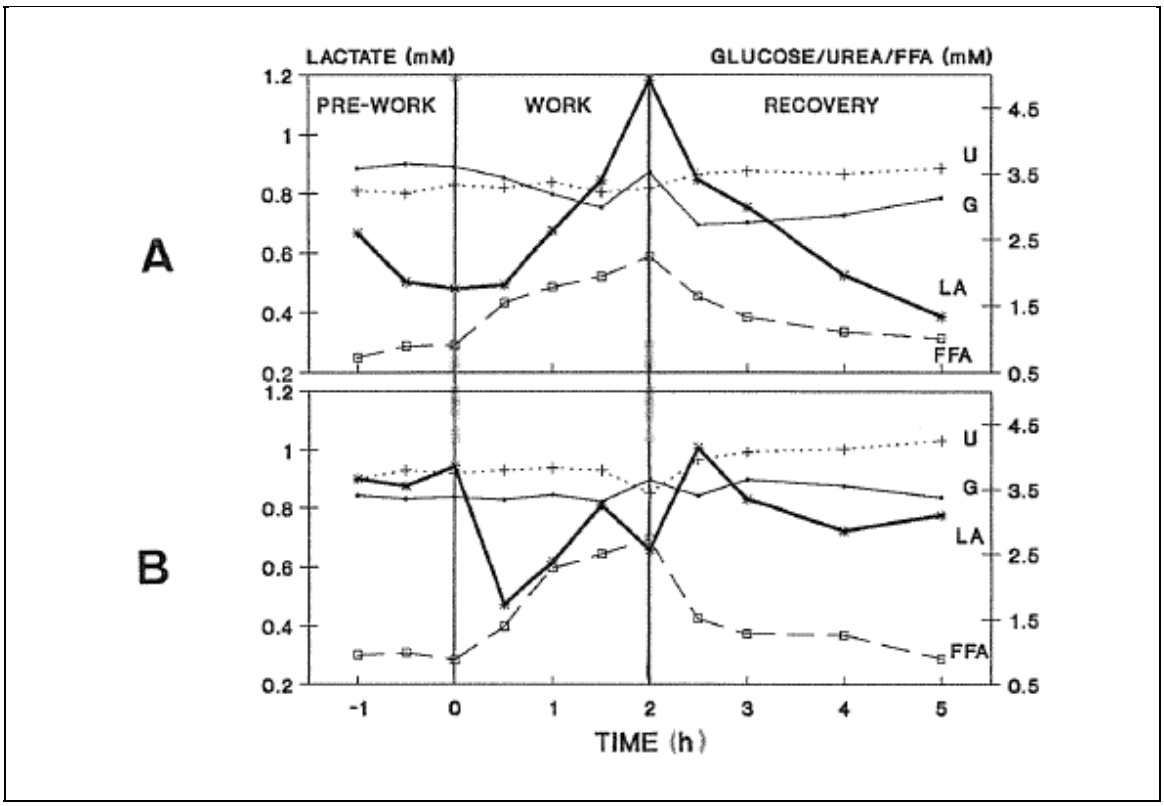


Figure 3A.40 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) concentrations of (A) untrained and (B) trained buffalo over work time across work status (*Pre-work, Work* and *Recovery*) at a load equivalent to 11% of live weight.

Oxygen

Data on O_2 concentrations in blood of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.41.

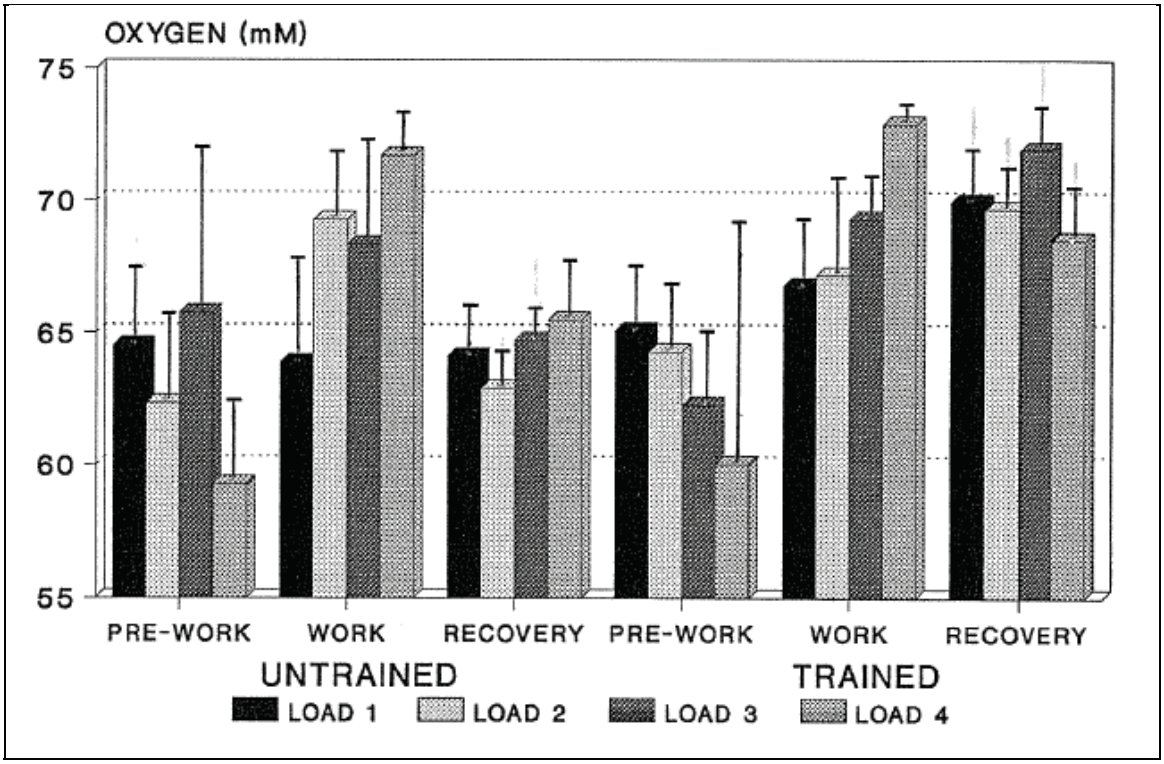


Figure 3A.41 Mean concentrations of oxygen in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean O_2 concentrations between physiological states, work loads and between work status are presented in table 3A.18.

Table 3A.18 Means of pooled data on oxygen concentrations in blood of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
Oxygen (mM)	64.84	68.39	66.25	66.08	67.55	66.58	62.94	68.66	67.16
± SE	0.67	0.76	2.56	2.50	2.97	3.08	1.98	1.30	0.81
Significance	P=0.0028		P=0.8498				P=0.0051		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

There were significant differences in O_2 concentrations between physiological states and work status (table 3A.18). The difference between work loads was not significant (table 3A.18).

The mean concentration of O_2 increased during the *Work* period by 8 and 10% respectively in both untrained and trained buffalo (figure 3A.41). Subsequent to work, O_2 concentrations in the untrained animals decreased during the *Recovery* period while corresponding values in the trained animals increased to above *Work* values. This is more clearly illustrated in figure 3A.42 where data for different work loads were pooled.

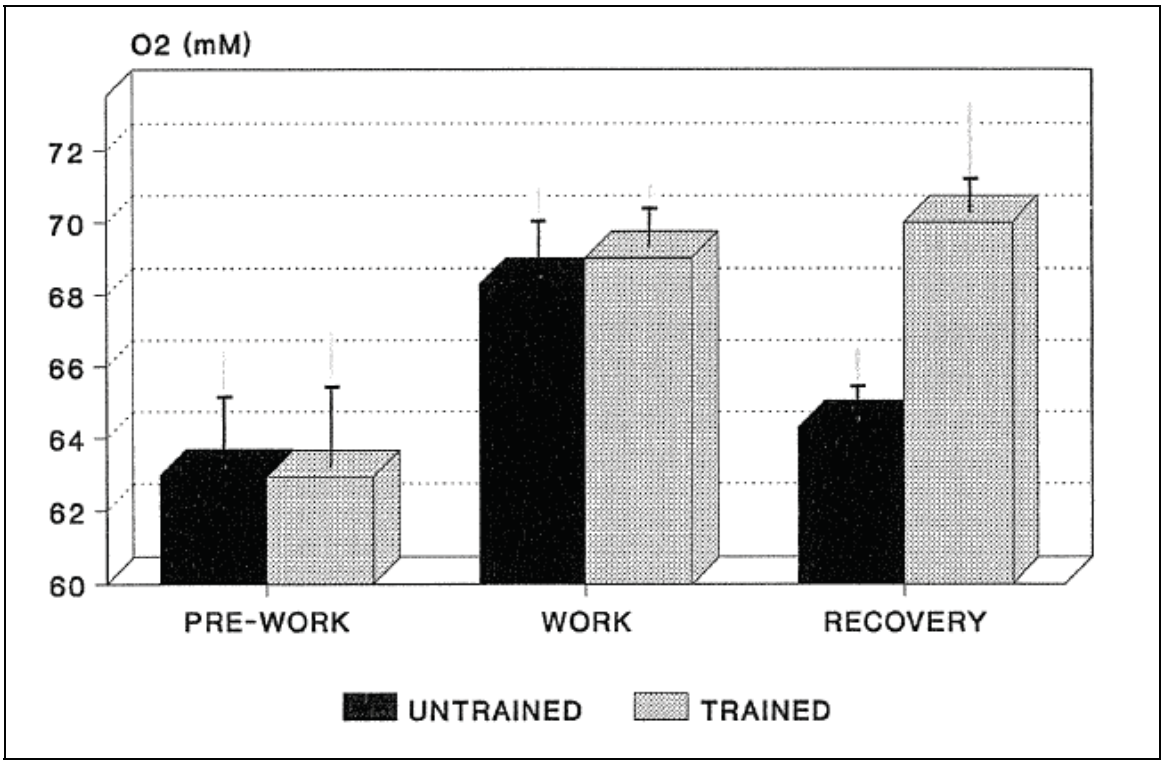


Figure 3A.42 Mean concentrations of oxygen in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

The changes in the pattern of concentration of O_2 in blood of buffalo over time across work status, are presented in figures 3A.49–3A.52.

Total carbon dioxide

Data on TCO_2 concentrations in blood of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.43.

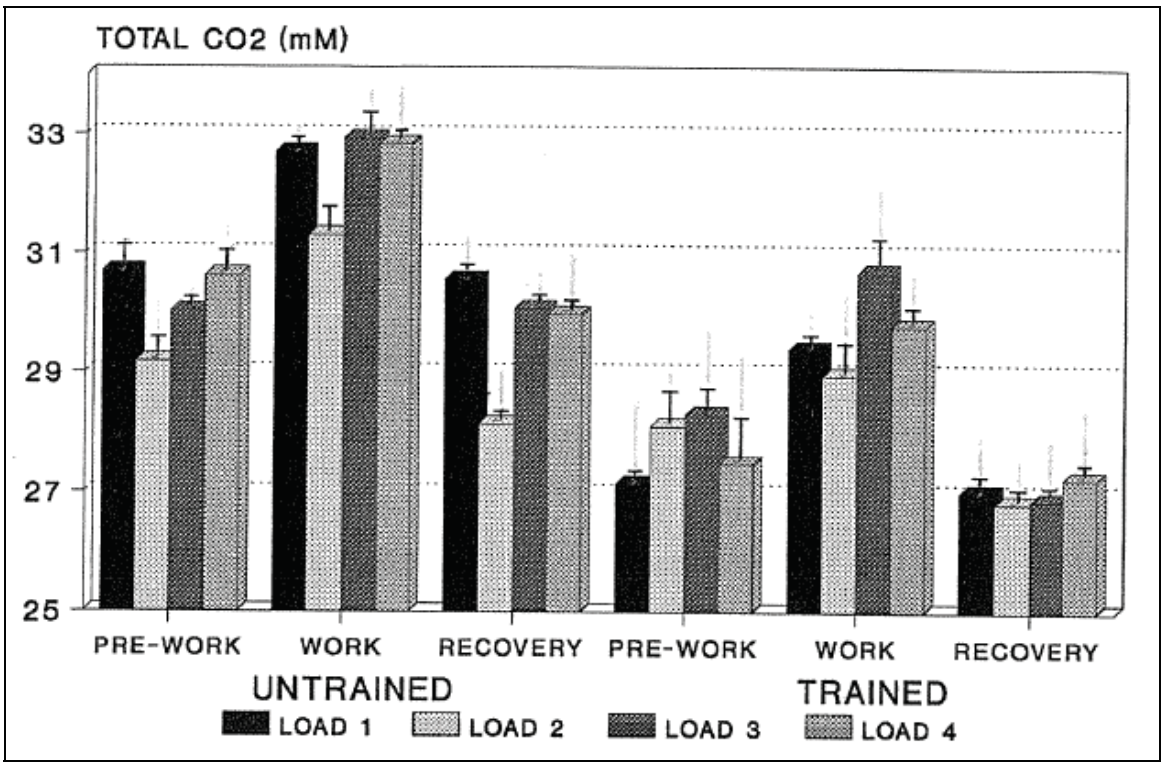


Figure 3A.43 Mean concentrations of total carbon dioxide in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean TCO_2 concentrations between physiological states, work loads and between work status are presented in table 3A.19.

Table 3A.19 Means of pooled data on total carbon dioxide (TCO_2) concentrations in blood of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
TCO_2 (mM)	30.33	27.69	29.27	28.25	29.28	29.24	28.94	31.07	28.34
± SE	0.22	0.25	0.78	0.88	0.97	1.04	0.53	0.45	0.26
Significance	P=0.0000		P=0.5492				P=0.0007		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

There were significant differences in TCO_2 concentrations between physiological states and work status (table 3A.19). The difference between work loads was not significant (table 3A.18). Overall TCO_2 concentrations were lower in the trained compared to untrained animals (figure 3A.43). The mean concentration of TCO_2 increased during the *Work* period by 8 and 7% respectively in both untrained and trained buffalo (figure 3A.43). Subsequent to work, TCO_2 concentrations in both the untrained and trained buffalo decreased to below *Pre-work* values. This is more clearly illustrated in figure 3A.44.

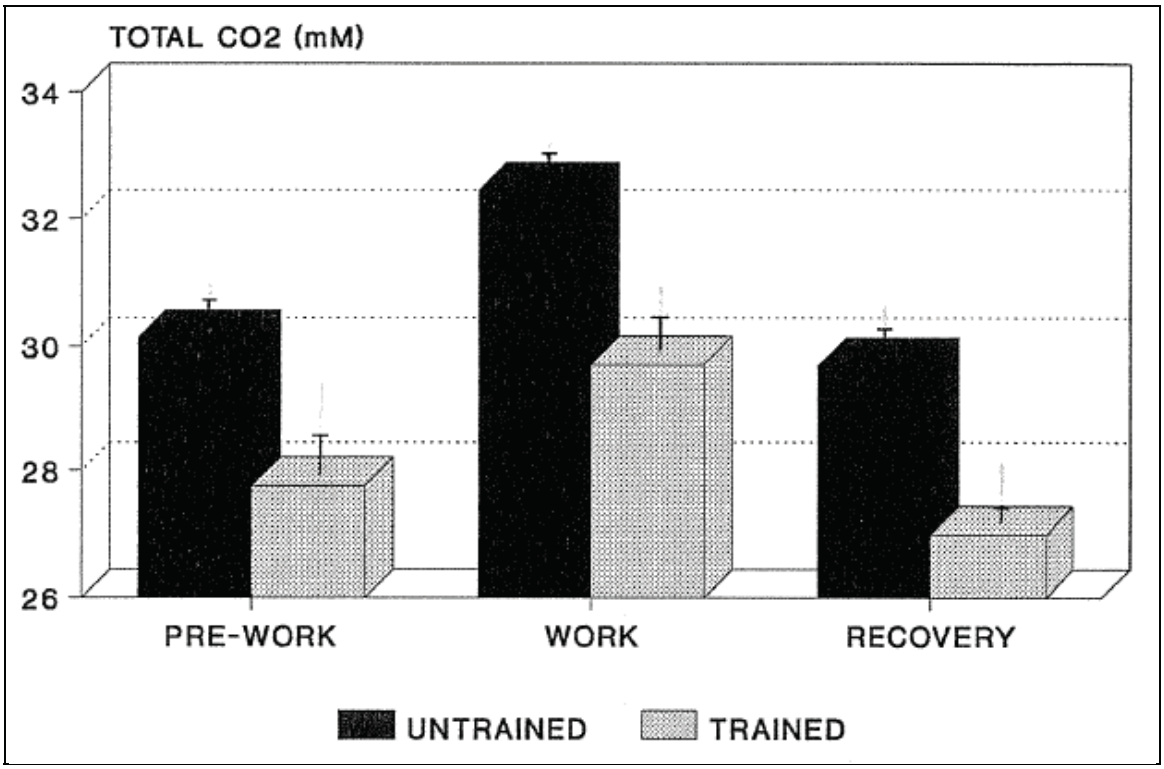


Figure 3A.44 Mean concentrations of total carbon dioxide in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

The changes in the pattern of concentration of TCO_2 in blood of buffalo over time across work status, are presented in figures 3A.49–3A.52.

Bicarbonate

Data on HCO_3 concentrations in blood of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.45.

Pooled data, comparing the difference in mean HCO_3 concentrations between physiological states, work loads and between work status are presented in table 3A.20.

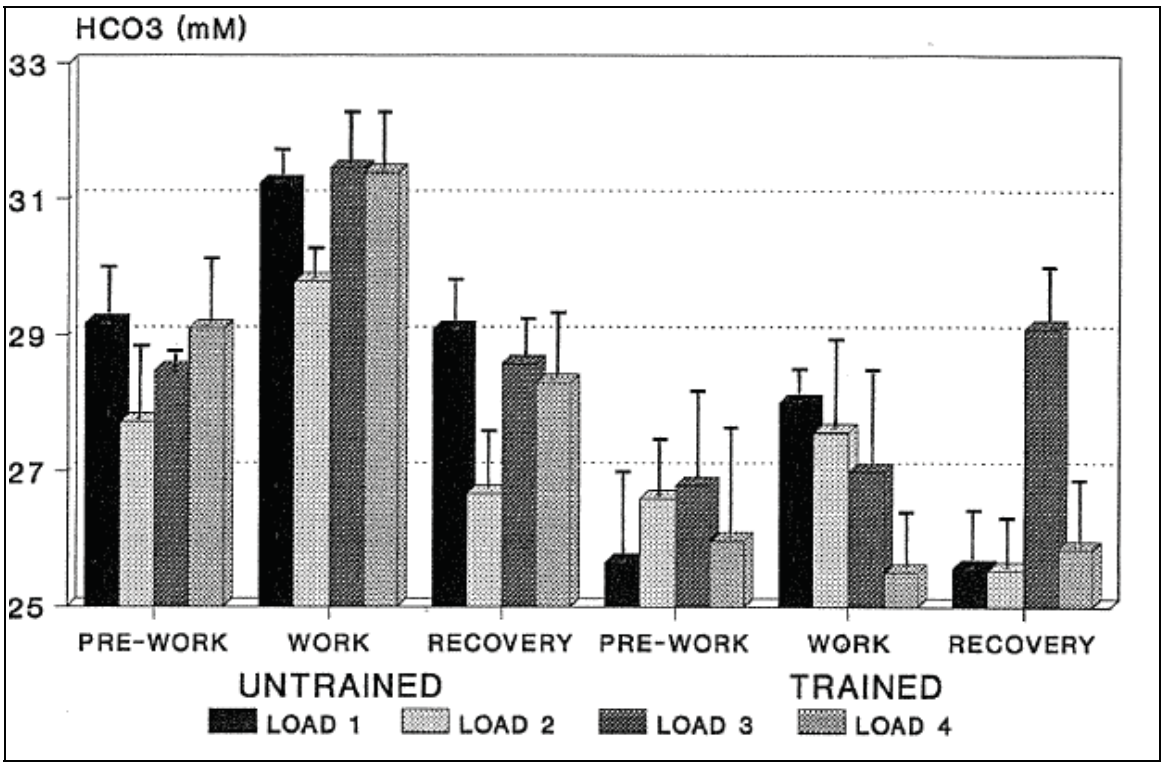


Figure 3A.45 Mean concentrations of bicarbonate in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Table 3A.20 Means of pooled data on bicarbonate (HCO_3) concentrations in blood of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
HCO_3 (mM)	28.81	26.16	27.80	26.80	27.60	27.74	27.44	29.39	26.87
± SE	0.22	0.24	0.75	0.86	0.86	1.02	0.52	0.44	0.25
Significance	P=0.0000		P=0.6092				P=0.0021		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

There were no significant differences in HCO_3 concentration between work loads (table 3A.20). There were significant differences in HCO_3 between physiological states and work status (table 3A.20). Overall blood HCO_3 concentrations were lower in trained compared to untrained animals. The mean HCO_3 concentration increased during the *Work* period by 8 and 6% respectively in both untrained and trained buffalo (figure 3A.45). The HCO_3 concentrations in both the untrained and trained buffalo decreased to below *Pre-work*

values during the *Recovery* period. This is more clearly illustrated in figure 3A.46 where data were pooled across work loads.

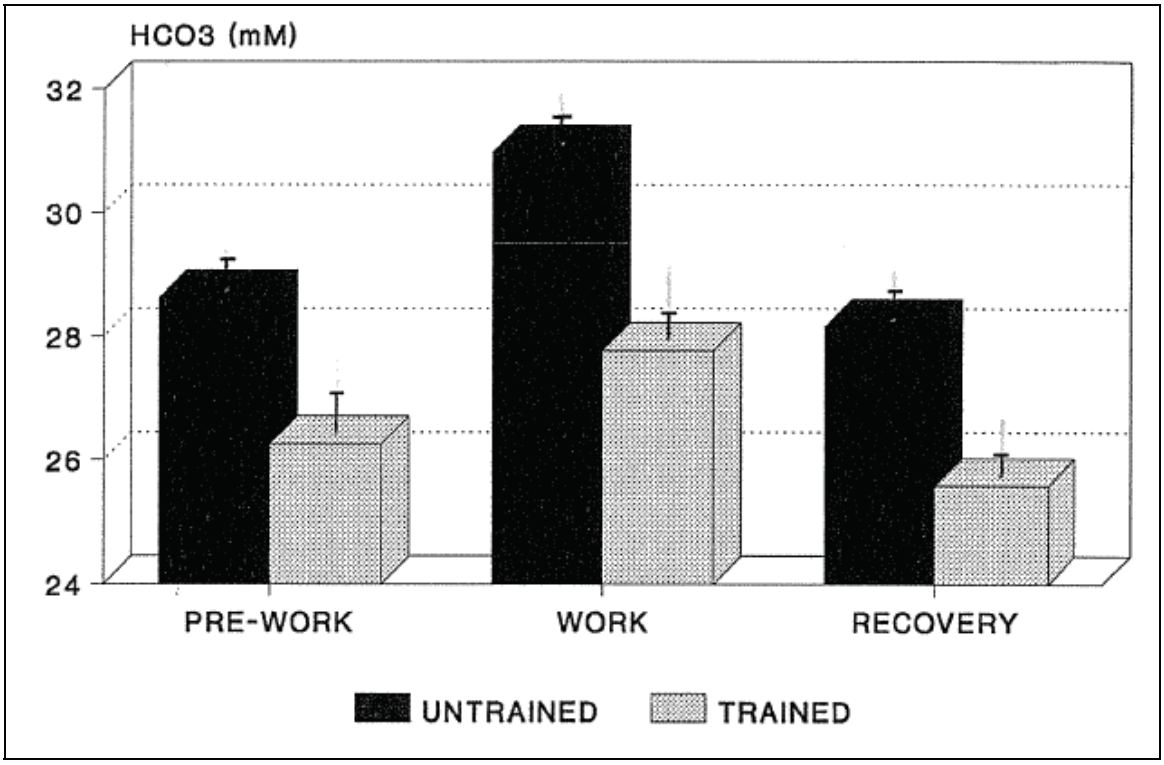


Figure 3A.46 Mean concentrations of bicarbonate in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

The changes in the pattern of concentrations of HCO_3 over time across work status are presented in figures 3A.49–3A.52.

Partial pressure of carbon dioxide

Data on pCO_2 in blood of untrained and trained buffalo under different work loads and work status are summarised in figure 3A.47.

Pooled data, comparing the difference in mean pCO_2 between physiological states, work loads and between work status are presented in table 3A.21.

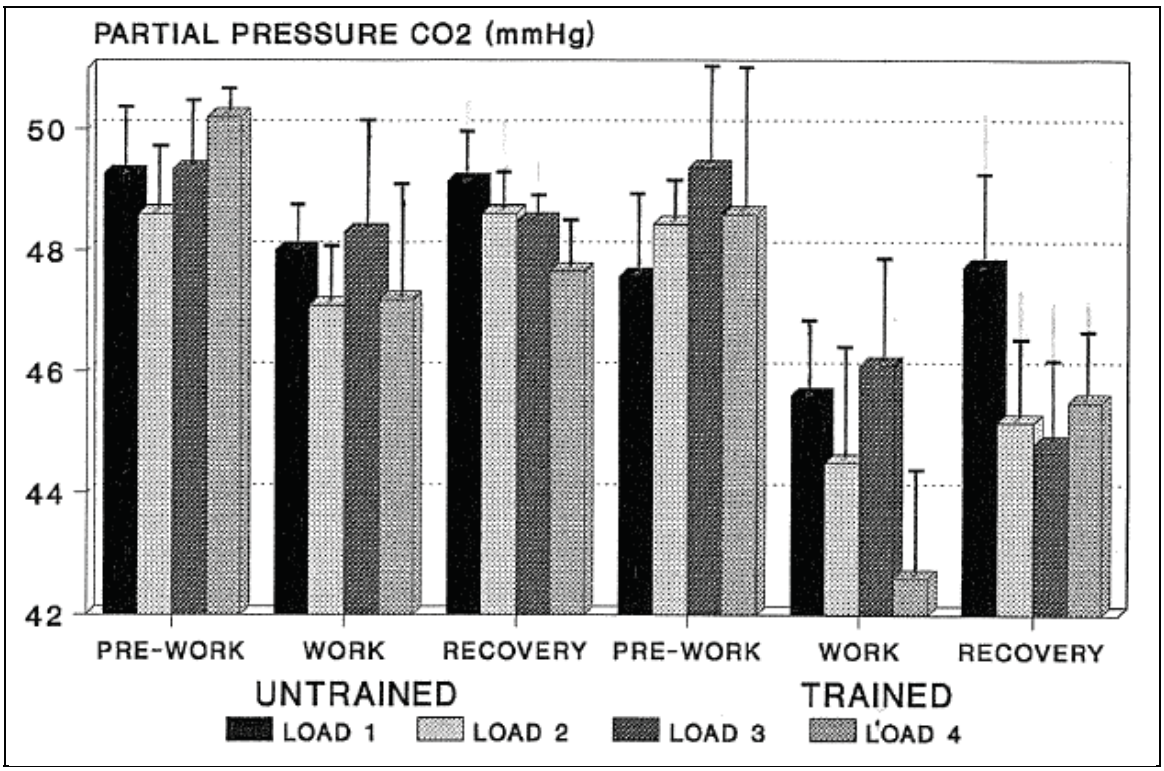


Figure 3A.47 Mean concentrations of partial pressure of carbon dioxide in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*) and work loads (1–4) where 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively. **Vertical lines above bars represent standard errors.**

Table 3A.21 Means of pooled data on partial pressure of carbon dioxide (pCO_2) in blood of buffalo for physiological states, work loads and work status
Values are means ± standard errors (SE)

	Treatments								
	Physiological states[1]		Work load[2]				Work status[3]		
	UNT	T	1	2	3	4	Pre-W	W	REC
pCO_2 (mm Hg)	48.48	46.11	48.10	46.99	47.29	46.80	48.92	46.18	47.13
± SE	0.26	0.46	1.20	1.18	1.65	1.45	0.66	0.73	0.50
Significance	P=0.0000		P=0.5035				P=0.0155		

[1] UNT = untrained; T = trained
[2] 1–4 are equivalent to 0, 5, 8 and 11% of live weight respectively
[3] Pre-W = pre-work period; W = work period; REC = recovery period

There were no significant differences in pCO_2 between work loads. Physiological states and work status had a significant effect on pCO_2. Overall, pCO_2 was lower in trained compared to untrained buffalo. The mean pCO_2 decreased by 3 and 8% respectively during the *Work* period in both untrained and trained buffalo (see figure 3A.47). During the *Recovery* period pCO_2 increased for both untrained and trained buffalo to below *Pre-work* values. This is more clearly illustrated in figure 3A.48 where data were pooled across work loads.

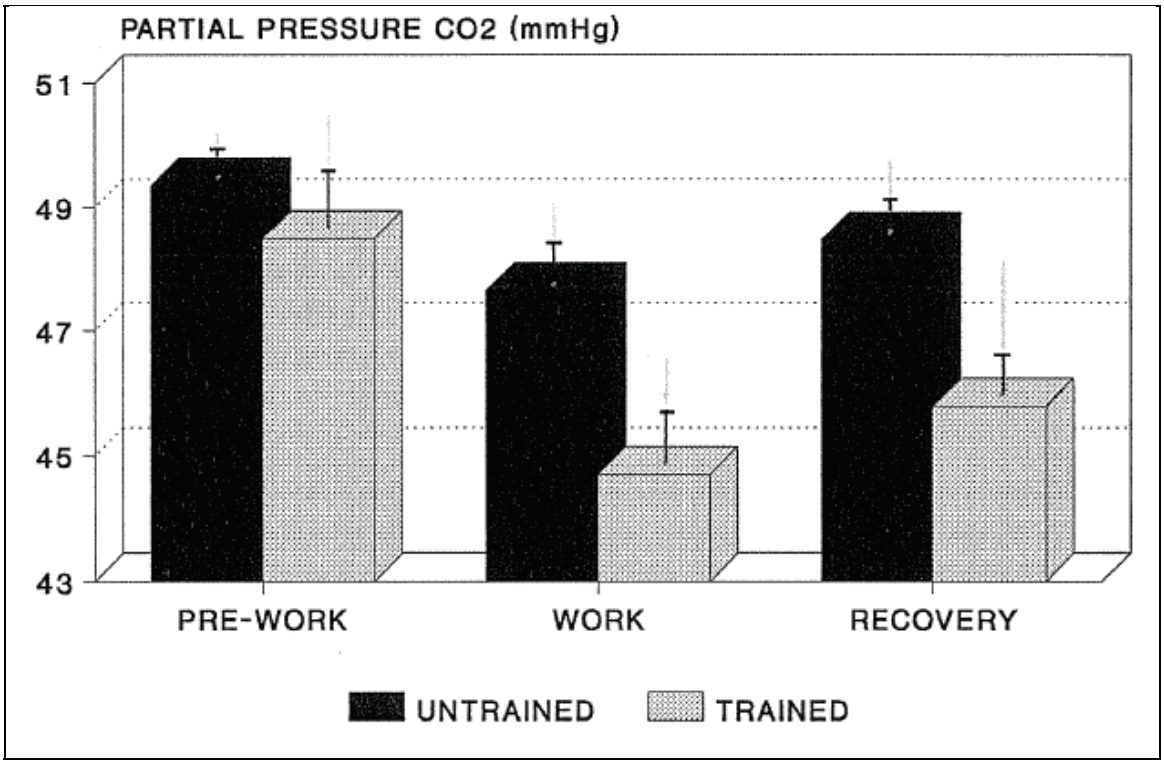


Figure 3A.48 Mean concentrations of partial pressure of carbon dioxide in blood of untrained and trained buffalo under different work status (*Pre-work*, *Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

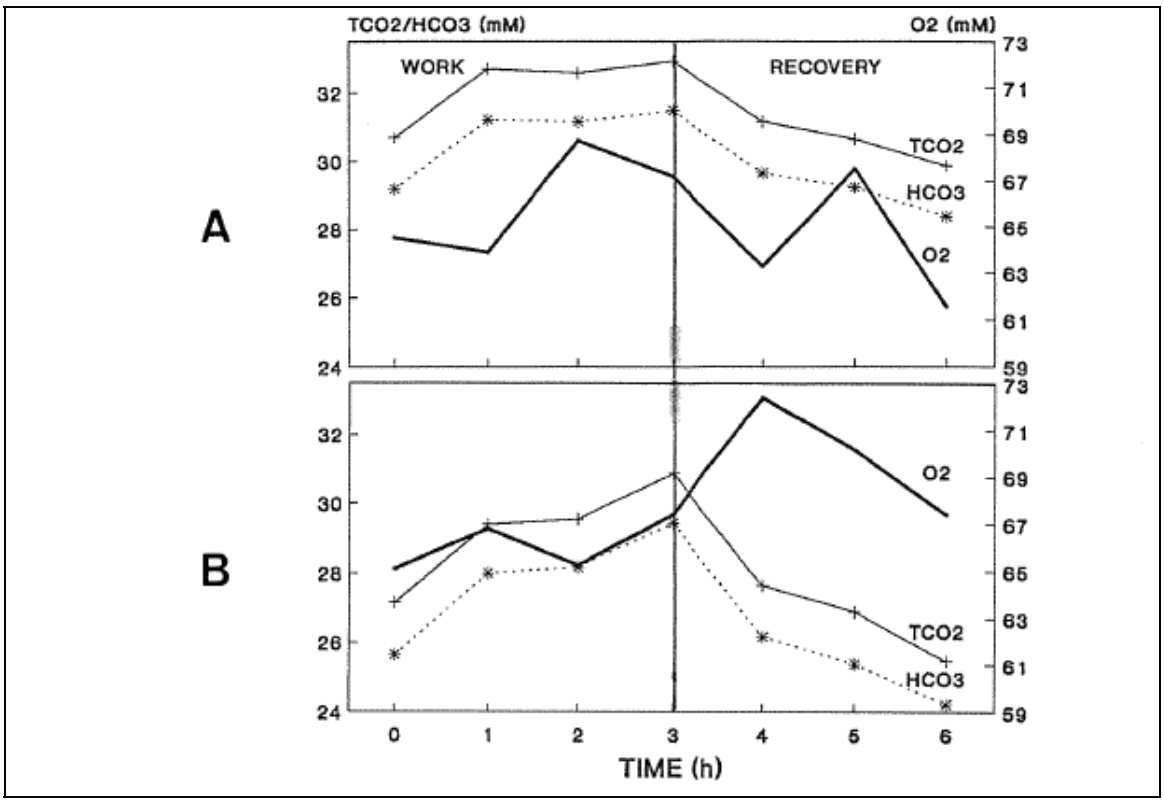


Figure 3A.49 Changes in concentrations of total carbon dioxide (TCO_2), oxygen (O_2) and bicarbonate (HCO_3) in blood of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 0% of live weight.

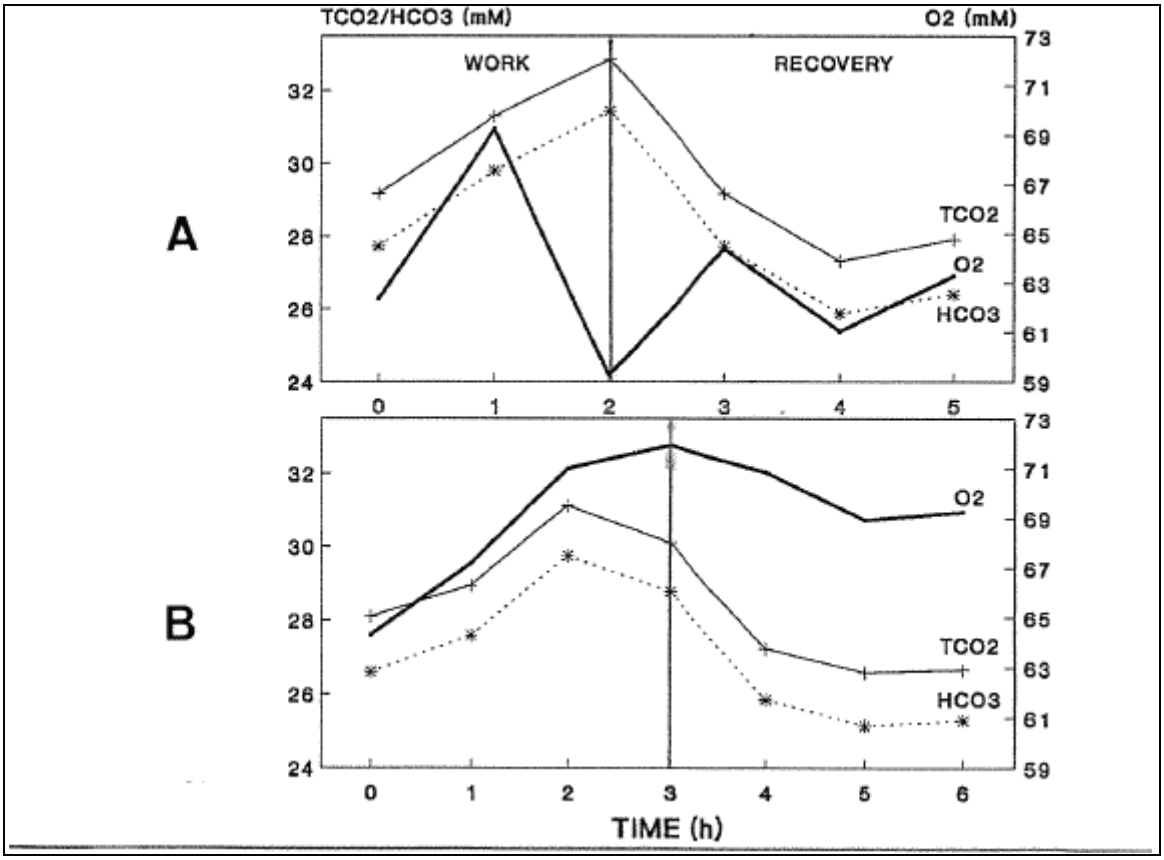


Figure 3A.50 Changes in concentrations of total carbon dioxide (TCO_2), oxygen (O_2) and bicarbonate (HCO_3) in blood of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 5% of live weight.

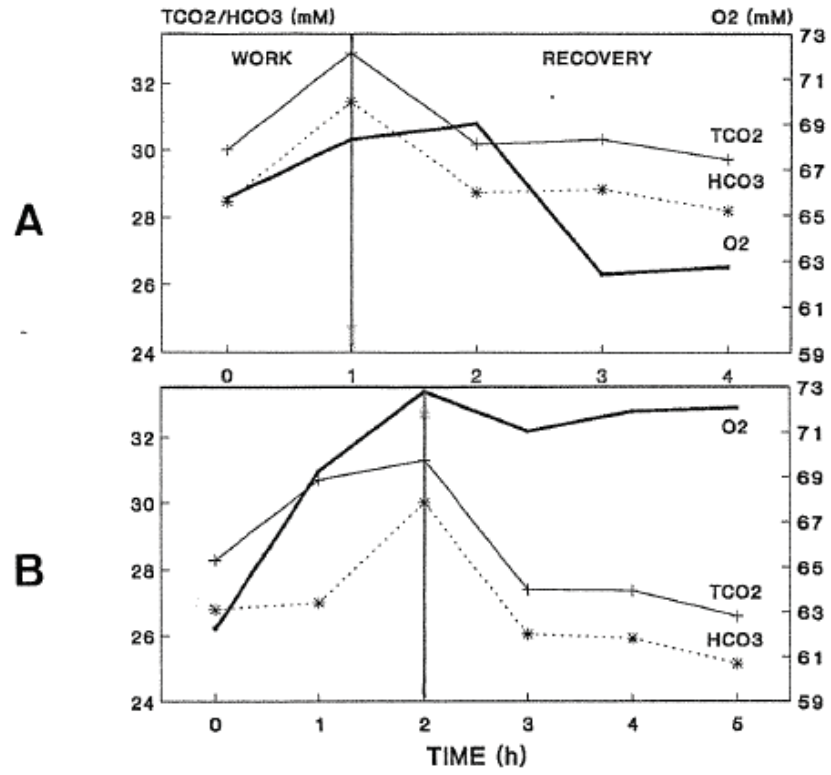


Figure 3A.51 Changes in concentrations of total carbon dioxide (TCO_2), oxygen (O_2) and bicarbonate (HCO_3) in blood of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 8% of live weight.

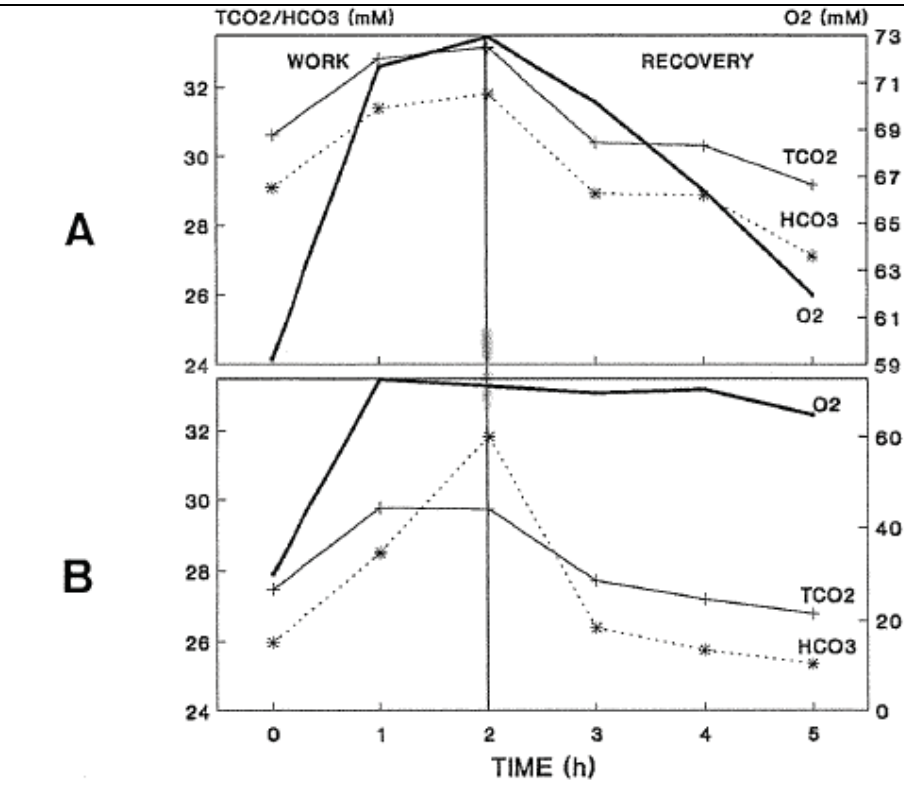


Figure 3A.52 Changes in concentrations of total carbon dioxide (TCO_2), oxygen (O_2) and bicarbonate (HCO_3) in blood of (A) untrained and (B) trained buffalo over work time across work status (*Work* and *Recovery*) at a work load equivalent to 11% of live weight.

3A.4 Discussion

Work capacity

In this experiment, it is clear that training for a period of about 25 d can increase work capacity. The untrained buffalo was not able to sustain pulling a draught load equivalent to 11% of its live weight for more than 107 minutes, whereas its trained counterpart was able to pull the same load for approximately 128 minutes; an increase of work capacity of 20%. This acquired capacity by the trained animal allowed it to pull a heavier draught load (11% of live weight) for a similar period of time as an untrained animal would pull a lighter draught load (8% of live weight).

Lactic acid and acid-base balance

The accumulation of lactate has been shown to induce acidosis (Metzger & Fitts 1987; Allen et al. 1989). In the untrained animal, the OBLA was observed after 30 minutes of work when the animals were subjected to work loads 3 and 4 [figures 3A.39(A) and 3A.40(A)]. The OBLA was also observed in the trained animals subjected to load 4 [figure 3A.40(B)]. However, it was in the untrained animals on load 4 that the OBLA was most sharply defined [figure 3A.40(A)]. The reduction in concentrations of plasma lactate during the work period in animals on loads 1 and 2 for example, would indicate the increased clearance rate of the metabolite by the liver. It would appear, in general, that the clearance rate of lactate from blood of the trained animals was greater than that from blood of the untrained animals. This is consistent with reports by MacRae et al. (1992), Donovan and Brooks (1983) and Oyono-Enguelle et al. (1990).

That lactate production was decreased to a greater extent in the trained animals is also a possibility (see MacRae et al. 1992). It is highly likely, however, that lactate production was significantly increased in the untrained animals on load 4. Despite the OBLA observed, there was a mild respiratory alkalosis occurring across all loads and physiological states (see figures 3A.25 and 3A.45 for pH and HCO_3^- concentration). There was no acidosis due to the buffering by the bicarbonate system. Respiratory alkalosis was also observed in working sheep (Pethick et al. 1991) and in working cattle and buffalo (Martin et al. 1989). Evidence for respiratory alkalosis include reduced pCO_2, increased HCO_3^- and pH values (figures 3A.38, 3A.45 and 3A.25). Respiratory alkalosis is caused by hyperventilation resulting in excessive elimination of CO_2 and a reduction in blood H_2CO_2 concentration (reflected by the reduced pCO_2). It is not clear whether respiratory alkalosis increases work capacity by delaying the onset of fatigue. Alkalosis results in an increased concentration of plasma lactate. This is due to an increase in the rate of release of lactate from working muscles (Mainwood et al. 1972; McCartney et al. 1983). This increase in plasma lactate may be due

to an increased rate of lactate release or an enhanced rate of glycolysis, possibly leading to glycogen depletion.

Hyperthermia

Hyperthermia may be a factor contributing to fatigue. A rise in RT of 2.5☜C or greater above normal resting RT is considered to be intolerable to animals (Upadhyay & Madan 1987). Hyperthermia has also been shown (Edwards et al. 1972; Kozlowski et al. 1985) to cause increased rates of glycolysis, possibly leading to glycogen depletion, as well as reduced O_2SAT. In this experiment RT greater than 2.5☜C above normal resting values were observed in trained buffalo on loads 3 and 4 (2.6☜C and 2.76☜C respectively) and in the untrained animals on load 4 (2.73☜C). Since the trained animals worked for longer periods than did the untrained animals (table 3A.5) it appears that factors other than increased RT were important in the onset of fatigue, e.g. in the untrained animals on load 3.

The experiments were conducted under cover so no direct solar radiation contributed to the total heat load of the animals. Thermoregulatory mechanisms were stimulated to counteract increases in heat load in the working animals. Respiration rate increased as RT increased (figure 3A.53). This occurred at a rate of 15 breaths/min (RR) per 0.5☜C increase in RT. It might be suggested that the rate of heat generation by the working muscles of the buffalo was slow enough for RR to play a major role in cooling the animals.

Evaporative cooling of the respiratory passage was facilitated by increased RR. The parallel increase in PR (figure 3A.54) would have increased the blood flow to the skin and respiratory passages of the working buffalo. Blood flows to the skin and respiratory muscles have been shown to increase during exercise and while those to the hind-limb muscles decrease (Bell & Hales 1985).

As would be expected, the increased RT was reflected in increased ST ($r=0.6766$; $p=0.0000$).

The increased RT observed as work load was increased (see figure 3A.6) would have been due to the increased metabolic heat produced as metabolites were oxidised to supply energy to muscles.

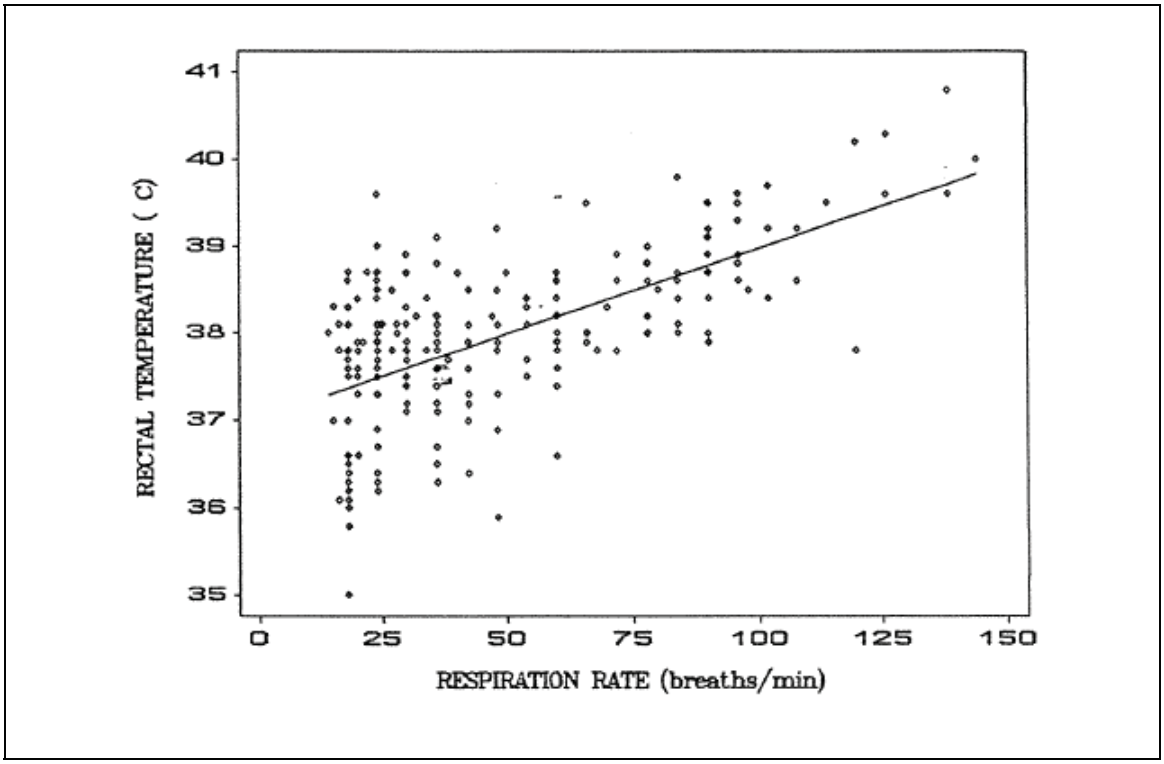


Figure 3A.53 The relationship between rectal temperature and respiration rate. **(y=0.02x + 37.03; r=0.9050; P=0.0000)**

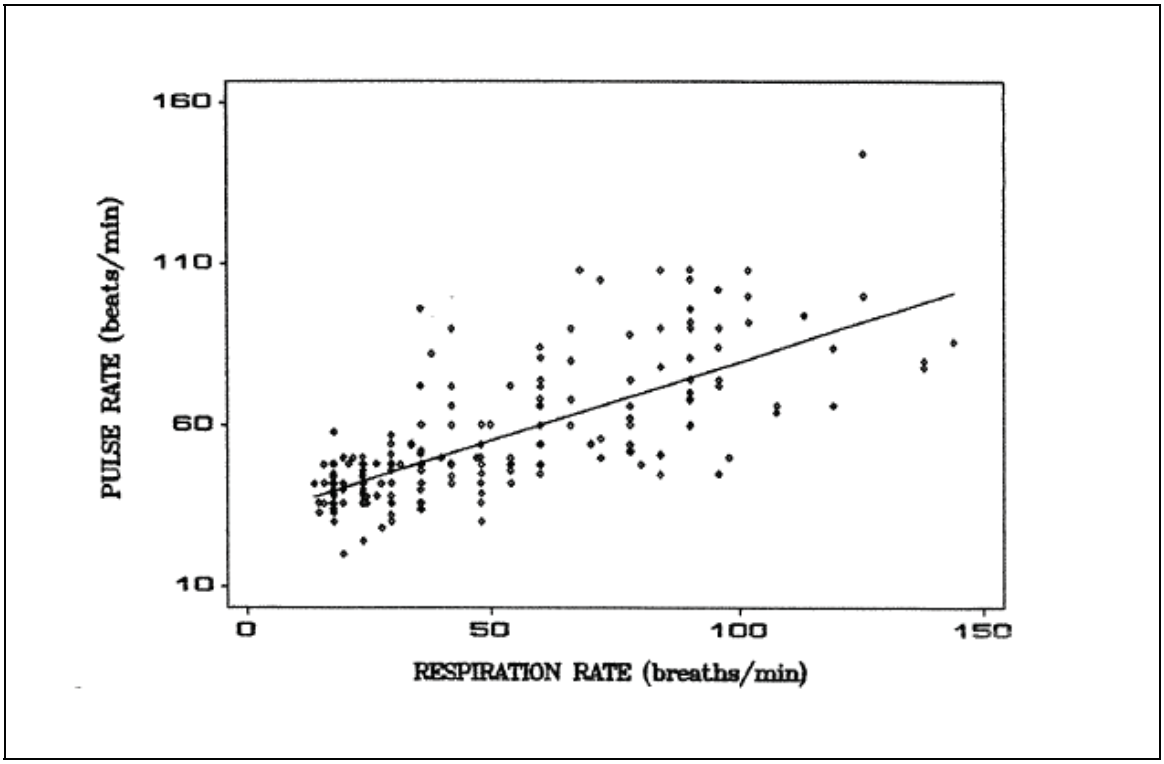


Figure 3A.54 The relationship between respiration rate and pulse rate. **(y=0.49x + 31.23; r=0.8965; P=0.0000)**

Substrate utilisation

Since blood samples were taken from the jugular vein, only general assumptions can be made on muscle metabolism in the animals studied. An increase in the concentration of a metabolite may indicate its release from the hind limb muscle or from other storage sites. A decrease in concentration may imply an uptake of the metabolite by the muscle or other tissues and/or a decreased release from storage sites.

In all animals except the untrained ones on load 4, lactate concentration in plasma decreased in the first 30 minutes of work, possibly indicating an uptake of the metabolite by the liver for gluconeogenesis and/or an uptake by working muscles for oxidation. In the untrained buffalo on loads 1 and 2 and in the trained ones on loads 1, 2, and 3, lactate concentration remained stable (after the initial reduction) throughout the *Work* period indicating that the rate of production of the metabolite was near equal to its clearance rate. A further reduction in lactate concentration in the last 30 minutes of work was observed in the trained animals on loads 2 and 3. Thus it might be suggested that in untrained buffalo on loads 1 and 2 and in the trained buffalo on loads 1, 2 and 3, muscle metabolism was predominantly aerobic.

As previously indicated (section 3A.3.4) the OBLA was observed in the untrained buffalo on loads 3 and 4 and in the trained buffalo on load 4. It is therefore likely that in these animals, on their respective work loads, there was a greater dependence on anaerobic metabolism for ATP supply to the working muscles. This is most likely in the untrained buffalo on load 4 in which the chances for the development of lactic acidosis would be greatest.

The general reduction in glucose concentration in plasma of the animals studied would suggest a net utilisation of this substrate by muscles. However, the strict narrow limits within which the concentration of blood glucose is maintained by homeostatic mechanisms is clearly illustrated by the relatively stable profile observed for glucose over time, across work status; particularly in the trained animals. This is consistent with the views of Donovan and Brooks (1983). There is the possibility also that in the trained animals, glucose was spared by the preferential use of FFA as the ATP-yielding substrate for the working muscle.

The fact that FFA is an important fuel in working muscle is indicated by the increased FFA concentration in plasma during the *Work* period. The increase was greater in animals on heavier loads. The greatest increases in FFA concentration occurred in the first hour of work when FFA mobilisation might be assumed to have exceeded uptake by muscles. The observed reduction in the rate of increase of FFA concentration (figures 3A.39 and 3A.40) probably indicate an increased uptake of FFA by skeletal muscle (see Pethick et al. 1983).

The continued increase in FFA concentration in the untrained buffalo on load 4 is probably due to the reliance by muscle on anaerobic metabolism rather than on FFA oxidation for ATP production. Lactate has been suggested to suppress FFA oxidation in dogs (see Issekutz et al. 1966).

Urea concentration increased in the plasma of trained buffalo on load 3 after 1 hour of work. This may indicate protein degradation in these animals. The reason why the same was not observed in untrained animals on loads 3 and 4 and in the trained animals on load 4 is probably the apparent dominance of anaerobic metabolism in muscles of these animals.

Cardiovascular system

The cardiovascular system was improved by training through an improved capacity to transport and distribute O_2 and other metabolites to relevant tissues. Evidence to support this can be seen in tables 3A.10 and 3A.11 in which the concentrations of haemoglobin and PCV were increased after training. The lower PR in the trained animals would allow it to achieve a higher maximum heart rate than would their untrained counterparts (see Rennie et al. 1974; Henriksson 1977). The lower capacity in the untrained animal for transportation of O_2 in blood might be a reason for the apparent dominance of anaerobic metabolism in muscles of these animals.

Conclusion

It might be concluded from the results of this experiment that

- work capacity is enhanced by training. This is due partly to the resulting increase in cardiovascular efficiency and to the greater clearance rate of lactate from the circulation.

- hyperthermia and alkalosis (may cause glycogen depletion) and not acidosis are probably the major factors causing the onset of fatigue as work load in buffalo is increased.

SECTION 3B

A COMPARISON OF WORK CAPACITY OF UNTRAINED AND TRAINED ONGOLE (*BOS INDICUS*), BALI (*BOS SONDAICUS*) AND MADURA COWS

3B.1 Introduction

In Indonesia, the breeds of cattle used for draught purpose in the Eastern provinces include the Ongoles (*Bos indicus*), the Bali [*Bos* (*Bibos*) *banteng* or *Bos sondaicus*] and the Madura. Largest of the breeds are the Ongoles (weighing up to 450 kg), next are the Bali (weighing up to 300 kg) and the Madura weighing up to 270 kg.

Despite the great variation in the sizes of these animals, they are all expected to perform the same amount of work in cropping land cultivation. That is, in ploughing land, the animals are expected to pull a draught load ranging from 40–80 kg (Teleni & Murray 1991). This can be critical to the capacity of an animal to perform work particularly in the case of the Madura where the work load relative to its live weight is greater than that for the Ongole.

Ways of increasing the work capacity of the smaller size breeds such as the Madura and Bali would be most important in the context of farming in Indonesia.

The experiment described in section 3A showed that a physically untrained buffalo (*Bubalus bubalis*) could not sustain pulling a draught load greater than that equivalent to 11% of its live weight for a typical working period of 3 h/d at a walking speed of 0.69 m/sec. Training, however, increased its work capacity and the animal was able to sustain pulling a the same draught load for a longer period of time.

Fatigue which occurred in the buffalo studied may have been due to hyperthermia, lack of substrate for ATP production or to indirect effects of lactate accumulation which was evident particularly in the untrained animals which were subjected to pulling draught loads equivalent to 11% of their live weights.

It might be suggested from the results of studies in section 3A that

- a short period of physical training would significantly increase the work capacity of an animal and

- since hyperthermia is probably the major cause of the onset of fatigue in animals working under normal field conditions, those animals with a greater capacity to maintain a lower body temperature would have a higher work capacity.

This study was undertaken to examine these suggestions by comparing the work capacity of Ongole, Bali and Madura cows under field conditions.

3B.2 Materials and Methods

3B.2.1 Location

This experiment was conducted in March/April 1991 at the branch of the Research Institute for Animals Production, Grati, Indonesia. Grati is a small village situated in East Java (figure 3B.1)

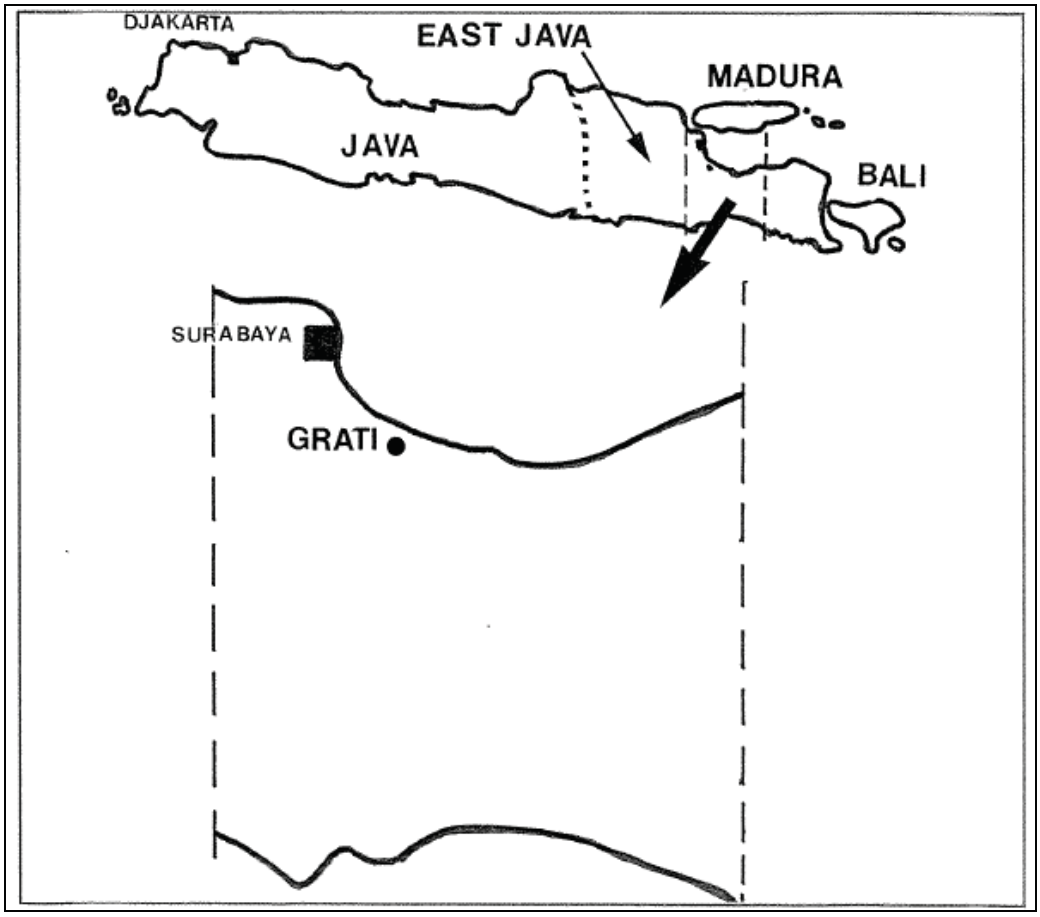
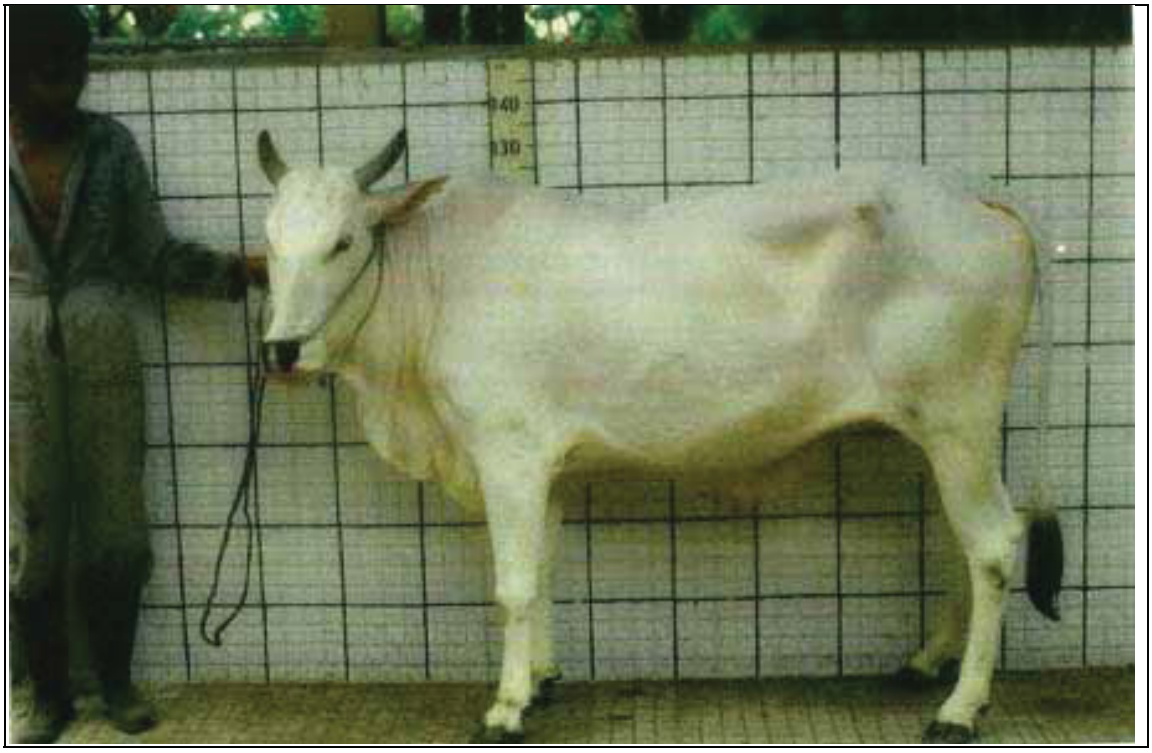


Figure 3B.1 The location of Grati in the province of East Java, Indonesia.

3B.2.2 Experimental animals

The Ongole is a white-coated animal which has its origin in India and is genetically identical to the Indian Brahman cattle (see photograph 3B.1) (Robinson 1977). The breed makes up approximately 65% of the cattle population of Indonesia.



Photograph 3B.1 An Ongole cow.

The Bali is a domesticated indigenous *banteng* (Meijer 1962) with a light brown coat and characteristic pale legs and rump (see photograph 3B.2). The breed comprises approximately 27% of the total cattle population of Indonesia.

The Madura is reddish-brown in colour and it makes up approximately 8% of the Indonesian cattle population (see photograph 3B.3). It is thought to be a cross between the Indian Brahman and the domesticated *banteng* (Robinson 1977).

The cows were obtained from surrounding farms but had not worked since the late ploughing season (approximately five months).

Photograph 3B.2 A Bali cow.

Photograph 3B.3 A Madura cow.

Management

Six Ongole, six Bali and six Madura cows with mean live weights of 257 ± 20 kg, 250 ± 22 kg and 220 ± 8 kg, respectively were used.

These animals were tethered in covered individual pens. The pens had cement floors and were cleaned daily. The animals were inspected daily for signs of ill-health. Fresh water was freely available.

Feeds and feeding

Each animal was fed a diet of chopped fresh elephant grass at a rate of 3.6 kg dry matter (DM) plus an amount of concentrate DM equivalent to 0.8% of its live weight. The elephant grass was fed at 1500 h and the concentrate at 1000 h and 1300 h daily.

The composition of the elephant grass and concentrate are shown in table 3B.1.

Table 3B.1 The dry matter (DM), protein, crude fibre (CF), ether extractives and ash content of elephant grass and concentrate fed to the experimental animals

	% of fresh weight	% of DM			
Feed	DM	Protein	CF	Ether extractives	Ash
Elephant Grass	16	10	31	2	12
Concentrate	84	17	10	5	6

Training

Training consisted of a 14 d period in which each pair of animals was made to walk for 2 h/d pulling a draught load (equivalent to 8% of the combined live weight of the animals) around a square track. All animals were accustomed to the work implements and conditions as well as to the sampling and measurement procedures by the time the first measurement period arrived.

Catheters

A catheter was inserted into an external jugular vein of each animal as described in section 3A.2.2. The catheter was located on the outer side of the neck of each animal in a pair to facilitate ease of sampling.

3B.2.3 Implements and equipment

Yokes

The yokes were made from bamboo which was as strong but much lighter than any other

wooden yoke (see photograph 3B.4).

Photograph 3B.4 A pair of working Ongole.

Draught Load

The draught load for each pair of animals was designed to be equivalent to approximately 12% of the total live weight of the pair. The components of the load included the relatively fixed weights of the metal sledge and the driver and the adjustable weights of concrete blocks (see photograph 3B.4).

The relationship between total weights of loads and amounts of draught force involved in moving the loads around a square dirt track at a constant speed was established in a preliminary study (see below). The relationship established allowed for the prediction of the approximate total load which would result in a draught force equivalent to 12% of the total live weight of a pair of animals.

The predicted weight was then tested under experimental conditions and adjusted, where necessary, by adding or subtracting concrete blocks.

The preliminary study

The measurement of draught force was conducted by Bakrie et al. (1991). Measurements were conducted on three pairs of each breed when pulling loads equivalent to 6, 8 and 12% of combined live weight around the square track (see figure 3B.2). A walking speed of 0.69 m/sec was adhered to as closely as possible. The angle force was recorded using a load cell (200–300 readings were recorded for each pair of cows). The duration of work and distance travelled were recorded at approximately every 100 m using a stop watch and an odometer. The angle of pull was measured for each pair using a protractor and a spirit level.

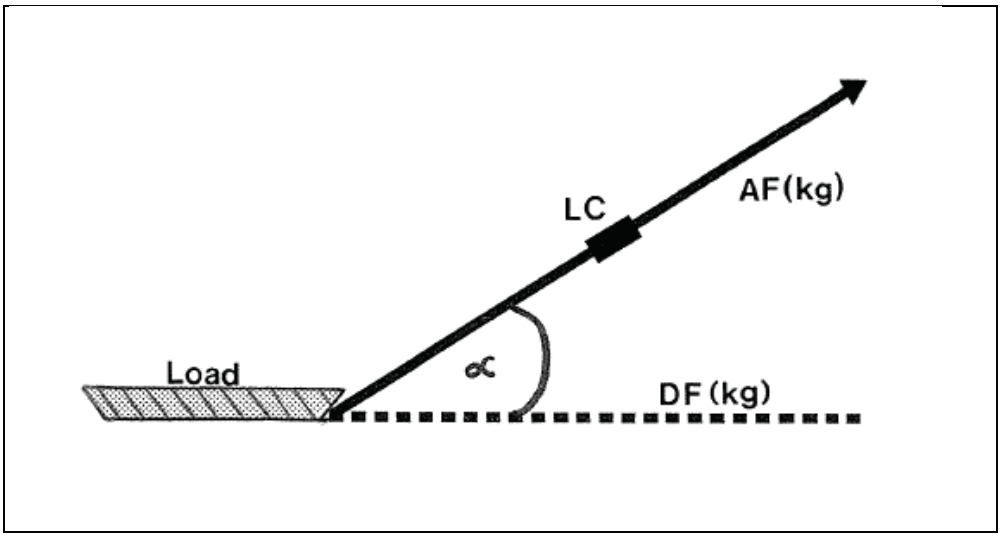


Figure 3B.2 The angle force (AF) measured by the load cell (LC) attached between the yoke and the load. **Draught force (DF) or the horizontal force component is calculated using AF and angle of pull (α).**

Draught force (DF) was calculated from the angle force (AF) and the angle of pull (α), using the equation

$$DF\ (kg) = AF\ (kg) \times \cos \alpha$$

The relationship established between draught force and load established using pooled data is illustrated in figure 3B.3.

Data recorded for angle of pull and walking speed for the three breeds are shown in table 3B.2.

Table 3B.2 The angle of pull (AP) and speed of walking (WS) while pulling a load equivalent to 12% of LW for Ongole, Bali and Madura cows **(Source: Bakrie et al. 1991)**

Breed	AP	WS
Ongole	22.0 ± 1.6	1.04 ± 0.060
Bali	21.0 ± 1.4	0.92 ± 0.004
Madura	21.7 ± 1.2	0.97 ± 0.009

Data are for dry track surface

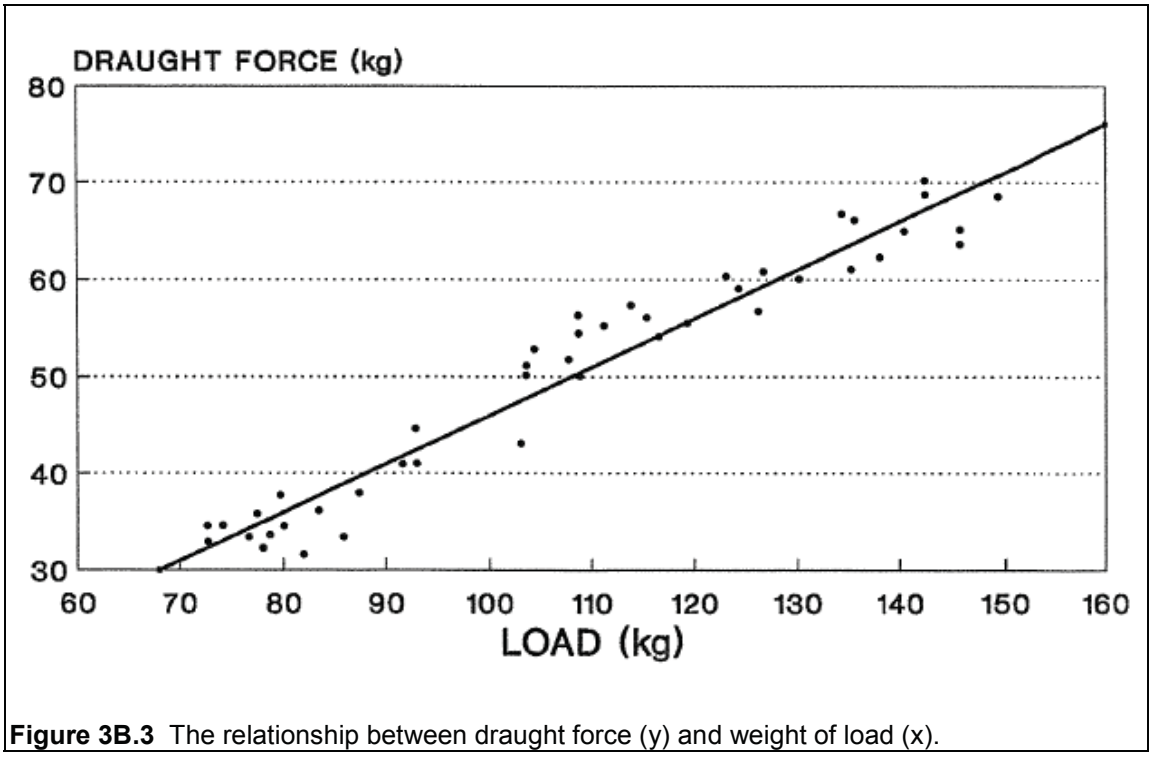


Figure 3B.3 The relationship between draught force (y) and weight of load (x).

y=0.49x – 3.6 (r=0.97; P=0.0000). (Source: Bakrie et al. 1991)

Draught Force Measuring Equipment

Angle force was measured using a load cell linked between the yoke and sledge which was connected to a digital readout meter. The walking speed of the animals was measured using a stop watch to record time and an odometer to record distance travelled. The angle of attachment of the load was measured using a protractor and spirit level.

3B.2.4 Experimental design

A total of 18 cows, six each of the Ongole, Bali and Madura breeds, were used. Their performances were compared within and between two measurement periods (Period 1 and Period 2). In Period 1, the cows were physically untrained and by Period 2, the same animals had undergone the 14 d training schedule described in section 3B.2.2. Each Period consisted of three days during which, three pairs (a pair from each breed) per day were subjected to the work regime and measurement procedure described in section 3B.2.5.

3B.2.5 Experimental procedure

Measurement periods

Measurement periods included the *Pre-work, Work* and *Recovery* periods which were from 0600–0700 h, 0730–1030 h and 1030–1330 h. During the *Pre-work* period, the animals were in their respective pens. After measurements and blood samples were taken from the six animals to be used for the day, they were walked gently to the walking track (figure 3B.4) where they were harnessed in breed pairs. Each pair was driven around the square walking track (figure 3B.4) while pulling its appropriate draught load (see section 3B.2.3). At each half hour approximately, during the *Work* period, the animals stopped briefly (approximately 3–5 minutes) at the sampling station (figure 3B.4) to allow measurements (if required) and blood samples to be taken.

In the *Recovery* period, the animals were tethered under the shade of a large tree beside the walking track, close to the sampling station. The animals did not have access to food or water during the measurement periods. Hence during the *Recovery* period, each animal had a woven cane basket over its mouth and nose to prevent it from eating grass but to allow it to pant.

Blood samples and sampling

A 10 mL blood sample was drawn through the jugular catheter of each animal at half hourly intervals during the *Pre-work* and *Work* periods and at hourly intervals during the *Recovery* period.

The collection and treatment of blood samples were according to procedures described in section 3A.2.5 except that sodium fluoride/potassium oxalate were used as the anticoagulant instead of lithium heparin.

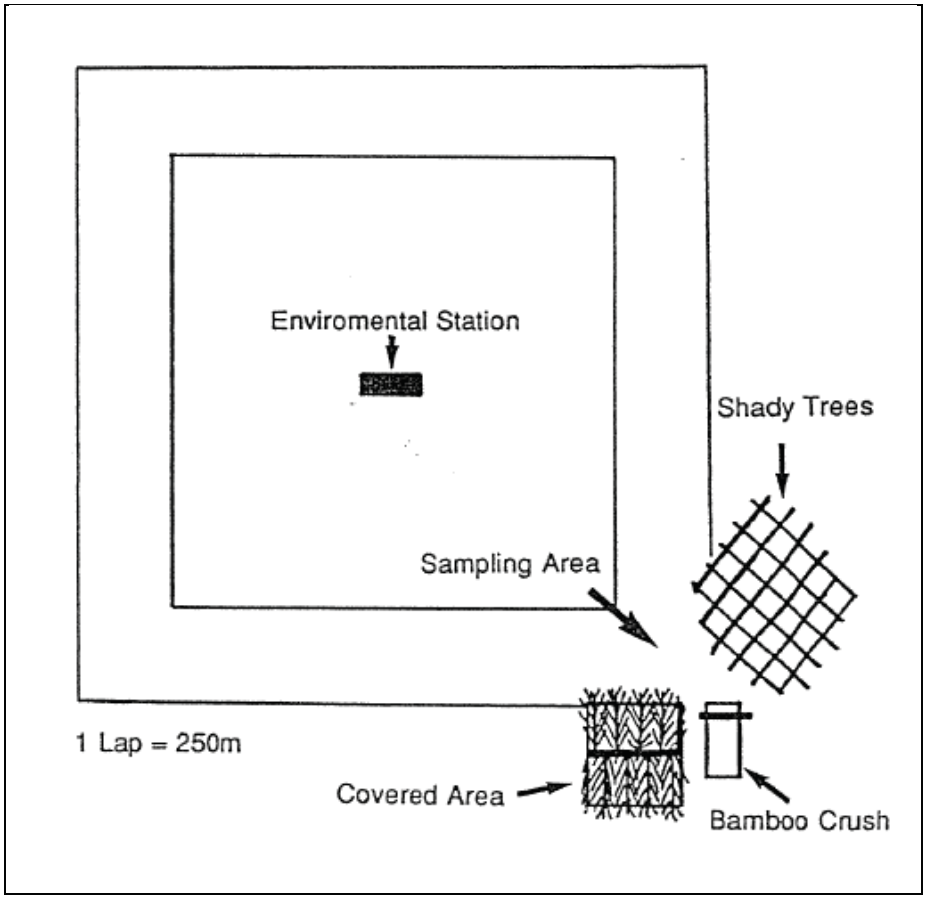


Figure 3B.4 Diagram of square track showing location of sampling and environmental stations.

Measurements

Environmental

Environmental parameters were measured at hourly intervals.

Ambient temperature

The thermometer (described in section 3A.2.5) was attached to a retort stand on a table positioned inside the square track.

Black bulb

The BB (described in section 3A.2.5) was also attached to a retort stand on the table located inside the square track.

Relative humidity

A wet and dry bulb hygrometer located at the sampling station was used. The bulbs were graduated from 0–50☜C with 0.1☜C increments (Brannan, England) and the relative humidity values were calculated using readings from these.

Physiological

Physiological parameters were recorded at hourly intervals. During the *Work* period the animals were stopped briefly (3–5 mins) while their rectal and skin temperatures and respiration and pulse rates were recorded. Pulse rates were also recorded immediately after the work period at 60 second intervals for 10 minutes, then at 5 minute intervals for 20 minutes.

Rectal temperature

Rectal temperature was measured as described in section 3A.2.5.

Skin temperature

Skin temperature was measured as described in section 3A.2.5, however the rectal probe was used because the skin probe was not functioning.

Respiration rate

Respiration rate was measured as described in section 3A.2.5 or by listening, through a stethoscope, to the inhalation and exhalation of air through the trachea.

Pulse rate

Pulse rate was measured as described in Section 3A.2.5.

Fatigue assessment

Fatigue was assessed as described in section 3A.2.6.

3B.2.6 Laboratory Analysis

Packed Cell Volume

The PCV value was determined on each hourly blood sample as described in section 3A.2.7.

Glucose

A manual method using a Sigma glucose kit number 510–A (Sigma-Aldrich Pty Ltd, Castle Hill, NSW, Australia) was used to determine plasma glucose concentration. The principle of the method is the same as that described in section 3A.2.7 using the glucose oxidase, peroxidase enzymes. The concentration of glucose was determined colorimetrically at 450 nm in a spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY).

Urea

The concentration of urea in plasma was also determined by a manual method using a Sigma kit. The principle of the urea nitrogen kit (535–B) is described in section 3A.2.7. The spectronic 20 spectrophotometer was used as described above for glucose, however, samples were read at 530 nm.

Free Fatty Acids

The FFA concentration in plasma was determined using the technique described in section 3A.2.7 except that the tetrabutyl ammonium hydroxide was diluted in heptane and not methanol. The methanol obtained via chemical agents in Indonesia appeared to contain some impurities and was found unsuitable.

Lactic Acid

The lactate concentration in plasma was determined using the same principle described in section 3A.2.7 except that the analysis was totally manual. The samples were read in a UV-spectrophotometer (UV–VIS–NIR–Recording spectrophotometer, UV–365; Shimadzu Corporation, Japan).

3B.2.7 Calculations

Energy expenditure was calculated using equations shown in section 3A.2.8 with the following changes:

For Bali and Madura cows
A = 2.0……(ARC 1980)

This value was used in the absence of any published values for the two breeds.

and for Ongoles
A = 2.9……..(Lawrence 1985)
C = 0.29 ± 0.0006……..(Lawrence 1985).

The draught force and distance travelled were calculated as described in section 3B.2.3.

3B.2.8 Statistical Analysis

Data were computed and subjected to analysis of variance using the Statistics 3.0 program (NH Analytical Software).

3B.3 Results

The mean duration of work which at least two of the three pairs of untrained and trained cattle completed was two hours. Untrained Bali and Madura were the only groups in which two and not three pairs completed the work schedule. A comparison has therefore been made for a two hour *Work* period.

3B.3.1 Environmental conditions

The mean AmT during the experimental period was 28.9°C (minimum 24.0°C; maximum 39.5°C). Relative humidity averaged 77% (minimum 51%; maximum 96%). and BB averaged 35.09°C (minimum 22.6°C; maximum 50.5°C).

3B.3.2 Work capacity and energy expenditure

Duration of work

The mean duration of work, distance travelled and EE are presented in table 3B.3.

Table 3B.3 Means of duration of work, distance travelled, energy expenditure (EE) and EE:Maintenance energy requirement (Mm) ratio of untrained (UNT) and trained (T) Ongole, Bali and Madura cows
Values are means ± standard errors (n=18)

	Duration of work (min)		Distance travelled (km)		Energy expenditure (MJ/d)[1]	
	UNT	T	UNT	T	EE	EE:Mm
Ongole	186 ± 14	192 ± 2	7.71 ± 0.06	7.96 ± 0.09	64.07 ± 0.62	2.04 ± 0.003
Bali	154 ± 20	169 ± 9	6.38 ± 0.83	7.01 ± 0.38	61.30 ± 0.72	1.99 ± 0.003
Madura	144 ± 23	167 ± 8	5.95 ± 0.94	6.90 ± 0.34	56.44 ± 0.10	2.01 ± 0.029

[1]n=36

Duration of work

The maximum working time allowed was approximately three hours. Duration of work generally exceeded three hours due to the position of the sampling site on the track. A full lap of the track had to be completed before work ceased even if three hours had elapsed.

Two pairs of untrained Ongole cows and all of the trained pairs of Ongole completed the three hours work. For Bali and Madura cows only one pair of untrained and trained cow of each pair completed the three hours. Physiological state and breed had no significant effect

on duration of work (table 3B.4). The respective increases in duration of work by the trained animals compared with the untrained animals were 10 and 16% for Bali and Madura respectively (table 3B.4). It should be noted that the percentage increases do not take into account the fact that some of the animals which completed the three hour work schedule would have been able to continue working for a longer period.

Distance travelled

Corresponding to the duration of work, the percentage increases in distance walked by the trained animals were 0, 10 and 16% for Ongole, Bali and Madura cows respectively. Physiological state and breed had no significant effect on distance travelled (table 3B.4).

Table 3B.4 Means of pooled data on duration of work and distance travelled in cattle for physiological states and breeds
Values are means ± standard errors (SE)

	Treatments				
	Physiological states[1]		Breed[2]		
	UNT	T	Ongole	Bali	Madura
Duration of work (min)	161	176	189	162	155
± SE	6	2	3	5	5
Significance	P=0.4591		P=0.3238		
Distance travelled (km)	6.68	7.29	7.84	6.69	6.42
± SE	0.25	0.10	0.19	0.30	0.33
Significance	P=0.4591		P=0.3242		

[1]n=9
[2]n=6

Energy expenditure

The data for EE in table 3B.3 were calculated from pooled data from 18 measurements (including untrained and trained animals) and indicates the rate of EE for the different breeds. The EE was lowest for Madura cows and highest for the Ongole cows (table 3B.3). In relation to Mm the increase in EE ranged from 1.99 for Bali to 2.04 for Ongoles.

Fatigue assessment

Fatigue scores recorded during work are summarised in table 3B.5.

Table 3B.5 Mean fatigue scores for untrained and trained Ongole, Bali and Madura cows Values are means ± standard errors (n=18)

Breed	Physical states		P (0.0415)
	Untrained	Trained	
Ongole	10.83 ± 1.00	7.22 ± 1.31	
Bali	17.93 ± 1.61	14.42 ± 0.97	0.0005
Madura	15.28 ± 2.66	12.00 ± 1.73	

Physiological states and breed significantly affected fatigue score (table 3B.5). Mean fatigue scores were higher for all the three breeds of untrained animals compared to the trained animals (figure 3B.5). Bali cows had the highest fatigue score for both physiological states and the Ongole cows had the lowest (figure 3B.5).

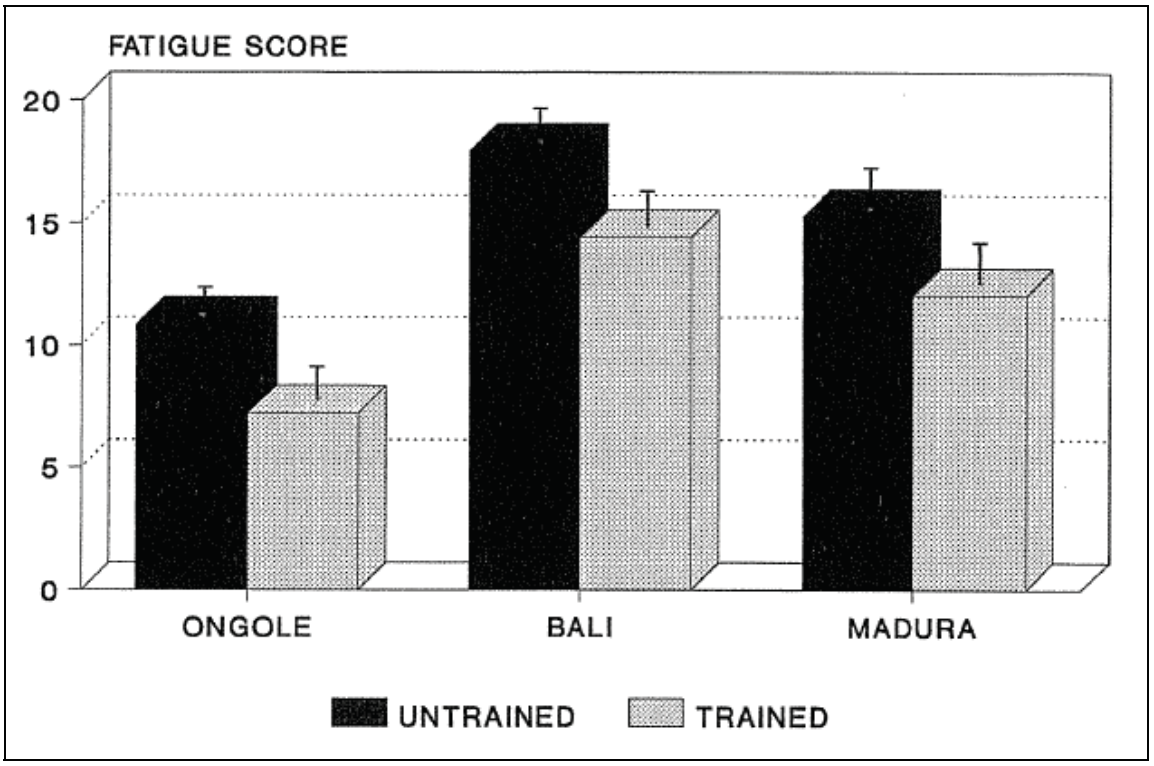


Figure 3B.5 Mean fatigue score (0–20) for untrained and trained Ongole, Bali and Madura cows **Vertical lines above bars represent standard errors.**

3B.3.3 Physiological variables

Skin temperature

Data on ST of untrained and trained cattle under different breeds and work status are summarised in figure 3B.6.

Pooled data, comparing the difference in mean ST between physiological states, breeds and between work status are presented in table 3B.6.

All treatments, *viz,* physiological state, breeds and work status significantly affected ST (table 3B.6). Skin temperature was higher in the trained animals during the *Work* period compared to the untrained animals across all breeds (figure 3B.6).

Table 3B.6 Means of pooled data on skin temperature (ST) of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
ST (☜C)	34.9	33.8	33.7	34.8	34.5	32.3	36.9	34.4
± SE	0.2	0.2	0.4	0.4	0.4	0.2	0.3	0.2
Significance	P=0.0055		P=0.0355			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Pre-work values for ST are lowest for Ongole cows and highest for Madura cows (figure 3B.6).

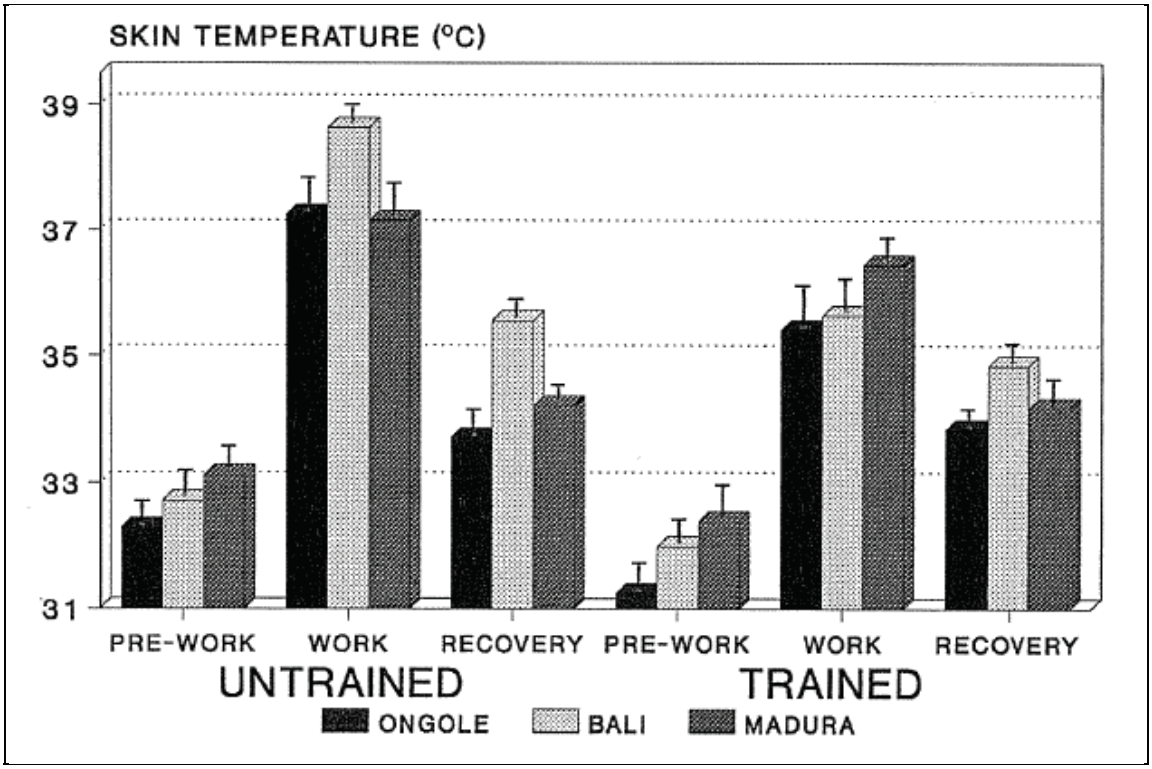


Figure 3B.6 Mean skin temperature of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Skin temperature increased by 4.97, 5.93 and 4.04☜C during the *Work* period in the untrained Ongole, Bali and Madura cows and by 4.16, 3.64 and 4.04☜C respectively in the trained Ongole, Bali and Madura cows. Skin temperature decreased during the *Recovery* period but remained above *Pre-work* values (figure 3B.6).

The changes in the pattern of ST over time across work status are presented in figures 3B.11 to 3B.13.

Rectal temperature

Data on RT of untrained and trained cattle under different breeds and work status are summarised in figure 3B.7.

Pooled data, comparing the difference in mean RT between physiological states, breeds and between work status are presented in table 3B.7.

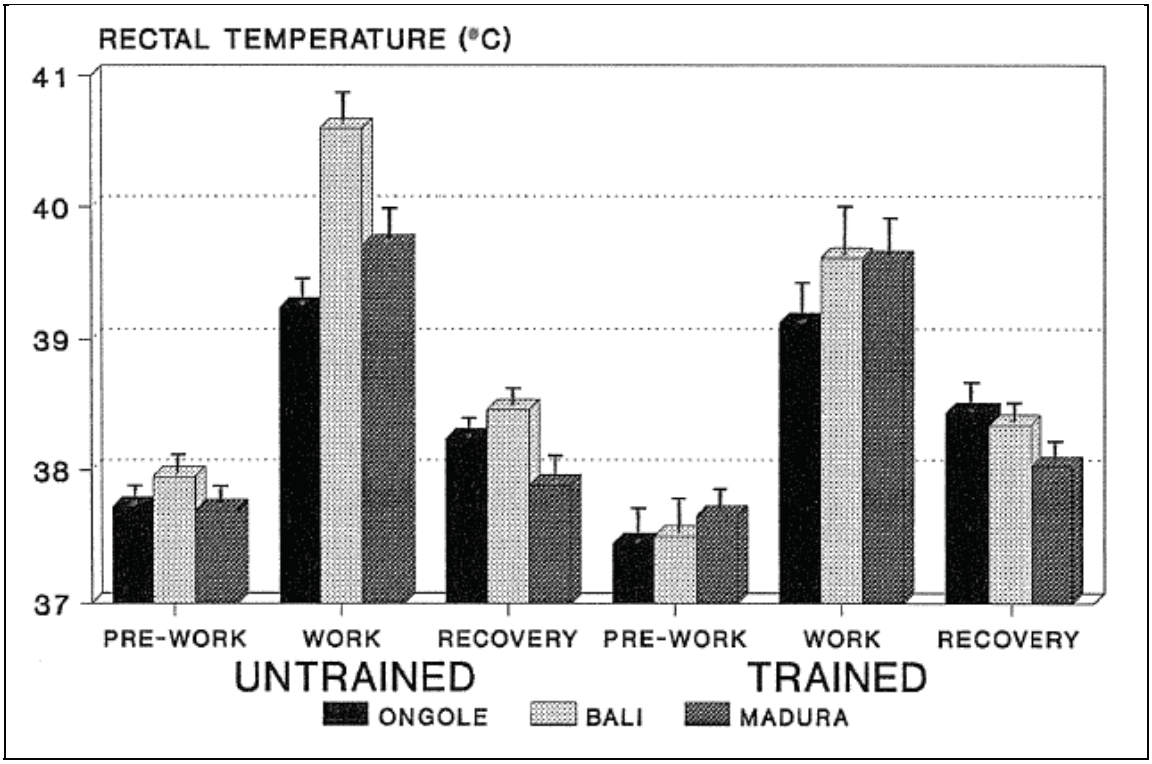


Figure 3B.7 Mean rectal temperature of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Table 3B.7 Means of pooled data on rectal temperature (RT) of cattle for physiological states, breeds and work status**.**Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
RT (☜C)	38.6	38.3	38.3	38.7	38.4	37.7	39.7	38.2
± SE	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1
Significance	P=0.3624		P=0.1320			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

There were no significant differences in RT between the physiological states or breeds (table 3B.7) although RT was lower for Ongole cows compared to the other two breeds (figure 3B.7). There is also a trend for RT to be lower in the untrained animals compared to the trained animals (table 3B.7). The RT was higher in the trained physiological state during *Work* for Ongole cows only.

Work status significantly affected RT (table 3B.7). RT increased by an average of 2.0☜C during the *Work* period and decreased during *Recovery* to remain above *Pre-work* values

(table 3B.7).

The changes in the pattern of RT over time across work status are presented in figures 3B.11 to 3B.13.

Respiration rate

Data on RR of untrained and trained cattle under different breeds and work status are summarised in figure 3B.8.

Pooled data, comparing the difference in mean RR between physiological states, breeds and work status are presented in table 3B.8.

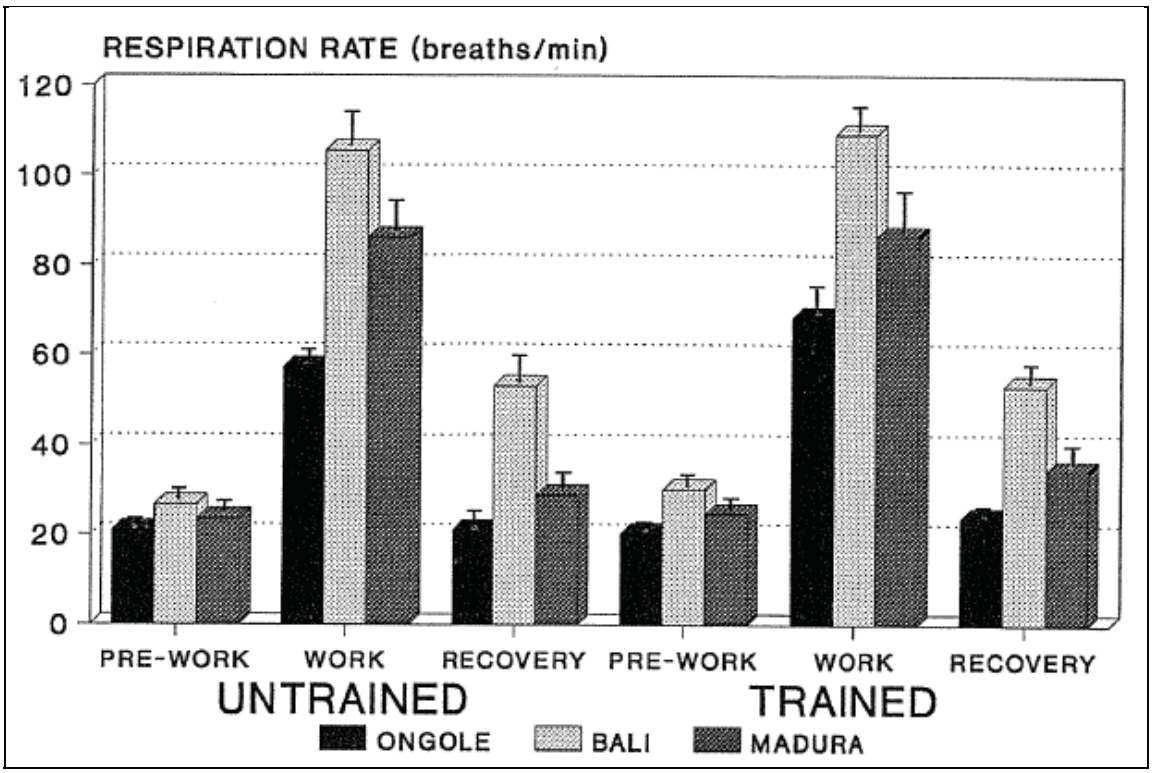


Figure 3B.8 Mean respiration rate of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Table 3B.8 Means of pooled data on respiration rate (RR) of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
RR (breaths/min)	47	45	32	60	45	24	87	36
± SE	2	3	2	4	4	1	5	3
Significance	P=0.2416		P=0.0000			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Respiration rate was not significantly affected by physiological state (table 3B.8), although there was a trend for RR to be lower in trained animals as compared to untrained animals (table 3B.8). Work status and breed significantly affected RR (table 3B.8).

Respiration rate was lowest for the Ongole and highest for the Bali cow across all work states (figure 3B.8).
Respiration rate increased by 200, 282 and 258% respectively, during *Work* in Ongole, Bali and Madura cows and decreased to *Pre-work* values for Ongoles but remained above *Pre-work* values for the other two breeds (figure 3B.8).

The changes in the pattern of RR over time across work status are presented in figures 3B.11 to 3B.13.

Pulse rate

Data on PR of untrained and trained cattle under different breeds and work status are summarised in figure 3B.9.

Pooled data, comparing the difference in mean PR between physiological states, breeds and work status are presented in table 3B.9.

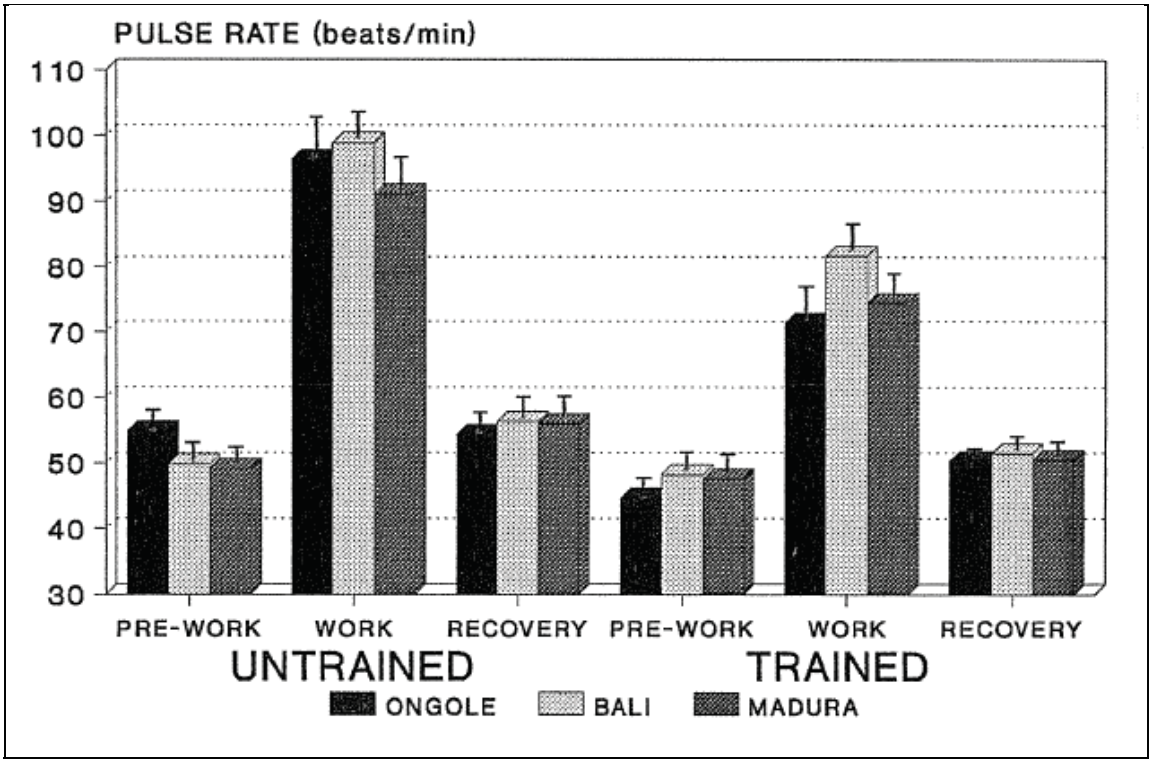


Figure 3B.9 Mean pulse rate of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Table 3B.9 Means of pooled data on pulse rate (PR) of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
PR (beats/min)	65	55	58	62	60	49	87	53
± SE	2	1	3	3	3	1	3	2
Significance	P=0.0004		P=0.6384			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Breed had no significant effect on PR (table 3B.9) although during *Work* PR was highest in Bali cows across both physiological states. Pulse rate was significantly affected by physiological states and work status (table 3B.9).

Pulse rate was lower in trained cattle as compared to untrained cattle (figure 3B.9). Pulse rate increased by 86 and 62% respectively, during *Work* in untrained and trained cows and decreased during *Recovery* to remain above *Pre-work* values (table 3B.9).

The changes in the pattern of PR over time across work status are presented in figures 3B.11 to 3B.13.

Pulse rate decline

Pulse rate decline during 30 minutes of *Recovery* immediately after work ceased across physiological states and breeds are presented in figure 3B.10.

There were significant differences in PR decline between physiological states (P=0.0000) and between breeds (P=0.0007). Pulse rate declined rapidly in the first 10 minutes of *Recovery* period across all breeds and physiological states (figure 3B.10). Pulse rate decline was fastest in the untrained Bali and slowest in the untrained Madura (table 3B.10). In the trained cattle, the fastest decline in PR occurred in the Madura and slowest in the Bali cows (table 3B.10).

After 30 minutes of *Recovery* PR remained above *Pre-work* values for all physiological states and breeds (figure 3B.10).

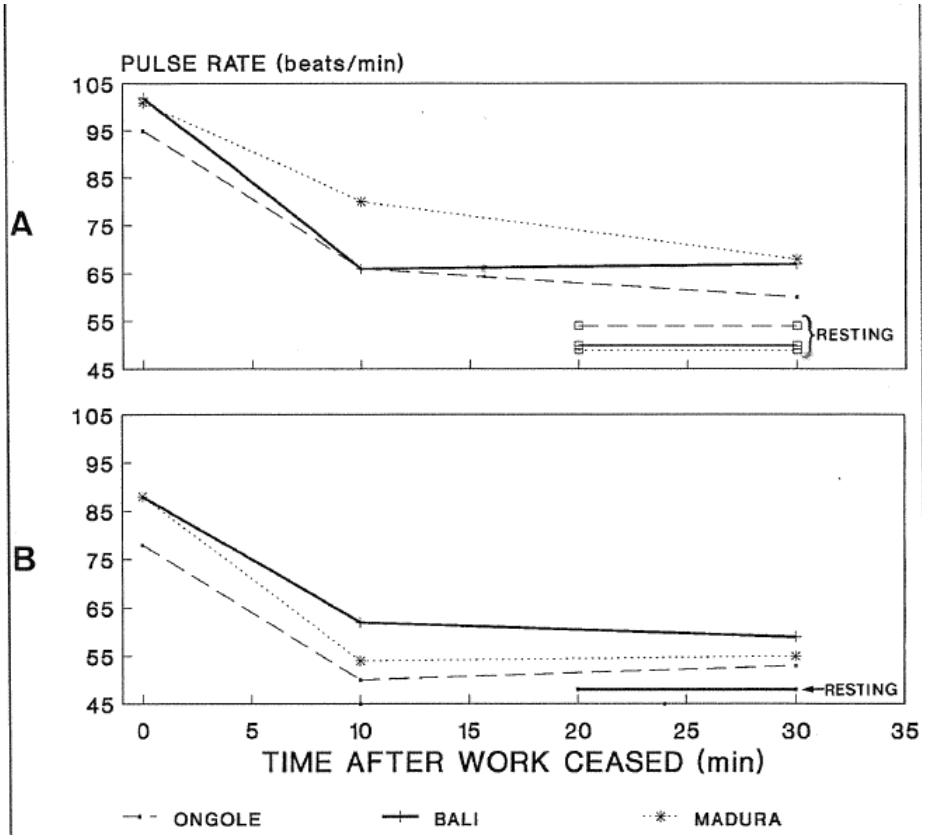


Figure 3B.10 Mean pulse rate decline during *Recovery* in (A) untrained and (B) trained Ongole, Bali and Madura cows.

Table 3B.10 Rate of decline of pulse rate and slope 10 minutes immediately work ceased across physiological states and breeds

	Physiological states			
	Untrained		Trained	
	Slope	Rate of decline (beats/min)	Slope	Rate of decline (beats/min)
Ongole	-0.67	3.2	-0.59	3.1
Bali	-0.82	4.0	-0.61	2.9
Madura	-0.52	2.3	-0.76	3.8

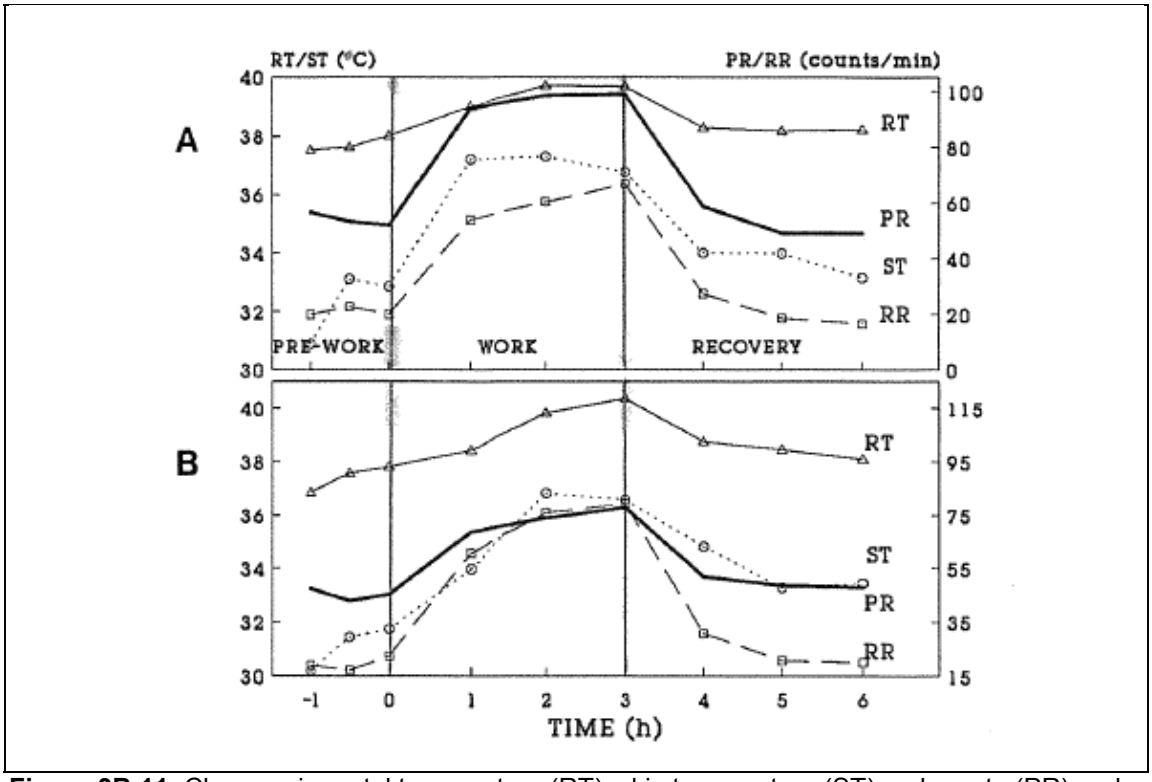


Figure 3B.11 Changes in rectal temperature (RT), skin temperature (ST), pulse rate (PR) and respiration rate (RR) of (A) untrained and (B) trained Ongole cows over work time across work status (*Pre-work, Work* and *Recovery*).

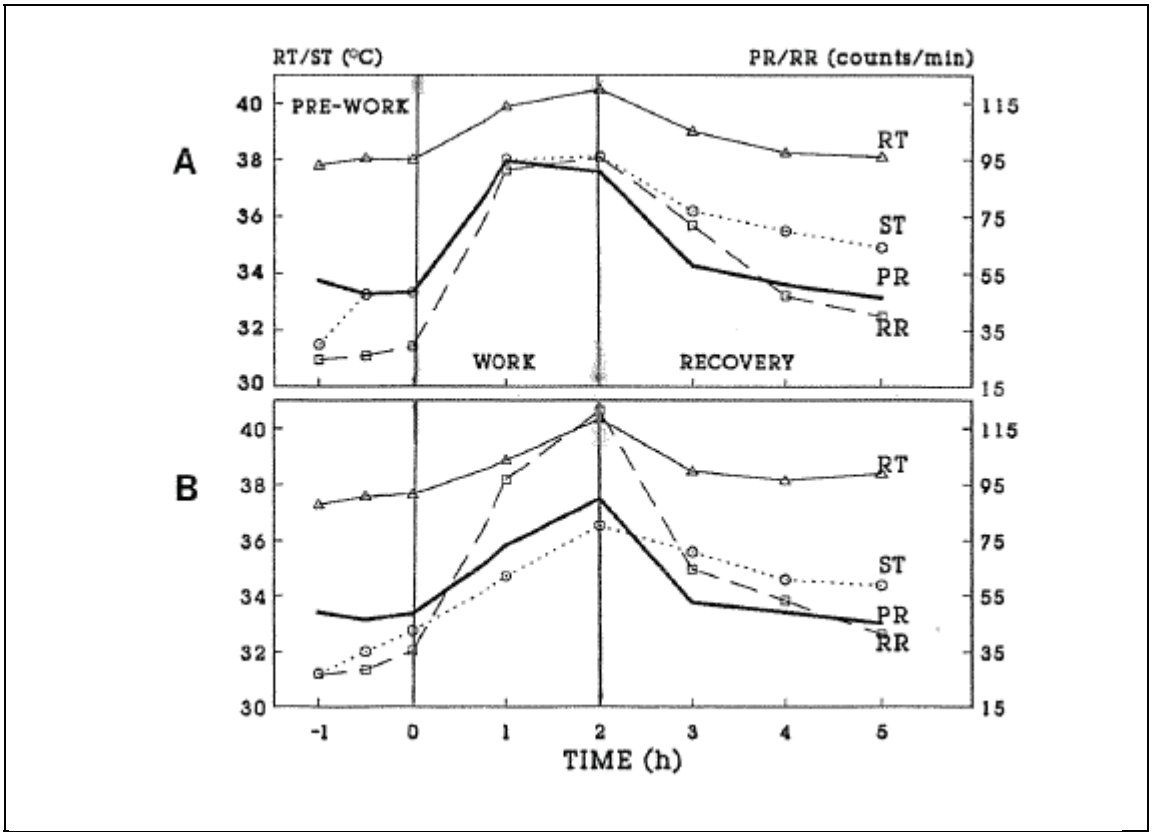


Figure 3B.12 Changes in rectal temperature (RT), skin temperature (ST), pulse rate (PR) and respiration rate (RR) of (A) untrained and (B) trained Bali cows over work time across work status (*Pre-work, Work* and *Recovery*).

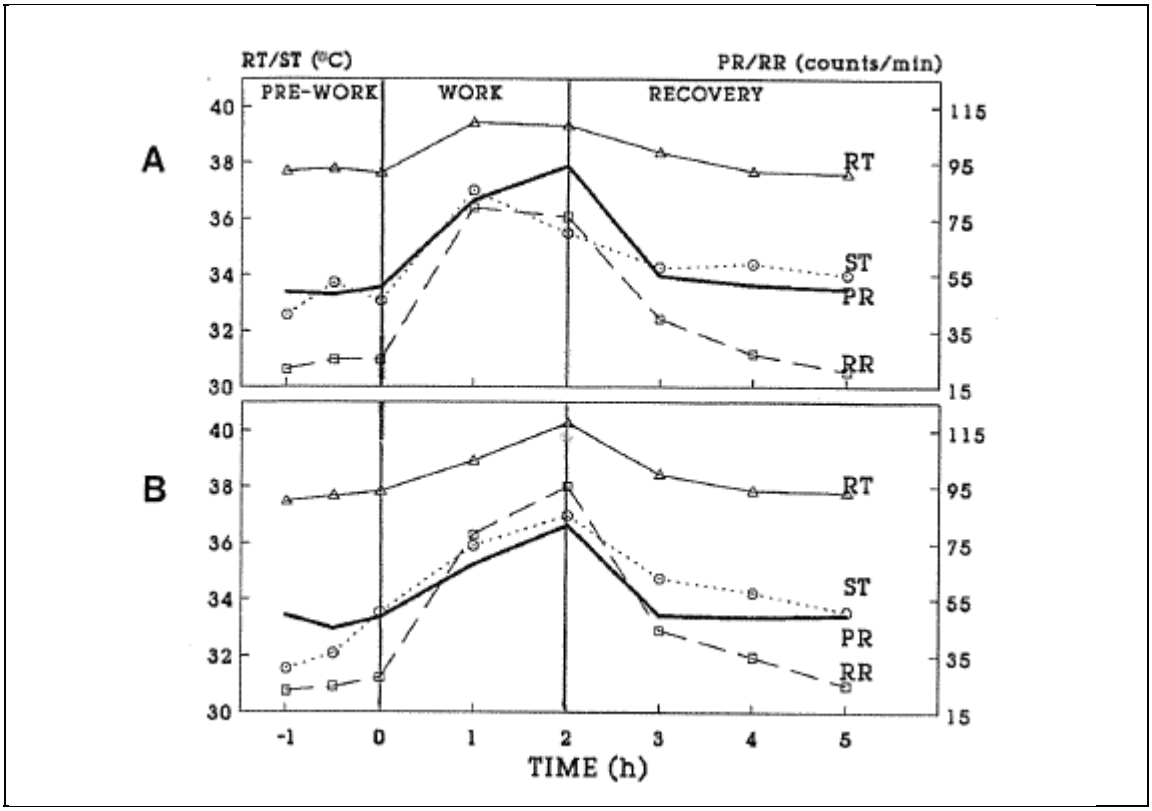


Figure 3B.13 Changes in rectal temperature (RT), skin temperature (ST), pulse rate (PR) and respiration rate (RR) of (A) untrained and (B) trained Madura cows over work time across work status (*Pre-work, Work* and *Recovery*).

3B.3.3 Packed cell volume

Data on PCV of untrained and trained cattle under different breeds and work status are summarised in figure 3B.14.

Pooled data, comparing the difference in mean PCV between physiological states, breeds and between work status are presented in table 3B.11.

Table 3B.11 Means of pooled data on packed cell volume (PCV) of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
PCV (%)	24	25	25	25	25	25	27	23
± SE	1	1	1	1	2	1	1	1
Significance	P=0.4744		P=0.8905			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

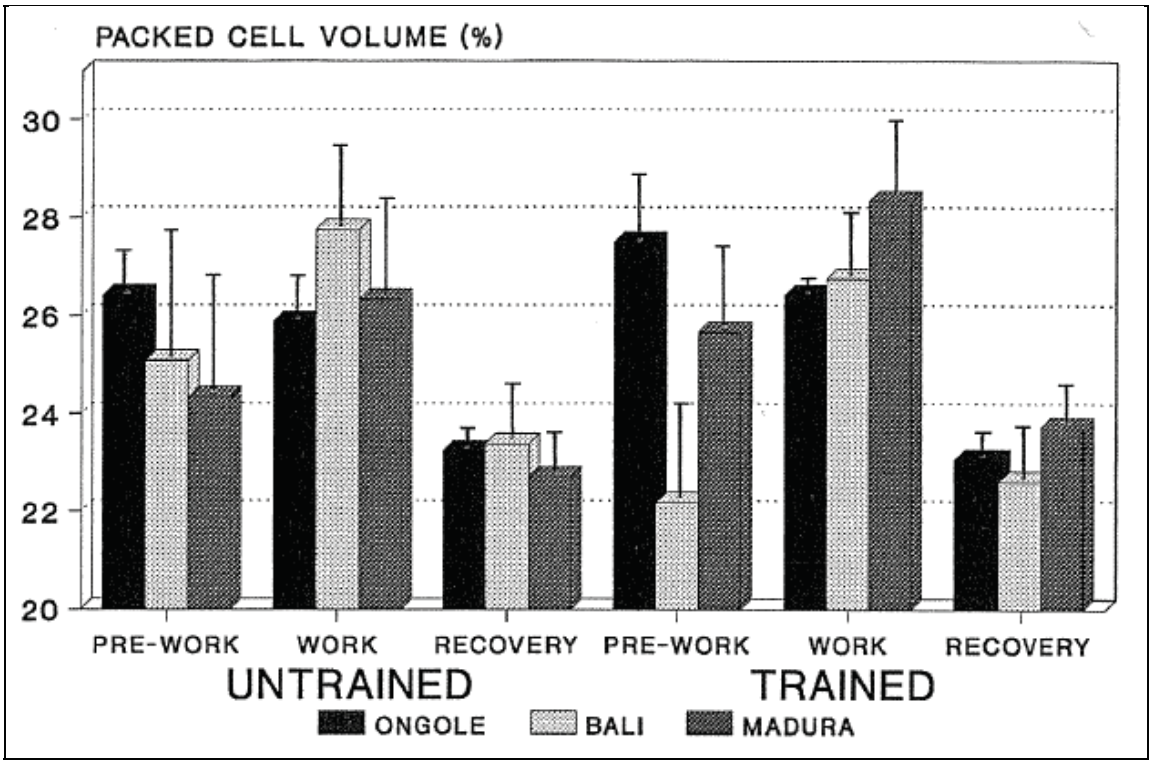


Figure 3B.14 Mean packed cell volume of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Packed cell volume was not significantly affected by physiological states or breed (table 3B.11), although there was a trend for PCV to decrease during *Work* for the Ongole in both physiological states compared to an increase during *Work* for the other two breeds (figure 3B.14). Work status significantly affected PCV (table 3B.11). Packed cell volume increased by 7% during *Work* and decreased to below *Pre-work* values in the *Recovery* period (figure 3B.15). The effect of work on PCV is clearly illustrated in figure 3B.15 where data were pooled across physiological states and breeds for the three work states.

The changes in the pattern of PCV over time across work status for physiological states are presented in figure 3B.16.

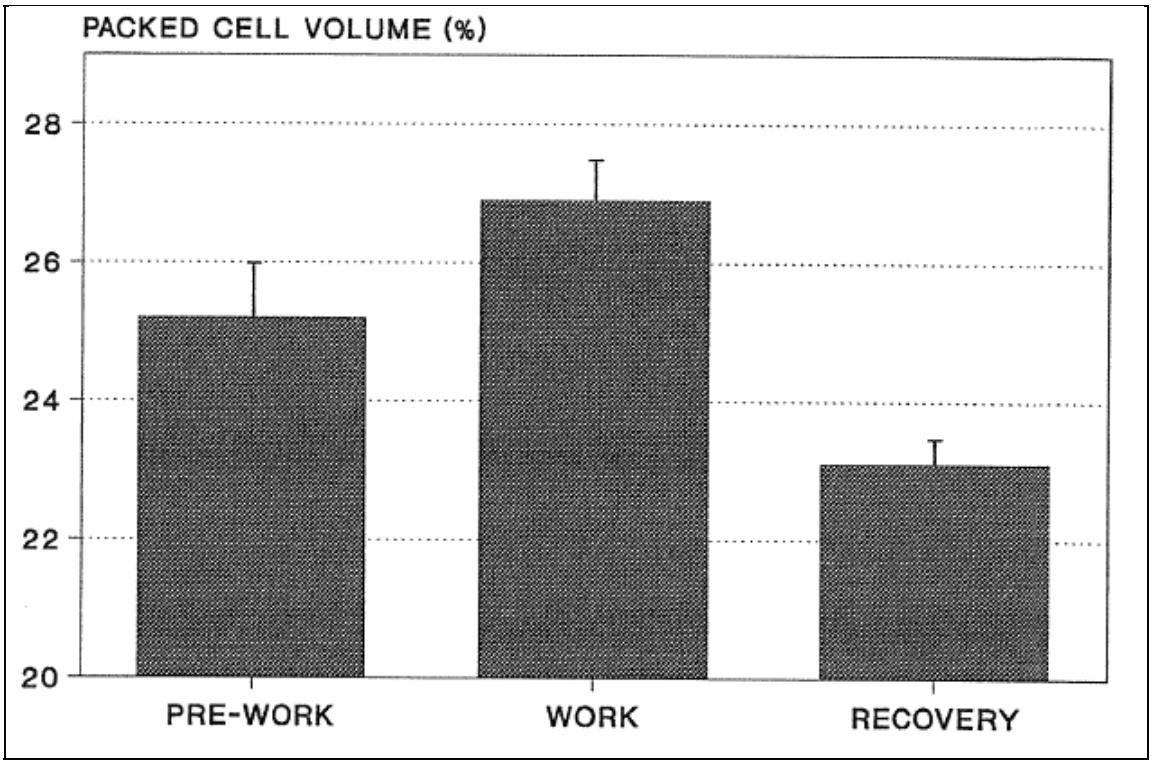


Figure 3B.15 Means of pooled data on packed cell volume of cattle during the *Pre-work, Work* and *Recovery* periods. **Vertical lines above bars represent standard errors.**

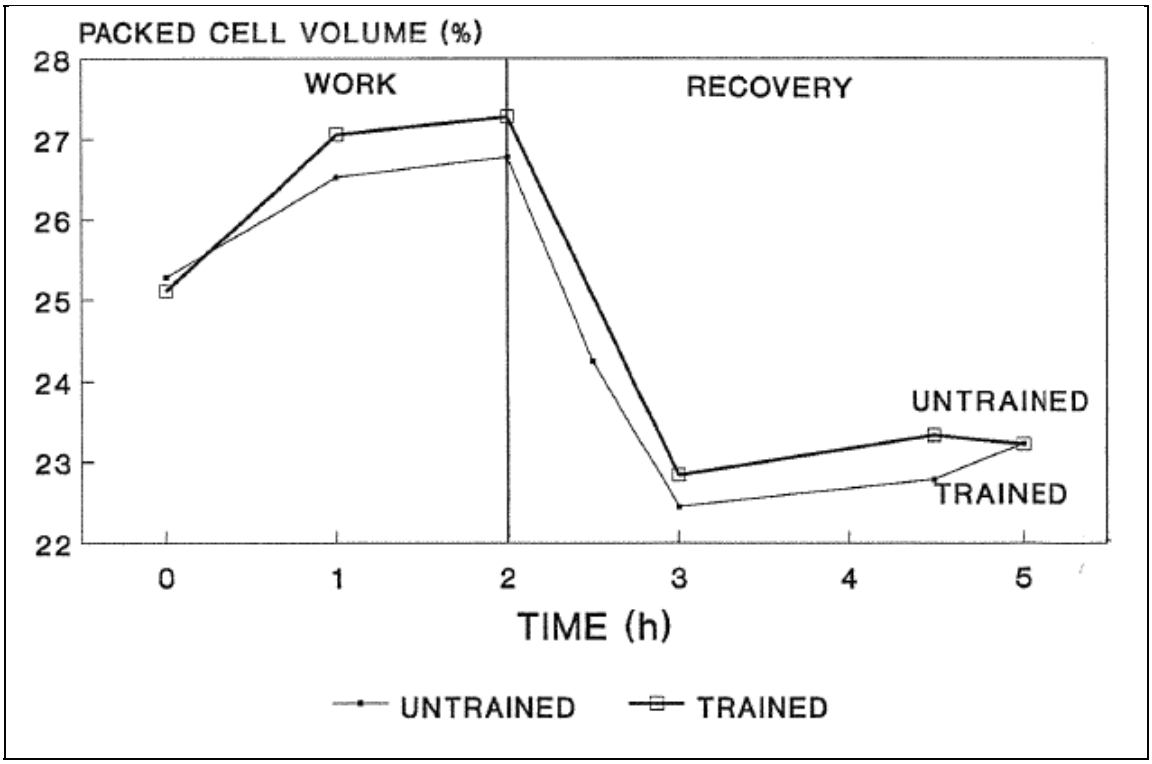


Figure 3B.16 Changes in packed cell volume of untrained and trained cattle, over work time across work status (*Pre-work, Work* and *Recovery*).

3B.3.4 Blood metabolites

Lactate

Data on lactate concentrations in plasma of untrained and trained cattle under different breeds and work status are summarised in figure 3B.17.

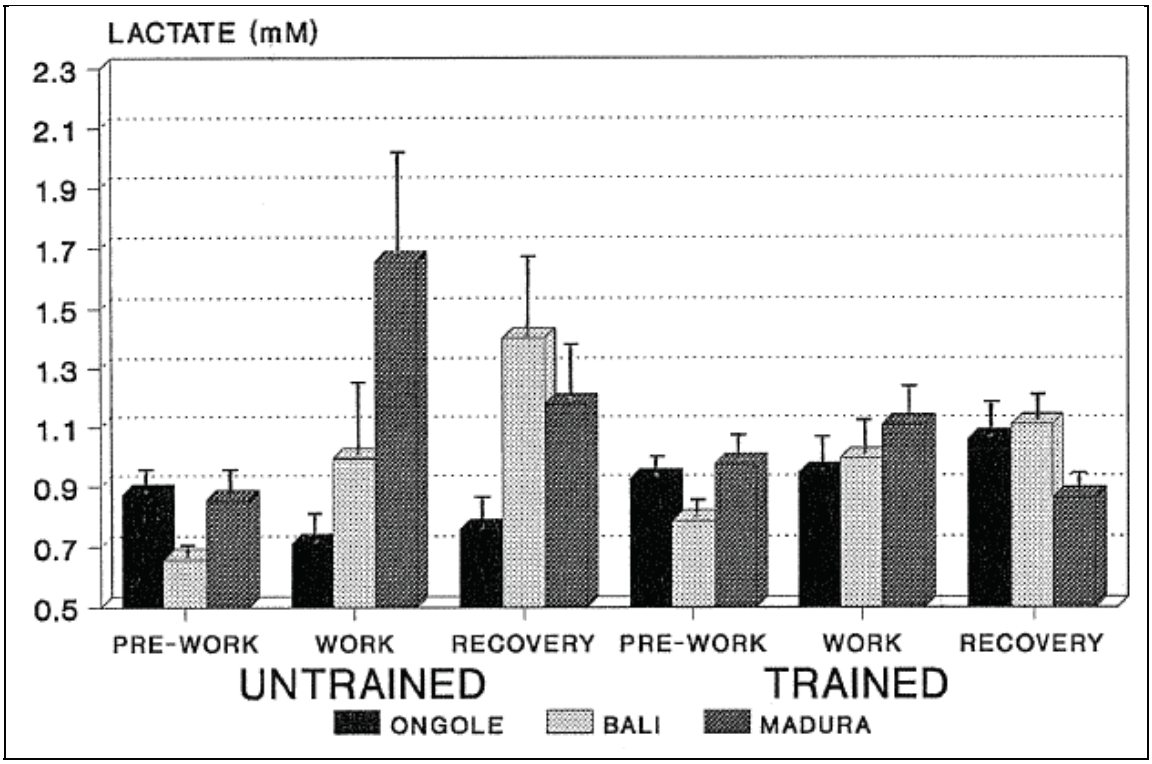


Figure 3B.17 Mean concentrations of lactate in plasma of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean lactate concentrations between physiological states, breeds and between work status are presented in table 3A.12.

Physiological states had no significant effect on plasma lactate concentration (table 3B.17) although overall, plasma lactate concentrations were higher in the trained animals as compared to the untrained animals (table 3B.17).

Plasma lactate concentrations were significantly affected by breed and work status (table 3B.17). Overall, plasma lactate concentrations increased during work by 27% with mean concentrations highest in the Madura cows and lowest in the Ongole cows (table 3B.12).

Table 3B.12 Means of pooled data on lactate concentration in plasma of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
Lactate (mM)	1.03	0.98	0.87	1.01	1.12	0.84	1.07	1.06
± SE	0.06	0.03	0.08	0.12	0.13	0.03	0.12	0.08
Significance	P=0.6643		P=0.0143			P=0.0212		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Plasma lactate concentrations during *Work* decreased by 9% in Ongole cows and increased by 38 and 52% respectively in Bali and Madura cows (figure 3B.17). The increase in plasma lactate during *Work* in the untrained Madura cows was much greater than all other breeds and physiological states (figure 3B.17). Plasma lactate concentrations increased during *Recovery* in the Ongole and Bali cows (figure 3B.17) and remained above *Pre-work* values with the exception of untrained Ongole cows. On the other hand plasma lactate concentrations decreased during *Recovery* in the Madura cows (figure 3B.17) and remained above *Pre-work* values.

The changes in the pattern of concentration of lactate in plasma of cattle over time across work status are presented in figures 3B.21 to 3B.23. The sharp increases in concentration of plasma lactate in the untrained Madura cows is clearly illustrated in figure 3B.23(A).

Glucose

Data on glucose concentrations in plasma of untrained and trained cattle under different breeds and work status are summarised in figure 3B.18.

Pooled data, comparing the difference in mean glucose concentrations between physiological states, breeds and between work status are presented in table 3B.13.

All treatments, *viz* physiological states, breed and work status, significantly affected the concentration of glucose in plasma of cattle (table 3B.13).

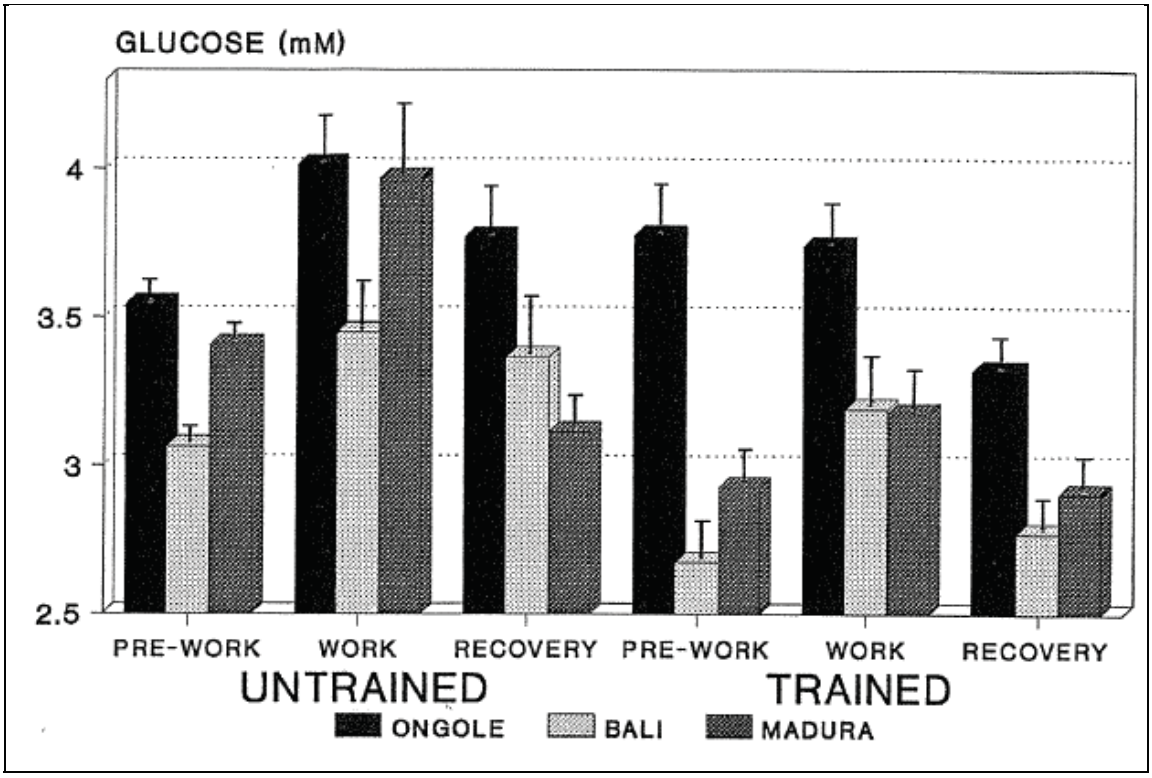


Figure 3B.18 Mean concentrations of glucose in plasma of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Table 3B.13 Means of pooled data on glucose concentration in plasma of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
Glucose (mM)	3.54	3.17	3.70	3.11	3.26	3.23	3.59	3.21
± SE	0.05	0.05	0.13	0.14	0.13	0.18	0.10	0.08
Significance	P=0.0000		P=0.0000			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

The trained animals had a lower mean glucose concentration in plasma than untrained animals (table 3B.13). Overall there was an increase in glucose concentration during the *Work* period. The exception was the decrease observed in trained Ongole cows. Subsequent to the *Work* period, values of glucose concentration in both untrained and trained animals decreased during the *Recovery* period (figure 3B.18).

Plasma glucose concentrations were highest in the Ongole cows and lowest in the Madura cows across all physiological states and work status (figure 3B.18). The exception was the lowest concentrations in untrained Madura cows during the *Recovery* period and trained Madura during the *Work* period (figure 3B.18). Plasma glucose concentrations were similar in the Bali and Madura cows during the *Work* period (figure 3B.18).

The changes in the pattern of concentration of glucose in plasma of cattle over time and across work status are presented in figures 3B.12 to 3B.23.

Urea

Data on urea concentrations in plasma of untrained and trained cattle under different breeds and work status are summarised in figure 3B.19.

Pooled data, comparing the difference in mean urea concentrations between physiological states, breeds and between work status are presented in table 3B.14.

All treatments, *viz* physiological states, breed and work status, significantly affected the concentration of urea in plasma of cattle (table 3B.14).

The trained animals had a higher mean urea concentration in plasma than untrained animals (table 3B.14). The exception was in the Bali breed which had similar plasma urea concentrations across physiological states (figure 3B.19).

Overall, there was an increase in plasma urea concentration during the *Work* period and a further increase during the *Recovery* period (table 3B.14). The exception was the decrease of 0.01 and 1% respectively, during *Work* observed in the trained Ongole and Bali breeds respectively.

Plasma urea concentrations in untrained cattle were lowest in the Madura breed and highest in the Bali breed across all work status (figure 3B.19). Plasma urea concentrations in trained cattle, however, were similar during the *Pre-work* and *Work* period across breeds (figure 3B.19).

The changes in the pattern of concentration of urea in plasma of cattle over time across work status are presented in figures 3B.21 to 3B.23.

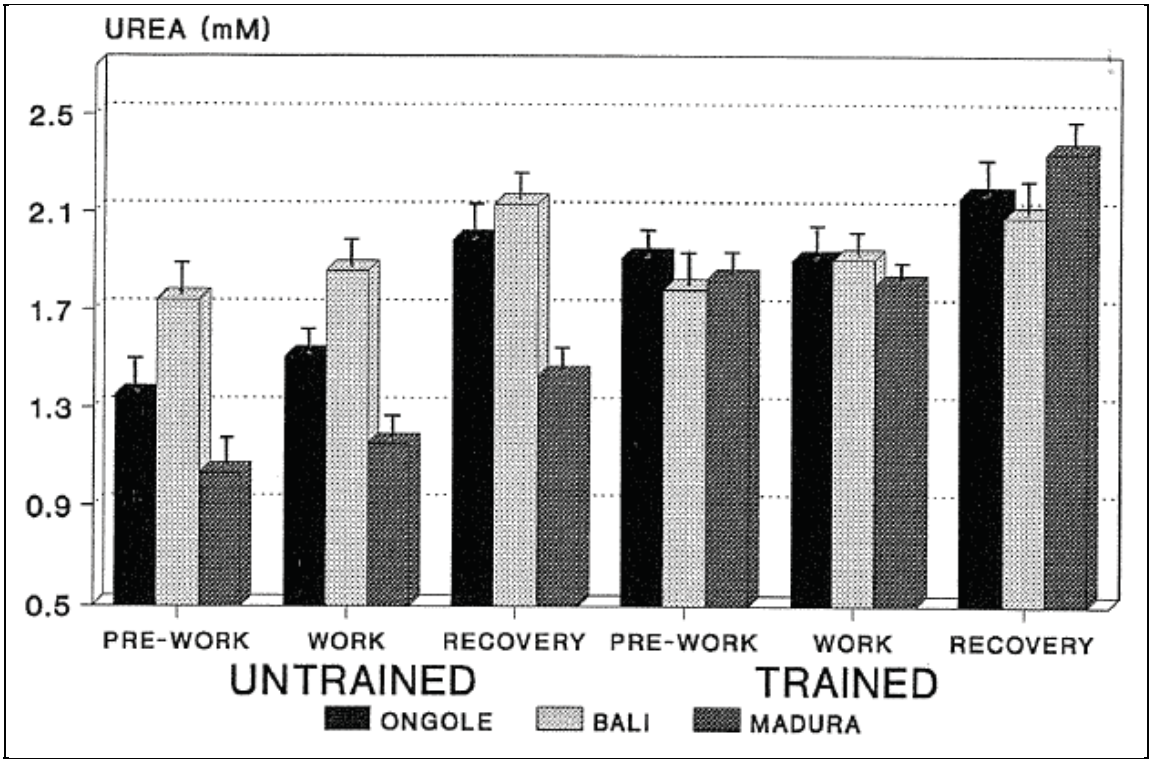


Figure 3B.19 Mean concentrations of urea in plasma of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Table 3B.14 Means of pooled data on urea concentration in plasma of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
Urea (mM)	1.6	2.0	1.82	1.93	1.61	1.61	1.69	2.02
± SE	0.04	0.04	0.11	0.11	0.09	0.07	0.06	0.07
Significance	P=0.0000		P=0.0001			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Free fatty acids

Data on FFA concentrations in plasma of untrained and trained cattle under different breeds and work status are summarised in figure 3B.20.

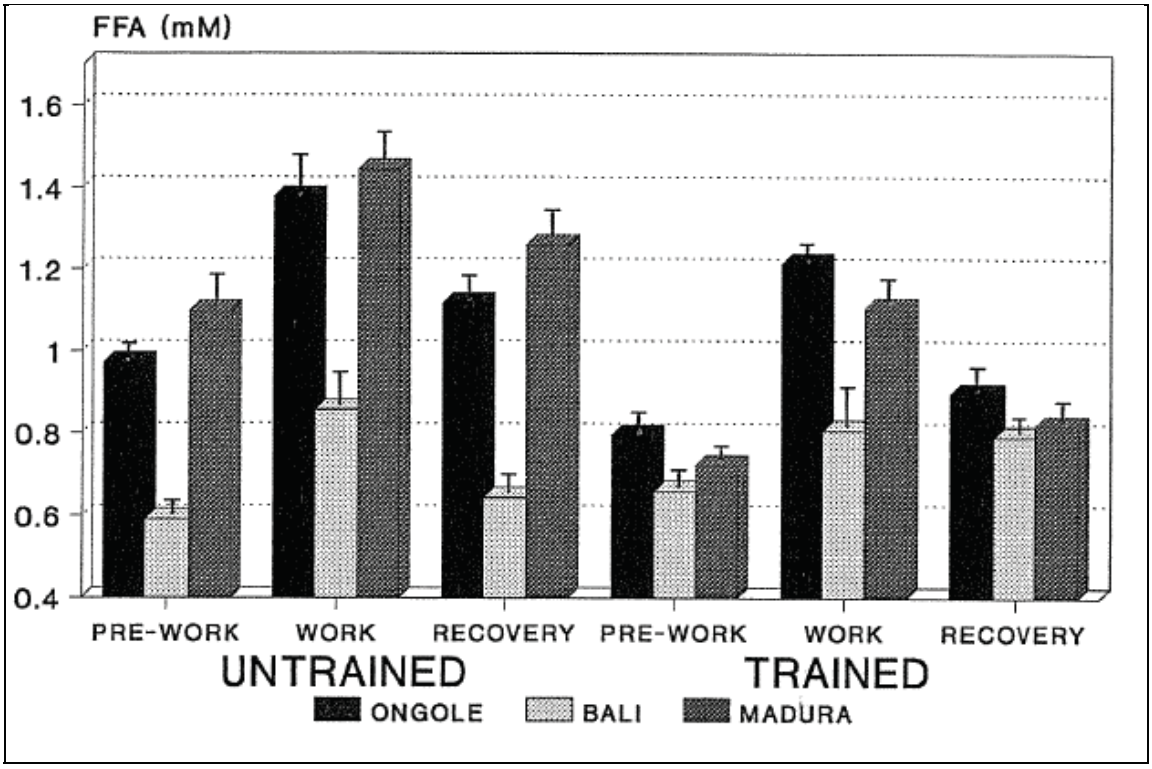


Figure 3B.20 Mean concentrations of free fatty acids in plasma of untrained and trained Ongole, Bali and Madura cows under different work status (*Pre-work, Work* and *Recovery*). **Vertical lines above bars represent standard errors.**

Pooled data, comparing the difference in mean FFA concentrations between physiological states, breeds and between work status are presented in table 3B.15.

All treatments, *viz* physiological states, breed and work status significantly affected the concentration of FFA in plasma of cattle (table 3B.15).

Table 3B.15 Means of pooled data on free fatty acids (FFA) concentration in plasma of cattle for physiological states, breeds and work status
Values are means ± standard errors (SE).

	Treatments							
	Physiological states[1]		Breed			Work status[2]		
	UNT	T	Ongole	Bali	Madura	Pre-W	W	REC
FFA (mM)	1.05	0.88	1.09	0.73	1.09	0.81	1.13	0.92
± SE	0.03	0.02	0.03	0.04	0.07	0.04	0.05	0.04
Significance	P=0.0000		P=0.0001			P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

The trained animals had a lower mean FFA concentration in plasma than untrained animals (table 3B.15). The exception was in the trained Bali breed during *Pre-work*, and *Recovery* periods (figure 3B.20).

Overall, there was an increase in plasma FFA concentrations during the *Work* period (table 3B.15). Plasma FFA concentration decreased but remained above *Pre-work* values during the *Recovery* period (table 3B.15).

Plasma FFA concentrations increased by 42, 45 and 31% respectively during work in untrained cattle and by 52, 22 and 53% in trained cattle (figure 3B.20). Overall, the concentration of FFA in cattle was similar in the Madura and Ongole breeds but lower in the Bali breed (table 3B.15). The difference between breeds was highlighted in untrained animals across work status when FFA concentrations were highest in the Madura breed and lowest in the Bali breed (figure 3B.20). Plasma FFA concentrations in trained animals, however, across work status were highest in the Ongole breed and lowest in the Bali breed (figure 3B.20).

The changes in the pattern of concentration of FFA in plasma of cattle over time across work status are presented in figures 3B.21 to 3B.23.

3B.4 Discussion

Work capacity and energy expenditure

Training increased work capacity of Bali and Madura cows but the increase was not detectable for the Ongole cows because, in both physiological states, the 3 h of work scheduled was completed. The Ongole breed therefore had the greatest endurance or work capacity followed by the Bali cow with the Madura being the breed with the least endurance. This superior work capacity was reflected in the fatigue score which was much lower for the Ongole compared with the other two breeds (see figure 3B.5). As expected the fatigue score was much lower for the trained than the untrained cows.

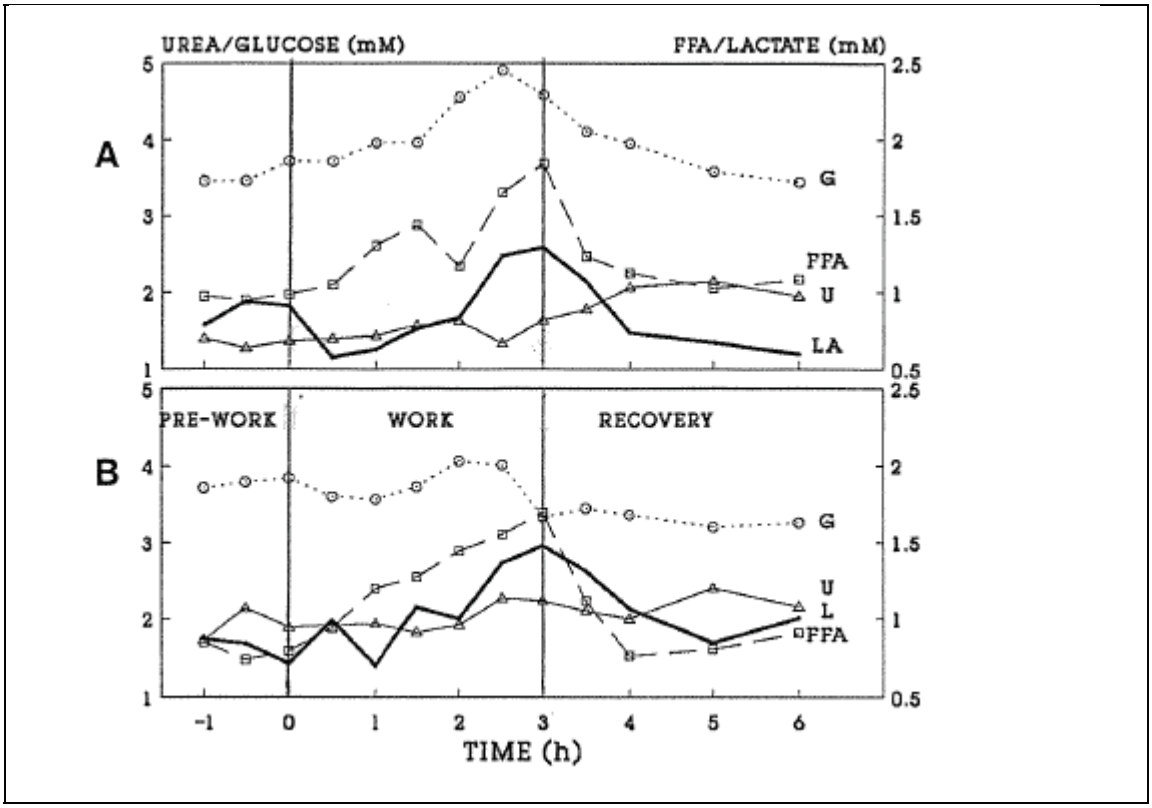


Figure 3B.21 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acid (FFA) concentrations of (A) untrained and (B) trained Ongole cows over work time across work status (*Pre-work, Work* and *Recovery*).

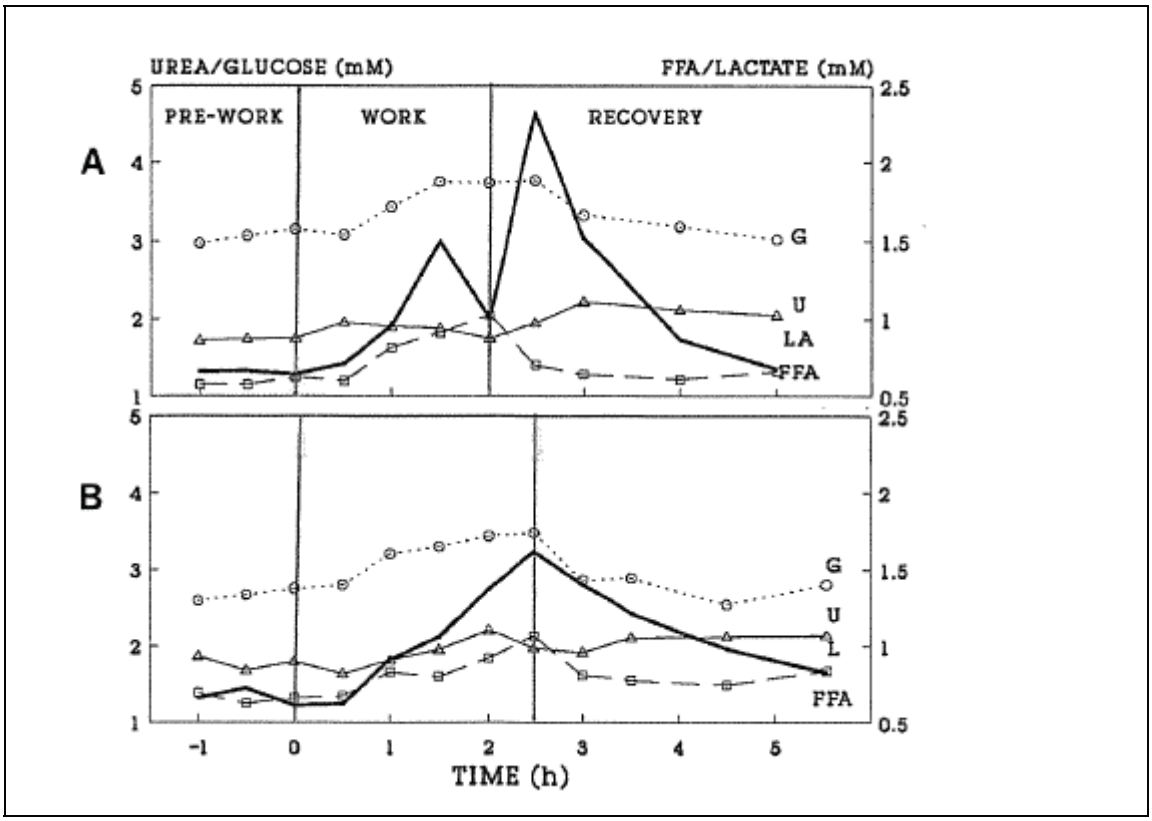


Figure 3B.22 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acid (FFA) concentrations of (A) untrained and (B) trained Bali cows over work time across work status (*Pre-work, Work* and *Recovery*).

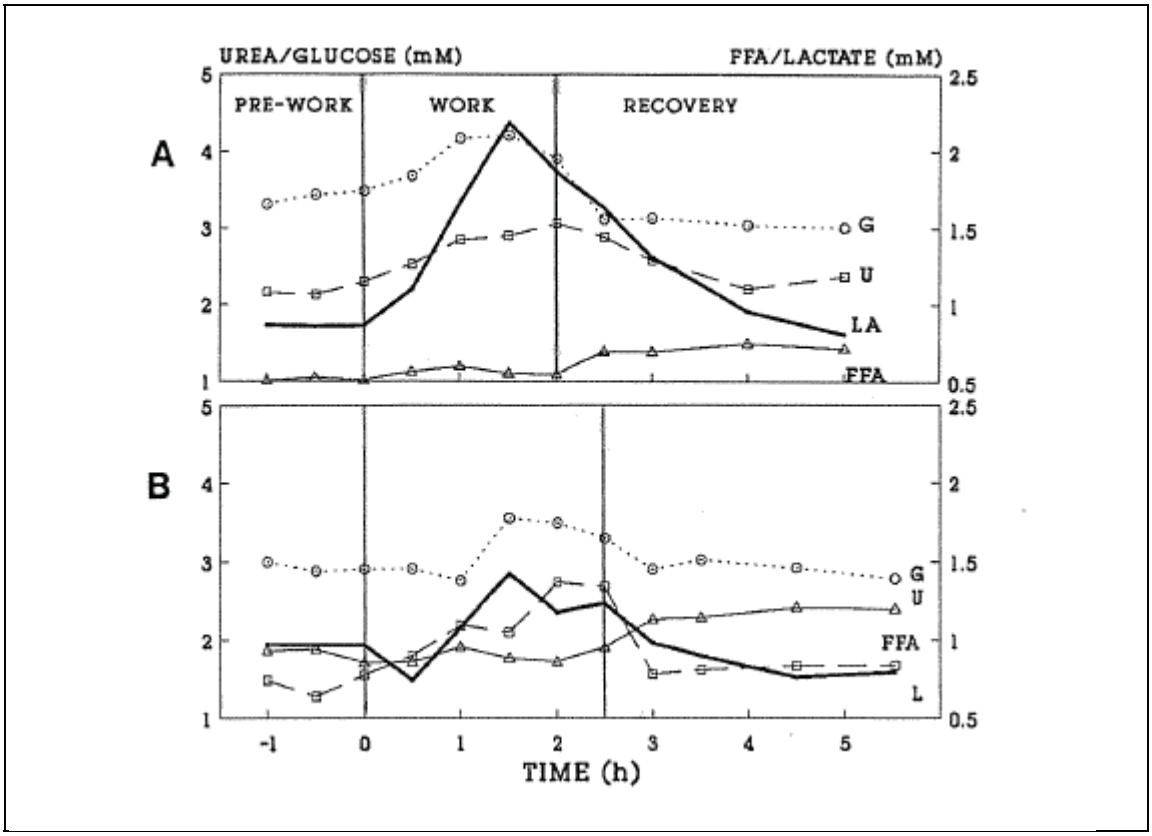


Figure 3B.23 Changes in plasma lactate (LA), glucose (G), urea (U) and free fatty acid (FFA) concentrations of (A) untrained and (B) trained Madura cows over work time across work status (*Pre-work, Work* and *Recovery*).

The energy expenditures during work were 2.2, 2.2 and 2.0x maintenance for Ongole, Bali and Madura cows respectively. These values are slightly lower than that reported for buffalo (2.3–2.7x maintenance) walking at the same speed (Teleni et al. 1991) but higher than that reported by Lawrence (1985) for oxen (1.67x maintenance). It should be noted that the values calculated in this experiment used the values for the coefficient for doing work derived from Brahman cattle (Lawrence 1985) and *Bos taurus* cattle (ARC 1980).

The Ongole had a much lower RT which may be due to the white coat colour of the breed compared to the other breeds or due to the type of substrate metabolised as fuel for the working muscle. The Ongole also had the lowest peak plasma lactate (table 3B.16).

Table 3B.16 Peak values for plasma lactate in Ongole, Bali and Madura cows and time of onset of blood lactate accumulation (OBLA)

	Peak plasma lactate		Time of OBLA (hours after work)	
	Untrained	Trained	Untrained	Trained
Ongole	1.29	1.48	2	2
Bali	1.50	1.62	0.5	0.5

Madura	2.19	1.42	0.5	0.5

The Ongole must have large oxidative capacity of cells with no change occurring in PCV during work or after training.

Physiological variables

The average increase in RT of 2.03☜C across all breeds in this experiment was similar to values reported by Upadhyay and Madan (1985c) for Haryana and Crossbred cattle after 3 h of work. The actual RT values recorded in this experiment, however, were lower than those recorded by Upadhyay and Madan (1985c) for their cattle. The exception was the RT values for Bali cows which averaged 40.6☜C.

The untrained Bali cows were the only animals which had increases in RT exceeding 2.5☜C (table 3B.17) suggesting that FFA was utilised at a greater rate in this breed.

Table 3B.17 The increases in skin and rectal temperatures of untrained and trained Ongole, Bali and Madura cows during the *Work* period

	Rectal temperature (☜C)		Skin temperature (☜C)	
Breed	Untrained	Trained	Untrained	Trained
Ongole	1.52	1.68	4.97	4.16
Bali	2.65	2.11	5.93	3.64
Madura	2.01	1.93	4.04	4.04

The increases in RT were reflected in increases in ST (r=0.841; P=0.0000). Maximum values for RT and ST were 41.8☜C and 40.6☜C respectively. The rate of blood flow to the skin of the working animals must have increased dramatically in order to dissipate some of their body heat (see Bell & Hales 1985). in addition to such a mechanism for cooling, the increase in RR observed in the working animal is another cooling mechanism. This cools the animal through evaporative cooling of the respiratory tract. It is not surprising therefore to see a strong positive relationship between RR and RT (r=0.71;P=0.0000 – see figure 3B.24). The figure indicates that for every 1☜C increase there was an increase of 23 breaths/min in RR.

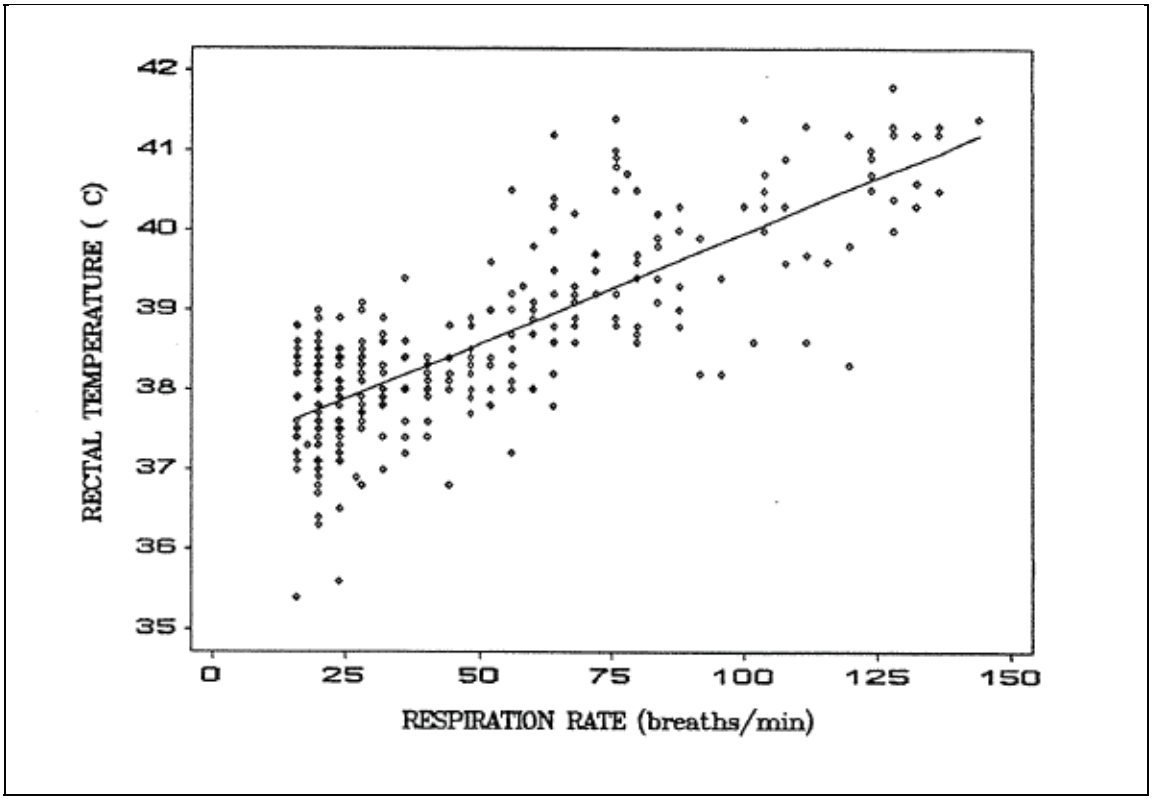


Figure 3B.24 The relationship between respiration rate and rectal temperature.

It is evident from the results of this experiment that the Bali cows were the most stressed of the three breeds, although the Madura had the lowest work capacity. There are also evidence (highest increases in RT, PCV and PR) which are consistent with the suggestion that the Bali cows had a high rate of FFA utilisation (see Kruk et al. 1987).

On the other hand, the Ongole cows appear to have been predominantly dependent on glycogen for their ATP supply during the *Work* period. It is possible that the breed had a larger total glycogen store than did the Bali and Madura. More importantly, however, the Ongole cows appear to have a greater oxidative capacity than the others.

Substrate utilisation

Mean lactate concentrations in plasma were not significantly different between breeds during the *Work* period, however, there was a difference in the pattern of lactate concentrations in Madura (figure 3B.21–3B.23). It would appear that the Bali and the Madura cows were more reliant on anaerobic metabolism than the Ongole cows for ATP supply during the *Work* period. The OBLA was observed after 30 minutes of work in both the untrained and trained Bali and Madura cows but the same was not observed in the Ongole cows in which OBLA occurred after 2 h of work. The mean lactate concentration was slightly higher at rest for trained cows, indicating a greater clearance rate from the trained muscle.

The increase in concentration of plasma FFA during the *Work* period is in agreement with the results reported by Kartiarso and Teleni (1988). In the Ongole and Madura cows, the increases in FFA concentration during the *Work* period in Period 2 were greater than those in Period 1. The same cannot be said for the Bali cows which in fact had a reduced increase in FFA concentration during the *Work* period in Period 2. The percentage increases in concentration of plasma FFA in the Ongole, Bali and Madura cows were 42, 45 and 31 respectively in Period 1 and 52, 22 and 53 respectively in Period 2. It would appear therefore, that the rate of utilisation of FFA, in relation to its rate of mobilisation, was greater in the trained Bali than in either the trained Ongole or Madura cows.

The reduction in FFA and the increase in glucose concentration in plasma of the untrained Ongole at 1.5–2.0 h of work, probably indicate a changeover from glucose to FFA as the predominant fuel for muscle contraction. It is interesting to observe also, the occurrence of the OBLA in the same animals at this point in the *Work* period, suggesting perhaps that anaerobic glycolysis may have been supporting the slower changeover process to FFA oxidation.

Glucose concentrations decreased in the last 30 minutes before work ceased. This may have been due to the depletion of glycogen stores in the liver. Overall, the Ongole cows had the highest mean peak concentration of glucose in plasma (table 3B.18).

Table 3B.18 Peak glucose concentrations in plasma of Ongole, Bali and Madura cows during the *Work* period

	Plasma glucose (mM)	
Breed	Untrained	Trained
Ongole	4.91	4.07
Bali	3.73	3.48
Madura	4.21	3.55

The general increase in concentration of plasma glucose in all the breeds during the *Work* period would indicate the mobilisation of the metabolite from the liver. In the trained Ongole cows, however, there was a decrease in the concentration of plasma glucose. It is probable in this case, that glucose was being taken up at a relatively high rate by the working muscles. The other possibility is that the release of glucose from glycogen stores in the liver was being suppressed by increasing rate of FFA oxidation (Costill et al. 1977). However, the comparatively lower RT values observed in the Ongole cows during the *Work* period would be consistent with a higher rate of glucose utilisation. Kruk et al. (1987) demonstrated in her

study with dogs, that enhanced glucose availability reduced exercise-induced rises in RT and also lowered the rate of O_2 uptake by the tissues. Furthermore, the observation that the blood PCV in the Ongole cows were not changed by work or by training is consistent with the suggestion that there was a higher rate of glucose utilisation in this breed. It would appear from the profile of urea concentrations (figures 3B.21–3B.23) that protein degradation occurred only in the untrained animals. It is possible that in the untrained animals, AA were utilised directly as energy substrates and/or as gluconeogenic precursors.

Conclusion

It might be concluded that because of its large size, white coat colour and its higher oxidative capacity, the Ongole is the superior of the three breeds, if all animals were subjected to a draught load equivalent to 12% of live weight.

The work capacity of the Bali and Madura cows was limited probably by hyperthermia as well as by the increasing accumulation of lactic acid in their muscles.

SECTION 3C

A COMPARISON OF WORK CAPACITY AND MUSCLE METABOLISM OF MERINO WETHERS WALKING AT DIFFERENT SPEEDS

3C.1 Introduction

In the previous two experiments, the work capacities of buffalo, Ongole, Bali and Madura cows were defined. It was shown that the work capacity of an animal can be increased by short periods of intensive training. Such an improvement in the performance of the trained animal is achieved through an improved oxidative capacity and cardiovascular system.

It was also shown that plasma concentrations of lactate were increased by work, particularly in the untrained cows. However, alkalosis but not acidosis occurred in these animals.

Since only jugular venous blood samples were taken from animals in the two experiments, the interpretation of the metabolic data obtained was largely speculative. It was suggested from the data of those experiments that the onset of fatigue in the working draught cows would largely be due to hyperthermia. An additional contributor to the onset of fatigue would be the relatively slow rate of ATP generation to meet the energy demand of the contracting muscles, but it is unlikely that acidosis would have a significant role.

This study was undertaken to examine the above suggestions, using the arterio-venous (A–V) concentration difference technique (see Teleni & Annison 1986) on Merino wethers.

3C.2 Materials and Methods

3C.2.1 Location

This experiment was conducted at the Department of Biomedical and Tropical Veterinary Sciences, James Cook University.

3C.2.2 Experimental animals

Management and feeding

Twelve Merino wethers, aged 2.5 years, with average live weight of 34 ± 3 kg were used. They were housed initially in a covered shed in individual pens which had slatted wooden floors. The pens were cleaned daily. The animals had been drenched regularly and were considered to be mostly free of intestinal parasites.

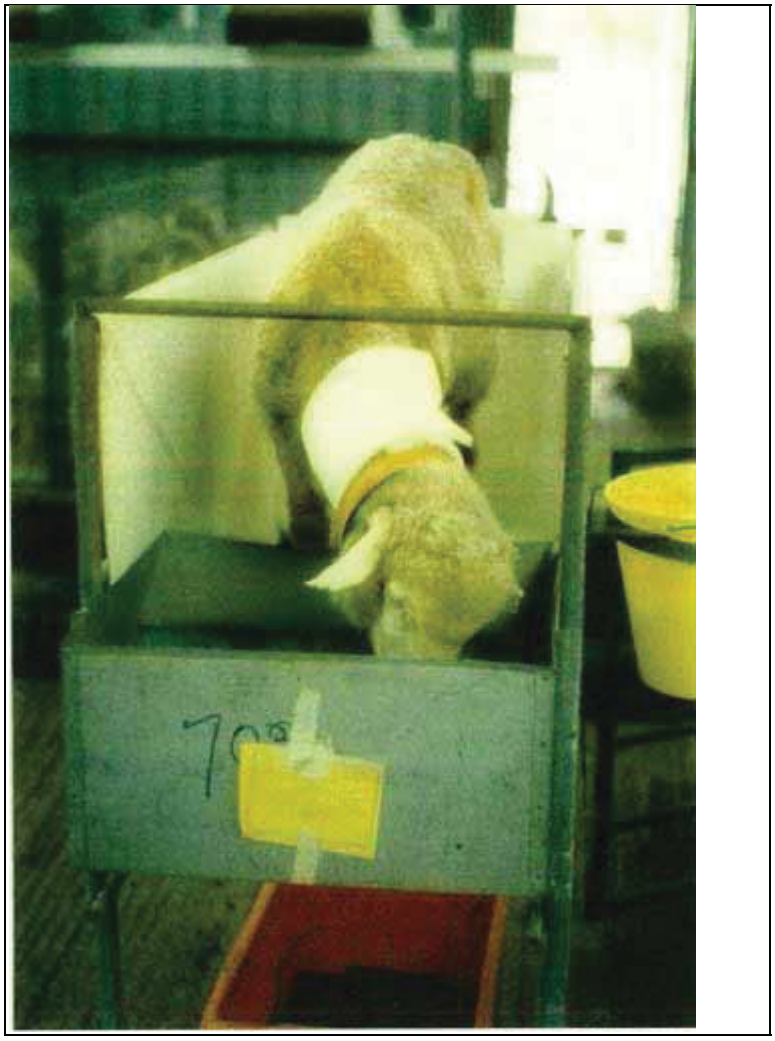
Two weeks before the measurement periods, the sheep were moved into individual metabolism crates (see photograph 3C.1) which facilitated ease of sampling during the *Pre-work* and *Recovery* periods.

The animals were fed at 1400 h daily with approximately 706 g/hd/d of hammermilled sorghum hay (*Sorghum sudanense* x *Sorghum bicolor*). A Rumevite mineral stock block (Rumevite, Cheetham Rural Division, Brisbane, Australia – see table 3C.2) and clean drinking water were made available to the animals at all times. Animals did not have access to feed or water during the measurement periods (see 3C.2.6).

All animals were inspected daily for signs of ill-health.

Training

Training consisted of a 15-d period (5 d/week for 3 weeks) in which a sheep was made to walk on a treadmill for 2 h/d at a speed of 0.69 m/sec while pulling a draught load equivalent to 8% of its live weight. The animal had been made accustomed to walking on the treadmill during a 5-d period prior to the 15-d training period. In that 5-d period, the animal was made to walk slowly for 15 minutes without a draught load. On each subsequent day the speed of walking and draught load were increased to those adopted in the training period. All animals were fully accustomed to all aspects of sampling and measurement procedures before the measurement period.



Photograph 3C.1 Experimental sheep in the fibreglass metabolism crates.

Catheters

Jugular vein

A jugular venous catheter, for pulse counting, was installed in each animal one day prior to the measurement period (see section 3A.2.2). The catheter, 70 cm in length, was inserted 40 cm into the external jugular vein. A protective foam collar (15 cm wide and 1 cm thick) was then fitted around the neck of the animal.

Lateral saphenous vein

A leg venous catheter was also installed one day prior to the measurement period. The catheter (PVC; 70 cm long; ID 0.88 mm x OD 1.27 mm – Dural Plastics and Engineering) was inserted percutaneously, approximately 30 cm into the vein with the aid of a wire guide (TSF 28 mm x 80 cm in length; WA Cook Australia Pty Ltd, Brisbane) via an 18 gauge

needle.

The catheter tip was fixed in position, 26 cm from the junction of the cranial and caudal branch of the lateral saphenous vein (see Teleni & Annison 1986). The catheter was treated, protected with a sleeve and secured as previously described (see section 3A.2.2).

Femoral artery

A femoral arterial catheter (polyethylene; 0.86 mm ID x 1.27 mm OD – Dural Plastics and Engineering) was installed surgically three days prior to the measurement period. The catheter was treated as for the leg venous catheter.

The medial side of the left thigh was clean-shaven, thoroughly cleansed and sterilised before an area (approximately 32 cm^2) above and around an appropriate portion of the femoral artery, was infiltrated, subcutaneously, with the local anaesthetic, Lignodren 2% (containing 20 mg/mL Lignocaine HCL and 0.01 mg/mL Adrenaline – Jurox Pty Ltd, Riverstone, Australia). An incision (approximately 4–5 cm) was made above and to the side (0.5 cm) of the artery. This artery was located and lignodren 2% was dripped continually onto it to prevent vasoconstriction. Thirty centimetres of the catheter was inserted with the aid of a wire guide (TSF 28 mm x 80 cm in length; WA Cook Australia Pty Ltd) and held in position by a soluble suture tied around the artery and catheter. Antibiotic powder (Penbritin) was sprinkled into the incision before stitching. Each animal was given an intramuscular injection of an antibiotic (Betamox; Norbrook Laboratories Limited, UK) immediately post-surgery.

All catheters were removed at the end of the last day of measurement.

3C.2.3 Feed

The composition of the sorghum hay and the mineral stock block fed to the experimental animals are shown in table 3C.1 and 3C.2.

Table 3C.1 The composition of sorghum hay

	%
Dry matter	95
Organic matter	89
Nitrogen	0.9

Source: K Mbwambo (1993), MSc thesis, unpublished

Table 3C.2 The composition of the Rumevite mineral stock block

Mineral	%
Calcium	16
Sulphur	4
Copper	0.03
Cobalt	0.003
Iodine	0.003
Zinc	0.05
Salt	30

3C.2.4 Equipment

Treadmill

The treadmill used has been described in section 3A.2.3 but with the following modifications to accommodate a pair of sheep:

Fencing mesh (70 cm high and 2.9 m long) was positioned along the centre and 5 cm above the belt of the treadmill. This allowed two sheep to walk side by side on the treadmill while pulling a load (see photograph 3C.2) or six sheep (three on either side) walking without a load (see photograph 3C.3). The draught load was applied to each of a pair of animals through a double pulley system as illustrated in figure 3C.1.

Photograph 3C.2 Treadmill adapted for two sheep to pull draught loads.

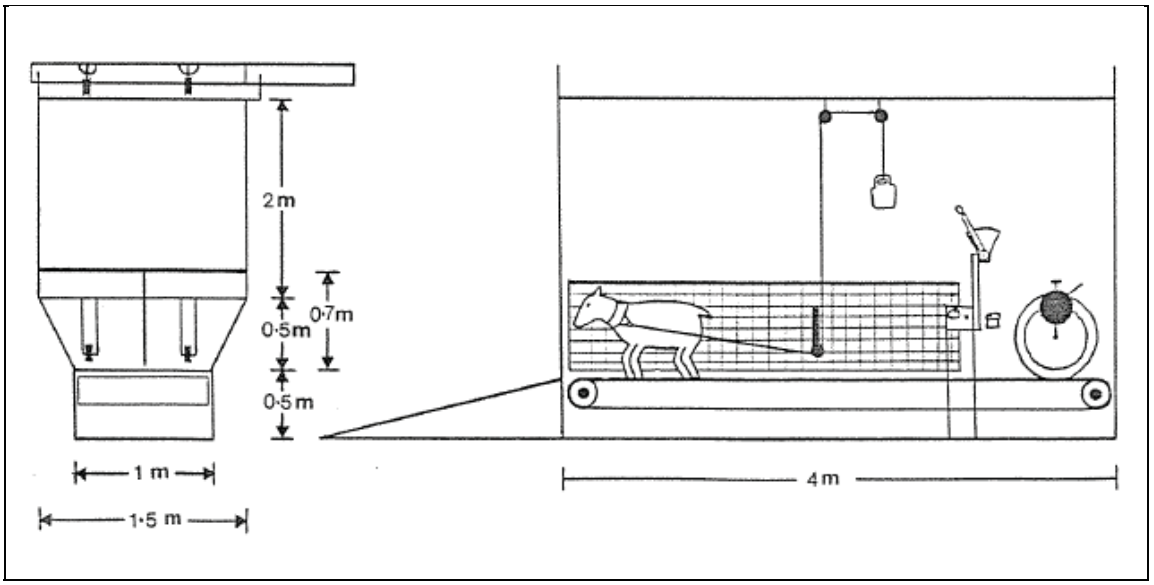


Figure 3C.1 Treadmill modified to accommodate sheep.

Photograph 3C.3 Six sheep walking on the treadmill.

Collar

A smaller version of the buffalo collar (see section 3A.2.3) was made using rubber from a car tyre, but no sheep skin covering was used. A leather dog collar was used for fastening the rubber collar around the shoulders of the sheep (see photograph 3C.2).

Load

The load was a 5-L plastic container filled to the required weight with water.

Draught force measuring equipment

The equipment used for measuring draught force was as described in section 3A.2.3 except that the load cell used was a Barlo TCS Load Cell (Barlo Instruments, Brisbane, Australia).

3C.2.5 Experimental Design

Two physiological states, untrained and trained were compared. Sheep were used as a model for draught animals due to ease of handling and economics of feeding and experimental procedures. Sheep are able to be used as a model due to the similarity with large ruminants in metabolic and physiological responses to work (Biswas et al 1991).

The animals were divided into six pairs, closely matched for body weight and frame size.

One of each pair was used as the untrained animal and the other was trained. Measurements were made on 1 pair per week for the first 2 weeks. Over the next 2 weeks, 2 pairs per week were used. Pairs 1, 2, 3 and 5 worked according to time 1 below and pairs 4 and 6 according to time 2 (see figure 3C.2).

The sheep were subjected to work by pulling a draught load of 11% of live weight while walking on a treadmill at 0.69, 1 and 1.4 m/sec. One trained and one untrained animal (a pair) were worked together for 3 h over three consecutive days, with the sequence of the three speeds being randomly selected.

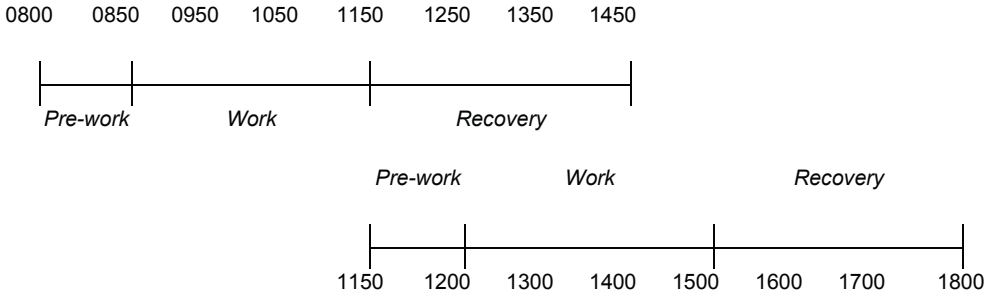


Figure 3C.2 The daily time schedule for the *Pre-work, Work* and *Recovery* periods for each pair of animals during the three day measurement period.

During the *Pre-work* period (30 minutes) measurements were taken while the sheep were standing quietly in their metabolism crates. The sheep then worked for 3 h on the treadmill or until fatigued and were placed back into their crate for the 3 h *Recovery* period. No feed or water was given during the measurement period.

Pulse rate was measured first followed by RR and then the blood sampling. Skin and RT were the last measurements taken. Environmental parameters (ambient and BB temperatures as well as wet and dry bulb readings) were recorded at the same time as each sample for sheep were taken.

3C.2.6 Experimental procedure

All animals were fully accustomed to the experimental procedure, well before the time for sampling and measurement arrived.

Samples and blood sampling

Blood

Six mL and 1 mL blood samples (for blood gas analysis – see details in section 3C.2.8) were collected half hourly during the *Pre-work* and *Work* periods. The samples were also collected half hourly for the first hour of the *Recovery* period and subsequently at hourly intervals for the remainder of the same period.

In addition, samples (2 mL x 10) were collected using a continuous sampling procedure for the first 20 minutes of the *Work* period. All samples were taken in A–V pairs; one from the femoral artery and the other from the lateral saphenous vein.

An additional A–V pair of samples was collected for bloodgas analysis 20 minutes into the *Work* period.

Blood samples were collected and processed as described in section 3A.2.5. The centrifugation of samples, however, was conducted using a Baird and Tatlock Bench Centrifuge (Chadwell Health, Essex, London; rotor diameter = 14 cm, 5000 *g* = 3300 rpm).

The *Pre-work* blood samples were collected while the sheep were standing in their metabolism crates. The animals were then carried to the treadmill where work proceeded. *Recovery* blood samples were taken when the animals were back in their metabolism crates. If a member of the working pair was fatigued before the other, the treadmill was stopped, the fatigued animal removed as quickly as possible and the remaining sheep continued to work.

Continuous sampling procedure

The continuous sampling of blood from the femoral artery and lateral saphenous vein was achieved by pumping blood from respective catheters using a Technicon Auto-Analyser II proportioning pump (Bran and Leubbe Pty Ltd, Homebush, NSW, Australia). The blood was pumped through PVC lines (1 mm ID x 1.5 mm OD) at a rate of 1 mL/min using Technicon flow rated pump tubes (Bran and Leubbe Pty Ltd). To prevent clotting of blood during continuous sampling, heparinised physiological saline (500 IU/mL) was pumped at a rate of 0.05 mL/min through a PVC line (0.86 mm ID x 1.27 mm OD) into the sampling line at the point where it joined the catheter (see figure 3C.3).

The sampling line was connected to its corresponding catheter, so that blood was flowing through it before the *Work* period started. The transit time of blood through each sampling line was 5 minutes. The first 2 mL aliquot of blood was therefore collected from the sampling line from the sixth minute into the *Work* period. Blood collection of 2 mL aliquots was continued for 20 minutes into the *Work* period although the sampling line was disconnected from the catheter on the sixteenth minute of the *Work* period. Blood samples were collected directly into 10 mL plain (non-heparinised) centrifuge tubes which were standing in an ice bath. Plasma was extracted from blood samples by centrifugation as described in section 3A.2.5.

Measurements

The values for physiological and environmental variables indicated below were recorded at hourly intervals. The treadmill was not stopped when measurements of these variables were taken.

Environmental

Ambient temperature

Ambient temperature was determined as described in section 3A.2.5.

Black bulb

The BB temperature was determined as described in section 3A.2.5.

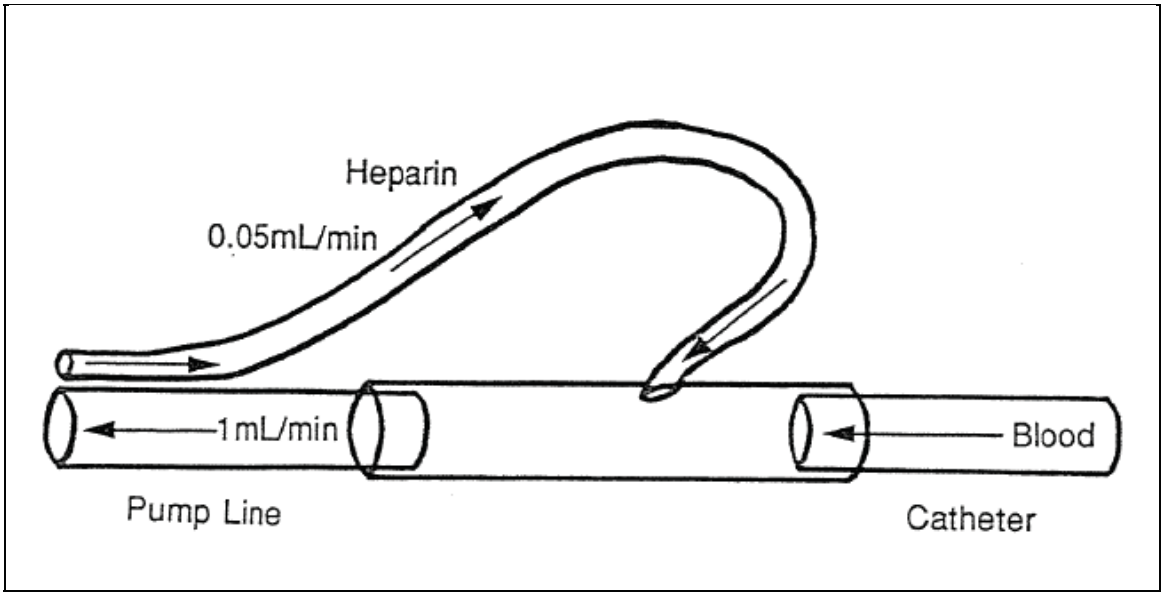


Figure 3C.3 Diagram illustrating the point at which the heparin enters the blood line.

Relative humidity

The wet and dry bulb used was the same as that described in section 3B.2.5. This was hung next to the BB position, on a table next to the treadmill.

Physiological

Rectal temperature

The RT was measured using a mercury clinical rectal thermometer (GH Zeal, London, England). The thermometer was graduated from 35–42☜C in 0.1☜C increments. The thermometer was inserted into the rectum and placed against the rectal wall for two minutes before the registered temperature value was recorded.

Skin temperature

The ST was measured according to the procedure described in section 3A.2.5.

Respiration rate

The RR was determined by counting the number of outward air movements from the nose in a 15–second interval. Air movements were registered by placing fingers close to the nose of the working animal. The registered value was multiplied by 4 to give breaths/minute.

Pulse rate

Pulse rate was determined as described in section 3A.2.5.

In addition to the hourly recordings, PR was also recorded at 30–second intervals for the first 10 minutes of the *Recovery* period.

3C.2.7 Fatigue assessment

Fatigue was considered to be the point when the sheep could no longer sustain the given work load. An animal considered to be fatigued was removed from the treadmill and returned to its metabolism crate. An animal was also removed from the treadmill when its RT reached or exceeded 41☜C.

3C.2.8 Laboratory analysis

Bloodgas

The 1 mL blood samples were analysed for TCO_2, O_2SAT and HCO_3 using a 278 Ciba Corning Bloodgas Analyser (Ciba Corning Diagnostics Corporation, Medfield, MA, USA).

Haemoglobin

The haemoglobin concentration in blood was determined as described in section 3A.2.7.

Packed cell volume

The blood PCV was determined as described in section 3A.2.7.

Glucose

Plasma glucose was analysed on a Technicon Auto-Analyser II (Bran & Leubbe Pty Ltd) using the Technicon colorimetric method, number 339–02 as described by Bittner and

Manning (1967). This technique is based on the reaction of cupric neocuprione chelate (2,9–dimethyl–1–,10 phenanthroline) with glucose. This cupric-neocuprione chelate is reduced by glucose in an alkaline medium, resulting in an intense, yellow-orange cuprous-neocuprione complex which is determined colorimetrically at 460 nm.

Urea

Plasma and urinary urea were analysed using the technique described in section 3A.2.7 except the Auto-Analyser II used was as described above.

Creatinine

Plasma creatinine was determined using the Technicon colorimetric method, number SM4–0141D91 on a Technicon RA–1000 (Bayer Diagnostics Australia Pty Ltd, Pymble, NSW, Australia). This method is based on the reaction of picric acid with creatinine in an alkaline medium as described in the original procedure of Jaffe (1886) and modified by Rossignol et al. (1984).

Potassium

Potassium was measured using the Ion Selective Electrode (ISE) module attached to the Technicon RA–1000 (Bayer Diagnostics Australia Pty Ltd. Pymble, NSW, Australia). The technique used was the Technicon method number SM4–0034C84.

Free fatty acids

The concentration of plasma FFA was determined as described in section 3A.2.5.

Lactate

Plasma lactate was analysed using a Stat–Pak Rapid Lactate Kit (Behring Diagnostics Inc, New Jersey, USA) on a Technicon RA–1000 (Bayer Diagnostics Australia Pty Ltd).

Total nitrogen

Two hundred µL of plasma was placed into a glass digestion tube with 6 mL of concentrated sulphuric acid and 1 catalyst tablet (Kjeltab – 3.5 g K_2SO_4 and 0.0035 g se). This was digested at 420☜C until clear in a Tecator 1016 Digestion System 40 (Tecator AB, Sweden) and analysed for total nitrogen using a Kjeldahl distillation unit (Tecator AB).

Ammonia

Plasma NH_3 was analysed using the Auto-Analyser Technique (Williams & Twine 1967) on a Technicon Auto-Analyser II (Bran & Leubbe Pty Ltd).

3C.2.9 Calculations

Energy expenditure was calculated as described in section 3A.7 with the following changes.

A = 2.5 j/kg/m……..(Clapperton 1964)
C = 0.389 (for buffalo)

had been used to calculate EE because no data were available for this value for sheep.

Calculations were made using both C for buffalo and C for cattle and there was not significant difference (P=0.0000) between EE calculated from both these coefficients.

Em (MJ/d) = 1.2 + 0.13 W…….(MAFF 1975)
Energy for maintenance (Mm) has been calculated using equation

where
Mm = energy used for maintenance
W = body weight

Protein/AA–N was calculated from data on urea–N, NH_3–N and total–N, where

Total-N = NH_3-N + Protein/AA-N + Urea-N

3C.2.10 Statistical analysis

Data were computed and analysed using the Statistix 4.0 program (NH Analytical Software).

3C.3 Results

Not all of the untrained or trained sheep were able to complete working through the 3 h *Work* period. Most of the trained animals, but none of the untrained animals completed 30 minutes of work. At the slow and medium speed of walking, all sheep were able to complete 60 minutes of work. A comparison has been made between physiological states, slow and medium walking speeds and work status over 60 minutes in the *Work* period. Comparisons have also been made for trained animals over 30 minutes in the *Work* period for the three walking speeds.

3C.3.1 Environmental conditions

The mean (and range) values for AmT and relative humidity were 31.4°C (26–37°C) and 55% (10–96%) respectively. Values for the BB or net radiometer temperature were 31.6°C (22.2–37.4°C). The profiles of values for ambient and BB temperatures and relative humidity are presented in figure 3C.4.

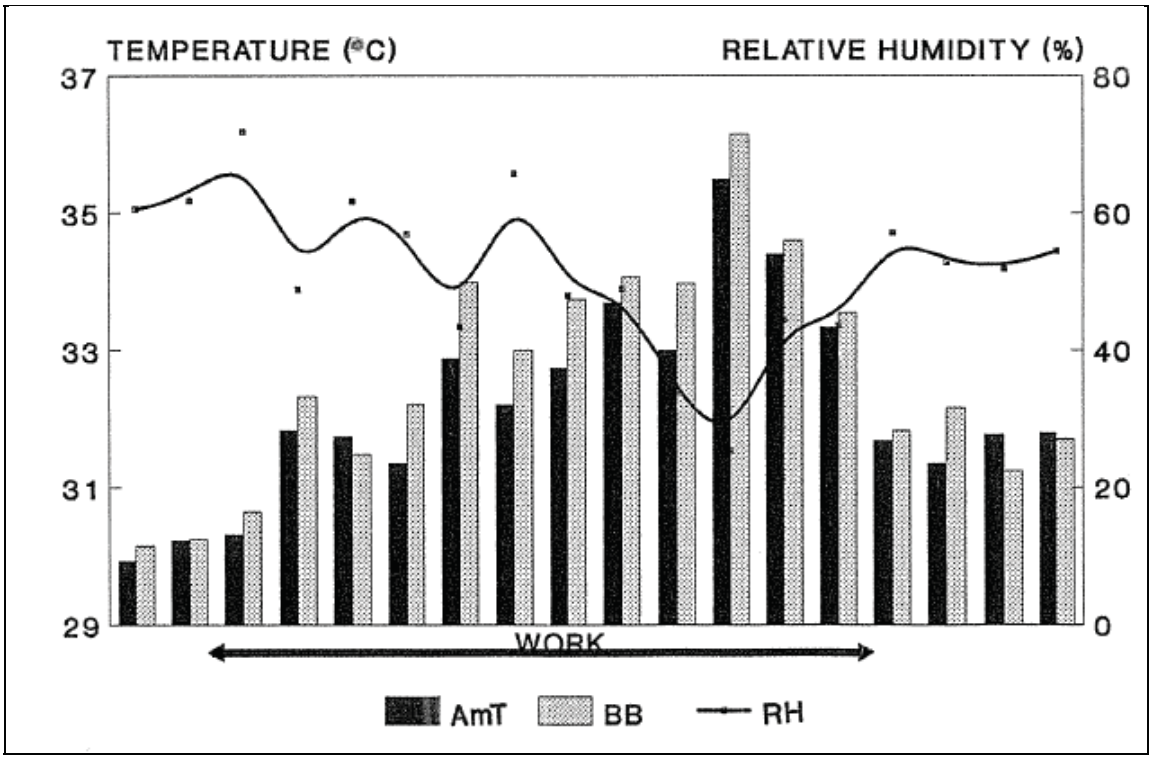


Figure 3C.4 Means of ambient temperature (AmT), black bulb (BB) temperature and relative humidity (RH) during the measurement period.

The AmT readings were highest during the *Work* period and lowest during the *Pre-work* period. The relative humidity values decreased as AmT increased (figure 3C.4).

3C.3.2 Work capacity

Work Duration and Distanced Travelled

The data on work duration and the total distance travelled by the untrained and trained sheep walking at the three different speeds are presented in table 3C.3.

Table 3C.3 Mean walking speed, duration and distance travelled by untrained and trained Merino wethers
Values are means ± standard errors (SE). (n=12)

	Work duration (min)			Distance travelled (km)		
Walking speed (m/sec)	Untrained	Trained	± SE	Untrained	Trained	± SE
0.66 (slow)	82	126	16	3.25	5.03	0.65
1.04 (medium)	57	136	17	3.50	8.45	0.10
1.38 (fast)				0.57	2.69	0.41
	7	32	5			

Work duration and distance travelled were significantly affected by physiological states and walking speeds (table 3C.4).

Table 3C.4 Means of pooled data on duration of work and distance travelled in sheep across physiological states and walking speeds

Values are means ± standard errors (SE).

	Treatments				
	Physiological states[1]		Walking speeds[2]		
	Untrained	Trained	Slow	Medium	Fast
Duration of work (mins)	48	98	104	96	19
± SE	4	5	5	5	1
Significance	P=0.0116		P=0.0002		
Distance travelled (km)	2.46	5.56	4.39	6.00	1.63
± SE	0.18	0.27	0.20	0.30	0.12
Significance	P=0.0019		P=0.0011		

[1] n=6
[2] n=12

Work duration decreased as the speed of walking increased. The exception to this general

trend was observed at the medium walking speed for the trained animals, which walked for 10 minutes longer, and therefore travelled further than they did at the slow speed of walking (table 3C.1). Training increased the work capacity of sheep on slow, medium and fast speeds by 54, 139 and 369% respectively.

Energy Expenditure

The estimated Mm of the sheep was 5.54 ± 0.04 (SE) MJ/d (see MAFF 1975). Using the published coefficient for efficiency for doing mechanical work by buffalo (Lawrence 1985) - see also section 3C.2.9, it was estimated that the total energy expended by the animals on the slow, medium and fast walking speeds are shown in table 3C.5.

Table 3C.5 Pooled data on energy expenditure (EE) and EE:Maintenance energy requirement (Mm) of sheep across walking speeds
Values are means ± standard errors (SE). (n=12)

Speed	EE	EE:Mm
Slow	10.70 ± 0.45	1.93 ± 0.06
Medium	13.10 ± 0.40	2.36 ± 0.01
Fast	15.73 ± 0.42	2.83 ± 0.01

Energy expended during work was equivalent to 1.93, 2.36 and 2.83 respectively x Mm by animals walking at slow, medium and fast speeds.

3C.3.3 Physiological variables

Skin temperature

Data for ST of untrained and trained sheep across work status and slow and medium speeds are presented in figure 3C.5.

Comparisons of mean differences of ST in sheep between physiological states, walking speeds and work status are presented in table 3C.6.

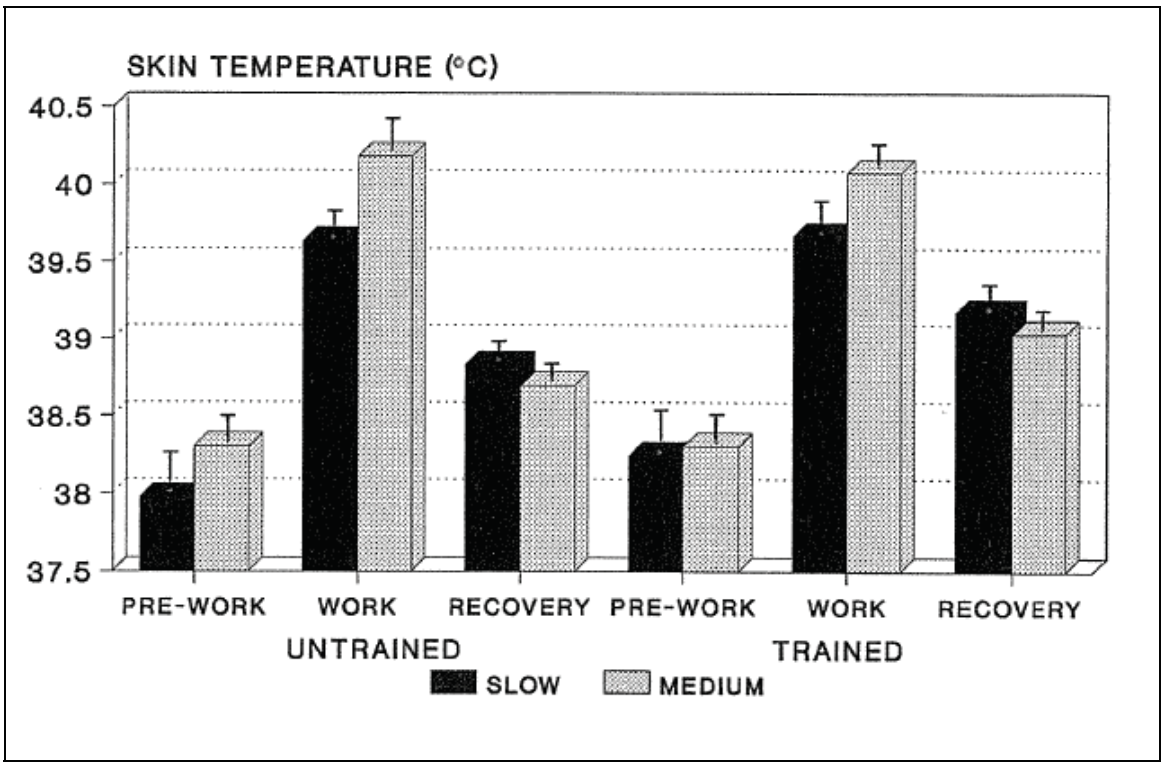


Figure 3C.5 Means of pooled data of skin temperature of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Table 3C.6 Means of pooled data on skin temperature (ST) of sheep for physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
ST (☜C)	39.1	39.2	39.1	39.3	38.2	39.9	38.9
± SE	0.1	0.1	0.2	0.1	0.7	1.2	0.5
Significance	P=0.5270		P=0.4595		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Skin temperature was not significantly affected by physiological states or walking speeds (table 3C.6). The ST increased with increasing speed and tended to be higher in the trained sheep which was mainly due to values in the *Recovery* period (figure 3C.5).
Work status had a significant effect on ST of sheep (table 3C.6). The ST increased by a mean of 1.69☜C during the *Work* period and decreased in the *Recovery* period but remained above *Pre-work* values (figure 3C.5).

Data on ST in trained sheep across work status and walking speeds are presented in figure 3C.6.

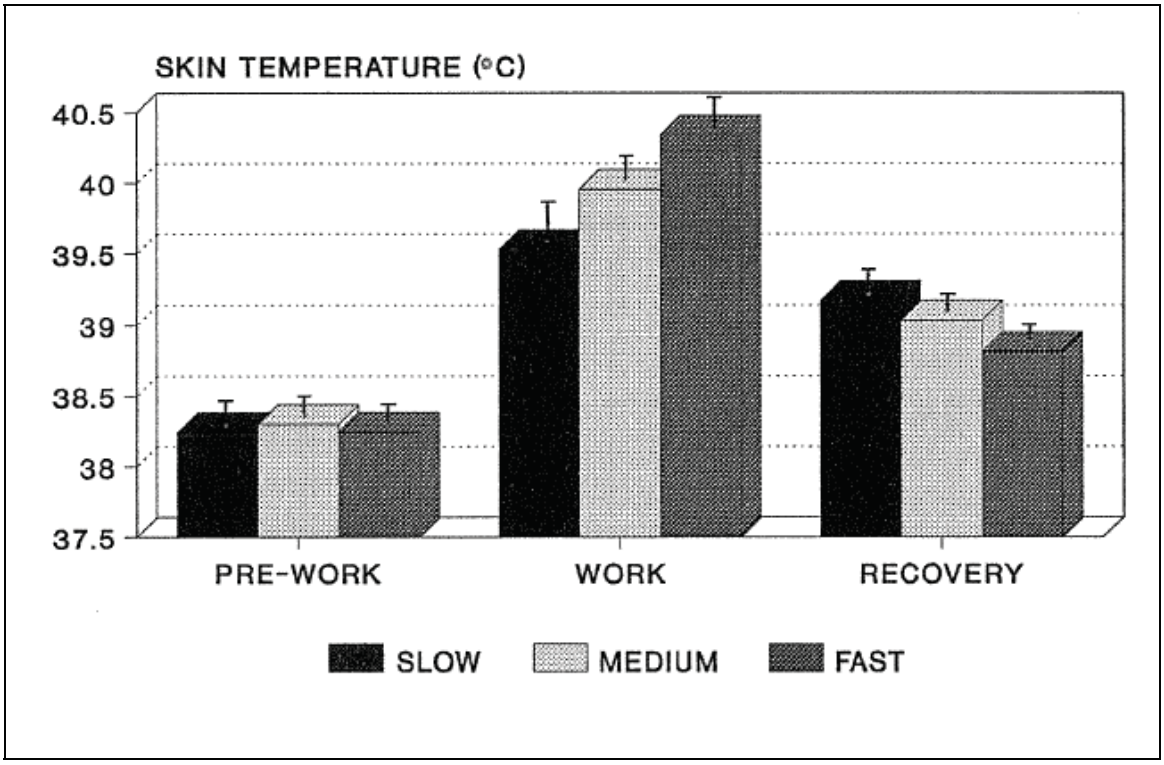


Figure 3C.6 Means of pooled data on skin temperature of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed had no significant affect (P=0.9896) on ST in sheep (figure 3C.6). Skin temperature was significantly affected (P=0.0000) by work status. There was an increase in ST by 1.7☜C during the *Work* period and a decrease during the *Recovery* period, to remain above *Pre-work* values.

The changes in the pattern of ST in untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.14–3C.16.

Rectal temperature

Data for RT of untrained and trained sheep at the three work status and slow and medium walking speeds are presented in figure 3C.7.

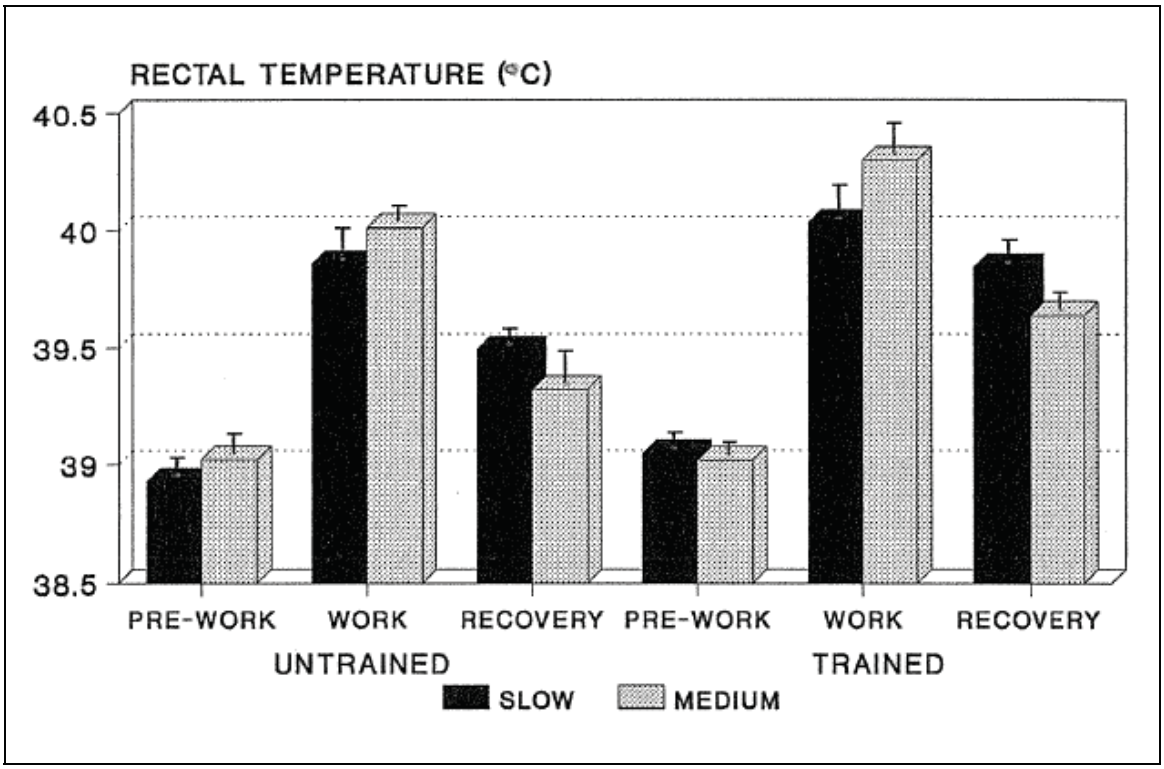


Figure 3C.7 Means of pooled data on rectal temperature of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Comparisons of mean differences of RT in sheep between physiological states, walking speeds and work status are presented in table 3C.7.

Rectal temperature was not significantly affected by physiological states or walking speeds (Table 3C.7).

There was a trend in RT to increase with increasing speed during the *Work* period and to be higher in the trained sheep as compared with the untrained sheep (figure 3C.6). Work status had a significant affect on RT (table 3C.7) The RT increased by a mean of 1.05☜C during the *Work* period and decreased in the *Recovery* period, to remain above *Pre-work* values (figure 3C.7).

Table 3C.7 Means of pooled data on rectal temperature (RT) of sheep for physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
RT (℃)	39.5	39.8	39.6	39.7	39.0	40.1	39.6
± SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Significance	P=0.1051		P=0.9140		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Data on RT in trained sheep across work status and walking speeds are presented in figure 3C.8.

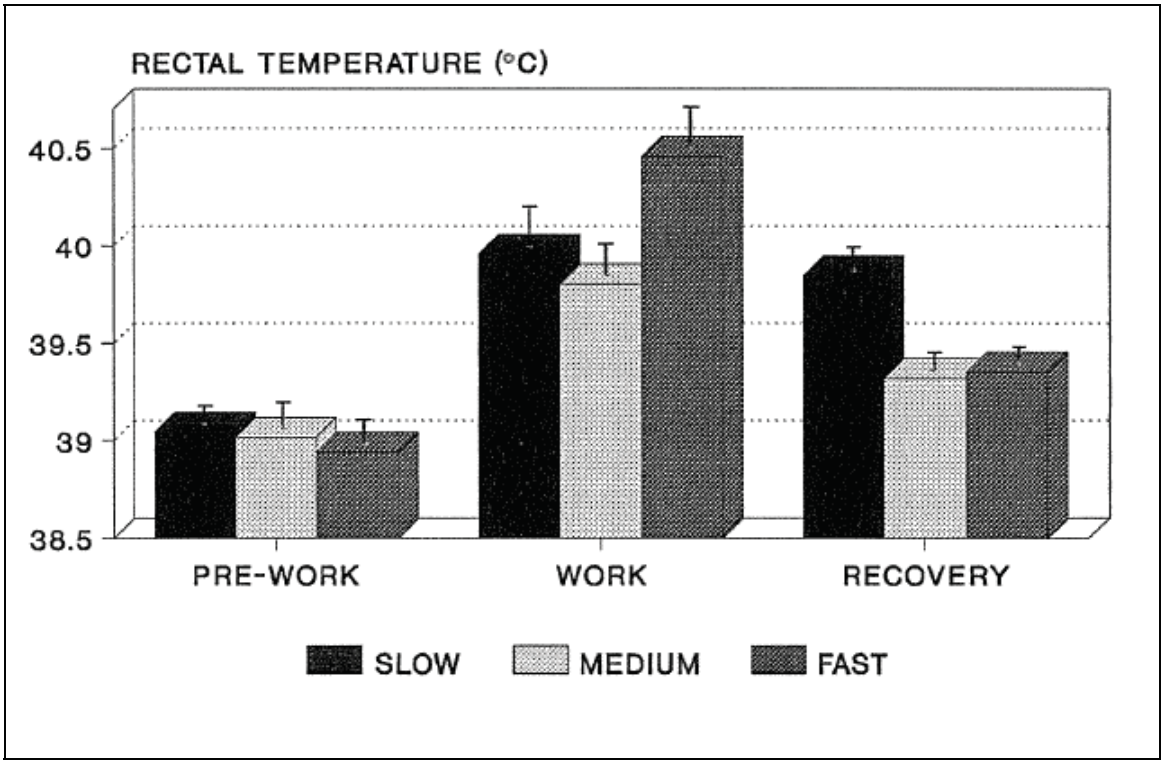


Figure 3C.8 Means of pooled data on rectal temperature of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed had no significant affect (P=0.8543) on RT in sheep although there was a trend of increased RT with increasing speed (figure 3C.8). Rectal temperature was

significantly affected (P=0.0000) by work status. Rectal temperature increased by a mean of 1.18☜C during the *Work* period and decreased during the *Recovery* period, to remain above *Pre-work* values.

The changes in the pattern of RT in untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.14–3C.16.

Respiration rate

Data for RR of untrained and trained sheep at the three work status and slow and medium walking speeds are presented in figure 3C.9.

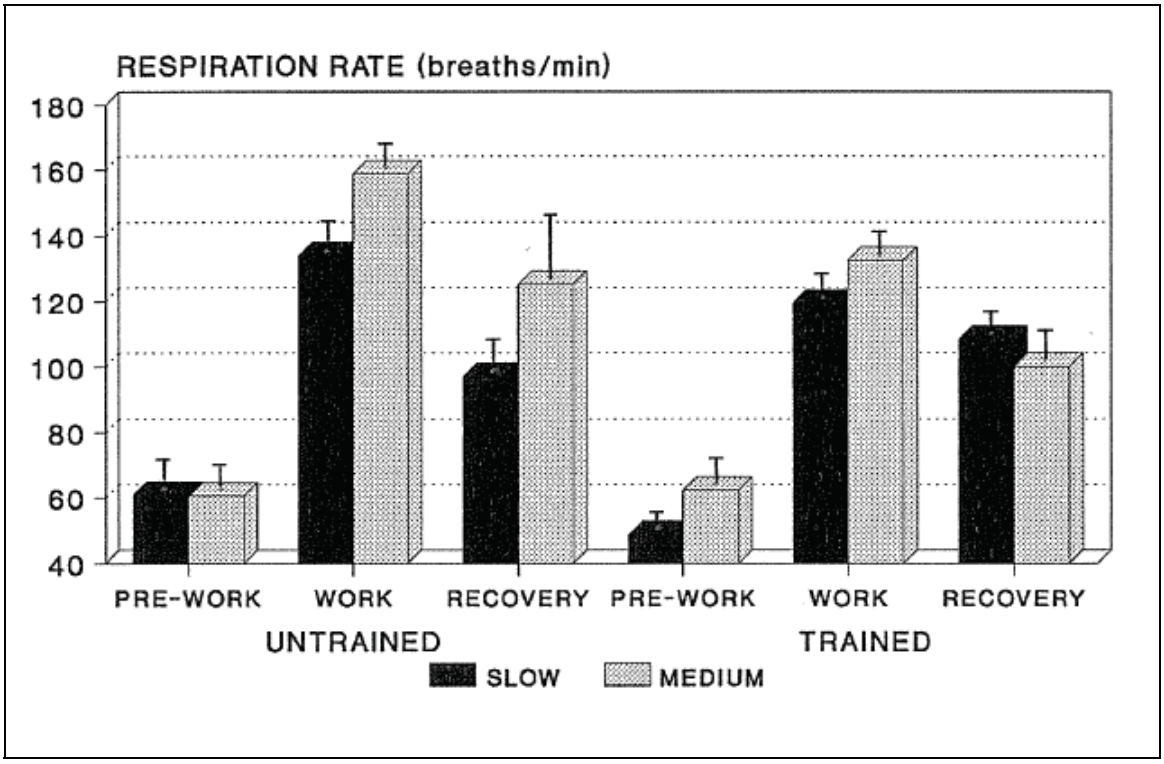


Figure 3C.9 Means of pooled data on respiration rate of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Comparisons of mean differences of RR in sheep between physiological states, walking speeds and work status are presented in table 3C.8.

Physiological states and walking speed had no significant effect on RR (table 3C.8). In the trained animals RR was lower than in the untrained animals (figure 3C.9). In the *Work* period there is a trend for RR to decrease with increasing walking speed (figure 3C.9). Respiration rate was significantly affected by work status (table 3C.8). RR increased by 136% during the *Work* period and decreased during the *Recovery* period (figure 3C.9).

Table 3C.8 Means of pooled data on respiration rate (RR) of sheep for physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
RR (breaths/min)	116	103	103	116	58	137	108
± SE	5	4	8	8	6	6	7
Significance	P=0.2597		P=0.2521		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Data on RR in trained sheep across work status and walking speeds are presented in figure 3C.10.

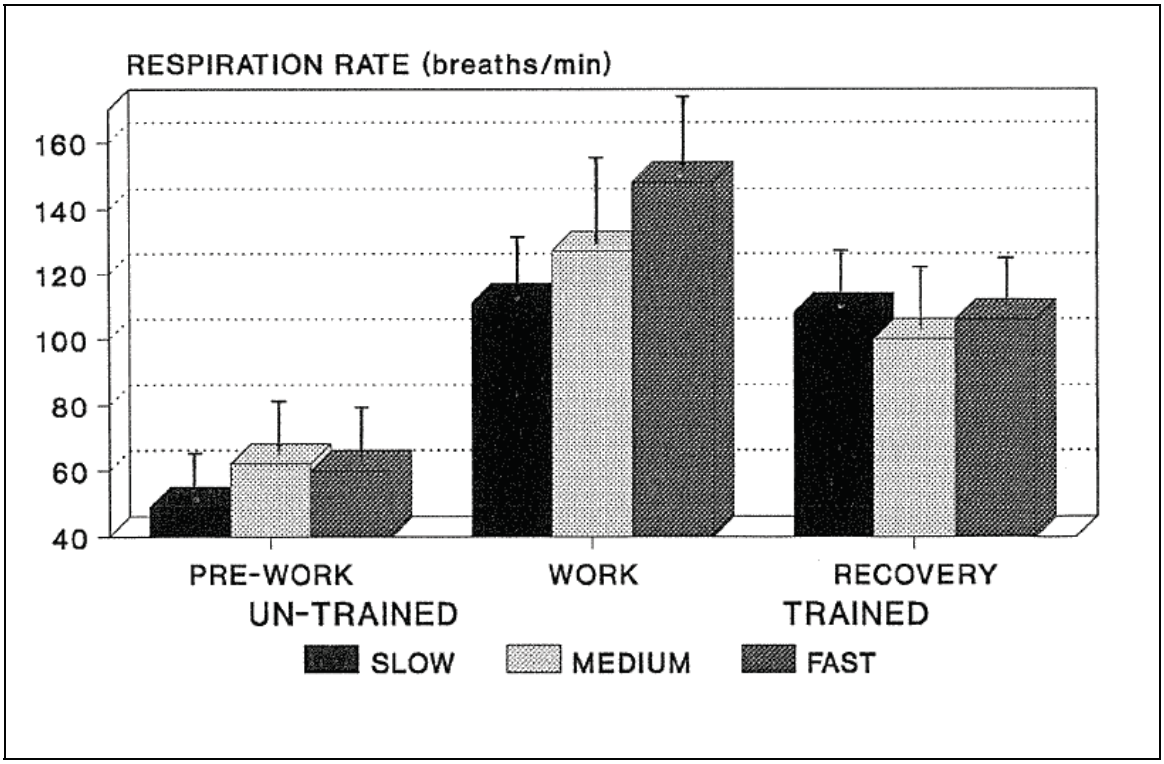


Figure 3C.10 Means of pooled data on respiration rate of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed had no significant effect (P=0.8028) on RR, however, there was a trend of increased RR with increasing speed (figure 3C.10). Work status significantly affected

(P=0.0000) RR with an increase of 125% during the *Work* period. The RR decreased during the *Recovery* period but remained above *Pre-work* values (figure 3C.10).

The changes in the pattern of RR in untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.14–3C.16.

Pulse rate

Data for PR of untrained and trained sheep across work status and slow and medium walking speeds are presented in figure 3C.11.

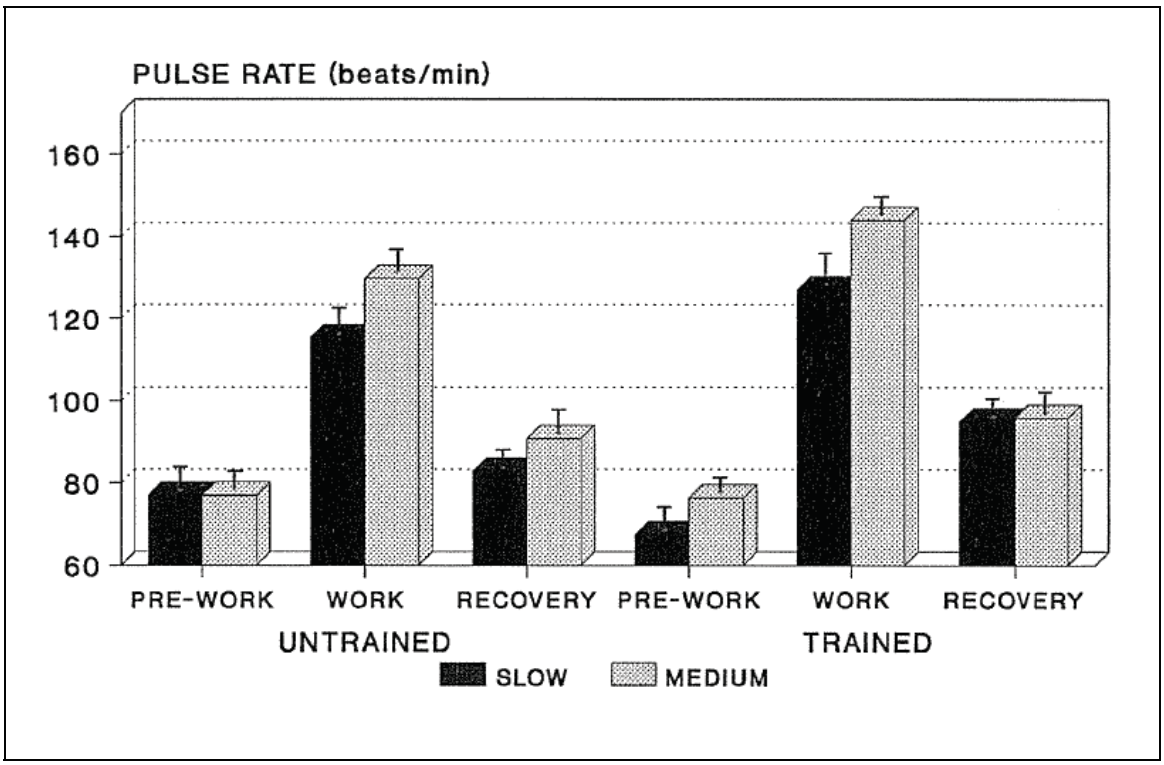


Figure 3C.11 Means of pooled data on pulse rate of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Comparisons of mean differences of PR in sheep between physiological states, walking speeds and work status are presented in table 3C.9.

Table 3C.9 Means of pooled data on pulse rate (PR) of sheep for physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
PR (beats/min)	99	107	98	107	74	129	91
± SE	3	3	5	4	4	4	3
Significance	P=0.3386		P=0.2603		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

The PR was not significantly affected by physiological states or walking speeds (table 3C.9). There was a trend for PR to be lower in the untrained animals as compared to the trained animals and at medium speed compared to slow speed (table 3C.9). Work status significantly affected PR (table 3C.9). Pulse rate increased by a mean of 74% during the *Work* period and decreased during the *Recovery* period, however, but remained above *Pre-work* values.

Data on PR in trained sheep across work status and walking speeds are presented in figure 3C.12.

Pulse rate was not significantly affected (P=0.7556) by walking speed, however, there was a trend of increased PR with increasing speed (figure 3C.12). Work status significantly affected (P=0.0000) PR, resulting in an increase by 96% during the *Work* period. Pulse rate decreased during the *Recovery* period but remained above *Pre-work* values.

The changes in the pattern of PR in untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.14–3C.16.

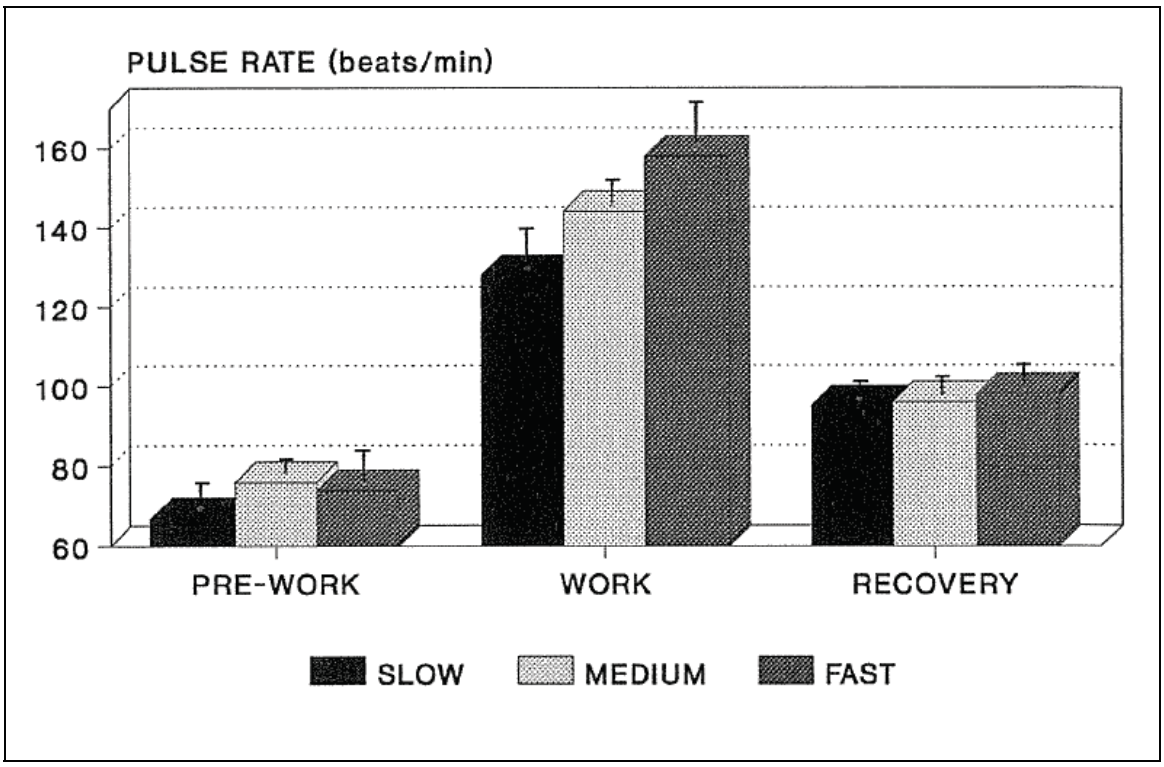


Figure 3C.12 Means of pooled data on pulse rate of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Pulse rate decline

Data for pulse rate decline during the first 10 minutes of the *Recovery* period across physiological states and walking speeds are presented in figure 3C.13.

Pulse rate declined rapidly in the first 4 minutes of the *Recovery* period and remained above *Pre-work* values after 10 minutes *Recovery* (figure 3C.13).

Physiological state did not significantly affect (P=0.1021) the rate of decline in PR. Pulse rate was significantly affected (P=0.0000) by walking speed prior to the cease of walking. In the untrained animals PR decreased at the fastest rate for medium walking speed and slowest for slow walking speed (table 3C.10). In the trained animals, however, PR decreased at a similar rate at medium and fast walking speeds, which were greater than the slow walking speed (table 3C.10).

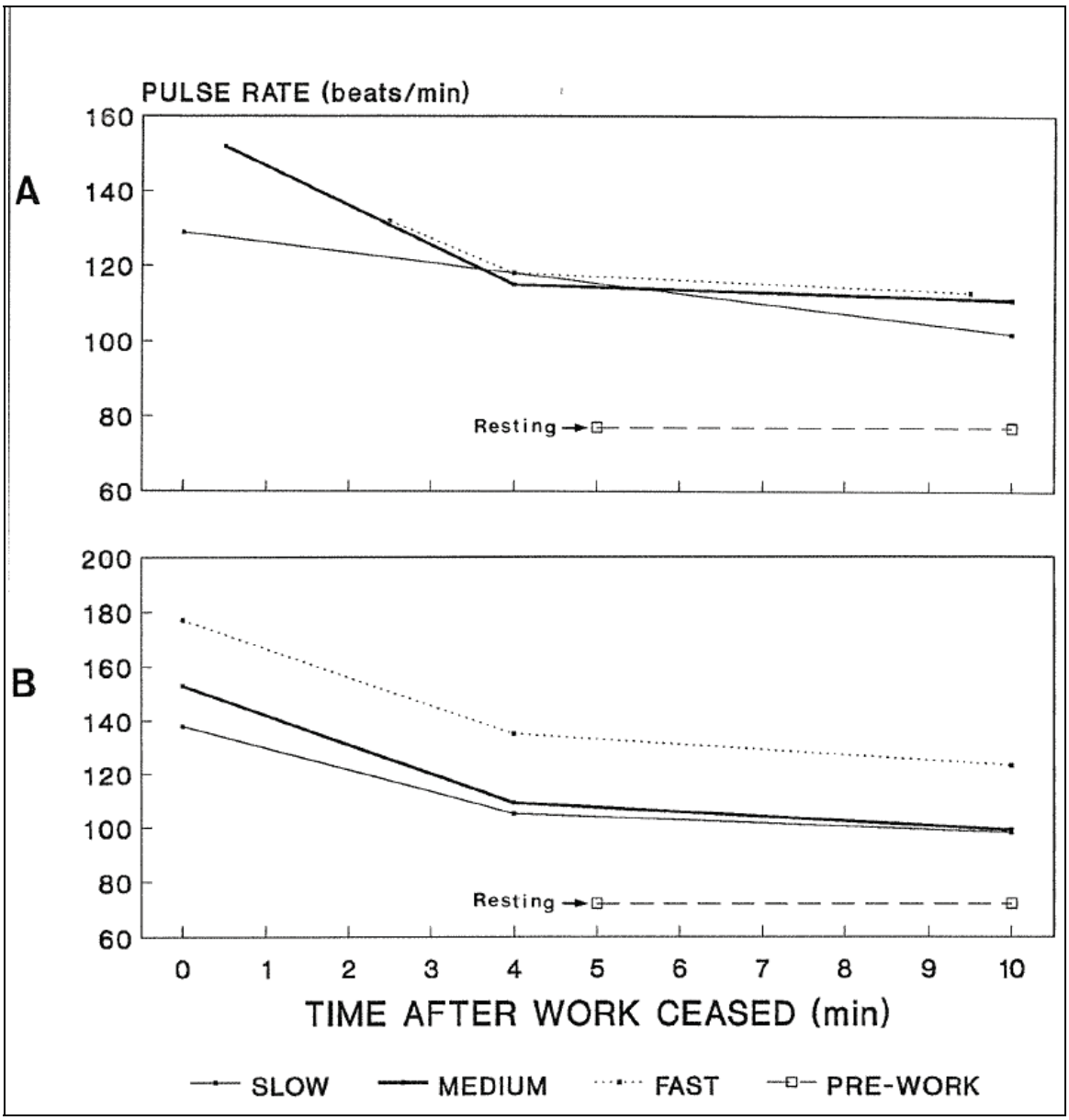


Figure 3C.13 Means of pooled data on pulse rate decline in (A) untrained and (B) trained sheep during the first 10 minutes of the *Recovery* period across walking speeds.

Table 3C.10 Rate of decline in pulse rate (PR) during the first 4 minutes of the *Recovery* period and shape of lines across physiological states and walking speeds

Speed	Untrained		Trained	
	Slope	Rate of decline in PR (beats/min)	Slope	Rate of decline in PR (beats/min)
Slow	-0.13	3	-0.27	8
Medium	-0.48	11	-0.34	11
Fast	-0.38	9	-0.33	11

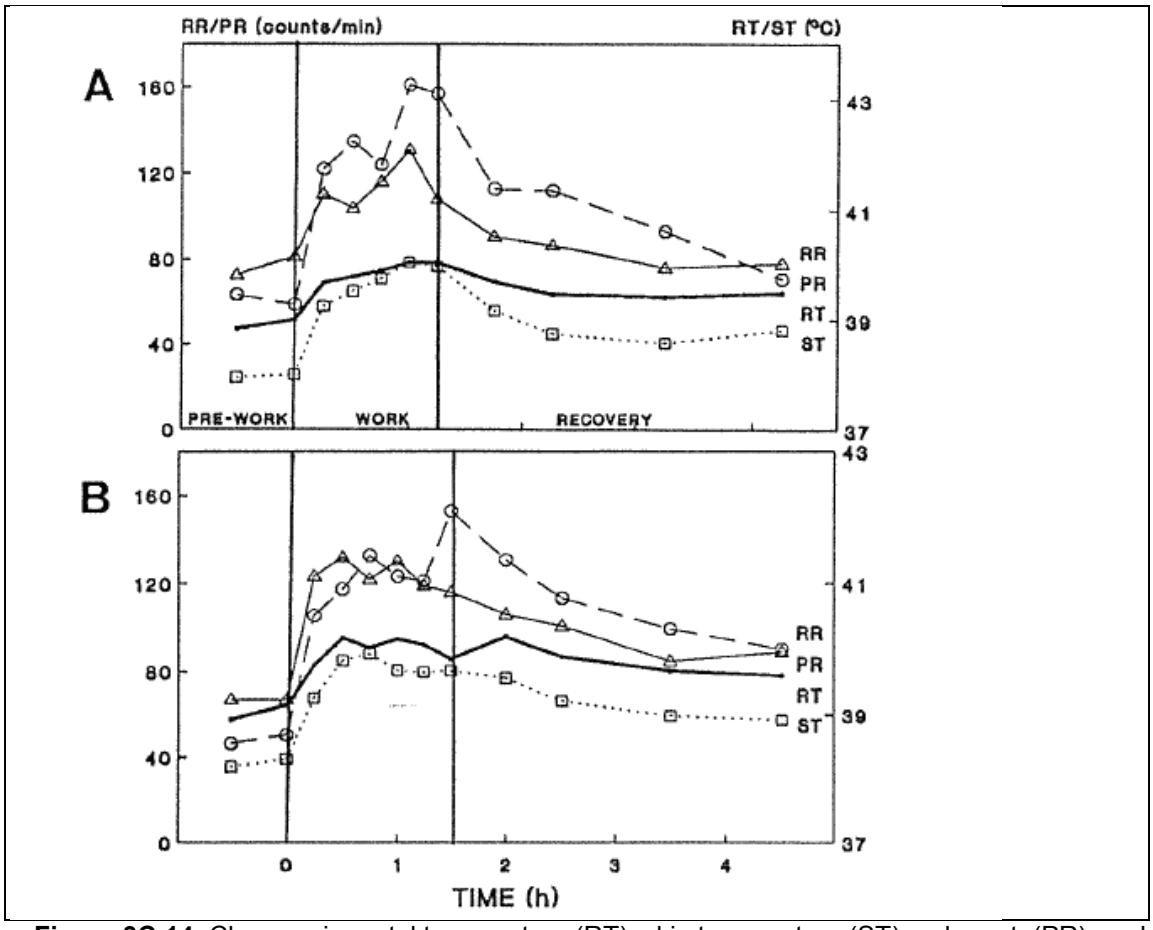


Figure 3C.14 Changes in rectal temperature (RT), skin temperature (ST), pulse rate(PR), and respiration rate (RR) in (A) untrained and (B) trained sheep over time across work status (*Pre-work, Work, and Recovery*) at slow speed.

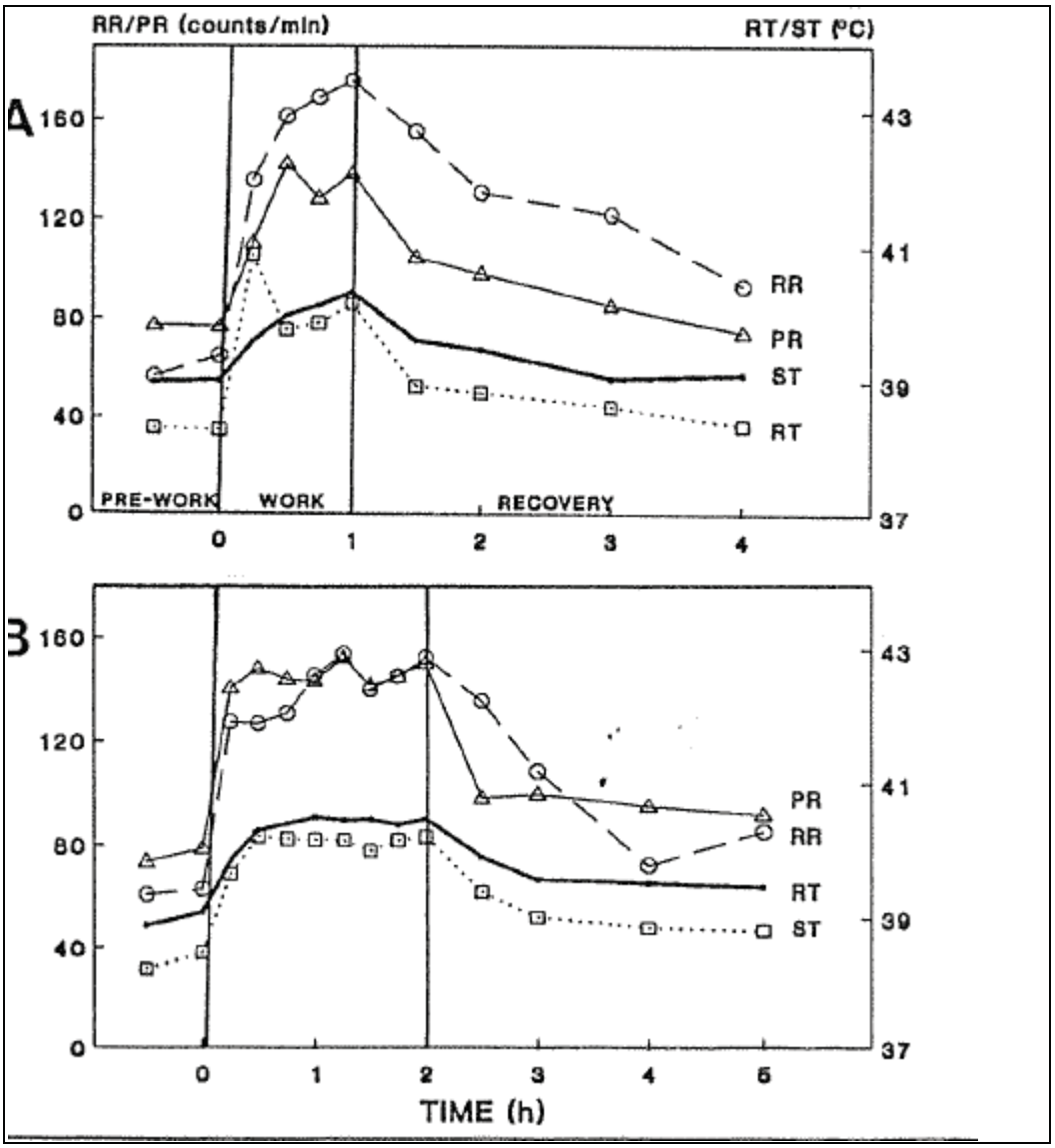


Figure 3C.15 Changes in rectal temperature (RT), skin temperature (ST), pulse rate (PR), and respiration rate (RR) in (A) untrained and (B) trained sheep over time across work status (*Pre-work, Work, and Recovery*) at medium speed.

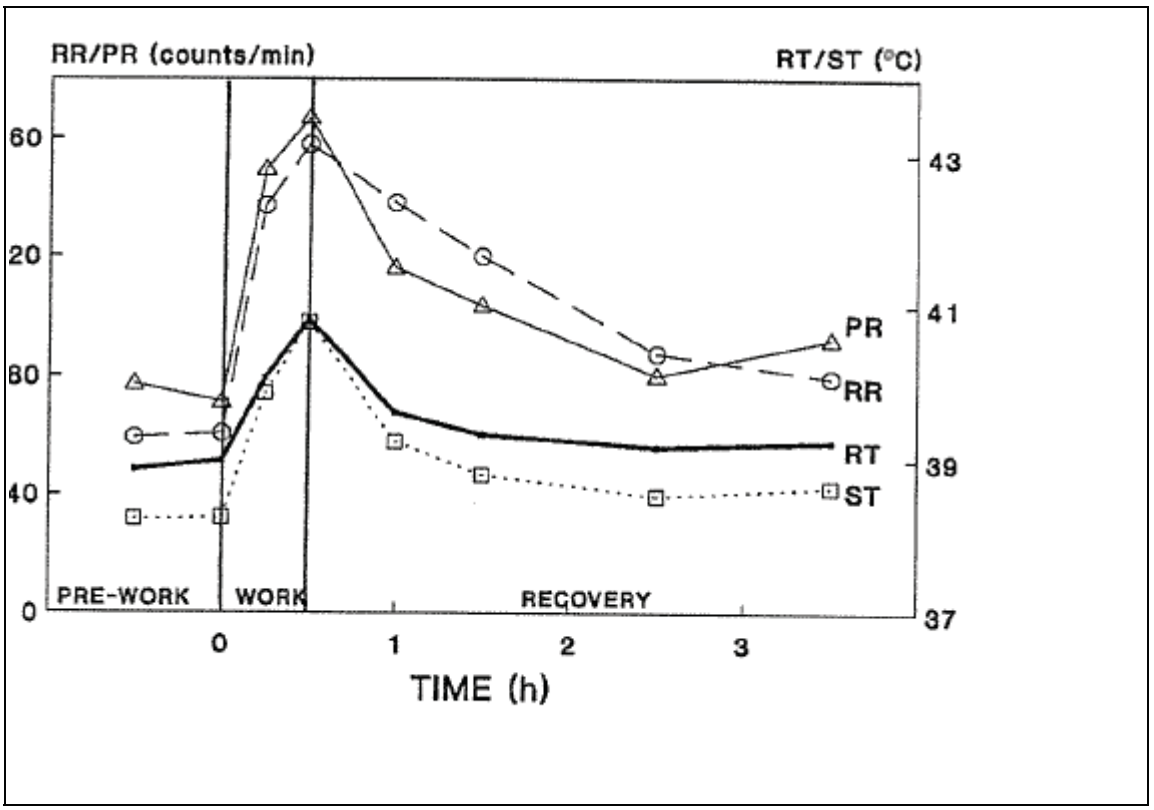


Figure 3C.16 Changes in rectal temperature (RT), skin temperature (ST), pulse rate (PR), and respiration rate (RR) in trained sheep over time across work status (*Pre- work, Work, and Recovery*) at fast speed.

3C.3.4 Blood metabolites

Lactate

Data for lactate concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.17. Corresponding data for A–V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.18.

Comparisons of mean arterial concentrations and A-V concentration differences of lactate in sheep between physiological states, walking speeds and work status are presented in table 3C.11.

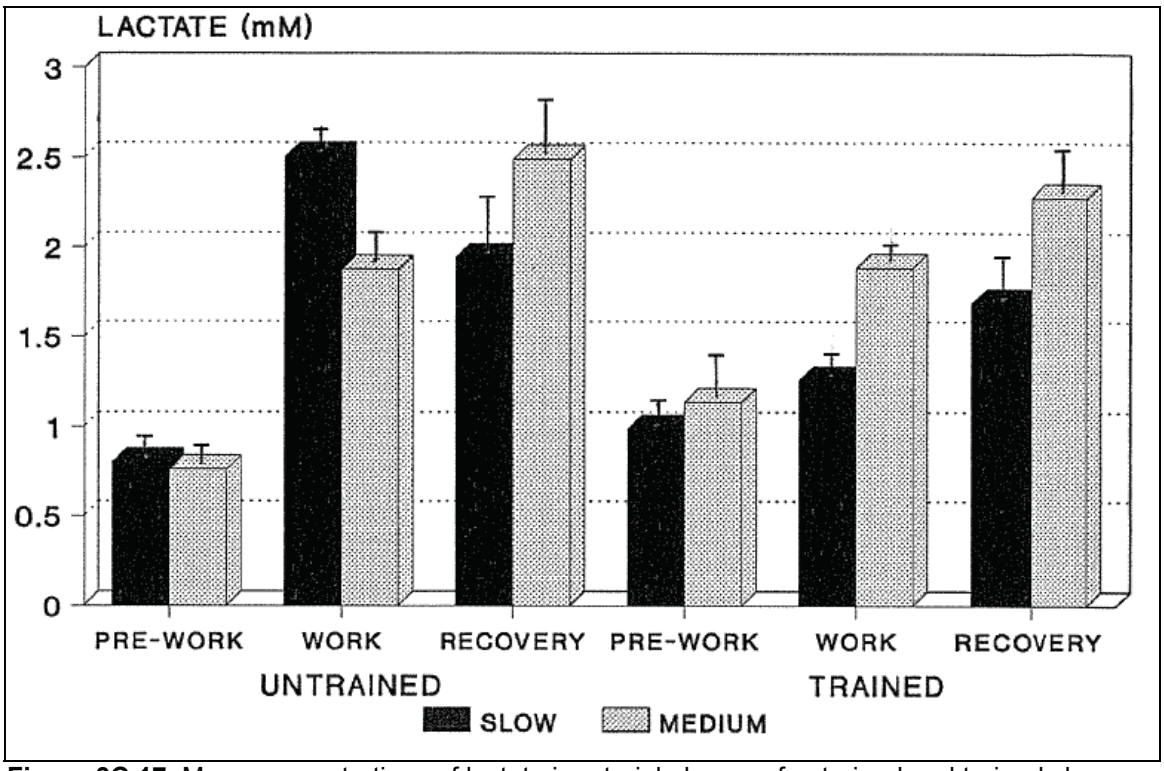


Figure 3C.17 Mean concentrations of lactate in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

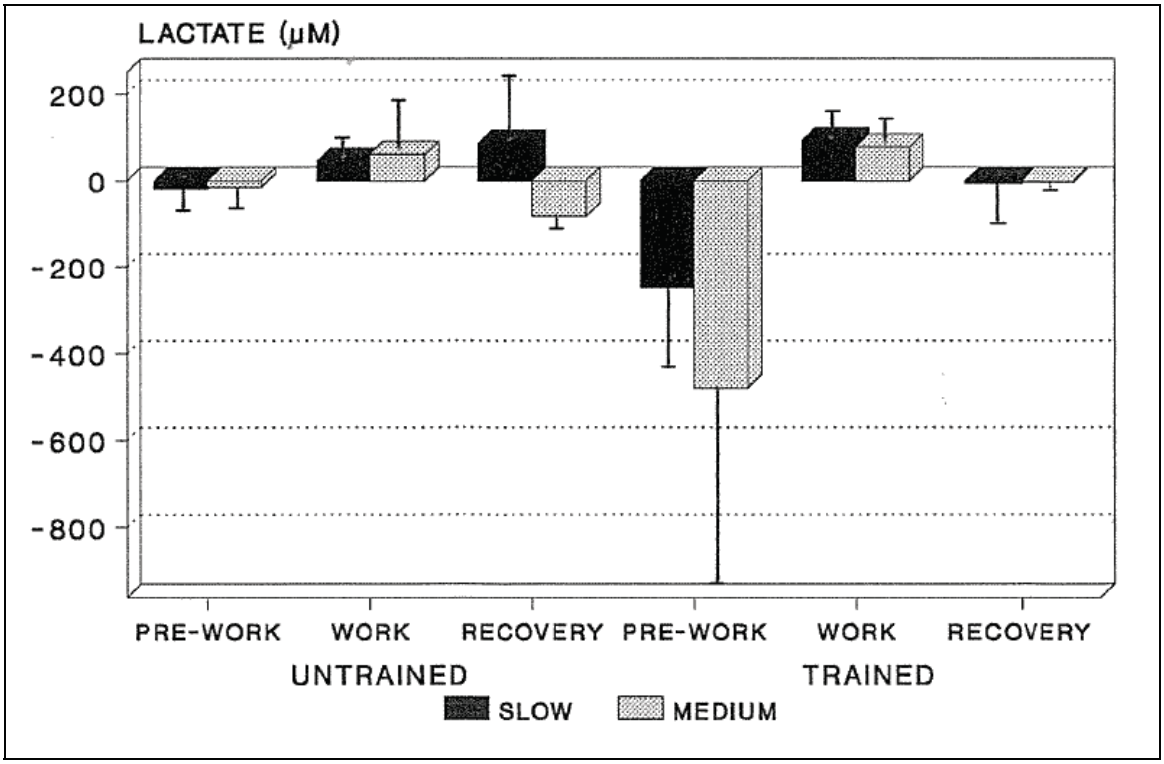


Figure 3C.18 Mean arterio-venous concentration difference of lactate in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Table 3C.11 Comparison of arterial concentration (A) and arterio-venous concentration difference (A-V) of lactate in plasma of sheep, between physiological states, walking speeds and work status Values are means standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Lactate (mM)							
A: Mean	2.08	1.65	1.76	1.96	0.96	1.92	2.13
± SE	0.09	0.06	0.16	0.18	0.09	0.09	0.19
Significance	P=0.0080		P=0.2522		P=0.0002		
Lactate (µM)							
A–V: Mean	38	-4	-15	48	-175	80	-80
± SE	41	38	101	133	135	46	76
Significance	P=0.3437		P=0.1547		P=0.0001		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

There was no significant effect of physiological states on lactate concentration in arterial plasma. Walking speeds and work status, however, had marked effects on the concentration of the metabolite (table 3C.11); work and increasing walking speed both increasing lactate concentration. Although the lactate concentration in plasma of the trained sheep was higher than that in the untrained sheep in the *Pre-work* period (figure 3C.17), the difference was not significant.

In the net movement of lactate across the hind-limb muscles, there was no significant difference between physiological states and between walking speeds (table 3C.11). Work status had a significant effect on the A-V concentration difference of lactate. Values indicate a net output, net uptake and a net output of lactate from the hind-limb muscles of sheep during the *Pre-work*, *Work* and *Recovery* periods respectively (table 3C.11).

Data on lactate concentration in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.19. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.19.

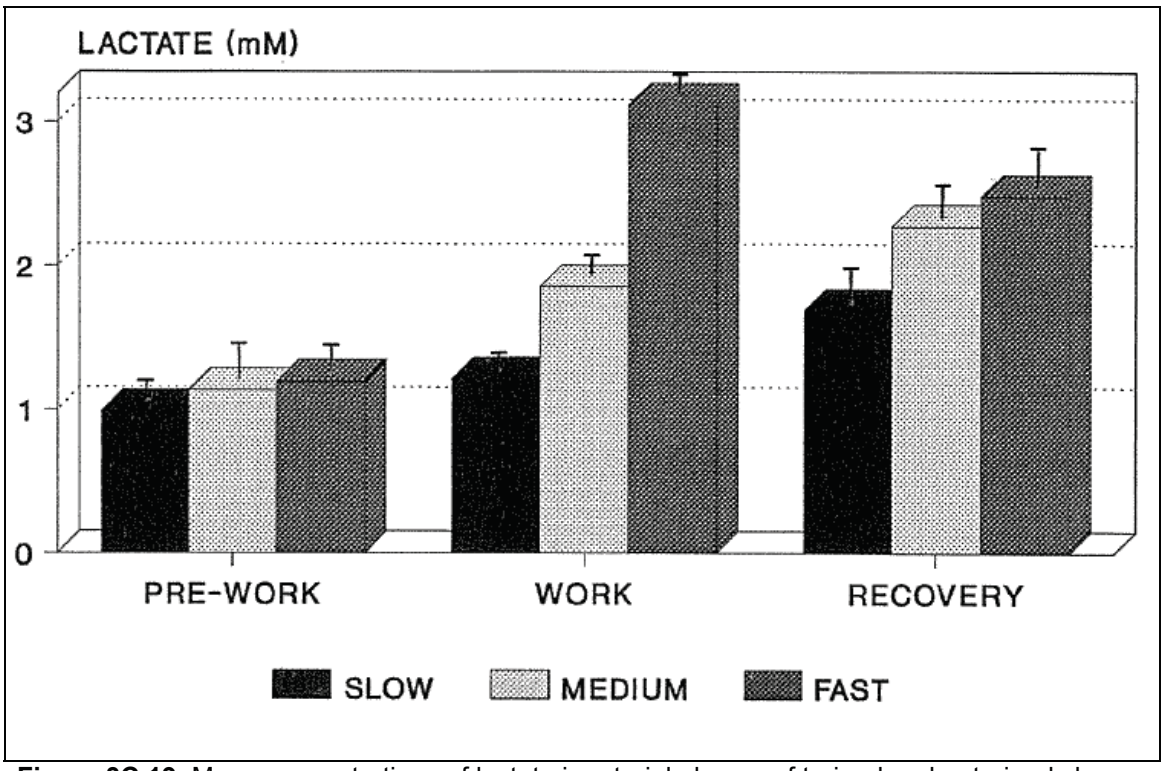


Figure 3C.19 Mean concentrations of lactate in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

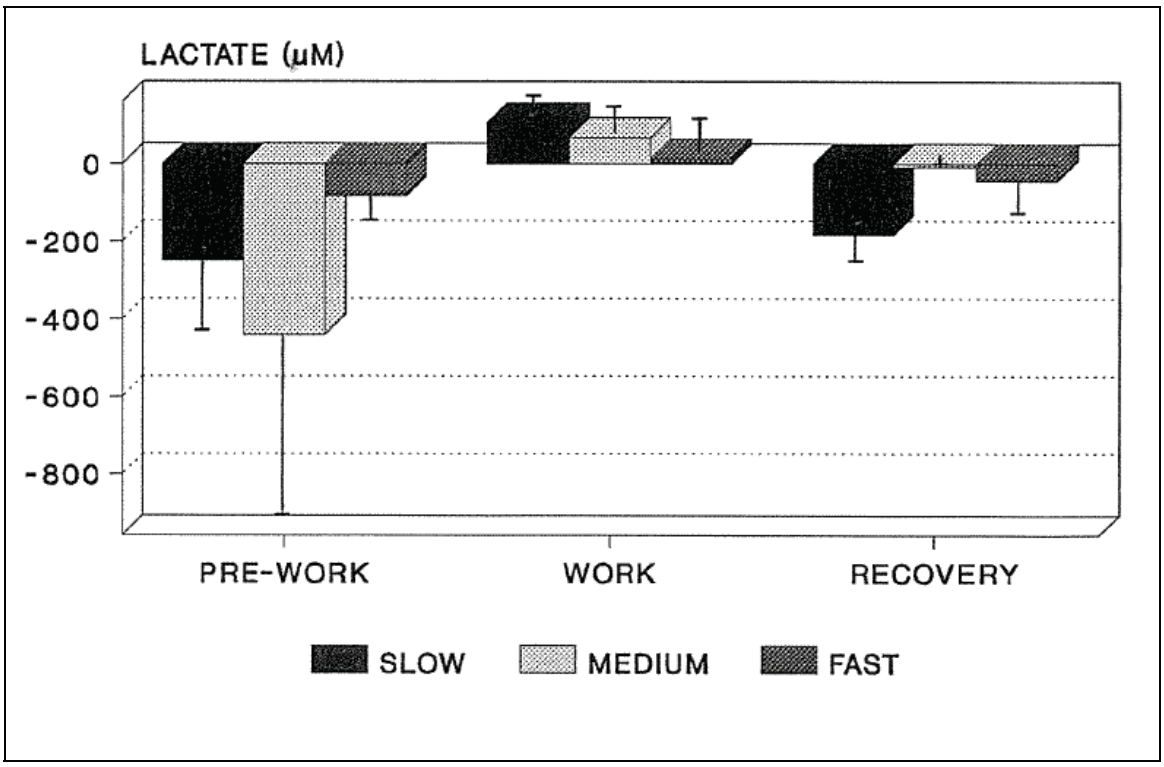


Figure 3C.20 Mean arterio-venous concentration difference of lactate in plasma of trained and untrained sheep during the *Pre-work, Work* and *Recovery* periods and at slow, medium and fast walking speeds. Vertical bars above lines represent standard errors.

Work status and walking speed both had a significant 3ffect (P=0.0001 and P=0.0120), respectively, on lactate concentration in arterial plasma. Lactate concentration in plasma increased with increasing speed during the *Work* period (figure 3C.19). The concentration of

the metabolite at slow and medium walking speeds increased during the *Recovery* period but at the fast walking speed it decreased during the *Recovery* period (figure 3C.19).

There was no significant difference (P=0.5729) in the effect of walking speed on the net movement of lactate across the hind-limb muscles. Work status had a significant effect (P=0.0000) on A-V concentration difference of lactate. Values indicate a net output, net uptake and a net output of lactate from the hind-limb muscles of sheep during the *Pre-work, Work and Recovery* periods respectively (figure 3C.20).

The changes in the pattern of the concentration of lactate in arterial and A-V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.32–3C.34.

The profiles of the concentration in arterial plasma and the A-V concentration difference of lactate in the untrained and trained sheep, in the first 20 minutes of the *Work* period, for the three different walking speeds, are shown in figure 3C.21.

The concentration of lactate in arterial plasma of the untrained sheep increased more quickly for the slow and medium speeds, than for the fast speed in the first two to three minutes of work. The concentration for the fast speed increased more slowly in the first two to three minutes but the rate of increase changed dramatically at the forth minute so that by the eighth minute, the concentration of lactate for the fast speed was higher than those for the slow and medium speeds, both concentrations of which had plateaued (figure 3C.21A).

In the untrained animals, the concentration of lactate in arterial plasma increased more quickly for the medium and fast speeds but more slowly in the case of the slow speed. As in the case of the untrained sheep, the lactate concentrations for the slow and medium speeds had plateaued by the sixth minute of work while that the fast speed continued to increase (figure 3C.21B).

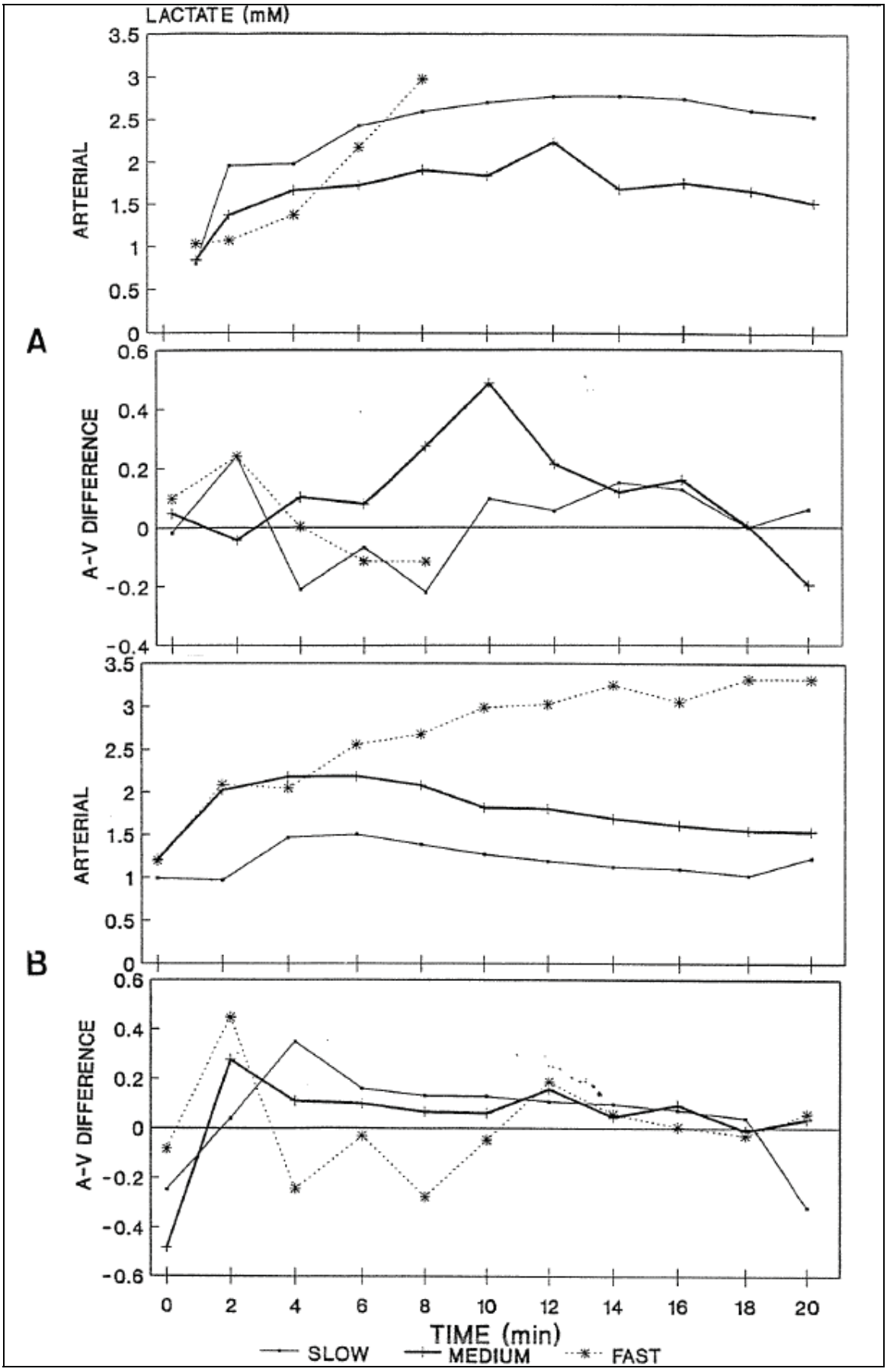


Figure 3C.21 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of lactate in untrained (A) and trained (B) sheep during the first 20 minutes of the *Work* period for slow, medium and fast walking speeds.

In both the untrained and trained sheep, there were transient outputs of lactate from the hind-limb muscles for the fast speed. Overall, however, there was a general net uptake of the metabolite. This was particularly expressed by the medium load in the untrained animals (figure 3C.21A).

Glucose

Data for glucose concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.22. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.23.

Comparisons of mean arterial concentrations and A-V concentration differences of glucose in sheep between physiological states, walking speeds and work status are presented in table 3C.12.

Table 3C.12 Comparison of arterial concentrations (A) and arterio-venous concentration difference (A-V) of glucose in plasma of sheep, between physiological states, walking speeds and work status Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Glucose (mM)							
A: Mean	4.09	4.40	3.76	4.17	3.56	4.12	4.10
± SE	0.11	0.06	0.16	0.23	0.20	0.23	0.16
Significance	P=0.1279		P=0.0062		P=0.0065		
A–V: Mean	0.05	0.07	0.07	0.05	0.20	0.23	0.23
± SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Significance	P=0.5048		P=0.4027		P=0.3164		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

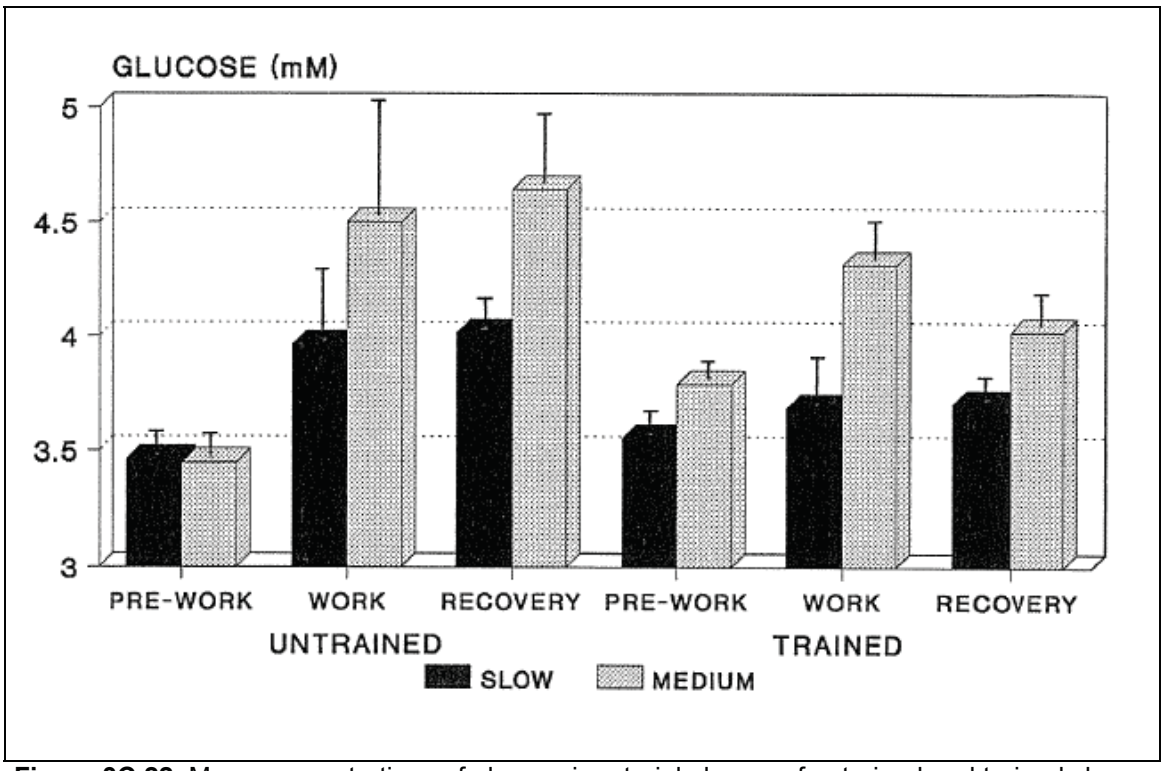


Figure 3C.22 Mean concentrations of glucose in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

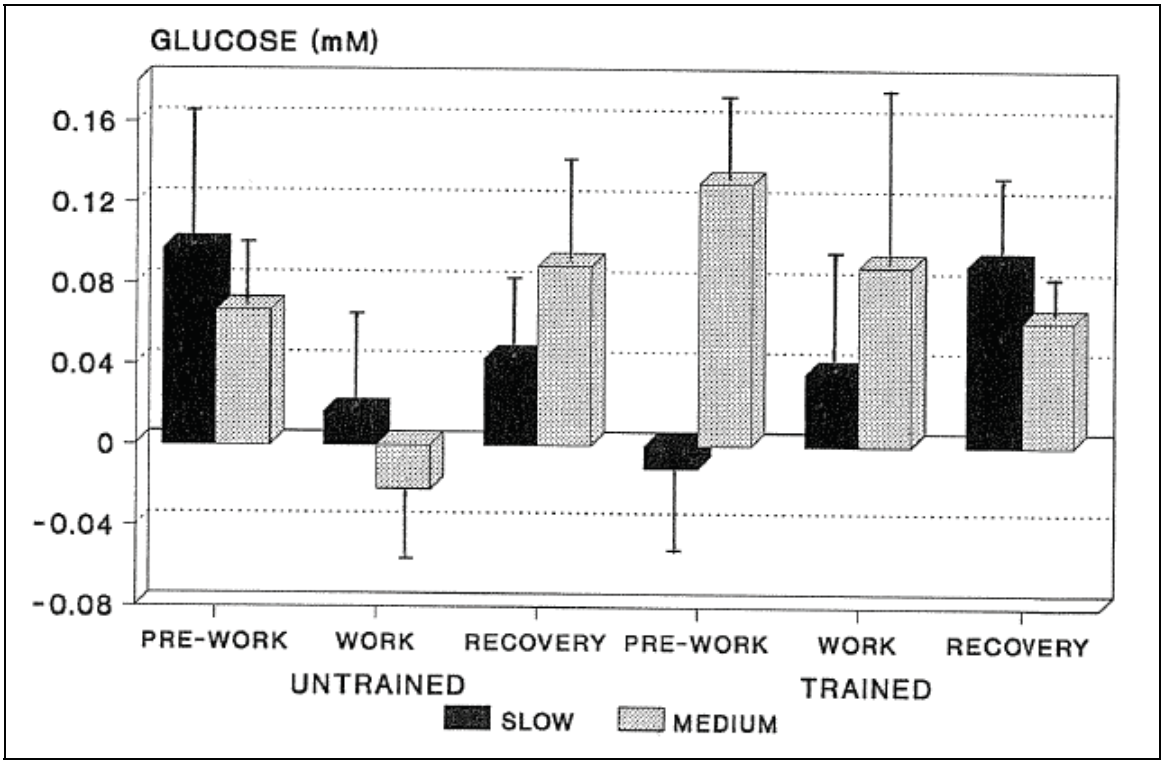


Figure 3C.23 Mean arterial-venous concentration difference of glucose in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represents standard errors.**

There was no significant effect of physiological states on glucose concentration in arterial plasma, although the concentration was higher in the trained than that in the untrained sheep (table 3C.12). Walking speeds and work status, however, had a significant affect on the concentration of the metabolite (table 3C.12); work and increasing speed both increasing glucose concentration.

In the net movement of glucose across the hind-limb muscle, there was no significant difference between treatments (table 3C.12). Values indicate a net uptake by the hind-limb muscles, across work status and there is a trend of increased glucose uptake by the untrained sheep as compared to a decreased uptake in the trained sheep (table 3C.12).

Data on glucose concentration in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.24. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.25.

Work status had no significant effect (P=0.2394) on glucose concentration in arterial plasma although there was a marked increase in concentration at fast speed during the *Work* period (figure 3C.24). Glucose concentration in arterial plasma was significantly affected (P=0.0022) by walking speed. Concentrations of the metabolite were higher with increasing speed during the *Work* and *Recovery* periods (figure 3C.24). There were no significant differences in the effect of walking speed or work status (P=0.1715 and P=0.8476) on the net movement of glucose across the hind-limb muscles. Values indicate a net uptake across walking speeds and work status (figure 3C.25).

The changes in the pattern of the concentration of glucose in arterial plasma and A–V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.32–3C.34.

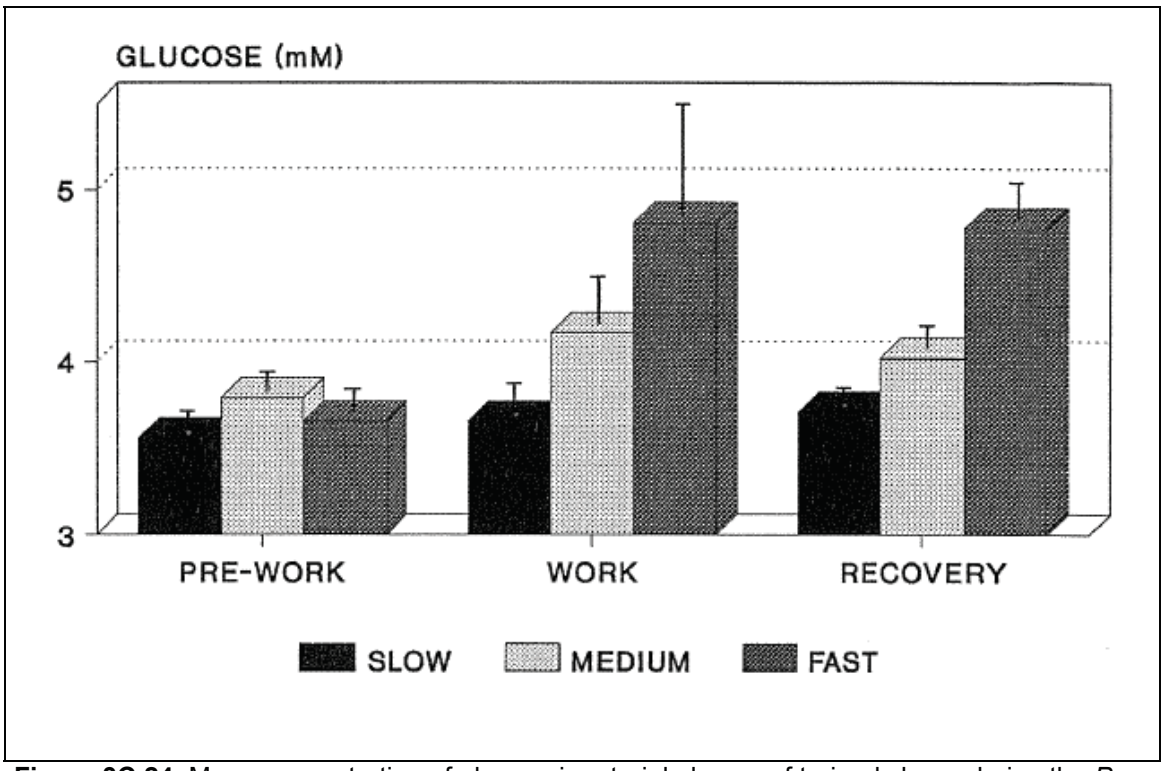


Figure 3C.24 Mean concentration of glucose in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. Vertical lines above bars represent standard errors.

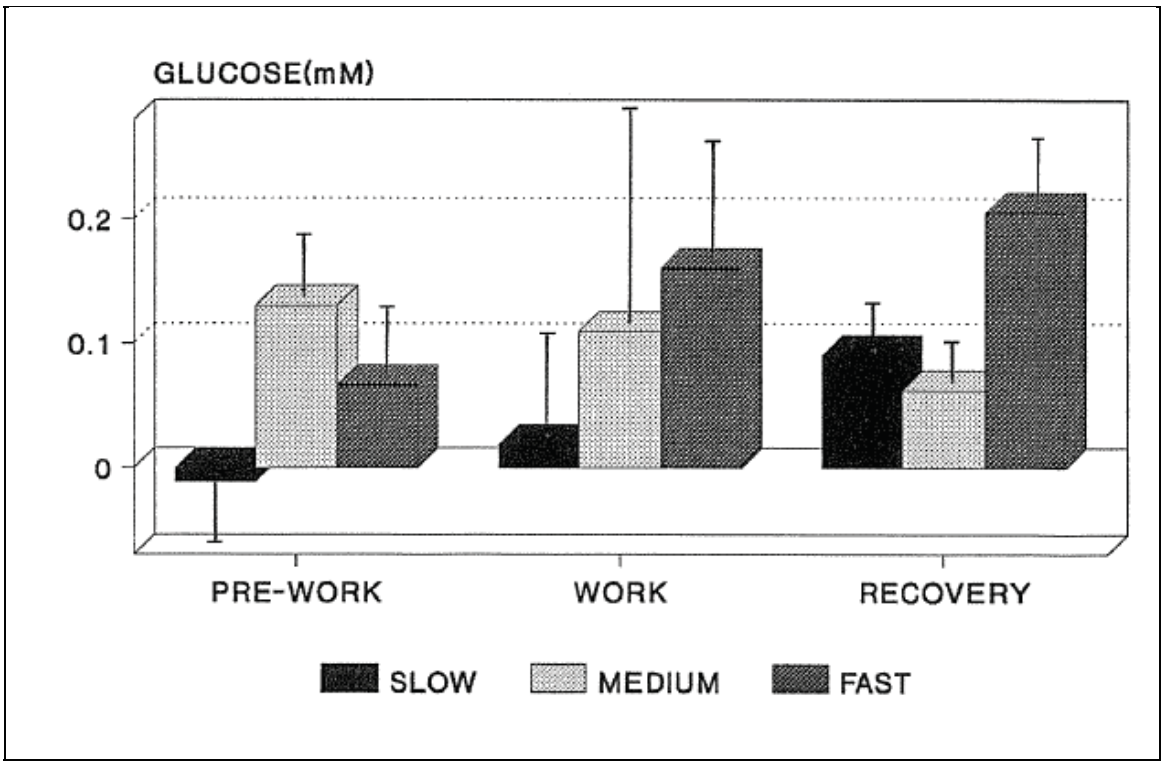


Figure 3C.25 Mean arterio-venous concentration difference of glucose in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Urea

Data for urea concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.26.

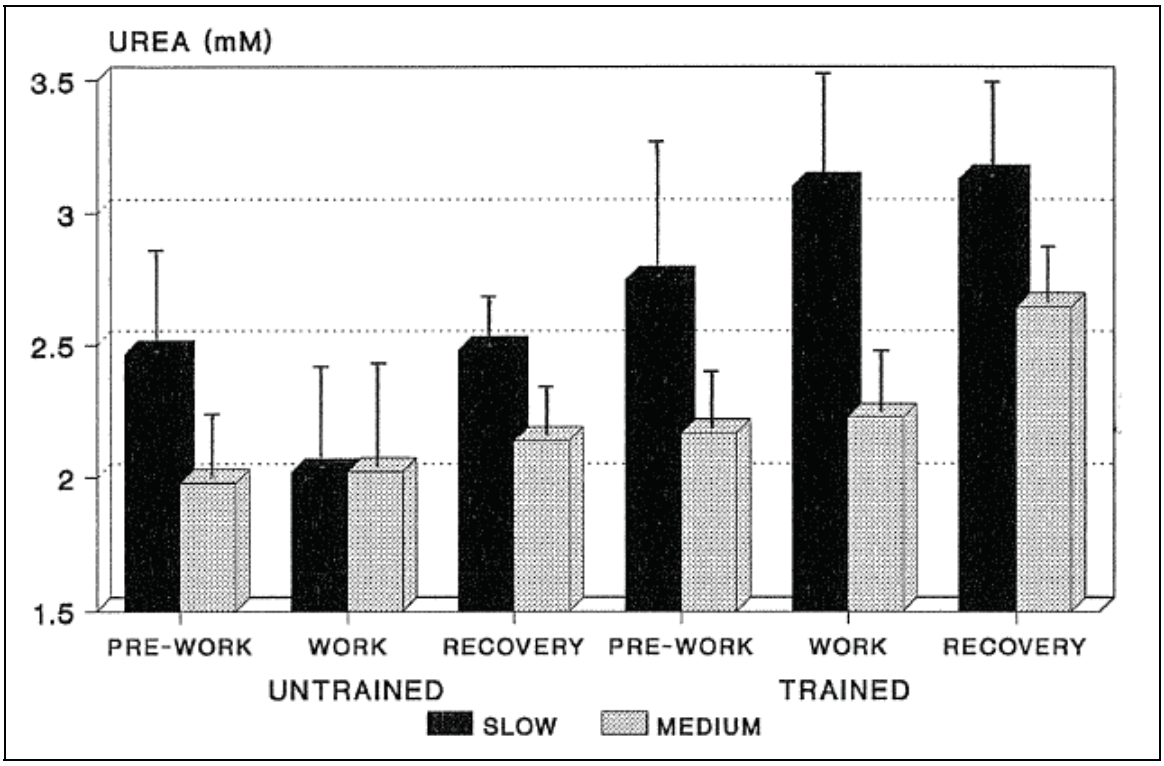


Figure 3C.26 Mean concentration of urea in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Comparisons of mean arterial concentrations of urea in sheep between physiological states, walking speeds and work status are presented in table 3C.13.

Table 3C.13 Comparisons of arterial concentration of urea in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Urea (mM)	2.22	2.72	2.69	2.25	2.33	2.34	2.60
± SE	0.02	0.02	0.06	0.05	0.04	0.04	0.03
Significance	P=0.0005		P=0.0020		P=0.0515		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

The concentration of urea in arterial plasma between work status approached significance (P=0.0515; table 3C.13) with the main difference occurring in the *Recovery* period with and increased concentration of the metabolite (figure 3C.26). Physiological states and walking speeds both significantly affected the concentration of urea in plasma (table 3C.13). The concentration of urea was higher in the trained animals as compared to the untrained animals and higher at slow speed than medium speed (table 3C.13).

Data on urea concentration in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.27.

Walking speed had no significant effect (P=0.1866) on the concentration of urea in arterial plasma. The concentration of the metabolite was significantly different (P= 0.0001) between work states with an increase in concentration during the work period (figure 3C.27).

The changes in the pattern of the concentration of urea in arterial plasma in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.32 - 3C.34.

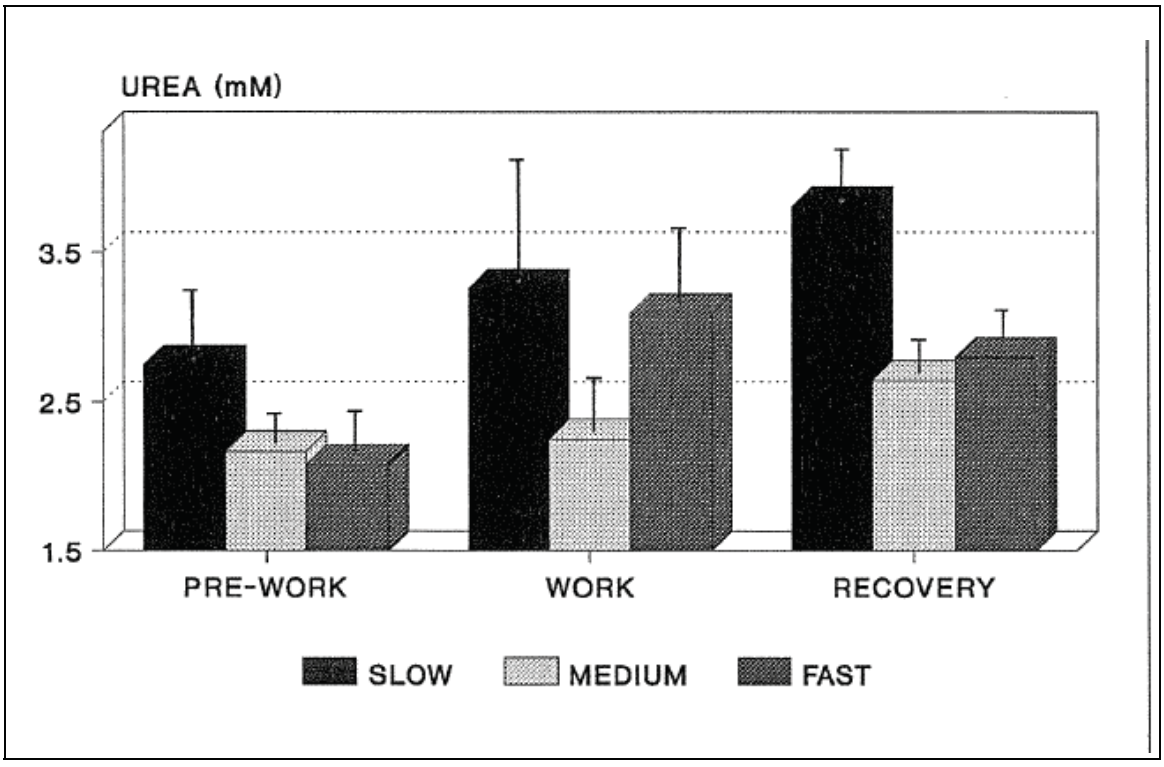


Figure 3C.27 Mean concentration of urea in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Free Fatty Acids

Data for FFA concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.28. Corresponding data for A–V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.29.

Comparisons of mean arterial concentration and A-V concentration difference of FFA in sheep between physiological states, walking speeds and work status are presented in table 3C.14.

Table 3C.14 Comparison of arterial concentration (A) and arterio-venous concentration difference (A-V) of free fatty acids (FFA) in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
FFA (mM)							
A: Mean	0.83	0.85	0.85	0.82	0.67	1.46	0.61
± SE	0.05	0.05	0.10	0.09	0.04	0.13	0.04
Significance	P=0.8606		P=0.8222		P=0.0000		
FFA (μM)							
A–V: Mean	22	10	13	20	48	8	5
± SE	13	18	64	31	14	30	25
Significance	P=0.5620		P=0.7032		P=0.1928		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Physiological state and walking speed had no significant effect on the concentration of FFA in arterial plasma (table 3C.14). There was a trend for the concentration of the metabolite to be higher during the *Work* period at medium speed compared to slow speed (figure 3C.28). Work status, however, had a marked effect on the concentration of FFA (table 3C.14). The concentration of FFA increased dramatically during the *Work* period and decreased to near *Pre-work* values during the *Recovery* period (figure 3C.28).

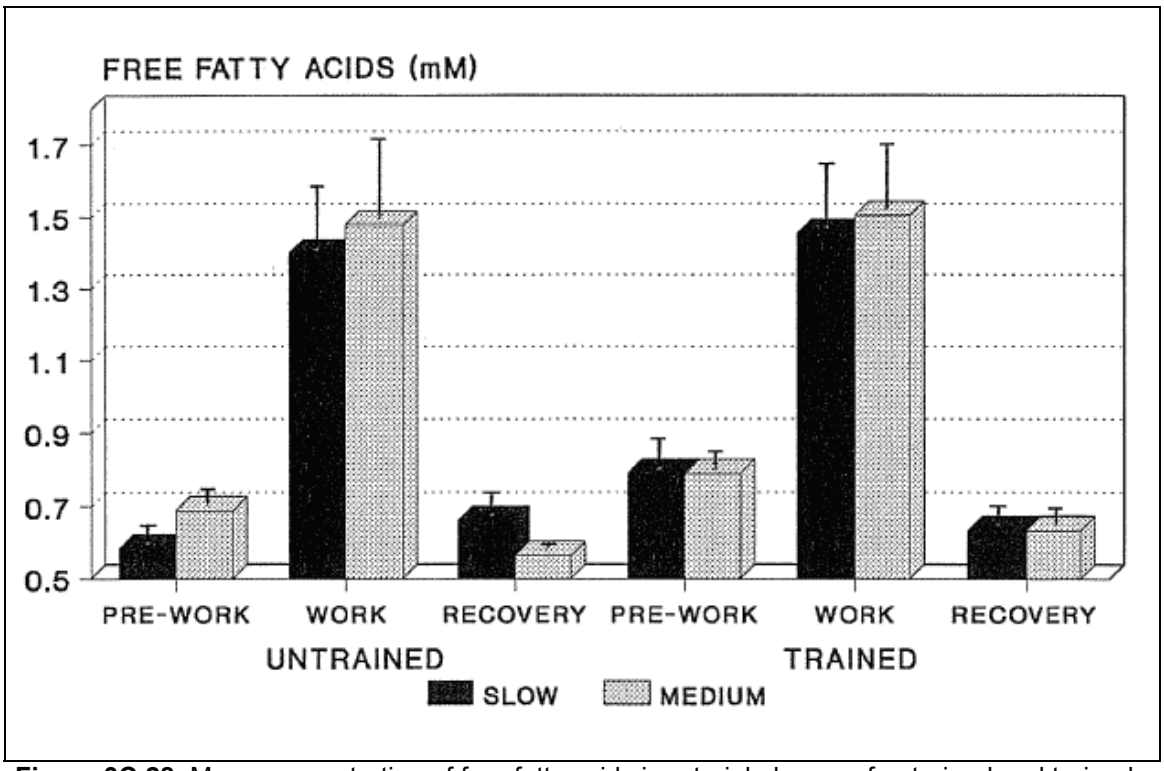


Figure 3C.28 Mean concentration of free fatty acids in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. Vertical lines above bars represent standard errors.

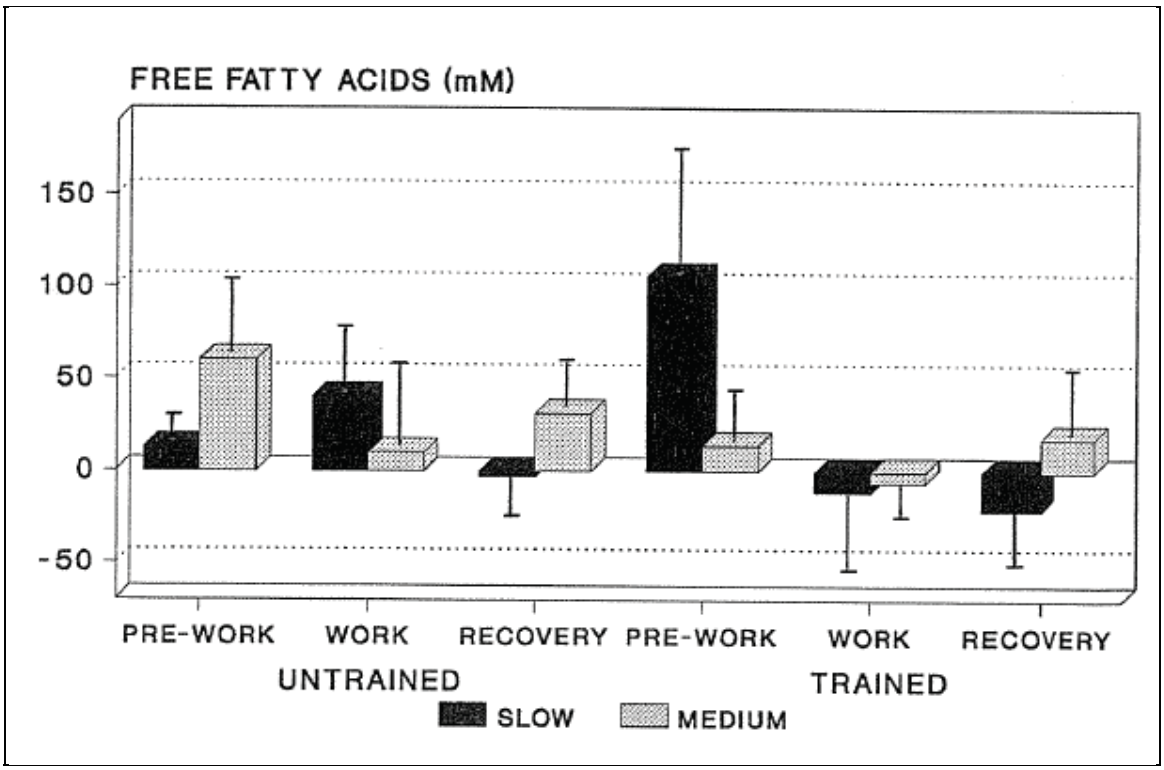


Figure 3C.29 Mean arterio-venous concentration difference of free fatty acids in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

The net movement of FFA across the hind-limb muscles, was not significantly affected by treatments (table 3C.14). There was, however, a trend of a net uptake across all treatments with the exception of a net output during the *Work* period for the trained animals (figure 3C.29).

Data on FFA concentration in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.30. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.31.

Walking speed had no significant effect (P=0.7754) on the concentration of FFA in arterial plasma, however, the concentration of the metabolite is higher during the work period at the fast speed (figure 3C.30). There were significant differences (P=0.0000) between work status. Plasma FFA concentrations increased dramatically during the *Work* period and decreasing during the *Recovery* period (figure 3C.30). The net movement of FFA across the hind-limb muscle, was not significantly affected (P=0.8664 and P=0.1978) respectively, by work status or walking speed. Values indicate a net uptake, net output and net uptake for the *Pre-work, Work and Recovery* periods, respectively (figures 3C.31).

The changes in the pattern of the concentration of FFA in arterial plasma and A-V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.32–3C.34.

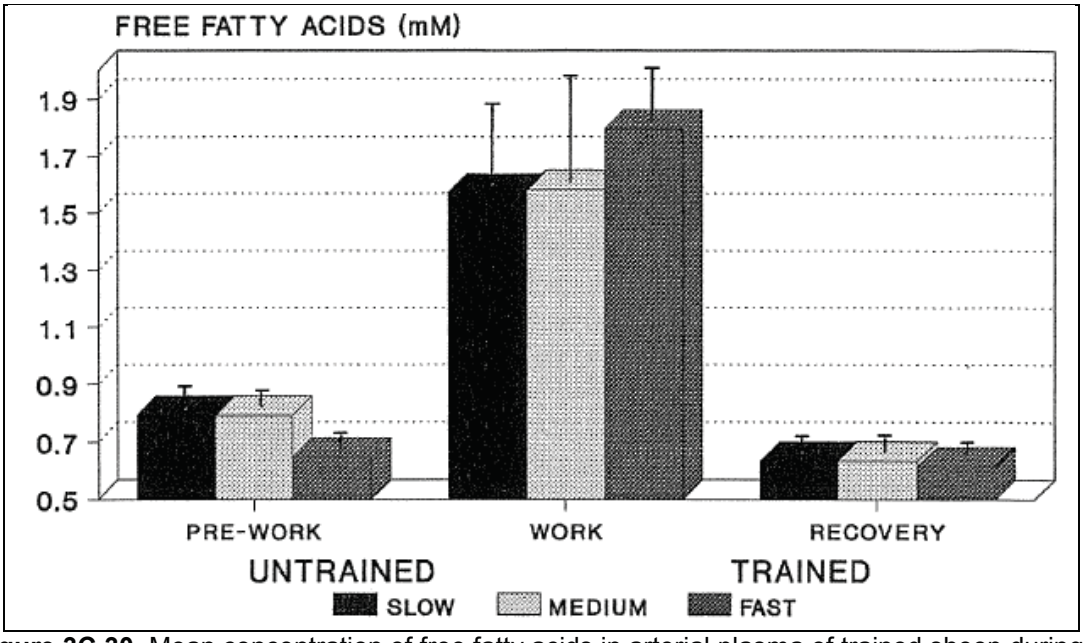


Figure 3C.30 Mean concentration of free fatty acids in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and of the slow, medium and fast walking speeds. Vertical lines above bars represent standard errors.

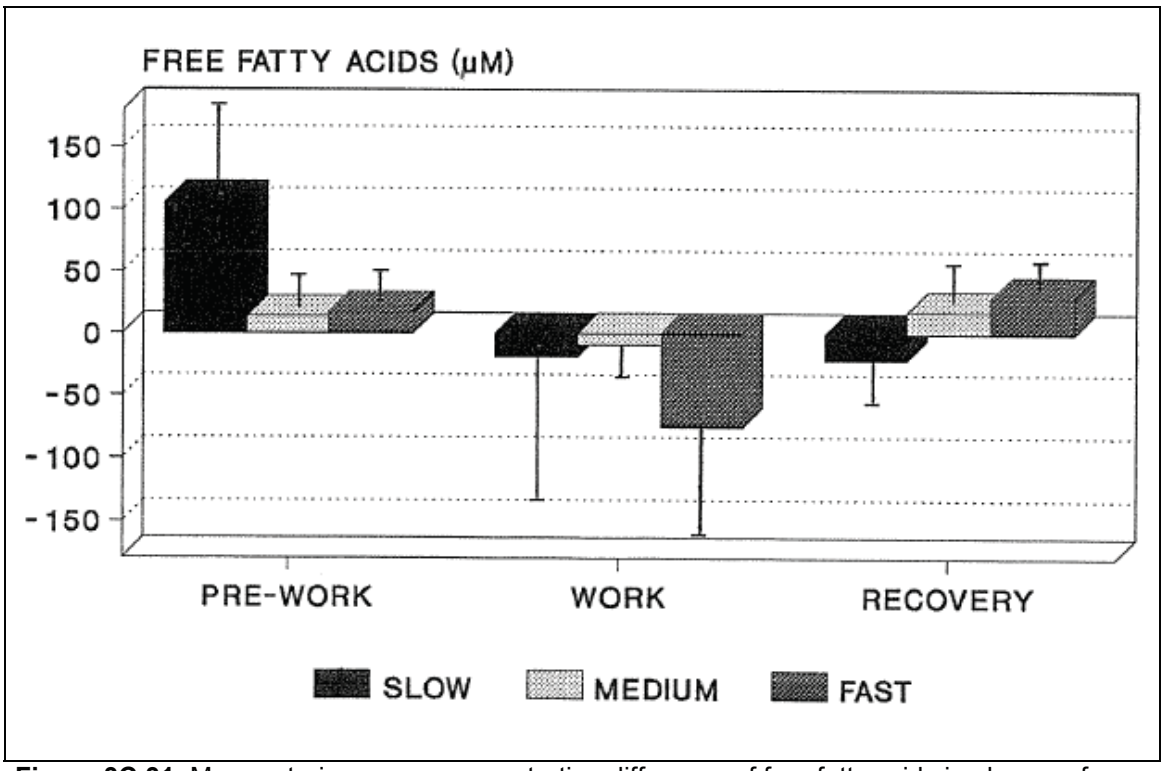


Figure 3C.31 Mean arterio-venous concentration difference of free fatty acids in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

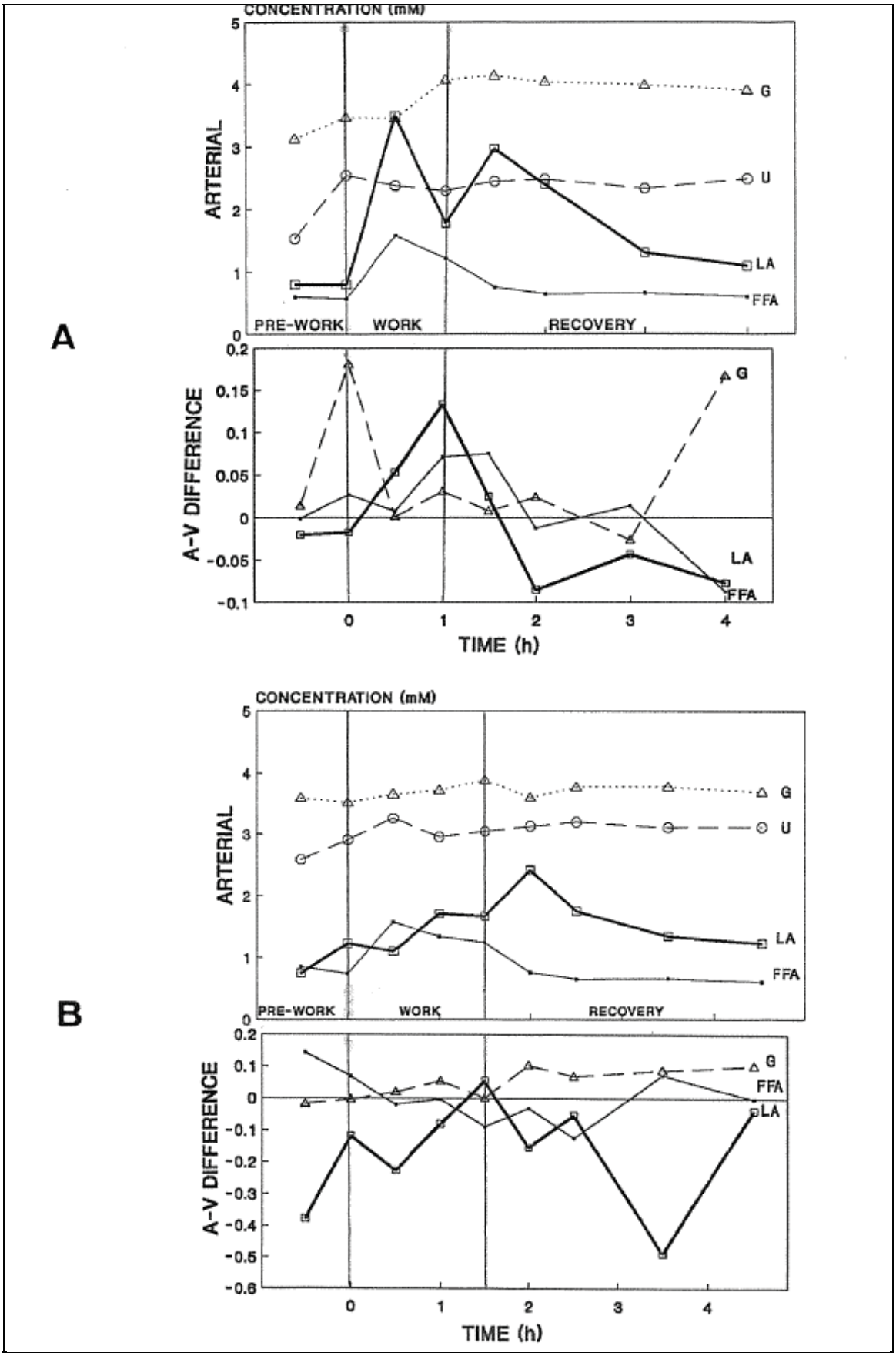


Figure 3C.32 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) at the slow walking speed.

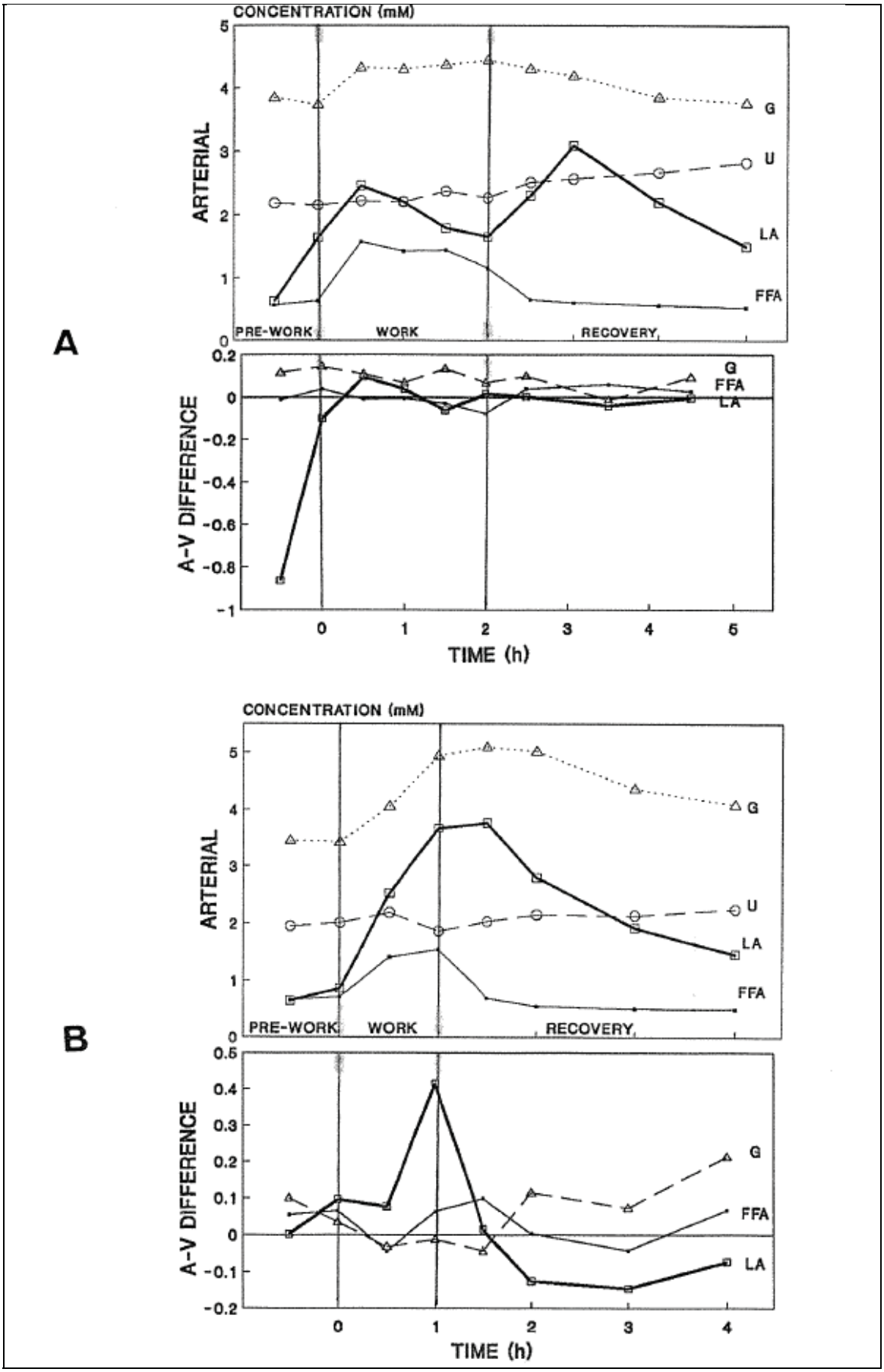


Figure 3C.33 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of lactate (LA), glucose (G), urea (U), and free fatty acids (FFA)in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) at the medium walking speed.

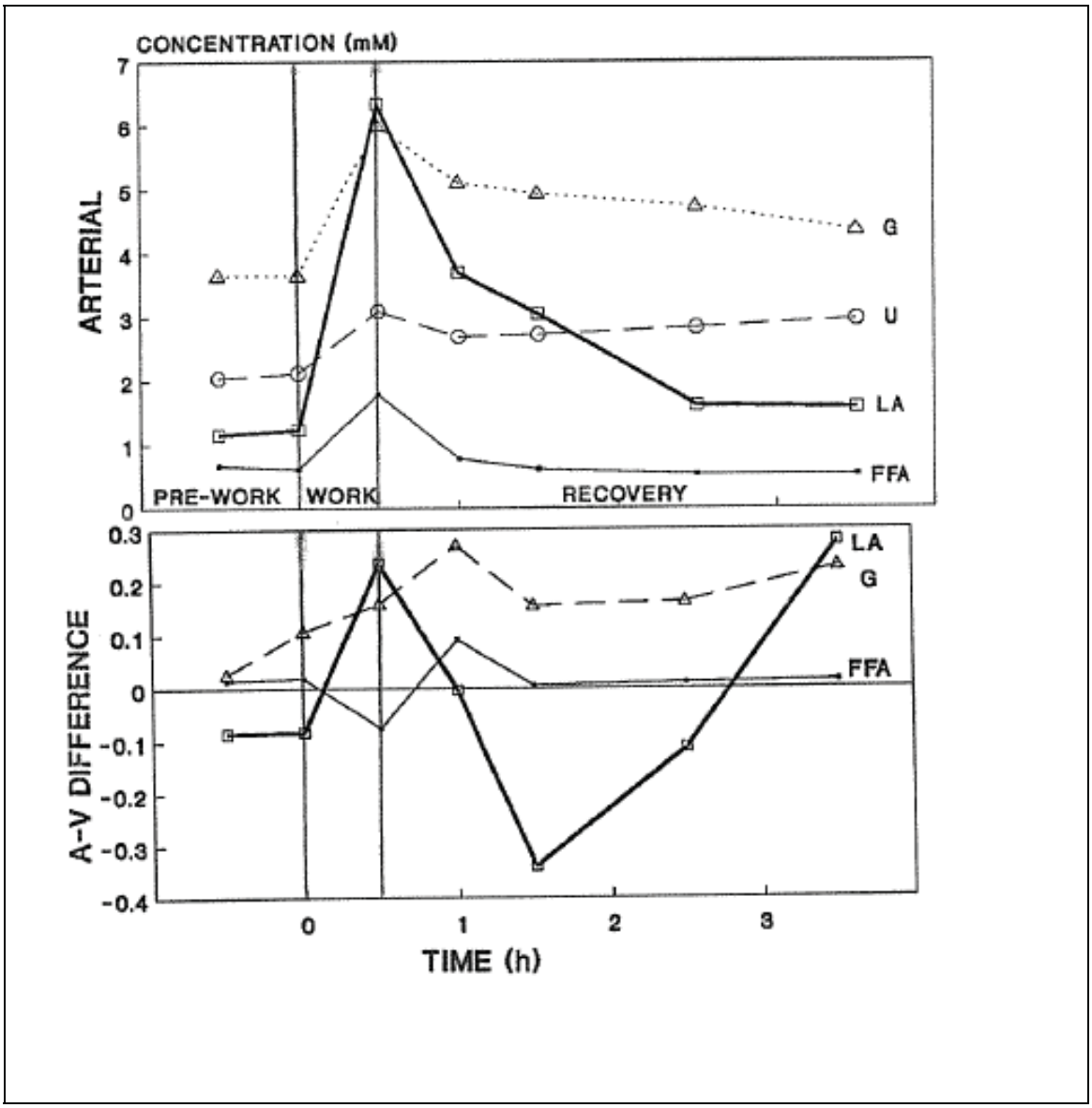


Figure 3C.34 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of lactate (LA), glucose (G), urea (U) and free fatty acids (FFA) in trained sheep over time across work status (*Pre-work, Work and Recovery*) at the fast walking speed.

Creatinine

Data for creatinine concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.35. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.36.

Comparisons of mean arterial concentrations and A-V concentration differences of creatinine in sheep between physiological states, walking speeds and work status are presented in table 3C.15.

Table 3C.15 Comparison of arterial concentrations (A)and arterio-venous concentration difference (A-V) of creatinine in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Creatinine (mM)							
A: Mean	0.98	0.99	1.02	0.95	0.94	0.96	1.02
± SE	0.02	0.03	0.03	0.09	0.05	0.06	0.03
Significance	P=0.6733		P=0.0330		P=0.0626		
Creatinine (μM)							
A–V: Mean	0	-17	11	-27	14	14	-30
± SE	30	10	48	65	70	50	20
Significance	P=0.5973		P=0.2265		P=0.3662		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

There was no significant effect of physiological states on creatinine concentration in arterial plasma (table 3C.15). Walking speed, however, had a significant effect on creatinine concentration and work status approached significance (table 3C.15). Creatinine concentration was higher for the slow walking speed and generally there was an increase during the *Work* period (figure 3C.35).

In the net movement at creatinine across the hind-limb muscles there were no significant differences between treatments (table 3C.15).

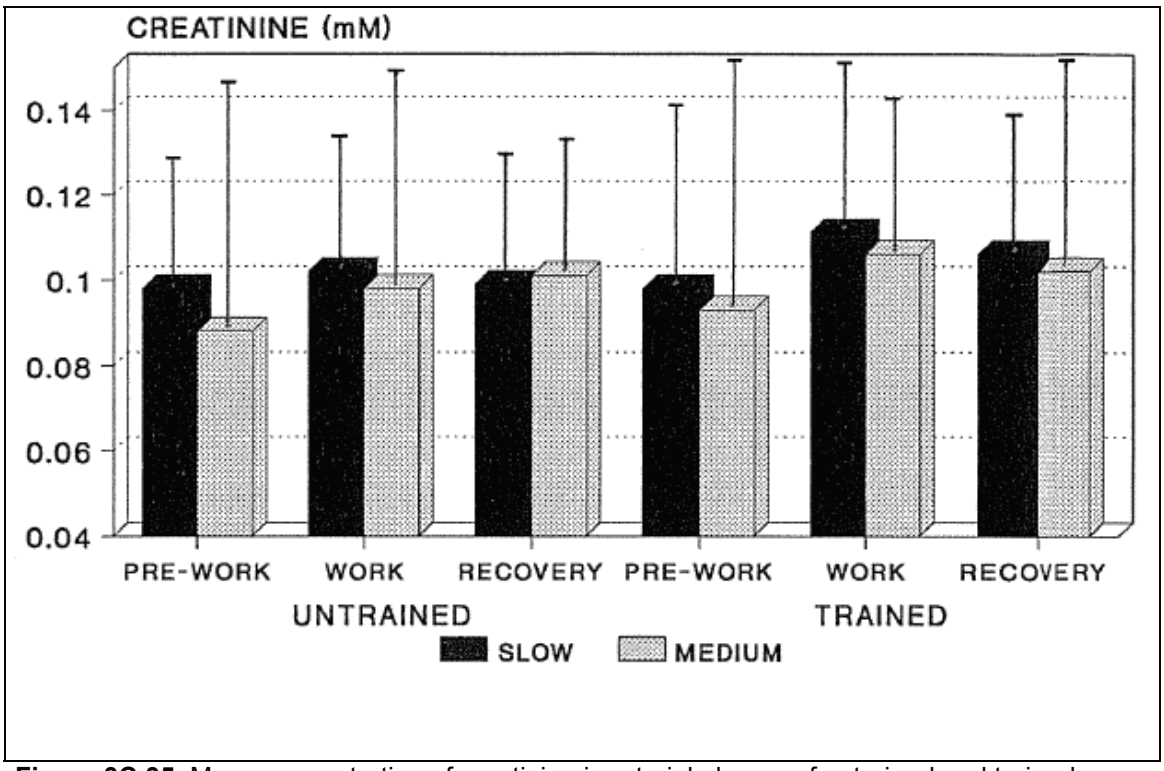


Figure 3C.35 Mean concentration of creatinine in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

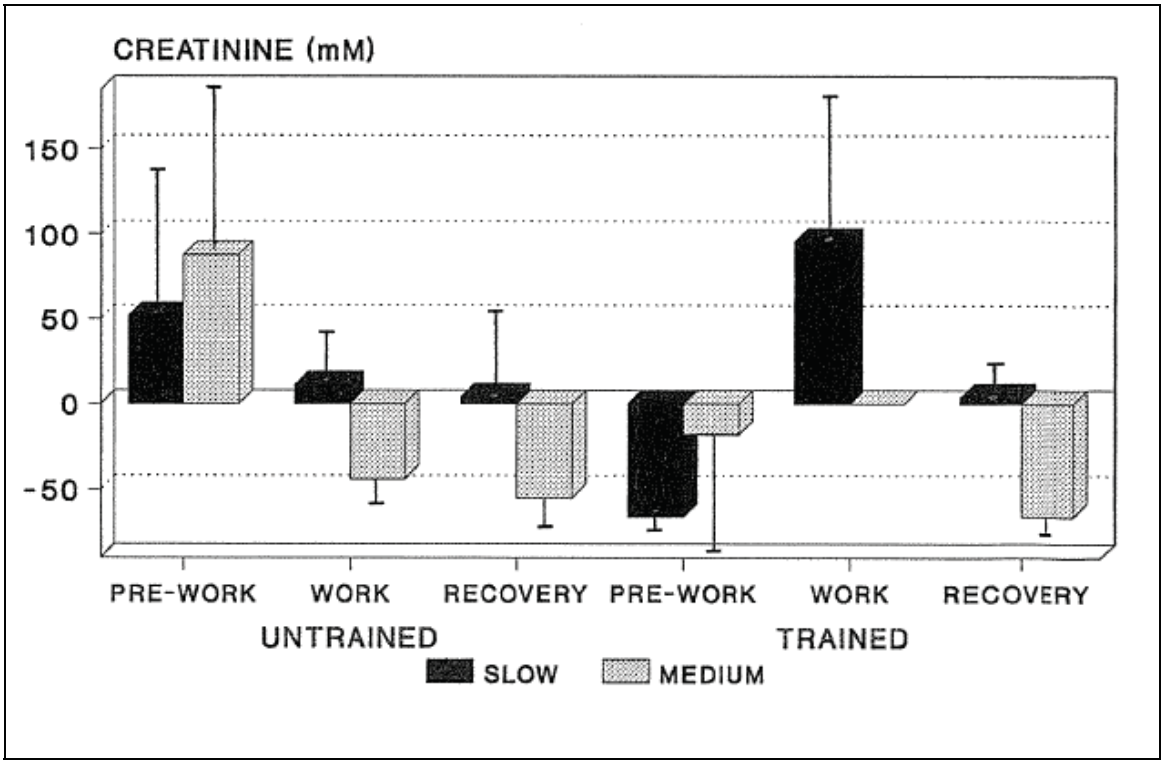


Figure 3C.36 Mean arterio-venous concentration difference of creatinine in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Data on creatinine concentrations in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.37. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.38.

There were no significant effects of work status (P=0.1826) or walking speed (P=0.0641) on creatinine concentration in arterial plasma, although, there was an increase during the *Work* period for the slow and fast walking speeds (figure 3C.37). There were no significant differences in the effect of walking speed (P=0.4617) or work status (P=0.5070) on the net movement of creatinine across the hind-limb muscles. Values indicate a net uptake for slow walking speed and output for fast walking speed during the *Work* period (figure 3C.38).

The changes in the pattern of the concentration of creatinine in arterial plasma and A-V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.39–3C.41.

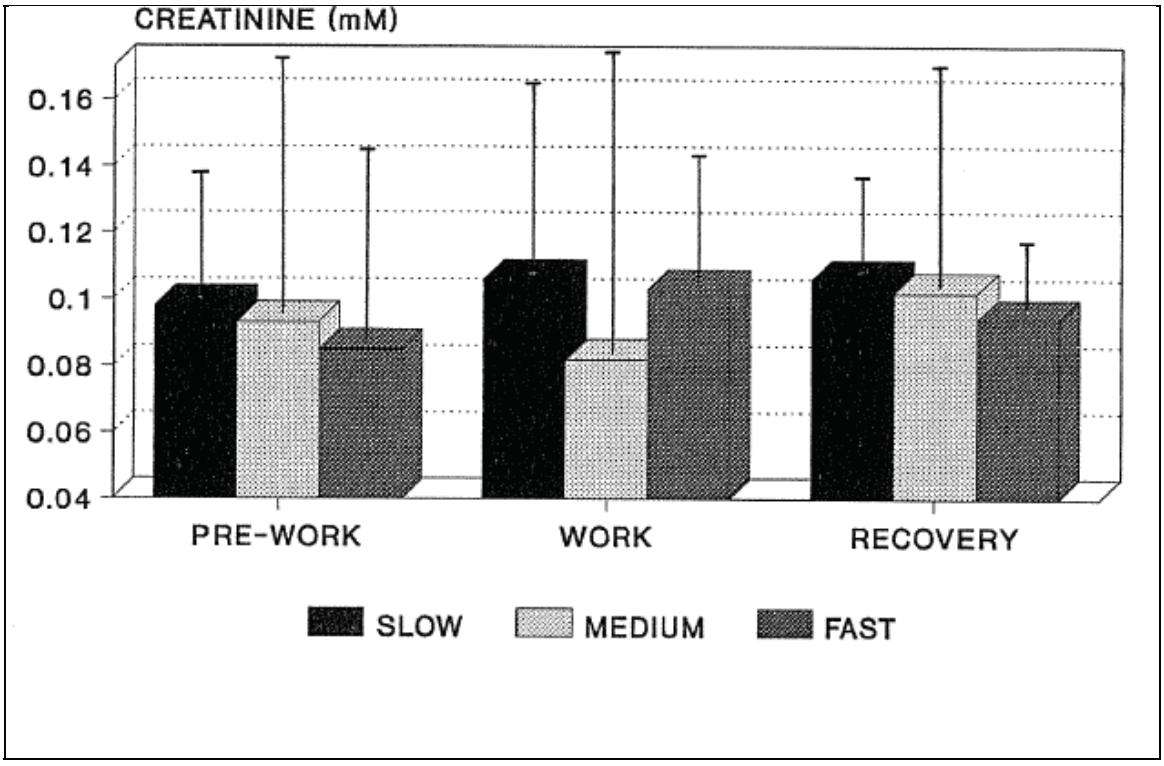


Figure 3C.37 Mean concentration of creatinine in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

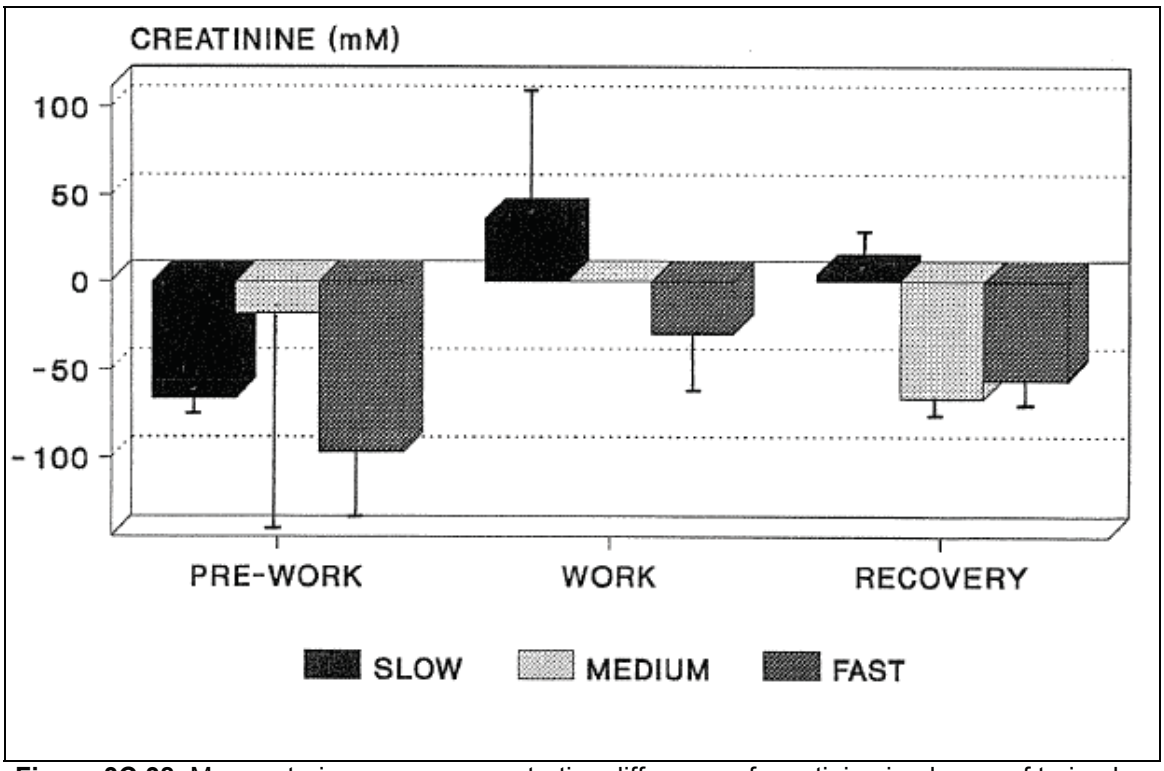


Figure 3C.38 Mean arterio-venous concentration difference of creatinine in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

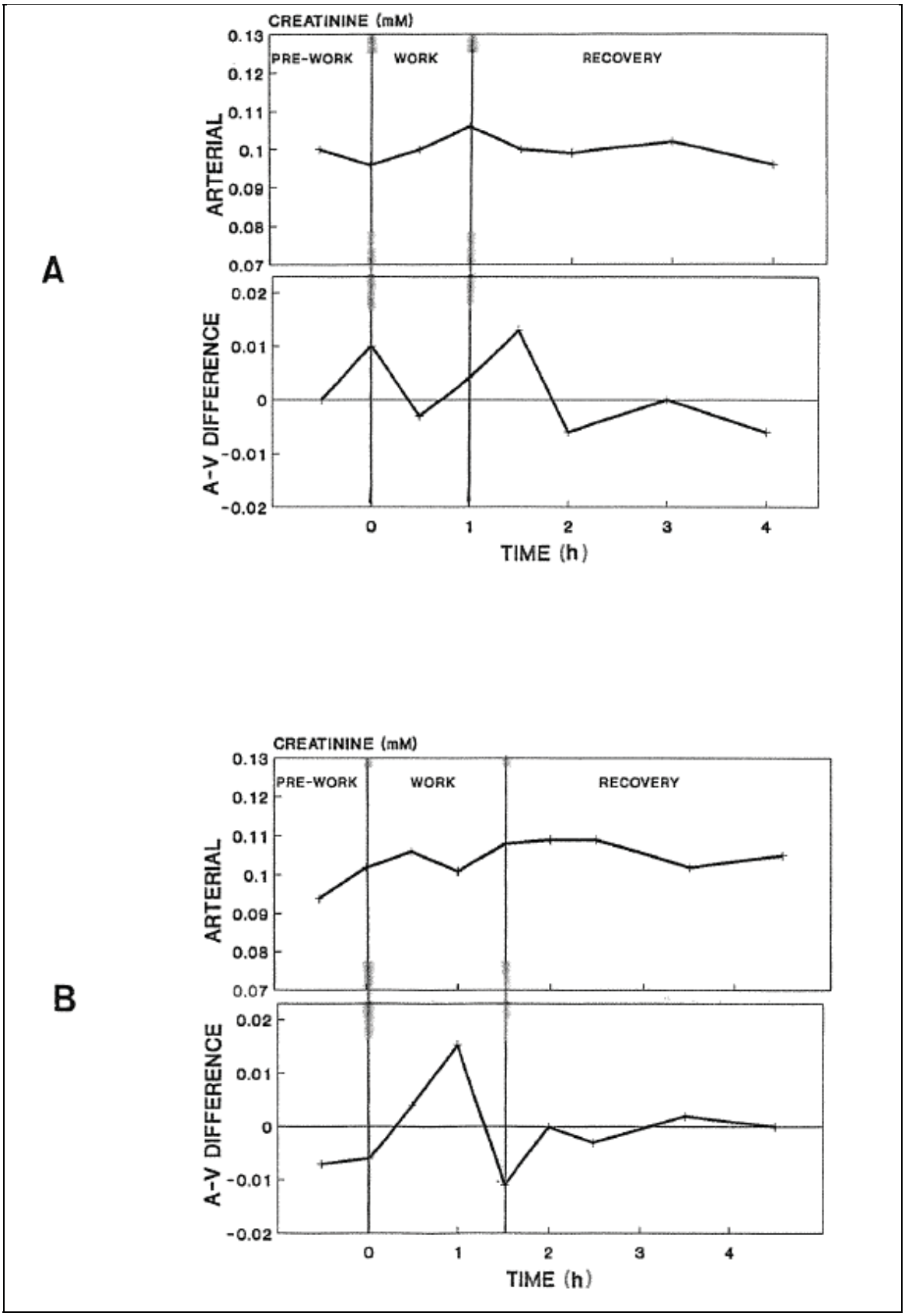


Figure 3C.39 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of creatinine in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) at the slow walking speed.

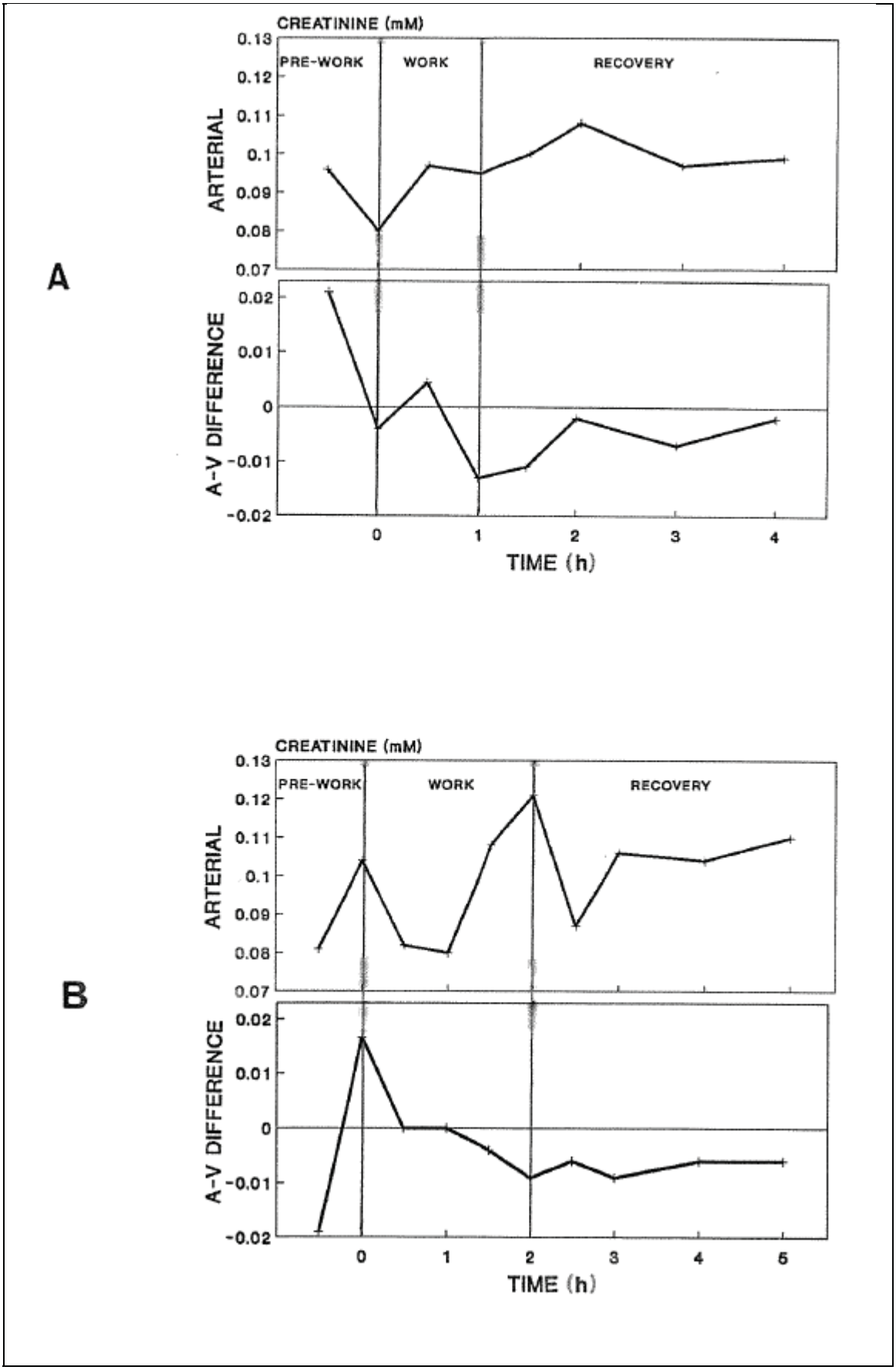


Figure 3C.40 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of creatinine in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) at the medium walking speed.

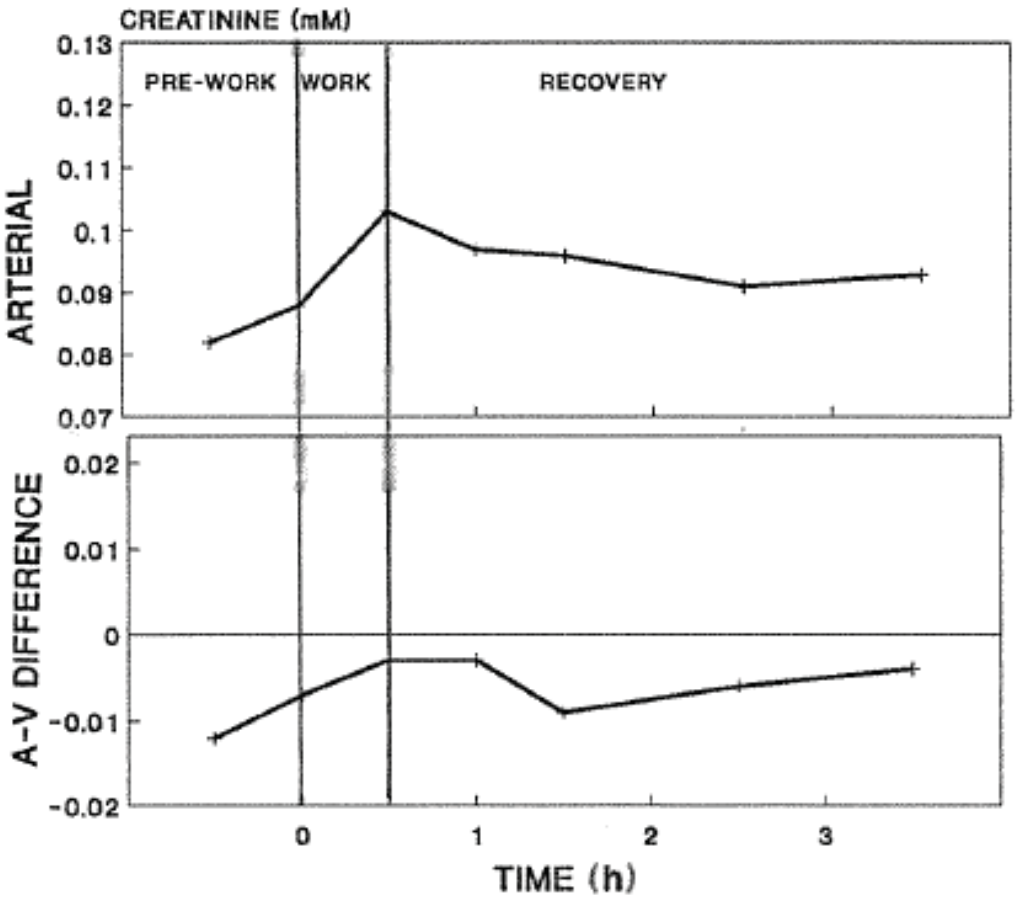


Figure 3C.41 The concentration in arterial plasma and arterio-venous (A-V) concentration difference of creatinine in trained sheep over time across work status (*Pre-work, Work and Recovery*) of the fast walking speed.

Potassium

Data for K^+ concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.42. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.43.

Comparisons of mean arterial concentrations and A-V concentration differences of K^+ in sheep between physiological states, walking speeds and work status are presented in table 3C.16.

Table 3C.16 Comparison of arterial concentrations (A) and arterio-venous concentration difference (A-V) of potassium in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Potassium (mM)							
A: Mean	6.82	6.15	6.40	6.56	6.47	7.58	5.94
± SE	0.27	0.12	0.30	0.64	0.26	0.51	0.26
Significance	P=0.0803		P=0.6910		P=0.0008		
A–V: Mean	0.45	-0.15	0.01	0.28	-0.36	0.29	0.32
± SE	0.22	0.12	0.24	0.59	0.24	0.28	0.28
Significance	P=0.0424		P=0.3700		P=0.1498		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Physiological states and walking speed had no significant effect on K^+ concentration in arterial plasma (table 3C.16). There was a trend, however, for potassium to be higher in the untrained as compared to the trained animals (figure 3C.42). Work status had a significant effect on K^+ concentration in arterial plasma (table 3C.16). Plasma K^+ increased during the *Work* period and decreased to below *Pre-work* values during the *Recovery* period (figure 3C.42).

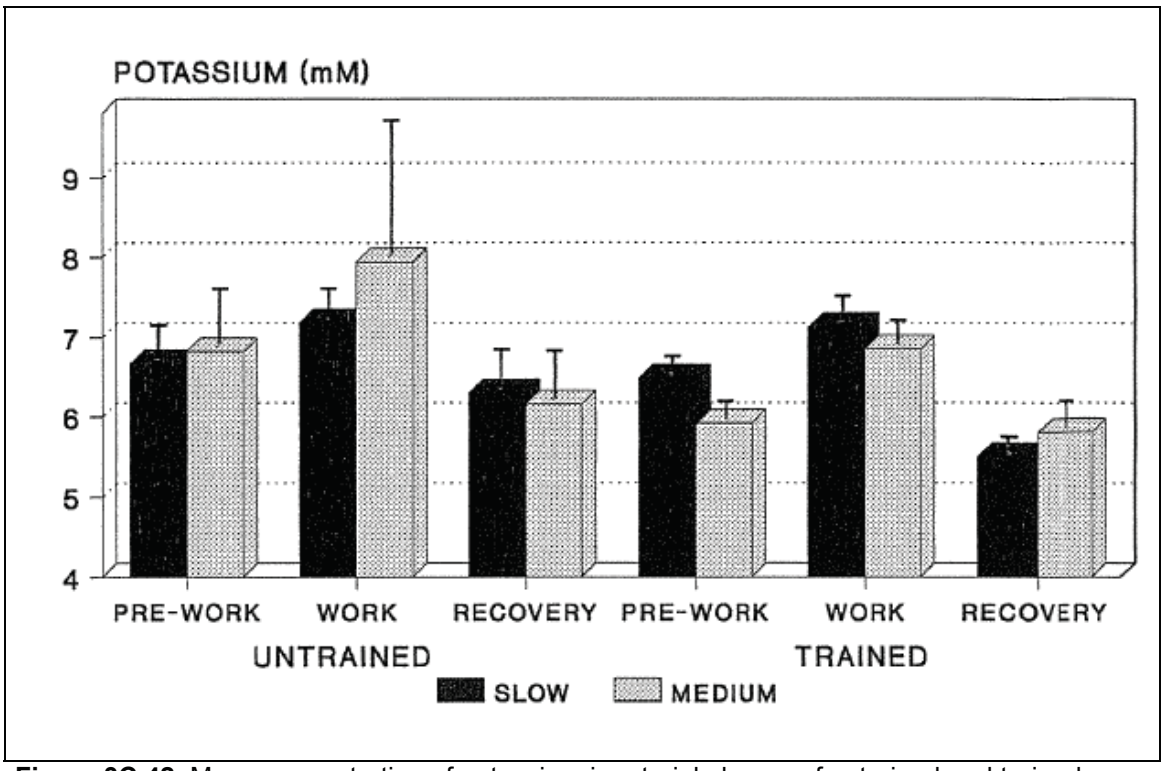


Figure 3C.42 Mean concentration of potassium in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

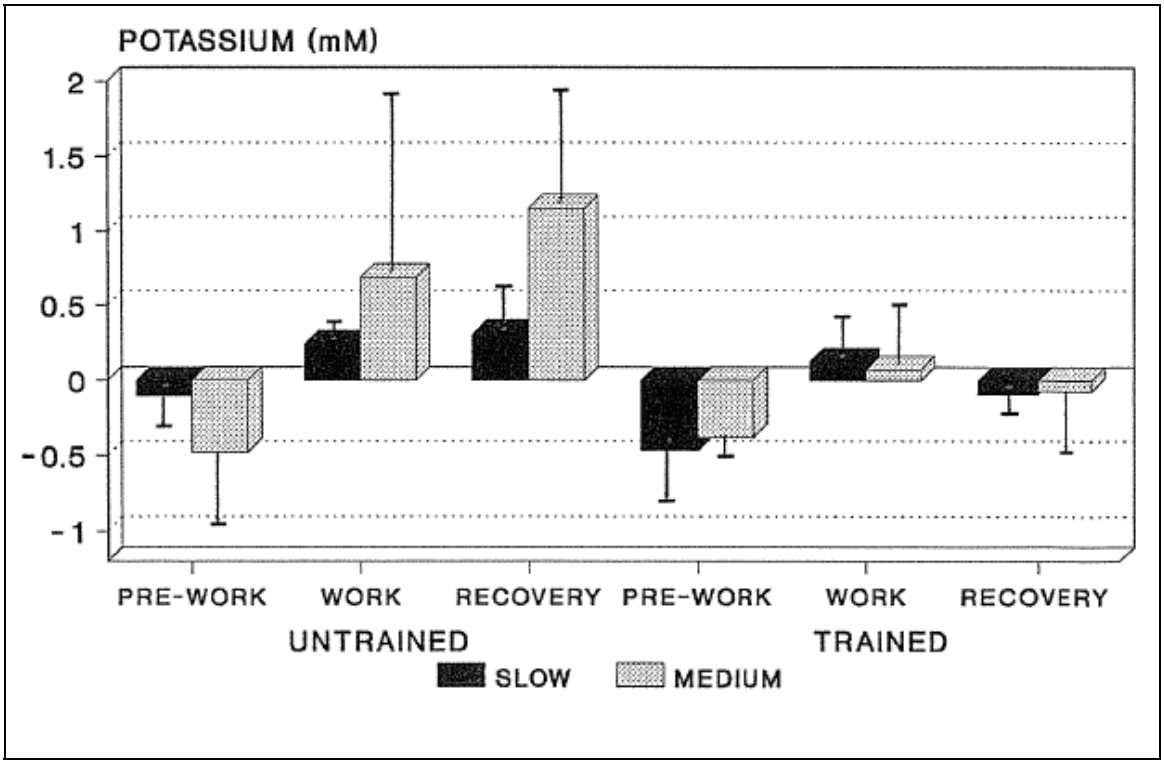


Figure 3C.43 Mean arterio-venous concentration difference of potassium in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

In the net movement of K^+ across the hind-limb muscle there was a significant effect due to physiological state but not due to walking speed or work status (table 3C.16). Values indicate a net uptake during the *Work* period (figure 3C.43). There was a net output for the trained animals as compared to an uptake for the untrained animals (table 3C.16).

Data on K^+ concentrations in arterial plasma of trained sheep of three different work status and walking speeds are presented in figure 3C.44. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.45.

Walking speed (P=0.0071) and work status (P=0.0020) both had a significant effect on K^+ concentration in arterial plasma of trained sheep. The K^+ concentration increases during the *Work* period and is similar for the slow and medium walking speeds but is higher for the fast walking speed (figure 3C.44). There were no significant effects of walking speed (P=0.9772) or work status (P=0.1295) on the net movement of K^+ across the hind-limb muscle.

The changes in the pattern of the concentration of K^+ in arterial plasma and A-V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.46–3C.48.

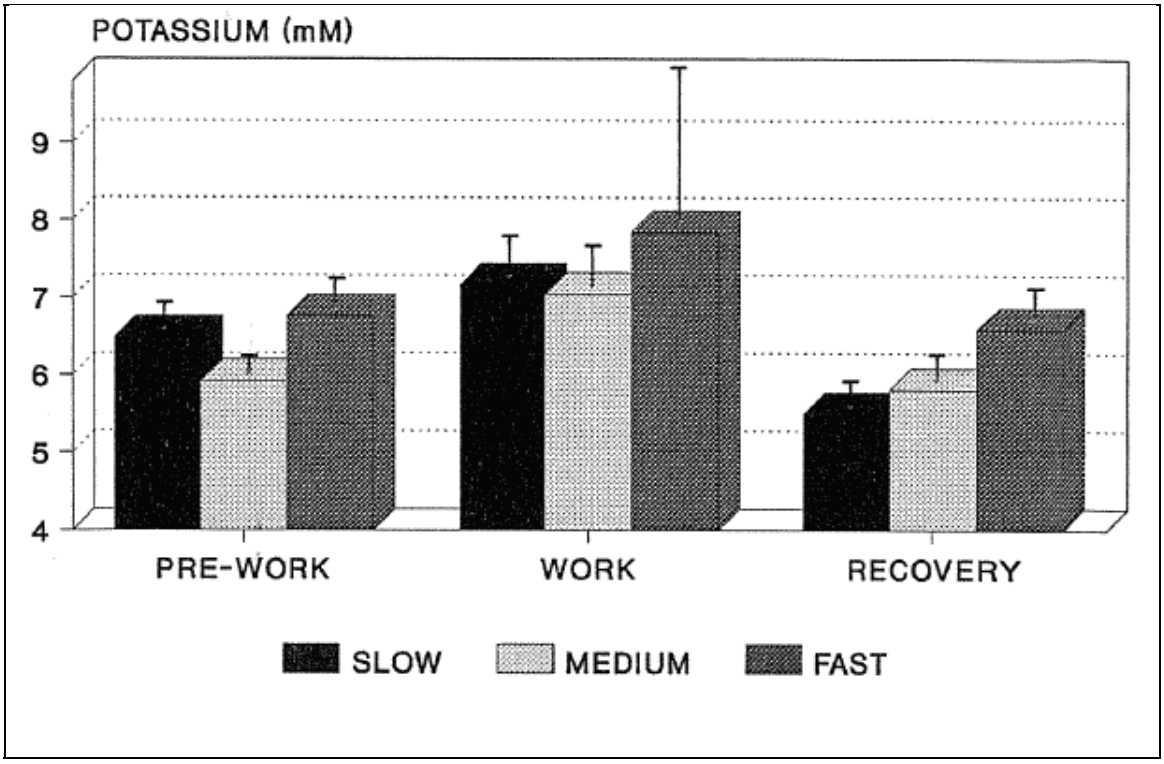


Figure 3C.44 Mean concentration of potassium in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

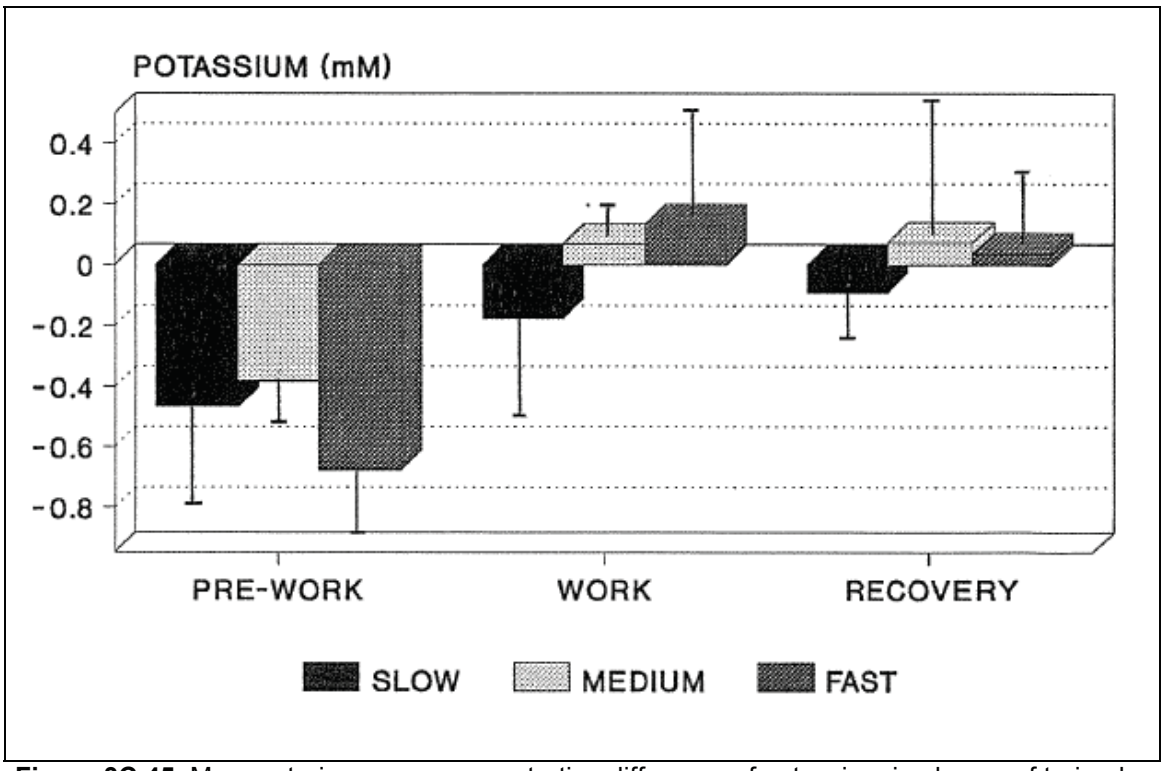


Figure 3C.45 Mean arterio-venous concentration difference of potassium in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

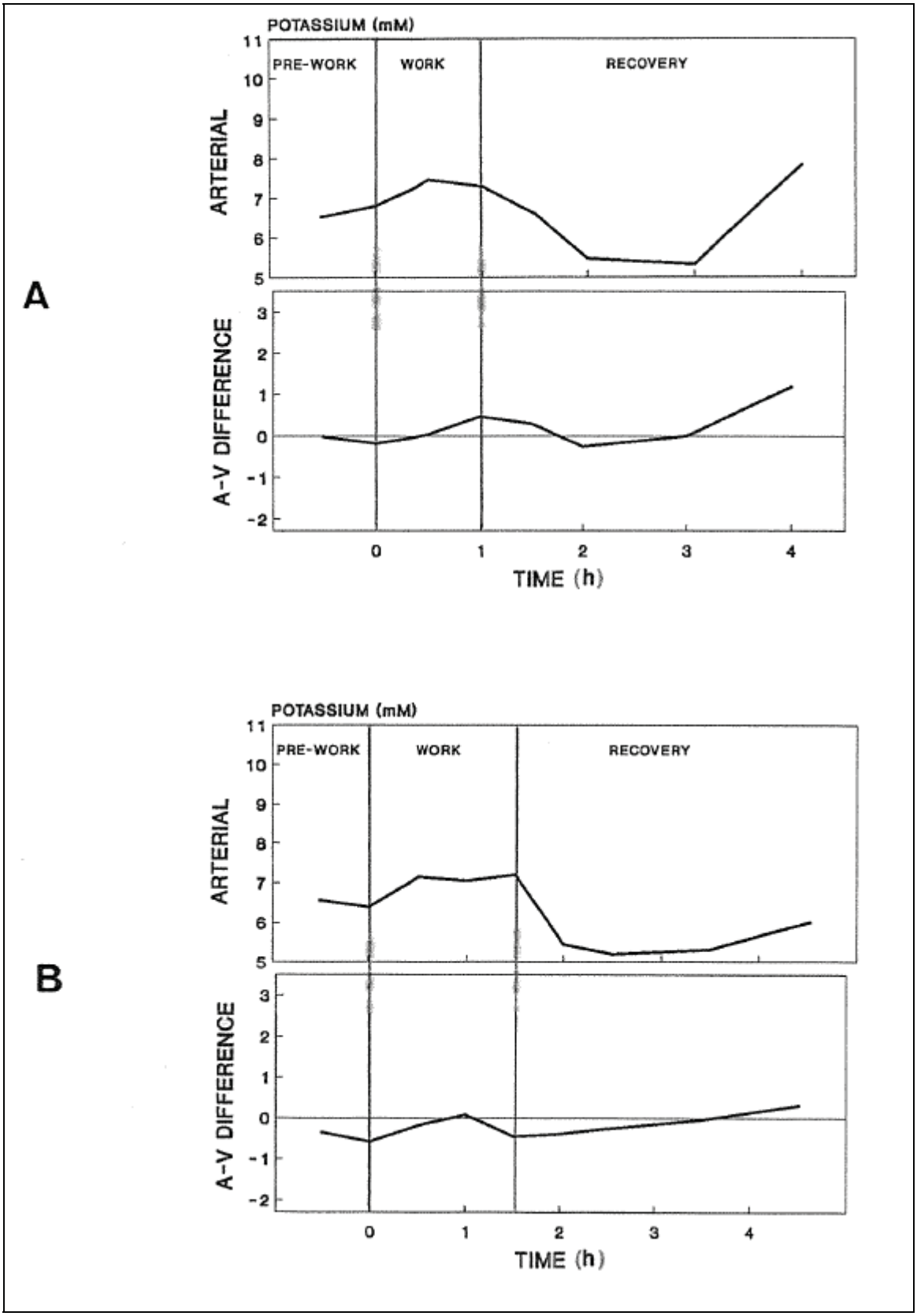


Figure 3C.46 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of potassium in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) of the slow walking speed.

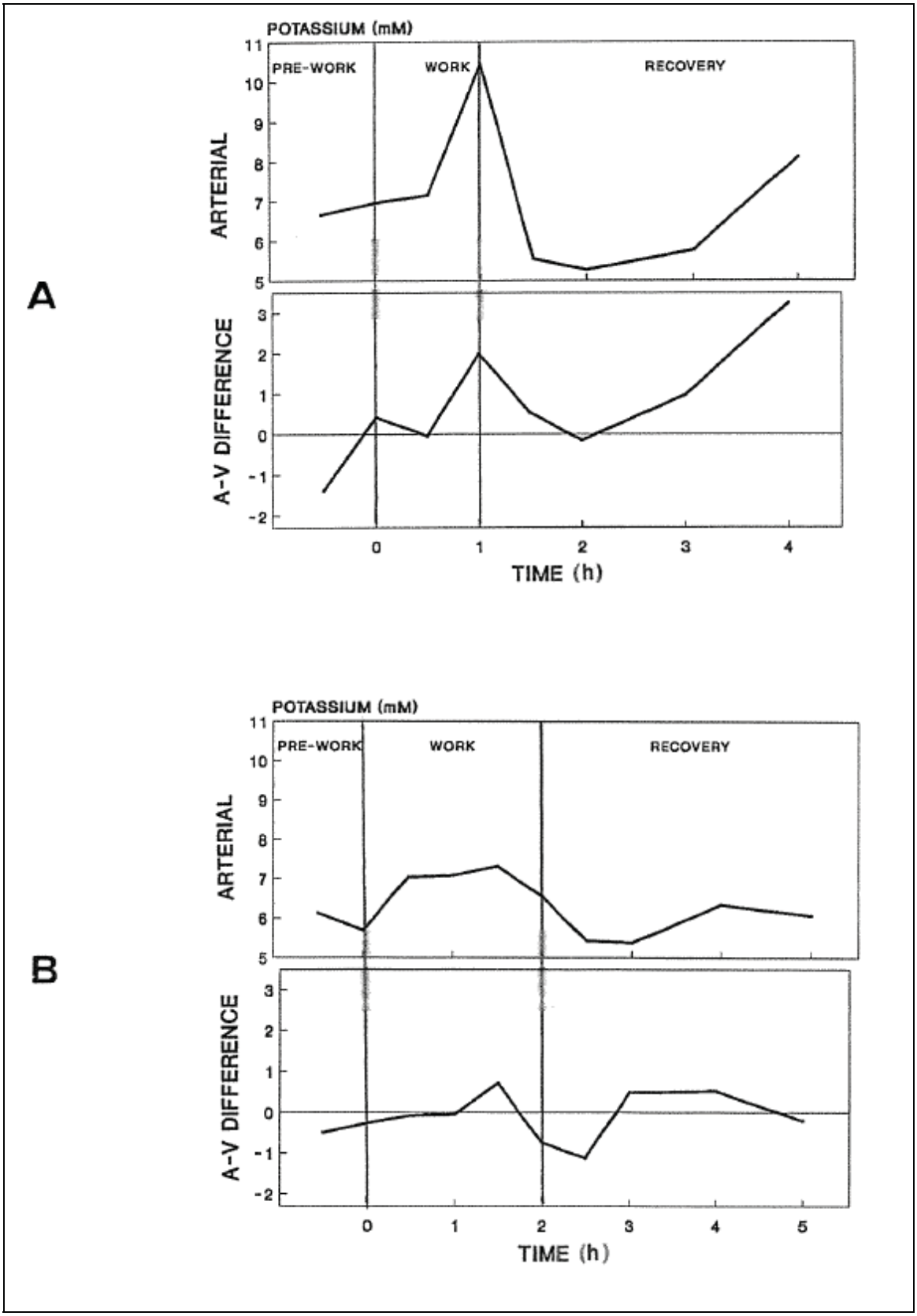


Figure 3C.47 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of potassium in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) of the medium walking speed.

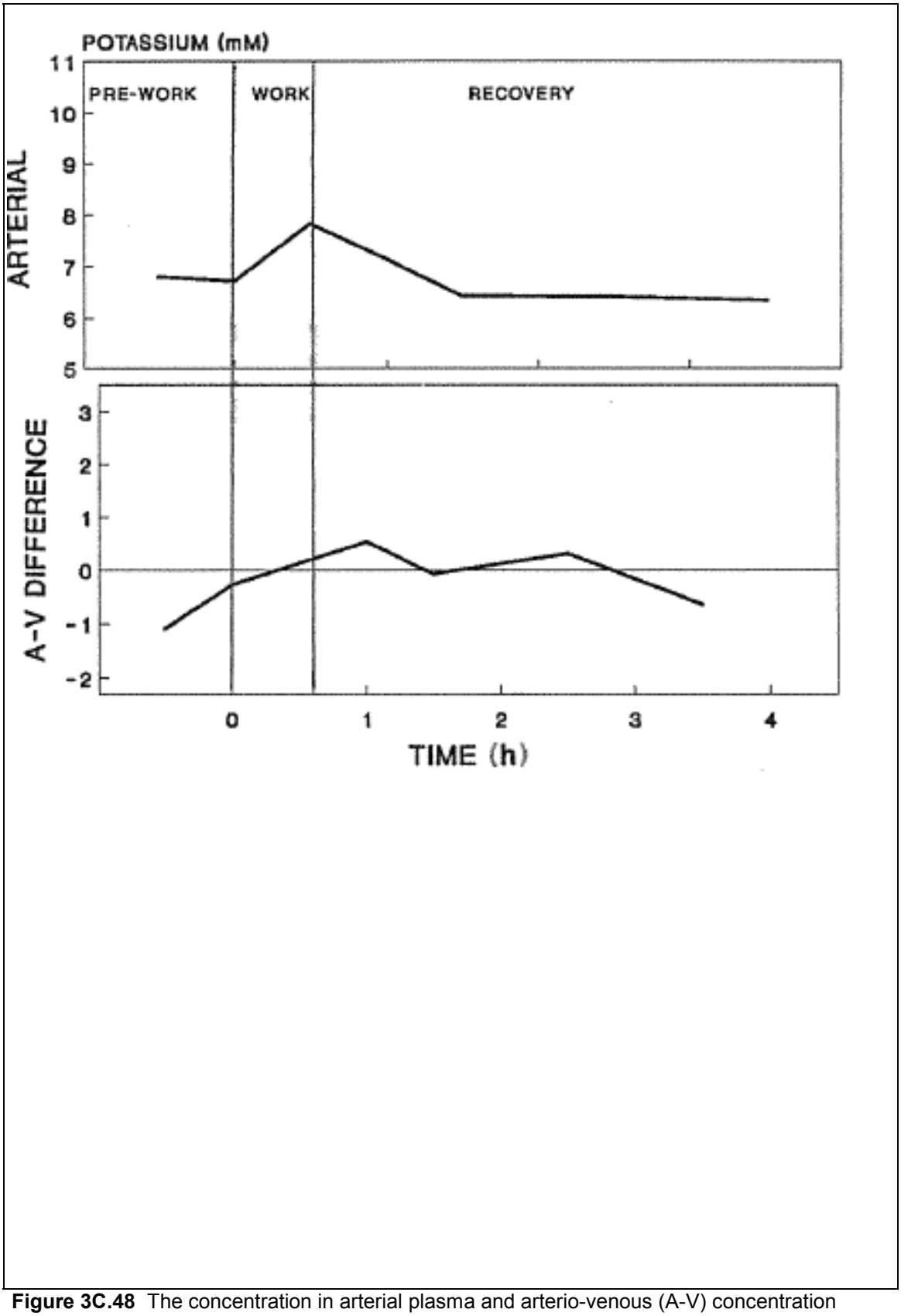


Figure 3C.48 The concentration in arterial plasma and arterio-venous (A-V) concentration difference of potassium in trained sheep over time across work status (*Pre-work, Work and Recovery*) at the fast walking speed.

Ammonia-Nitrogen

Data for NH_3-N concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.49. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.50.

Comparisons of mean arterial concentrations and A-V concentration differences of NH_3-N in sheep between physiological states, walking speeds and work status are presented in table 3C.17.

Table 3C.17 Comparisons of arterial concentrations (A) and arterio-venous concentration difference (A-V) of ammonia-nitrogen (NH_3-N) in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
NH_3 (mg/L)							
A: Mean	13.11	14.60	14.80	13.28	13.58	13.86	14.07
± SE	0.34	0.47	1.28	0.90	0.82	0.92	0.55
Significance	P=0.0115		P=0.0425		P=0.7824		
NH_3 (µg/L)							
A–V: Mean	-60	101	65	-35	22	173	-33
± SE	70	60	147	173	80	150	71
Significance	P=0.0843		P=0.2845		P=0.2617		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Work status had no significant effect on NH_3-N concentration in arterial plasma (table 3C.17), however, there was a general trend for the concentration to increase during the *Work* period, except for the untrained animals walking at slow speed (figure 3C.49).

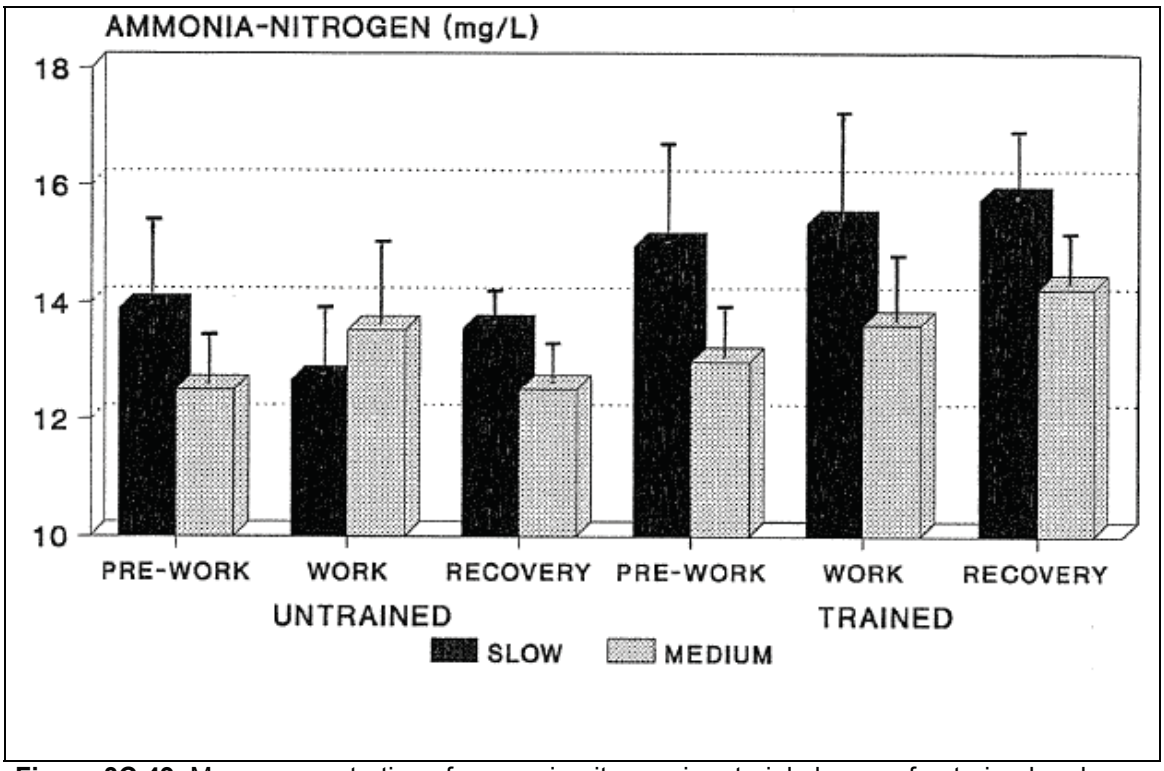


Figure 3C.49 Mean concentration of ammonia-nitrogen in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

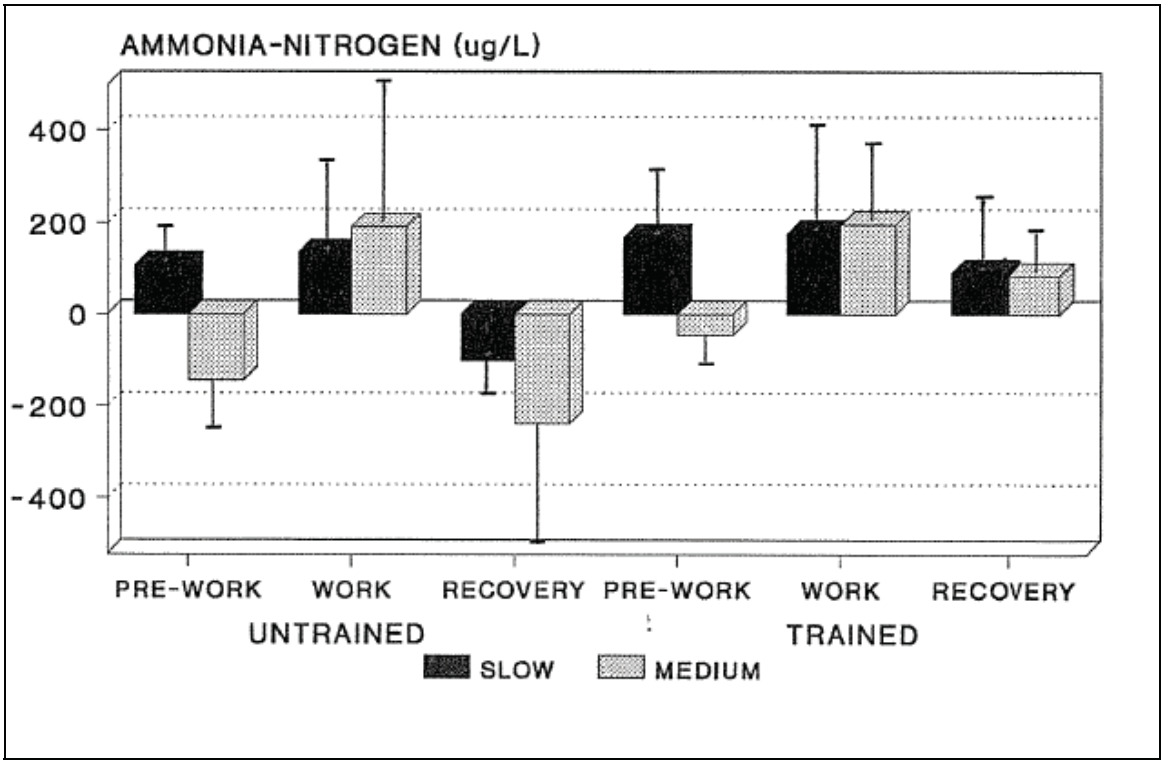


Figure 3C.50 Mean arterio-venous concentration difference of ammonia-nitrogen in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed and physiological state significantly affected NH_3-N concentration in arterial plasma (table 3C.17). The NH_3-N concentration was higher in the trained as compared to the untrained animals (figure 3C.49) and was also higher for the slow walking speed as compared to the medium walking speed (figure 3C.49). The exception being untrained animals at medium speed (figure 3C.49).

There were no significant effects of treatments on the movement of NH_3-N across the hind-limb muscle (table 3C.17), however, values indicate a net uptake of NH_3-N by the muscle (figure 3C.50).

Data on NH_3-N concentrations in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.51. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.52.

Walking speeds (P=0.2583) and work status (P=0.3076) had no significant effect on NH_3-N concentration in arterial plasma of trained sheep. There was an increase in NH_3-N during the *Work* period for the medium and fast walking speeds (figure 3C.51).

There was no significant effect of walking speed (P=0.4944) and work status (P=0.7329) on the net movement of NH_3-N across the hind-limb muscle of sheep. Values indicate a net output for fast walking speed during the *Work* period (figure 3C.52).

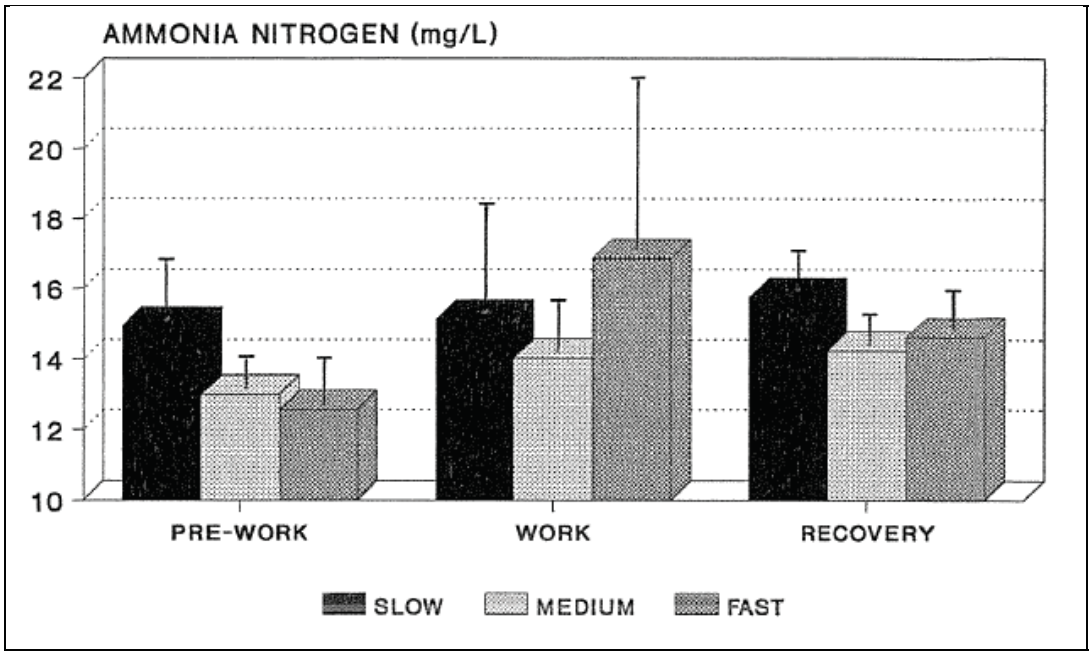


Figure 3C.51 Mean concentration of ammonia-nitrogen in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

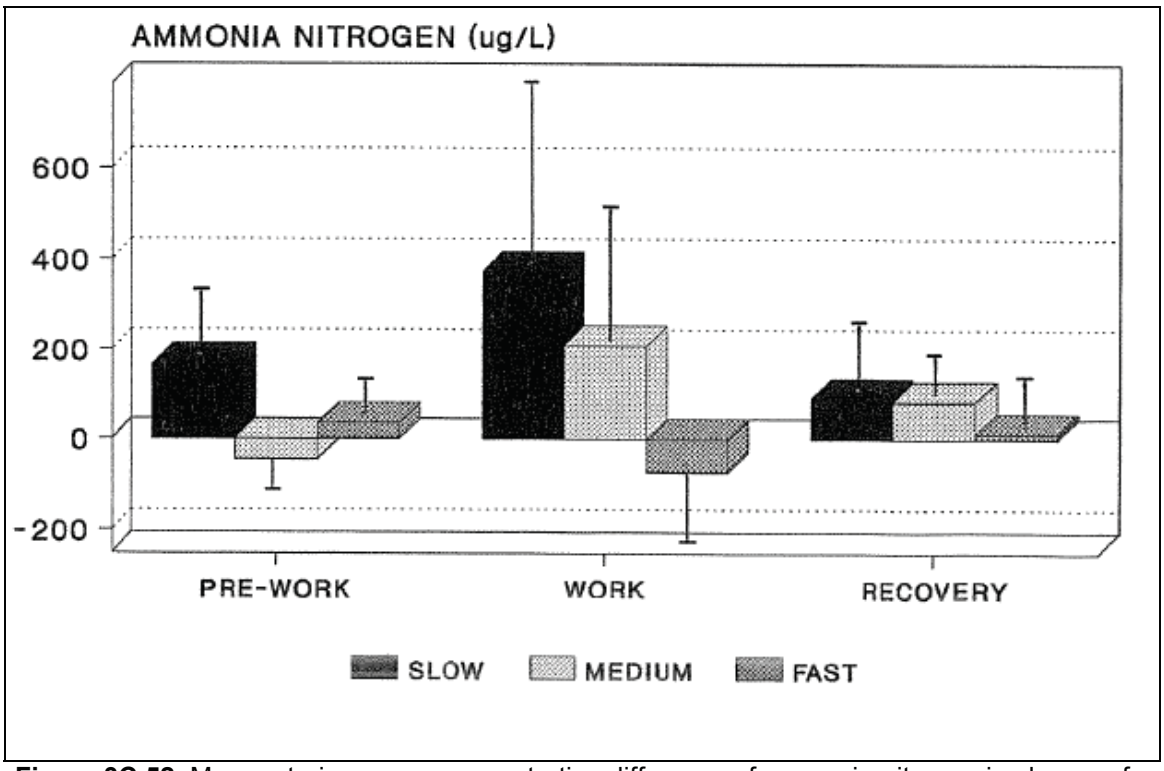


Figure 3C.52 Mean arterio-venous concentration difference of ammonia-nitrogen in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

Protein/Amino acid-Nitrogen

Data for protein/AA-N concentration in arterial plasma of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.53. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.54.

Comparisons of mean arterial concentrations and A-V concentration differences of protein/AA-N in sheep between physiological states, walking speeds and work status are presented in table 3C.18.

Table 3C.18 Comparison of arterial concentrations (A) and arterio-venous concentration difference (A-V) of protein/amino acid-nitrogen (Protein/AA-N) in plasma of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Protein/AA-N (mg/L)							
A: Mean	69.48	68.65	66.94	71.21	60.65	75.92	68.32
± SE	1.58	1.49	2.97	2.66	2.46	2.43	2.16
Significance	P=0.7036		P=0.0453		P=0.0000		
A–V: Mean	-0.11	-4.11	-1.85	-2.51	-11.92	3.16	0.63
± SE	1.60	1.69	2.06	2.07	1.80	3.24	1.49
Significance	P=0.0864		P=0.7813		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

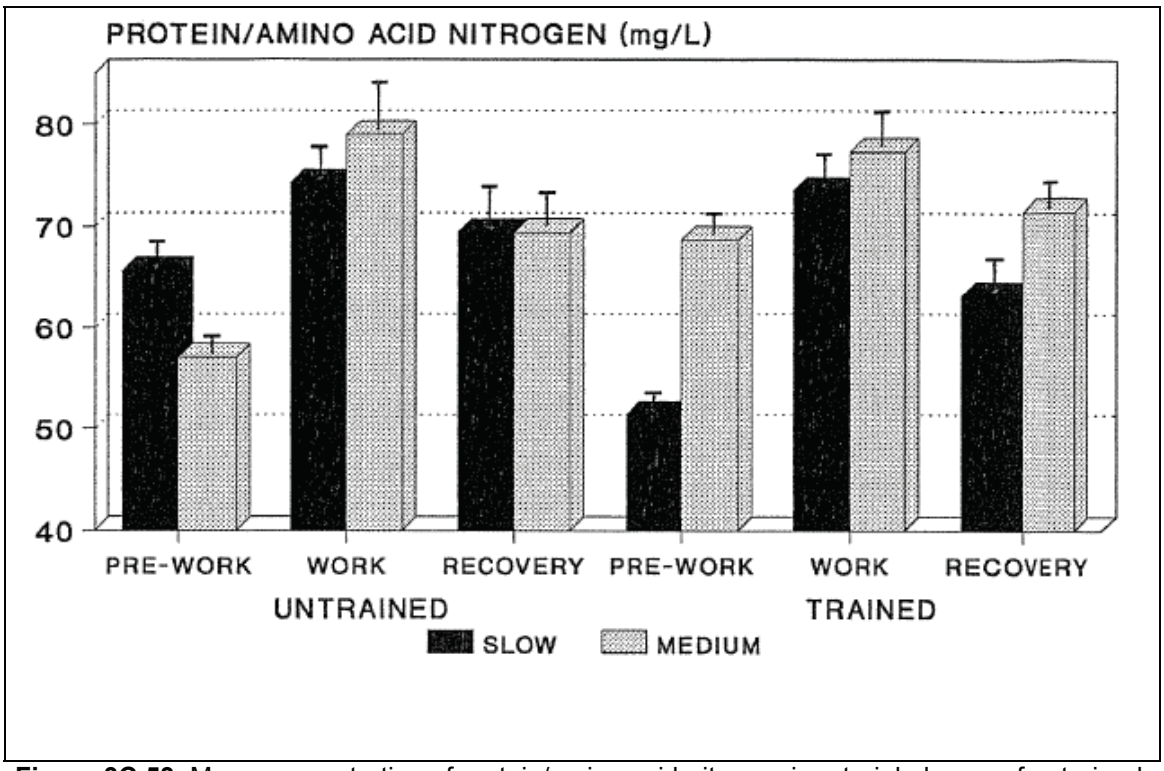


Figure 3C.53 Mean concentration of protein/amino acid-nitrogen in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

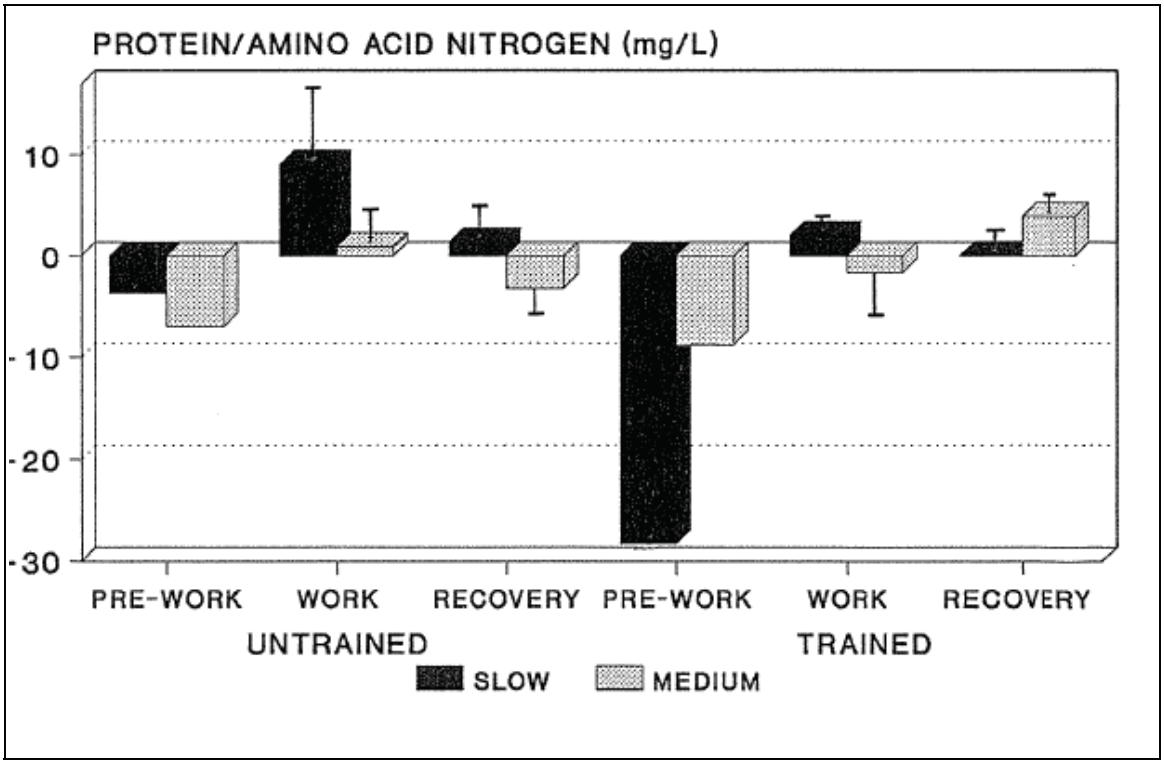


Figure 3C.54 Mean arterio-venous concentration difference of protein/amino acid-nitrogen in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Physiological state had no significant effect on protein/AA-N concentration in arterial plasma (table 3C.18). Walking speed and work status did have a significant effect on protein/AA-N concentration (table 3C.18). Arterial concentrations of protein/AA-N were higher for the medium walking speed as compared to the slow walking speed (figure 3C.53). The protein/AA-N concentrations increased during the *Work* period and decreased to remain above *Pre-work* values in the *Recovery* period (figure 3C.53).

In the net movement of protein/AA-N across the hind-limb muscle there was a significant effect due to work status only (table 3C.18). Physiological state approached significance and there was a greater output for the medium walking speed as compared to the slow walking speed. Protein/AA-N changed from an output during the *Pre-work* period to a large uptake during the *Work* period (table 3C.18).

Data on protein/AA-N concentrations in arterial plasma of trained sheep at three different work status and walking speeds are presented in figure 3C.55. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.56.

Walking speed (P=0.0232) and work status (P=0.0043) had a significant effect on protein/AA-N concentration in arterial plasma of trained sheep. Protein/AA-N increased concentration during the *Work* period and decreased during the *Recovery* period (figure 3C.55). During the *Work* period the concentration of protein/AA-N was higher for the slow and medium walking speeds as compared to the fast walking speed (figure 3C.55).

There was no significant effect on the net movement of protein/AA-N by walking speed (P=0.1750), however, there was a trend of a larger uptake for the slow walking speed as compared to the medium walking speed (figure 3C.56). Work status had a significant effect (P=0.0000) on the net movement of protein/AA-N across the hind-limb muscle. During the *Pre-work* period there was a net output of protein/AA-N, and during the *Work* period there was a net uptake for the slow walking speed, and a reduced output for the medium and fast walking speeds (figure 3C.56).

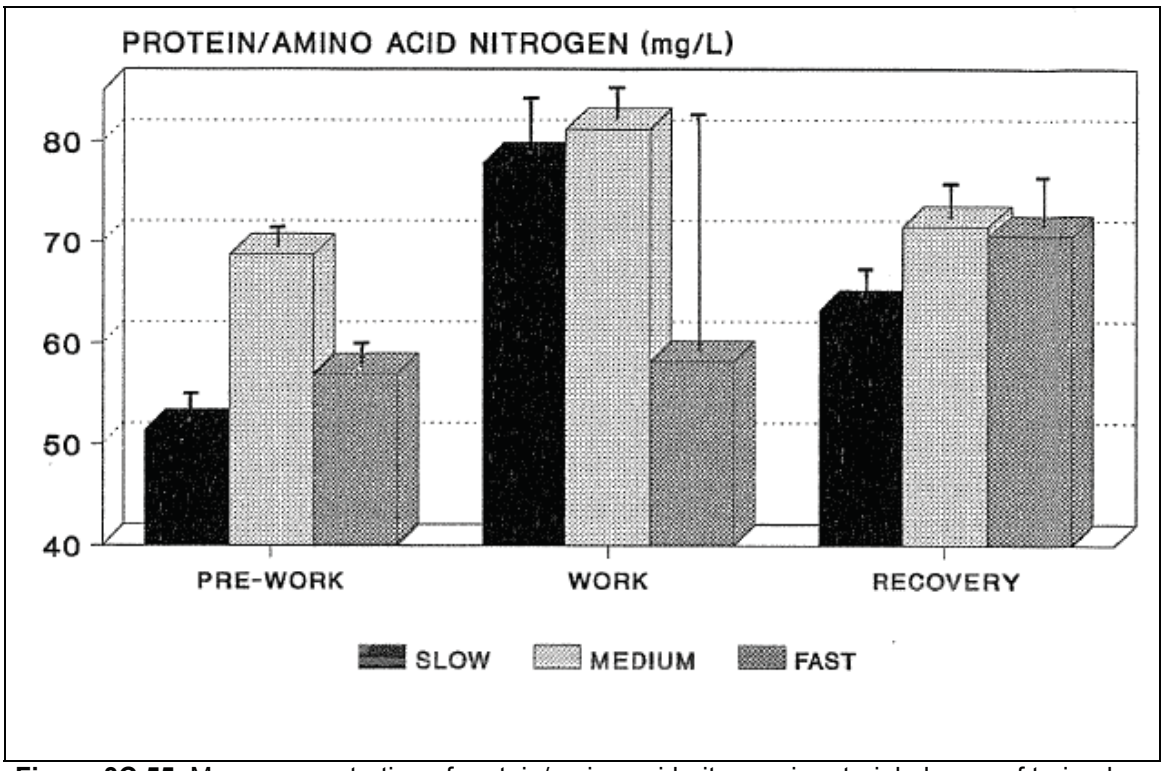


Figure 3C.55 Mean concentration of protein/amino acid-nitrogen in arterial plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

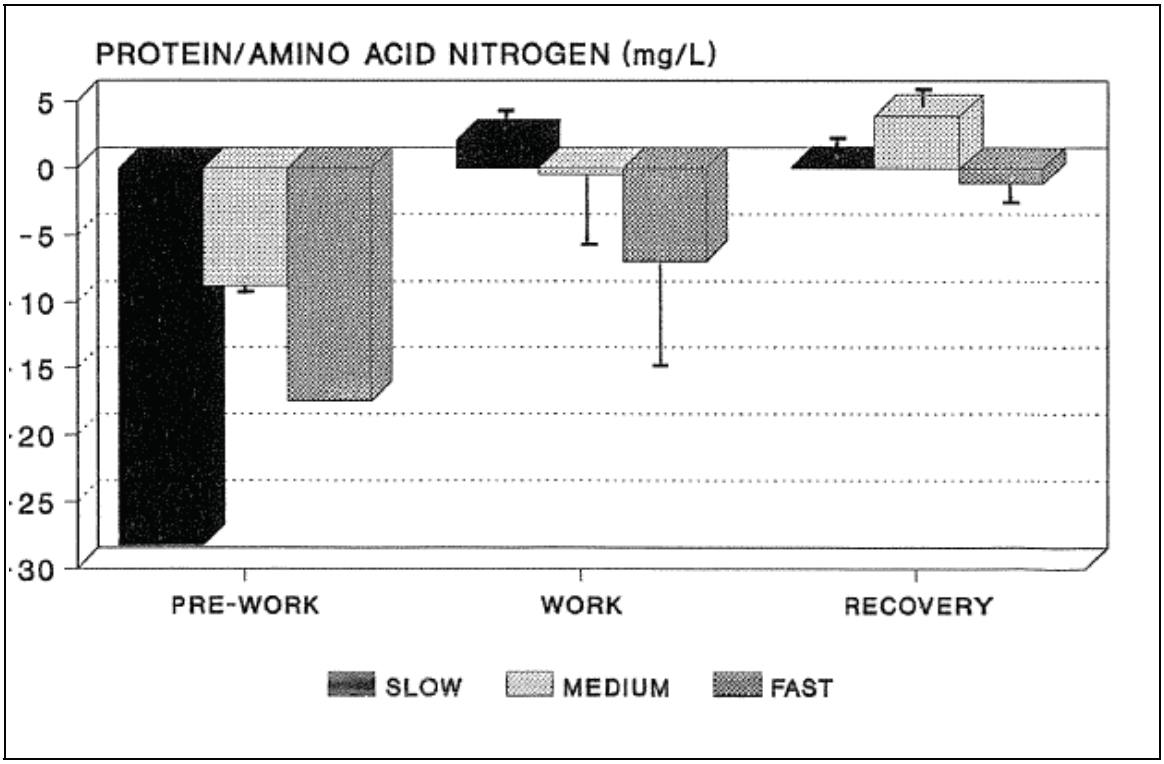


Figure 3C.56 Mean arterio-venous concentration difference of protein/amino-nitrogen in plasma of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

3C.3.5 Blood parameters

Packed cell volume

Data for PCV of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.57.

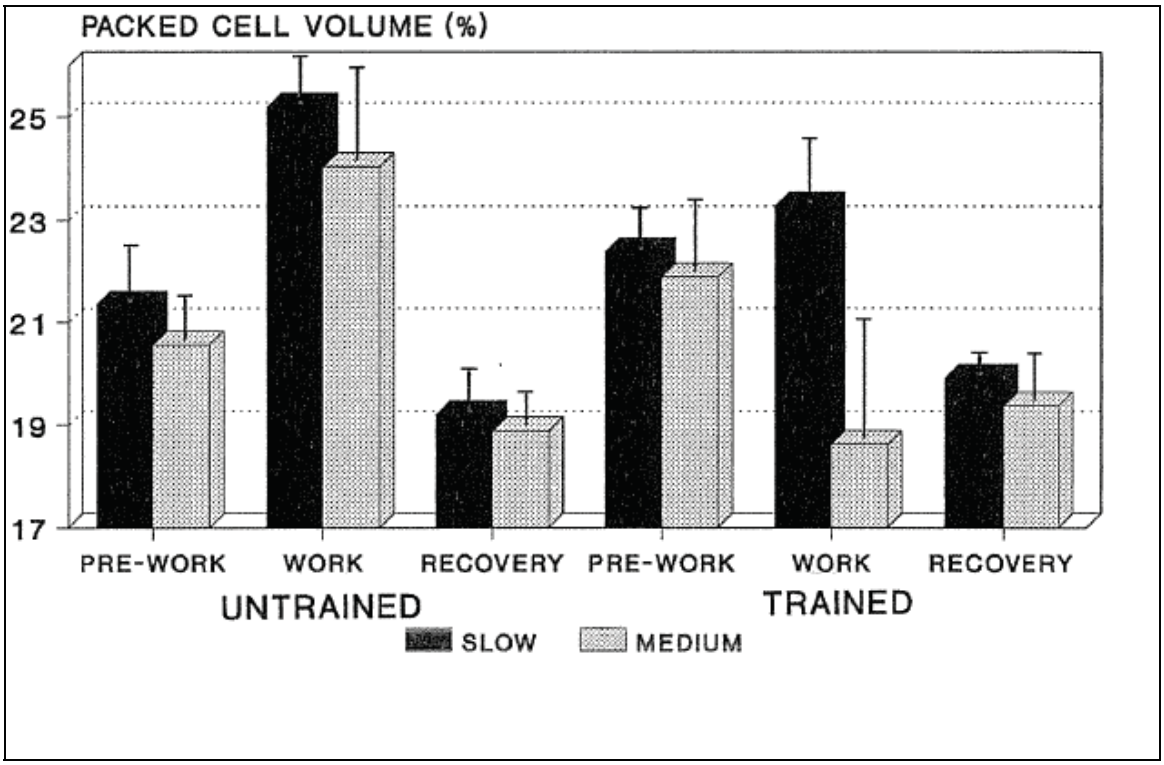


Figure 3C.57 Mean packed cell volume in untrained and trained sheep during the *Pre-work, Work* and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Comparisons of mean PCV in sheep between physiological states, walking speeds and work status are presented in table 3C.19.

Packed cell volume was not significantly affected by physiological states or walking speed (table 3C.19). Work status, however, significantly affected PCV in sheep (table 3C.19). The PCV increased by 14% during the *Work* period and decreased to below *Pre-work* values during the *Recovery* periods (figure 3C.57).

The changes in the pattern of PCV in untrained and trained sheep over time across work status and at slow and medium walking speeds are presented in figure 3C.58.

Table 3C.19 Means of pooled data on packed cell volume (PCV) of sheep, for physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
PCV (%)	20	21	21	20	22	25	19
± SE	1	1	1	2	1	1	1
Significance	P=0.5386		P=0.4582		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

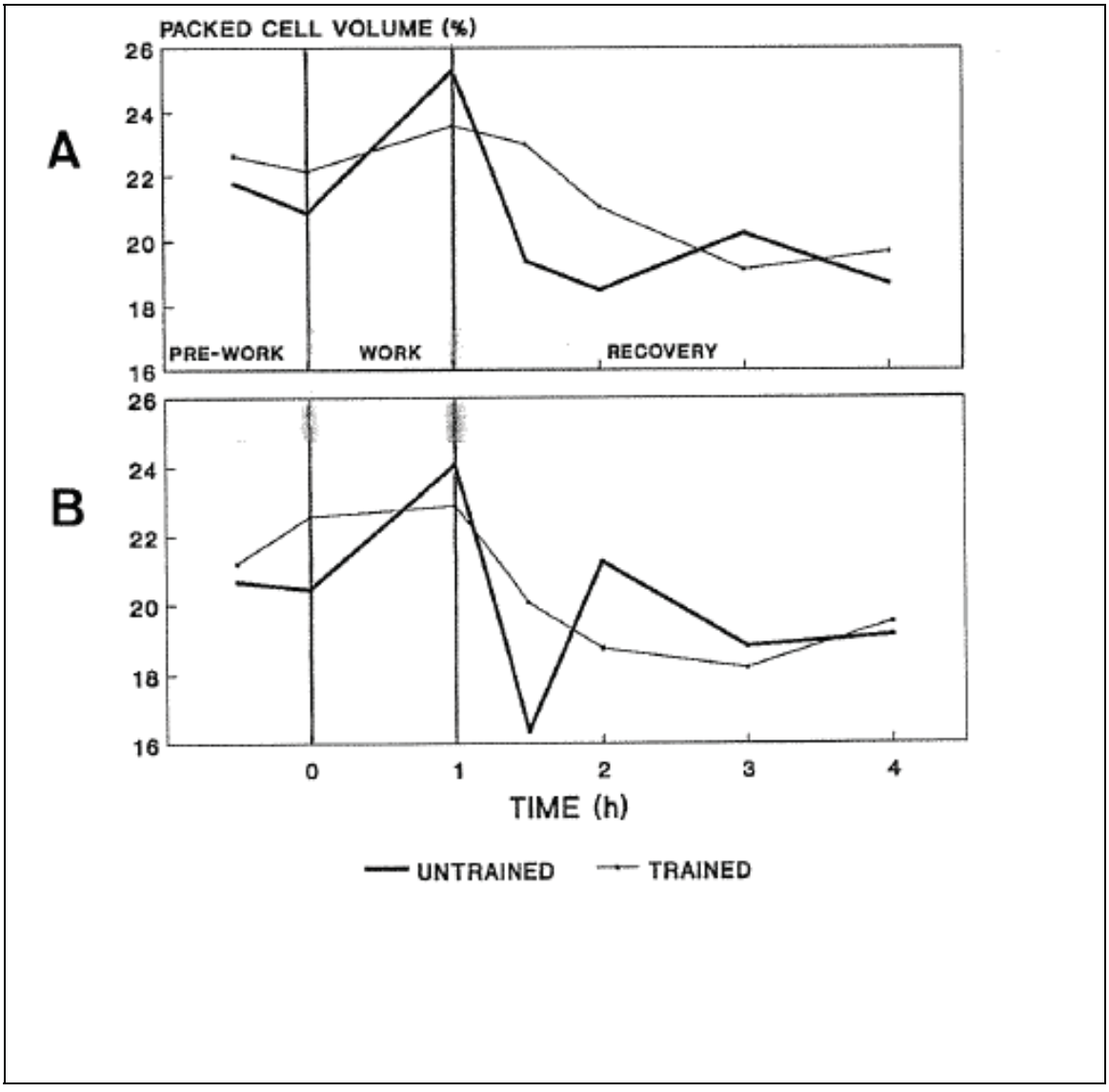


Figure 3C.58 Packed cell volume in untrained and trained sheep over time across work status (*Pre-work, Work* and *Recovery*) at slow (A) and medium (B) walking speeds.

Haemoglobin

Data for haemoglobin concentration in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.59. Corresponding data for A-V concentration differences of the metabolite across the hind-limb muscle of the animals are presented in figure 3C.60.

Comparisons of mean arterial concentrations and A-V concentration differences of haemoglobin in sheep between physiological states, walking speeds and work status are presented in table 3C.20.

Table 3C.20 Comparison of arterial concentrations (A) and arterio-venous concentration (A-v) of haemoglobin in blood of sheep, between physiological states, walking speeds and work status Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Haemoglobin (g/L)							
A: Mean	71.94	72.62	71.32	73.23	70.73	77.87	67.28
± SE	1.46	1.29	2.37	3.83	2.36	2.10	2.05
Significance	P=0.8064		P=0.4455		P=0.0008		
A–V: Mean	-1.09	-0.77	-1.42	-0.34	-2.40	-0.55	-0.29
± SE	1.09	0.99	1.70	2.60	1.89	1.90	0.81
Significance	P=0.7821		P=0.3500		P=0.3194		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Haemoglobin concentration was not significantly affected by physiological states or walking speed (table 3C.20). There was a trend, however, for haemoglobin concentration to be higher in the trained animals as compared to the untrained animals and at medium speed during the *Work* period (figure 3C.59). Work status significantly affected the concentration of haemoglobin in the animals (table 3C.20). The haemoglobin concentration increased by 10% during the *Work* period and decreasing to below *Pre-work* values during the *Recovery* period (table 3C.20).

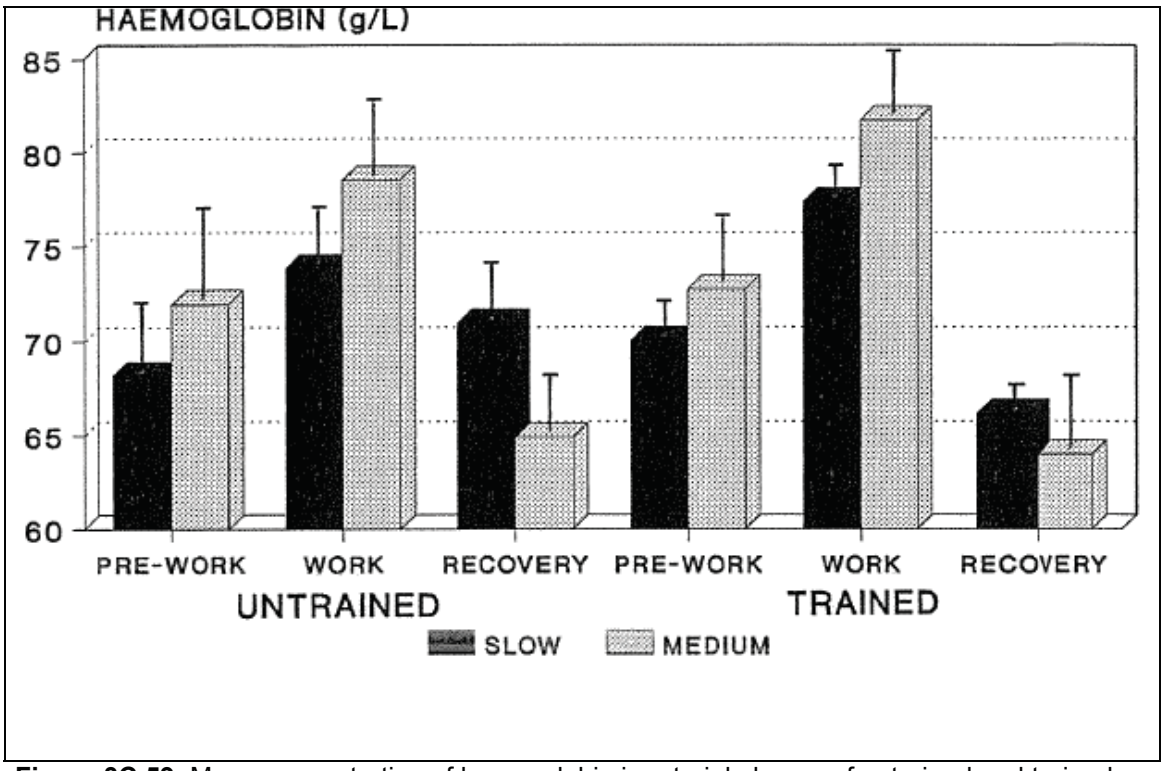


Figure 3C.59 Mean concentration of haemoglobin in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

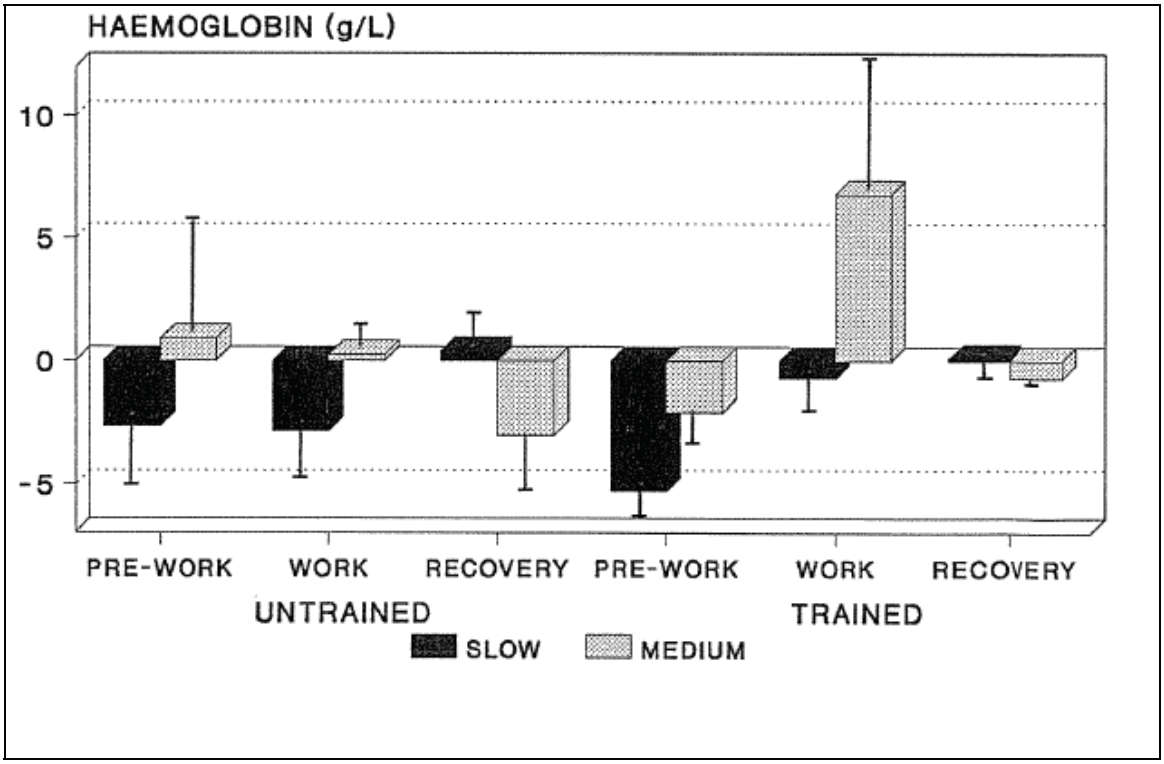


Figure 3C.60 Mean arterio-venous concentration difference of haemoglobin in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

All treatments, *viz* physiological states, walking speeds and work status had no significant effect on the net movement of haemoglobin across the hind-limb muscles (table 3C.20). Values indicate a net output across all treatments (table 3C.20).

Data on haemoglobin concentration in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.61. Corresponding data for A-V concentration differences of haemoglobin across the hind-limb muscle of the animals are presented in figure 3C.62.

Walking speed had no significant effect (P=0.6397) on the concentration of haemoglobin in sheep. Work status had a significant effect on haemoglobin concentration and decreased during *Work* at the fast speed compared to an increased concentration for slow and medium speeds (figure 3C.61). The concentration of haemoglobin decreased to below *Pre-work* values during the *Recovery* period (figure 3C.61).

There were no significant differences (P=0.4774 and P=0.8381, respectively) between walking speeds and work status in the net movement of haemoglobin across the hind-limb muscles.

The changes in the pattern of the concentration of haemoglobin in arterial blood and A-V concentration differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.63–3C.65.

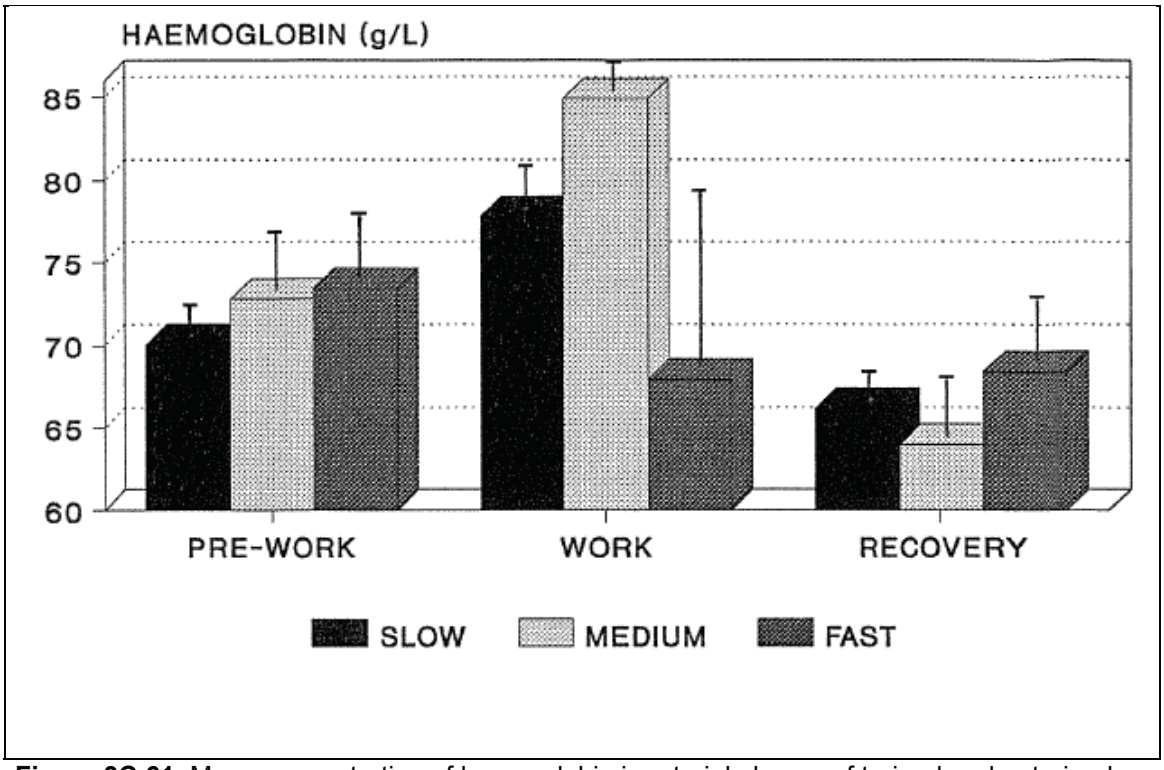


Figure 3C.61 Mean concentration of haemoglobin in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* p
eriods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

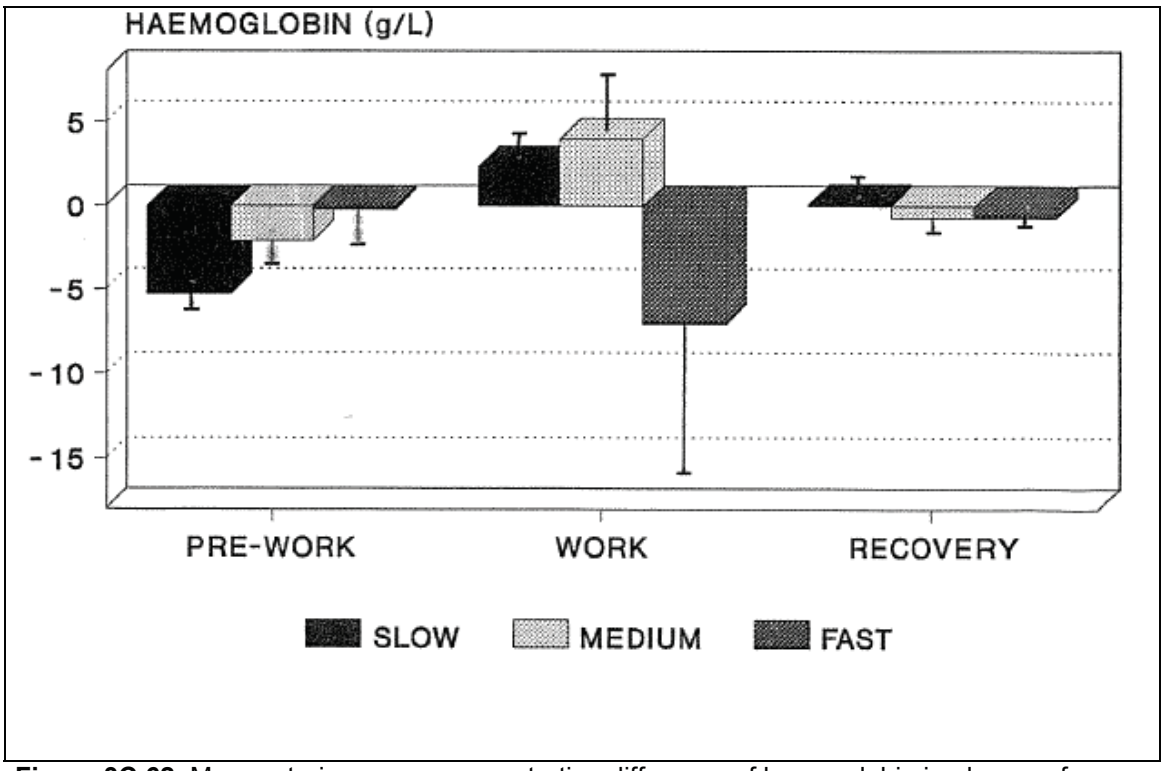


Figure 3C.62 Mean arterio-venous concentration difference of haemoglobin in plasma of trained and untrained sheep during the *Pre-work, Work* and *Recovery* periods and at slow, medium and fast walking speeds. Vertical bars above lines represent standard errors.

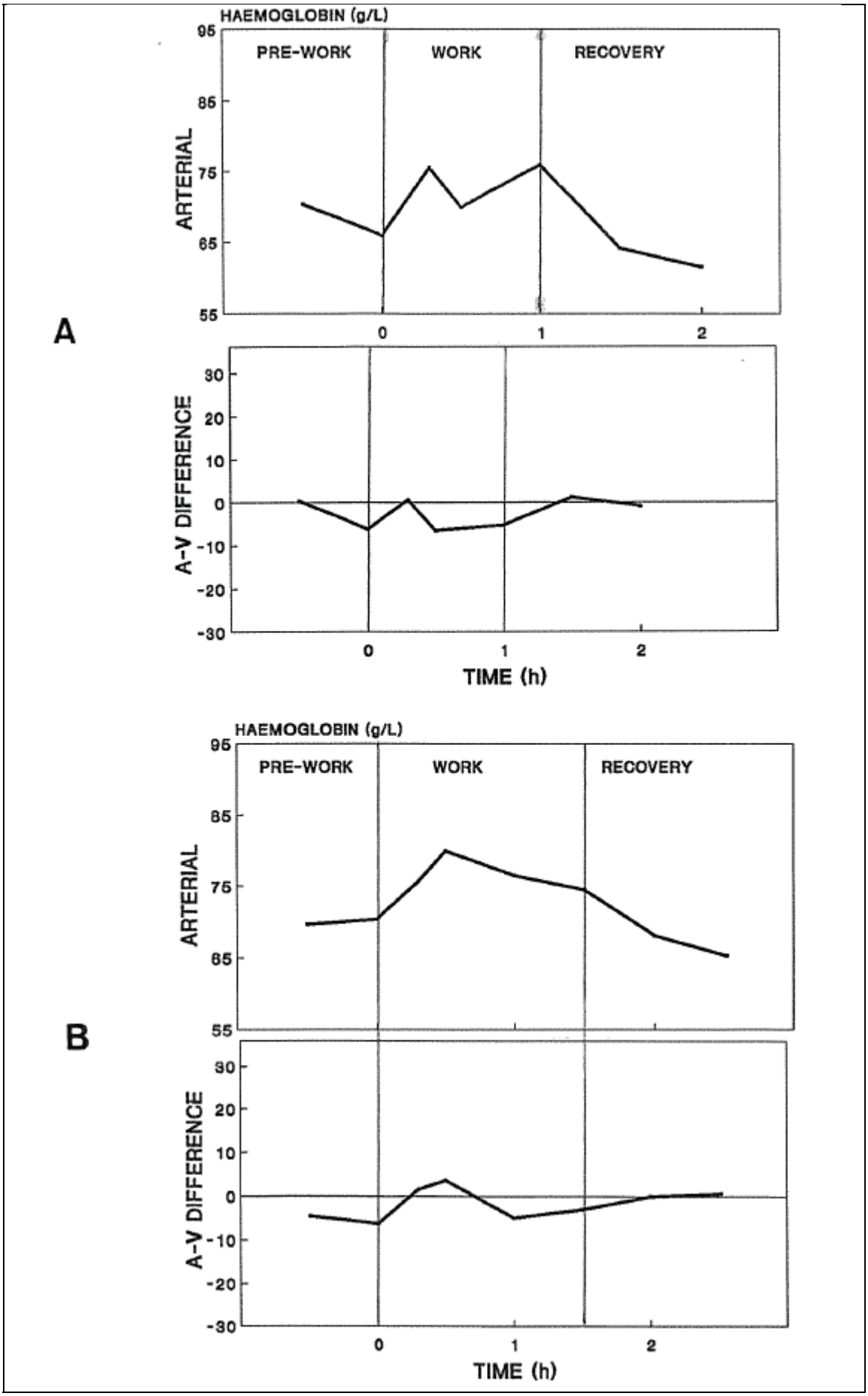


Figure 3C.63 The concentration in arterial blood and the arterio-venous (A-V) concentration difference of haemoglobin in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) at the slow walking speed.

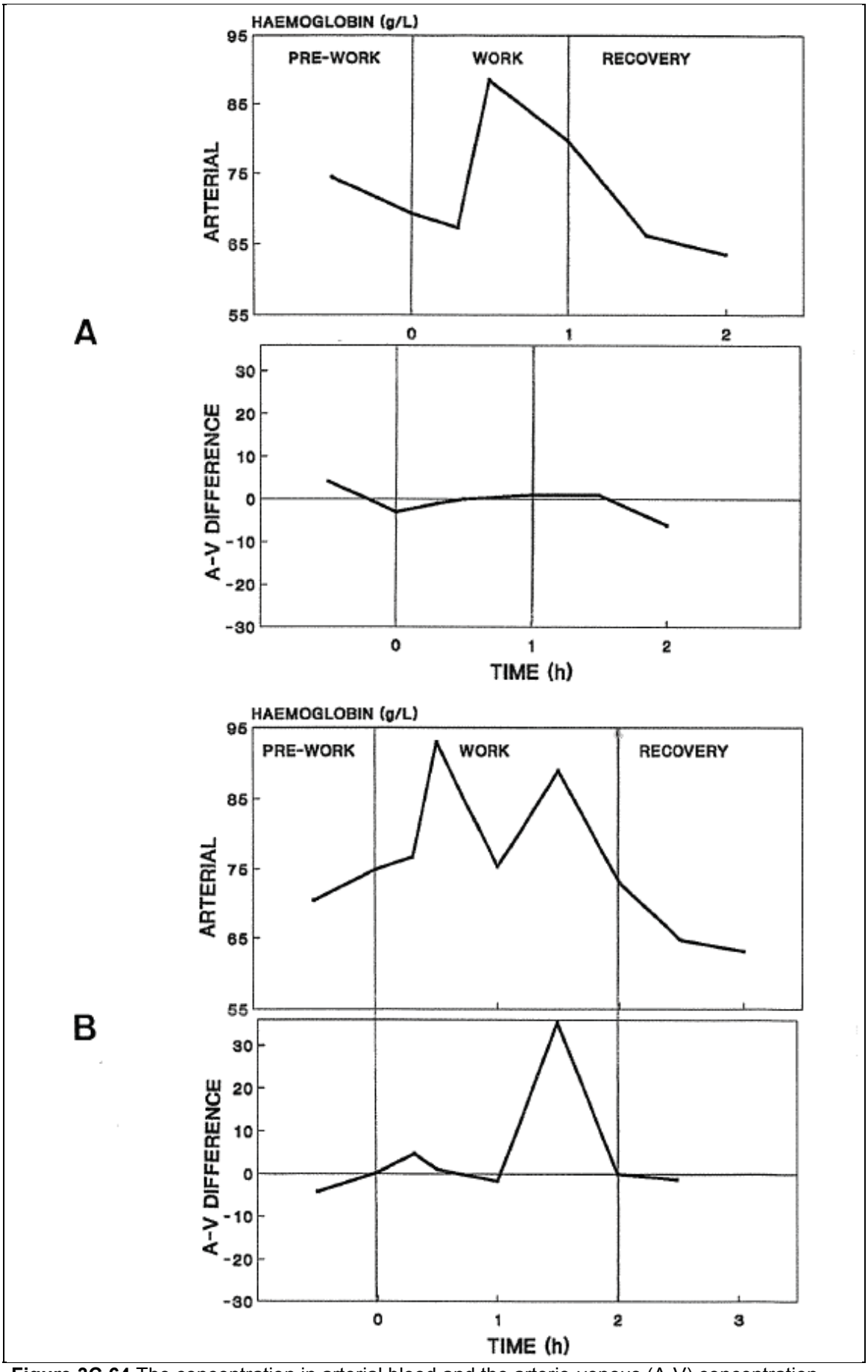


Figure 3C.64 The concentration in arterial blood and the arterio-venous (A-V) concentration difference of haemoglobin in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) at the medium walking speed.

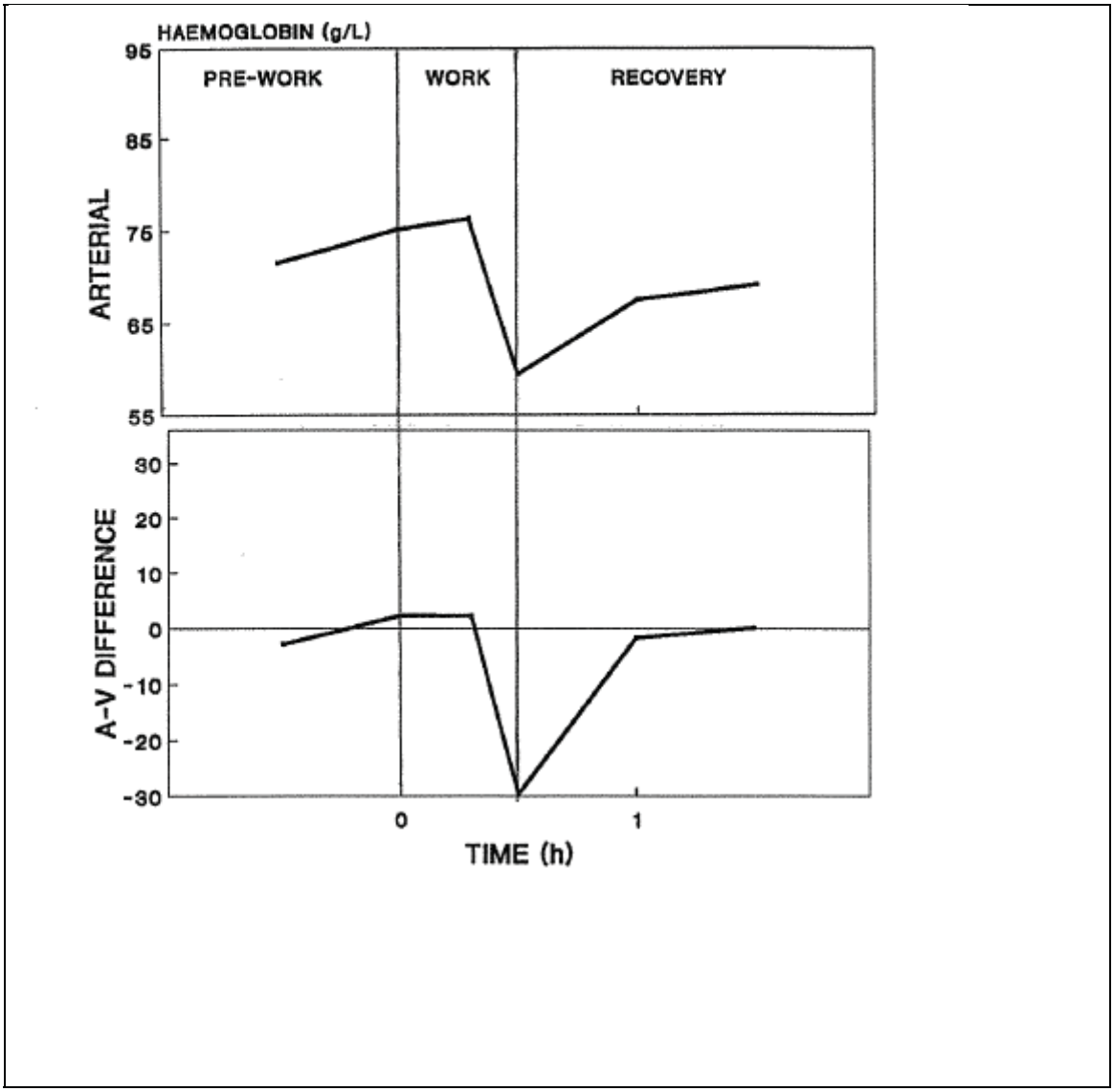


Figure 3C.65 The concentration in arterial blood and the arterio-venous (A-V) concentration difference of haemoglobin in trained sheep over time across work status (*Pre-work, Work* and *Recovery*) at the fast walking speed.

pH

Data for pH in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.66. Corresponding data for A-V differences of pH across the hind-limb muscle are presented in figure 3C.67.

Comparisons of mean arterial and A-V differences of pH in sheep between physiological states, walking speeds and work status are presented in table 3C.21.

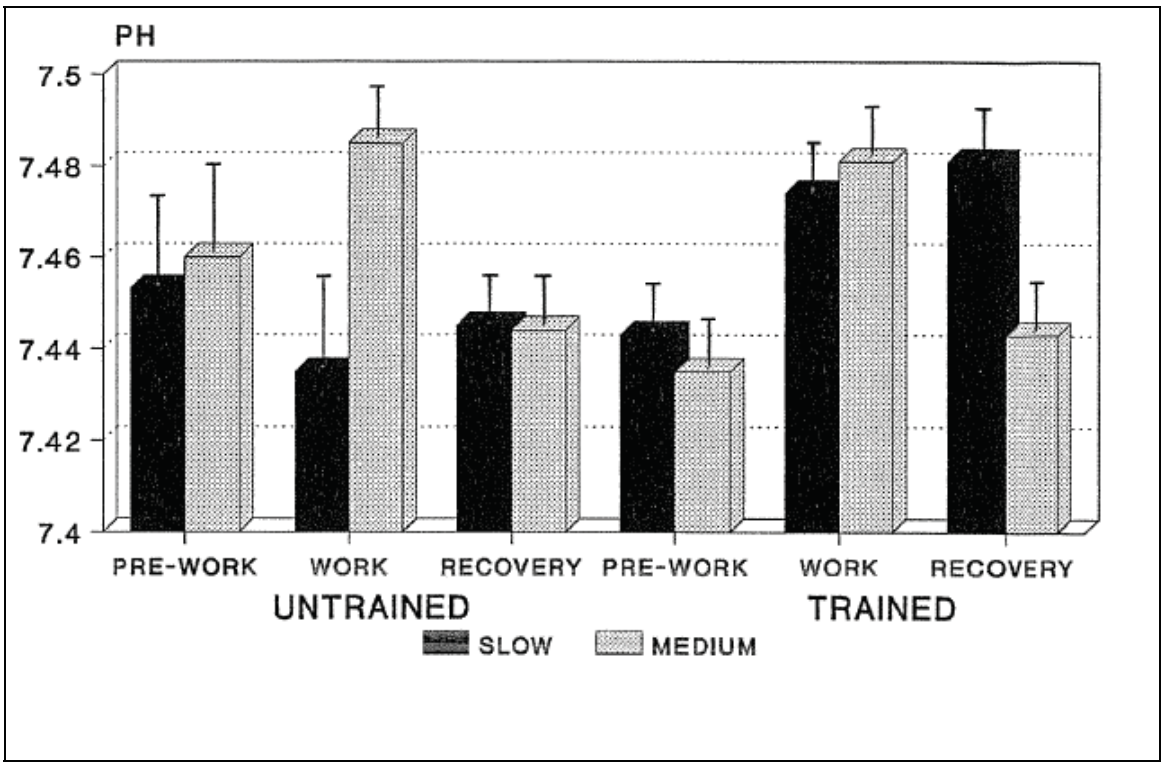


Figure 3C.66 Mean pH in untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

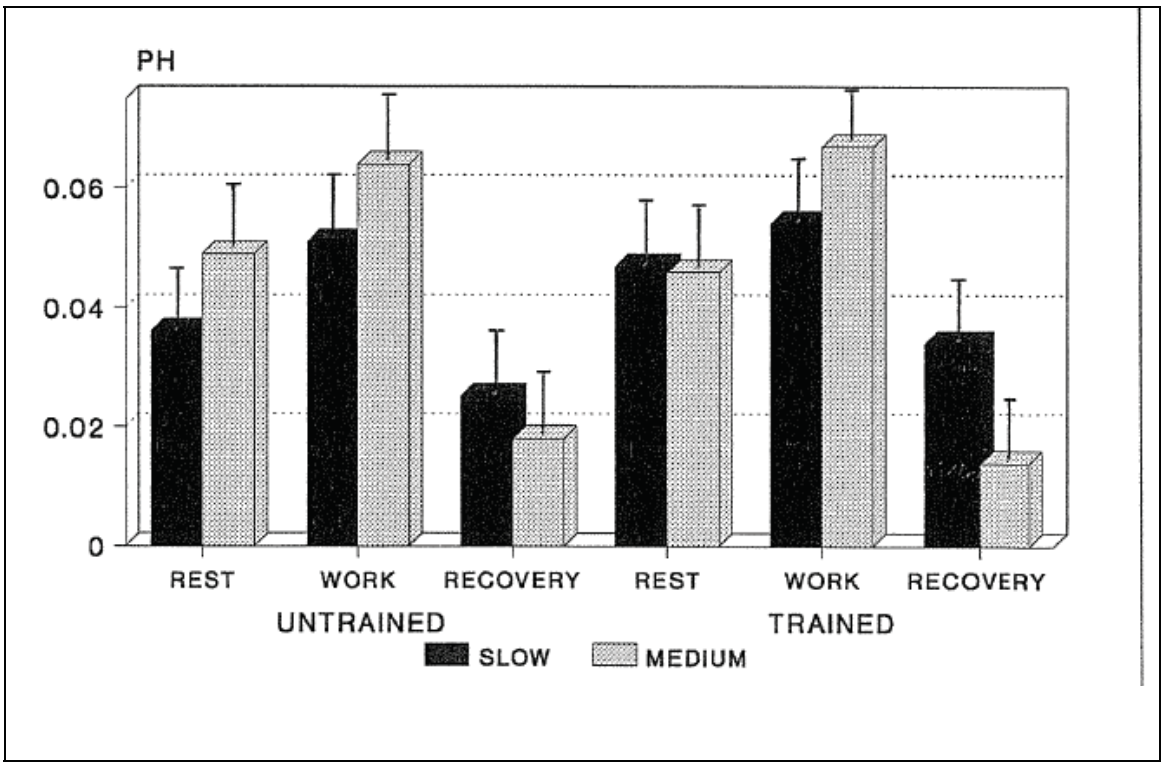


Figure 3C.67 Mean arterio-venous difference in pH of blood of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Table 3C.21 Comparison of arterial concentrations (A) and arterio-venous concentration difference (A-V) of pH in blood of sheep, between physiological states, walking speeds and work status Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
pH							
A: Mean	7.45	7.46	7.46	7.46	7.45	7.47	7.45
± SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Significance	P=0.3189		P=0.5376		P=0.1989		
A–V: Mean	0.04	0.05	0.04	0.05	0.04	0.06	0.02
± SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Significance	P=0.5373		P=0.4352		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

All treatments, *viz* physiological states, walking speed and work status had no significant effect on arterial pH, however, pH increased during the *Work* period (table 3C.21).

In the net blood pH across the hind-limb muscles, there were no significant differences between physiological states or walking speed (table 3C.21). Work status had a significant effect on blood pH with an increased net uptake during the *Work* period (table 3C.21).

Data on pH in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.68. Corresponding data for A-V differences of pH across the hind-limb muscle of the animals are presented in figure 3C.69.

Walking speed and work status had no significant effect (P=0.2077 and P=0.4121) respectively, on arterial pH. Work status had a significant effect (P=0.0004) on the A-V difference of pH across the hind-limb muscle with a net uptake increasing during the *Work* period (figure 3C.69). There were no significant differences (P=0.8001) between walking speeds for A-V difference of pH.

The changes in the pattern of pH in arterial blood and A-V differences in the untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.70 to 3C.72.

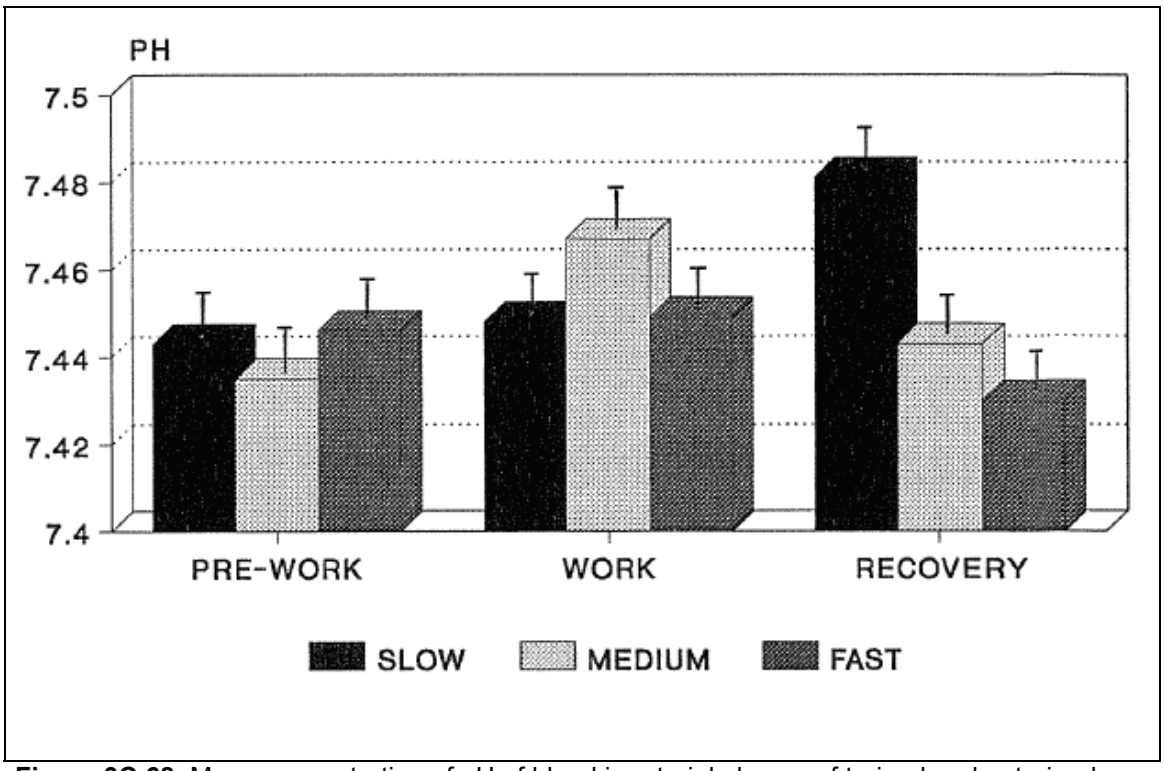


Figure 3C.68 Mean concentration of pH of blood in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

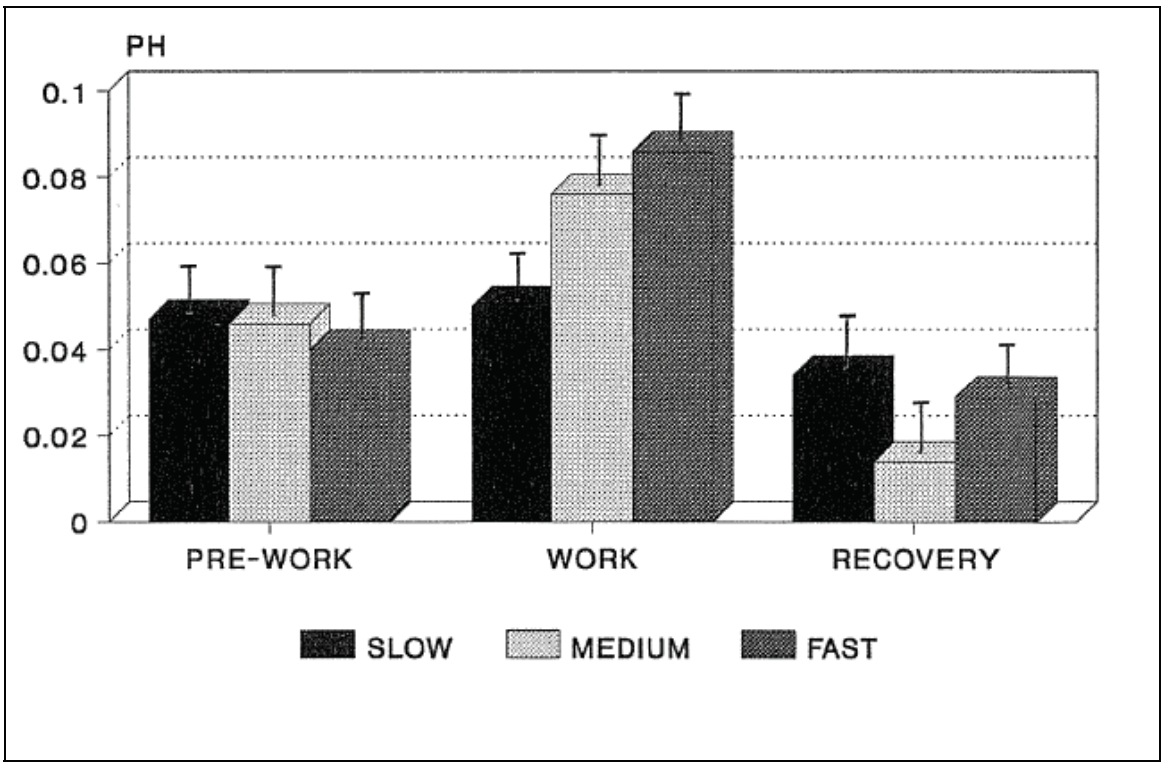


Figure 3C.69 Mean arterio-venous concentration difference in pH of blood in trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. **Vertical bars above lines represent standard errors.**

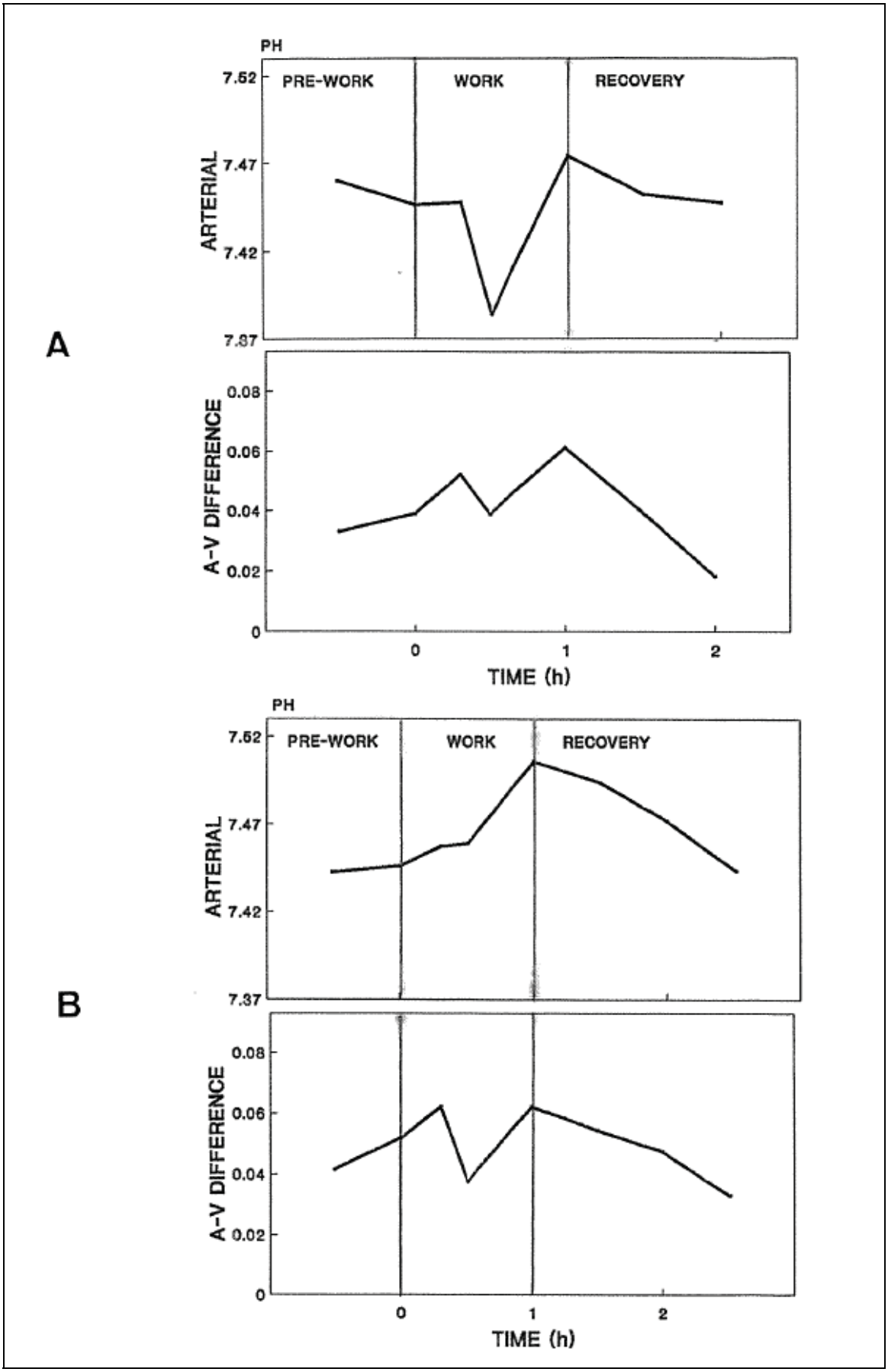


Figure 3C.70 The concentration of pH in arterial blood and the arterio-venous (A-V) concentration difference in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) at the slow walking speed.

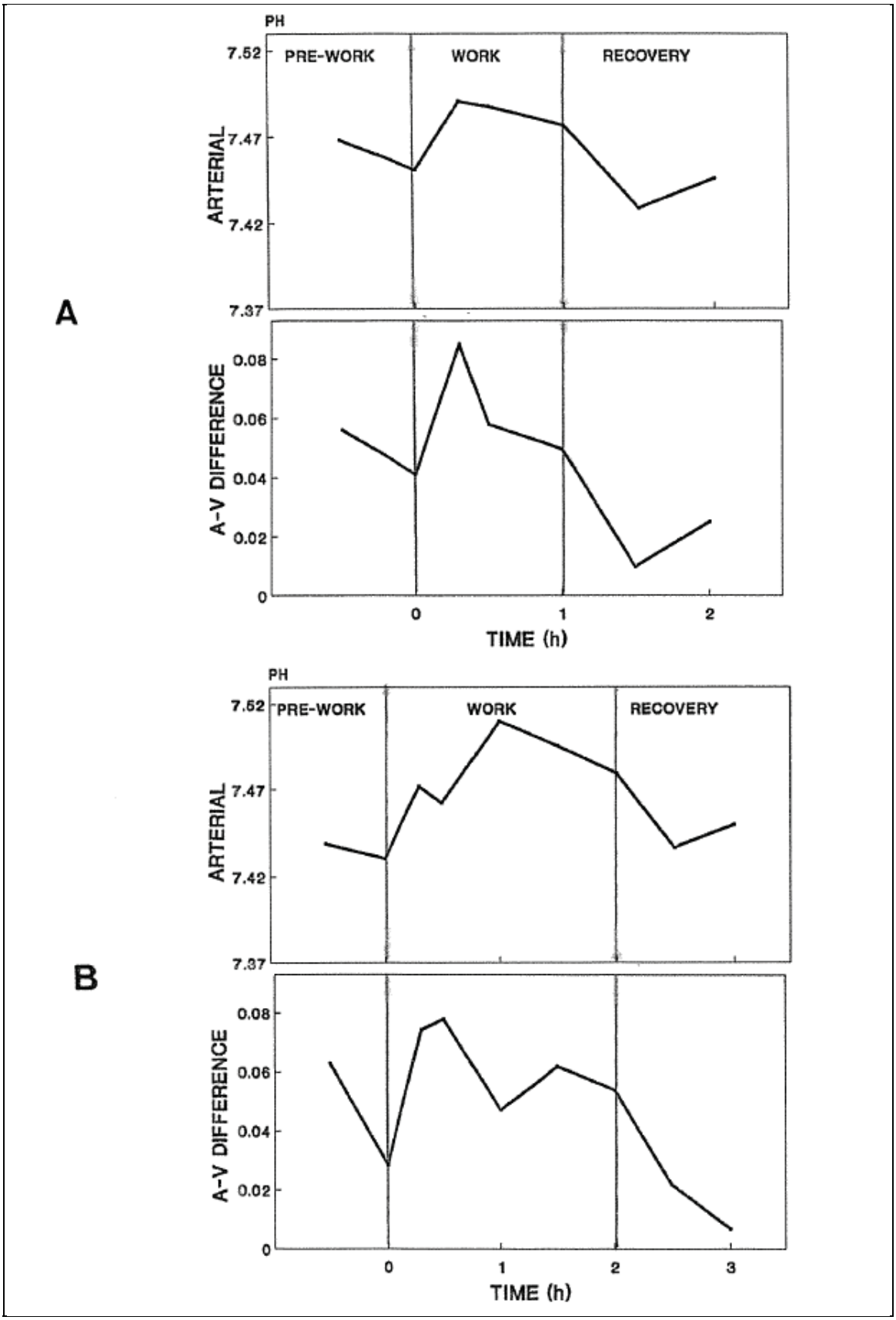


Figure 3C.71 The concentration of pH in arterial blood and the arterio-venous (A-V) concentration difference in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) at the medium walking speed.

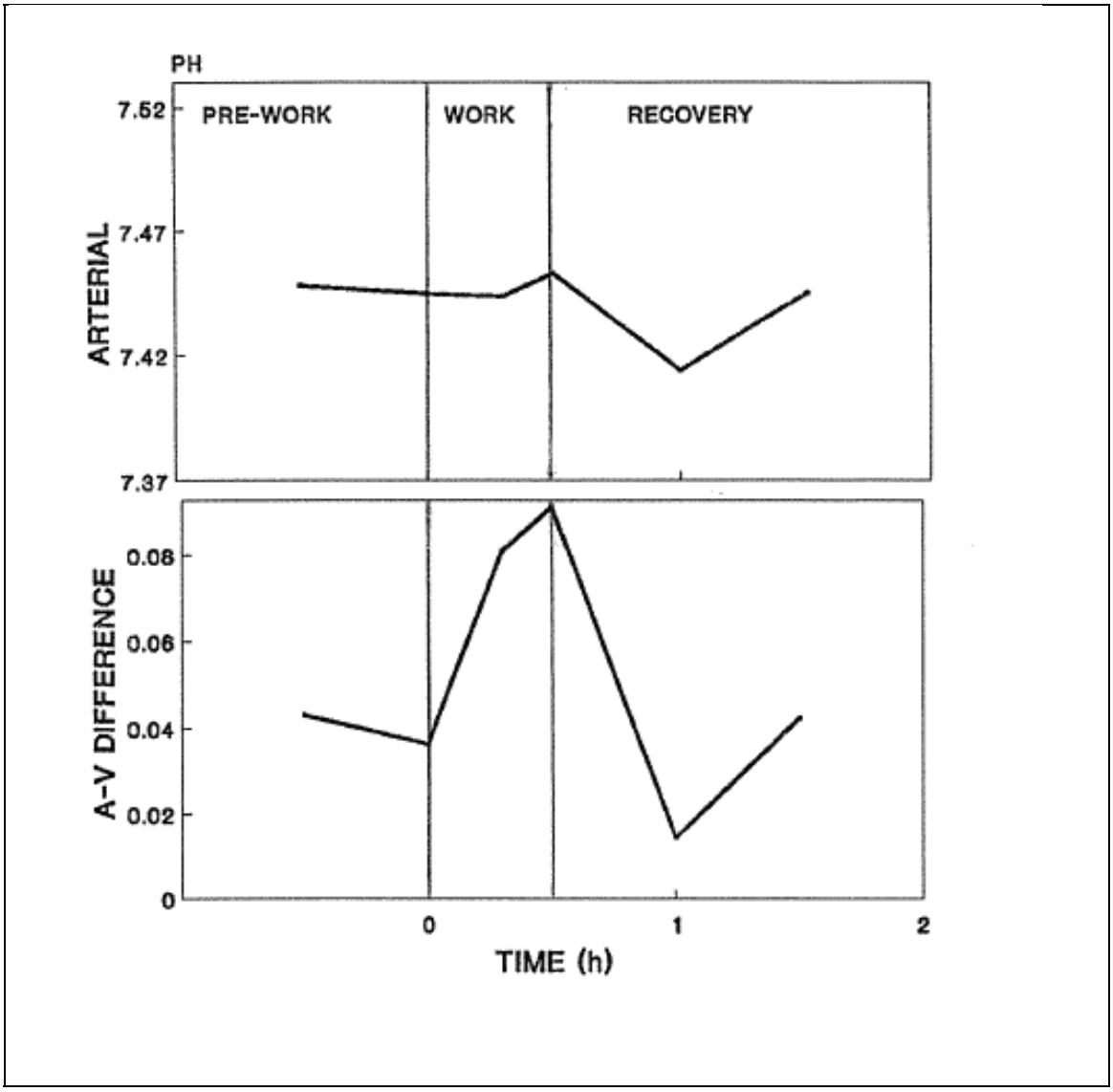


Figure 3C.72 The concentration of pH in arterial blood and the arterio-venous (A-V) concentration difference in trained sheep over time across work status (*Pre-work, Work* and *Recovery*) at the fast walking speed.

Oxygen saturation

Data for O_2SAT in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.73. Corresponding data for A-V differences of the parameter across the hind-limb muscle of the animals are presented in figure 3C.74.

Comparisons of mean arterial saturation and A-V differences of O_2SAT in sheep between physiological states, walking speeds and work status are presented in table 3C.22.

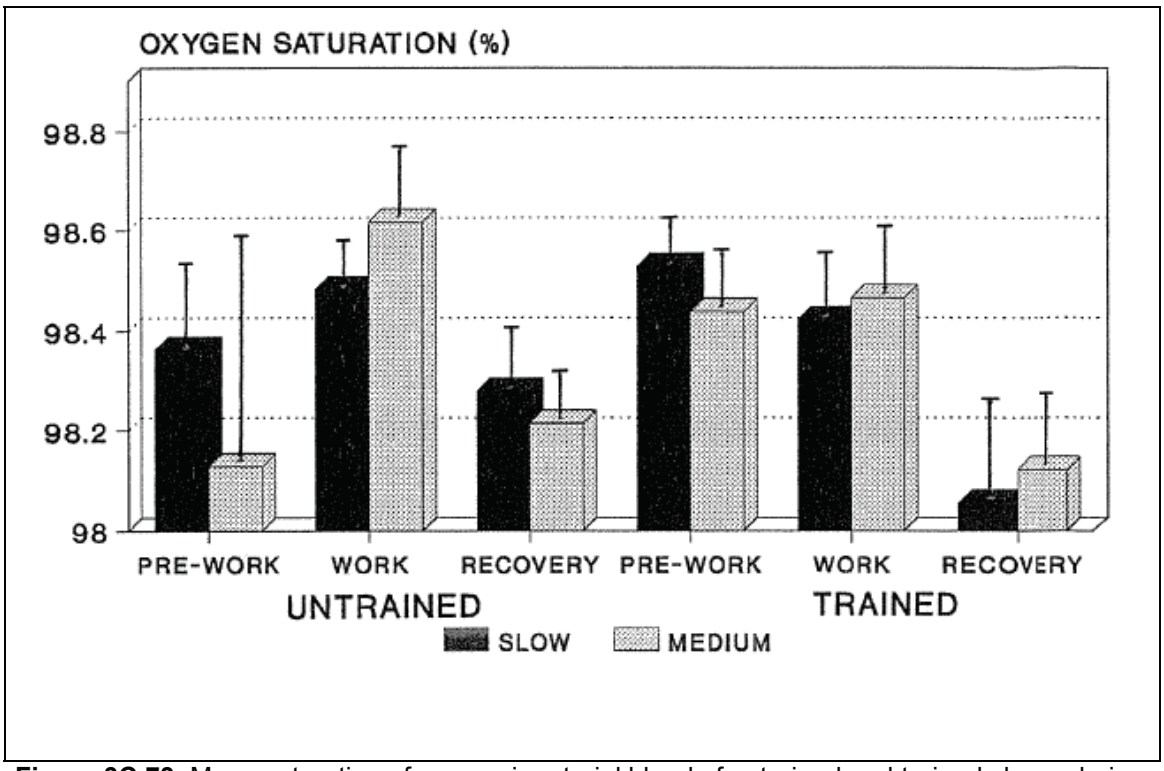


Figure 3C.73 Mean saturation of oxygen in arterial blood of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

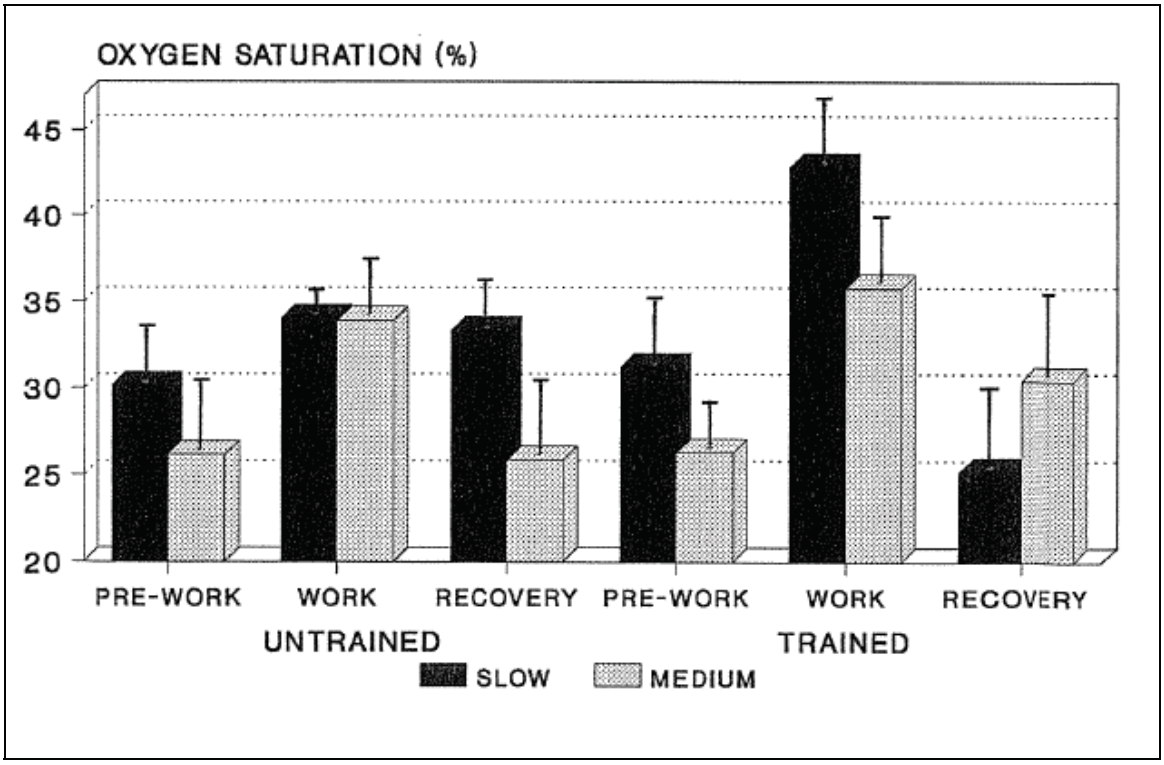


Figure 3C.74 Mean arterio-venous saturation difference of oxygen in blood of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical bars above lines represent standard errors.**

Table 3C.22 Comparison of arterial concentrations (A) and arterio-venous concentration differences (A-V) of oxygen saturation (O_2SAT) in blood of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
O_2SAT							
A: Mean	98.37	98.33	98.34	98.36	98.37	98.50	98.18
± SE	0.09	0.06	0.13	0.18	0.15	0.08	0.10
Significance	P=0.7758		P=0.8517		P=0.0265		
A–V: Mean	31.35	32.48	33.08	30.47	28.45	36.65	29.23
± SE	1.27	1.63	3.26	3.75	2.36	2.07	2.64
Significance	P=0.6818		P=0.3422		P=0.0137		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Physiological state and walking speed had no significant effect on O_2SAT in arterial blood (table 3C.22). In trained animals O_2SAT was lower than in untrained animals and this was mainly due to the low values in the *Pre-work* period of the trained sheep (figure 3C.73). Oxygen saturation was higher at medium walking speed as compared to slow walking speed and this was mainly due to the *Work* period (figure 3C.73). Oxygen saturation was significantly affected by work status (table 3C.22). The O_2SAT increased during the *Work* period (table 3C.22) with the exception of the trained animals at slow speed (figure 3C.73). There was a decrease in O_2SAT during the *Recovery* period (figure 3C.73).

Arterio-venous difference of O_2SAT across the hind-limb muscle was not significantly affected by physiological states or walking speeds (table 3C.22). Values indicate a net uptake across work status which increased during the *Work* period especially in the trained sheep (figure 3C.74).

Data on O_2SAT in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.75. Corresponding data for A-V saturation differences of oxygen across the hind-limb muscle of the animals are presented in figure 3C.76.

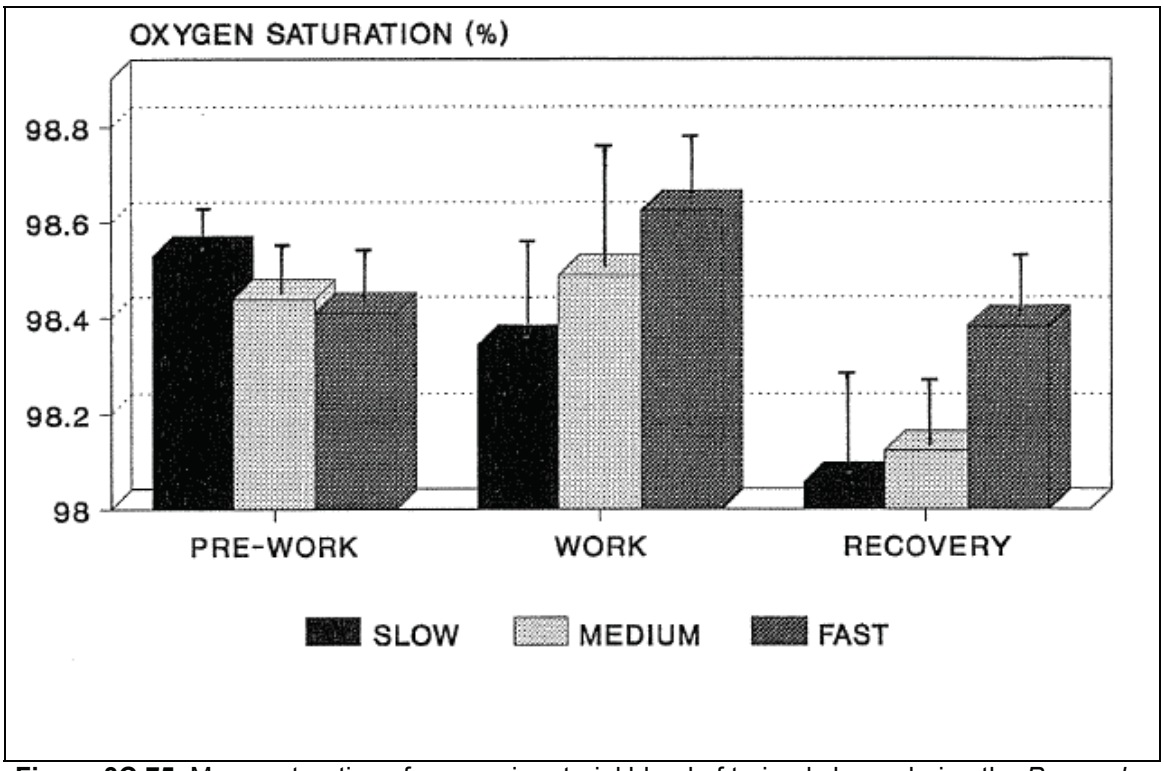


Figure 3C.75 Mean saturation of oxygen in arterial blood of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* period and at slow, medium and fast walking speeds. Vertical lines above bars represent standard errors.

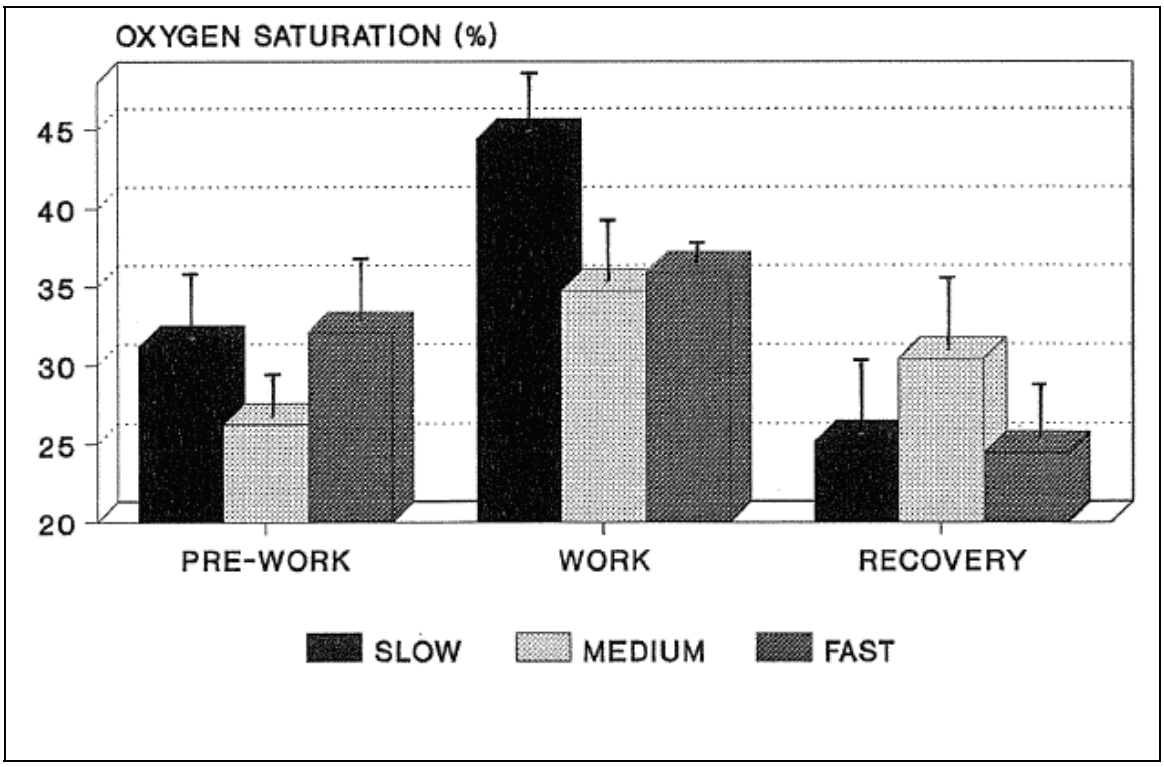


Figure 3C.76 Mean arterio-venous saturation difference of oxygen in blood of trained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed did not significantly affect O_2SAT in arterial blood (P=0.8195). Work status had a significant effect (P=0.0198) on O_2SAT. There was a trend for O_2SAT to increase with increasing walking speed during the *Work* period (figure 3C.74). Oxygen saturation

increased during the *Work* period at the medium and fast walking speeds as compared to a decrease at the slow walking speed (figure 3C.75).

The net uptake of O_2 across the hind-limb was not significantly affected by walking speeds (P=0.8903), however, work status had a significant effect (P=0.0041). The net uptake of O_2 increased during the *Work* period and decreased in the *Recovery* period (figure 3C.76).

The changes in the pattern of O_2SAT in arterial blood and the A-V saturation differences in untrained and trained sheep over time across work status and walking speeds are presented in figures 3C.85 to 3C.87.

Total carbon dioxide

Data for TCO_2 concentration in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.77. Corresponding data for A-V concentration differences of TCO_2 across the hind-limb muscle of the animals are presented in figure 3C.78.

Comparisons of mean arterial concentration and A-V concentration differences in sheep between physiological states, walking speeds and work status are presented in table 3C.23.

The TCO_2 concentration in arterial blood was not significantly affected by physiological states or walking speed (table 3C.23). At slow walking speed the TCO_2 for untrained animals was lower as compared to sheep walking at the medium speed (figure 3C.77). The opposite trend occurs in trained animals with TCO_2 concentration higher at slow walking speed compared to medium walking speed (figure 3C.77). Work status significantly affected the concentration of TCO_2 in arterial blood (table 3C.23). There was a decrease in the concentration of TCO_2 during the *Work* period and an increase during the *Recovery* period (figure 3C.77).

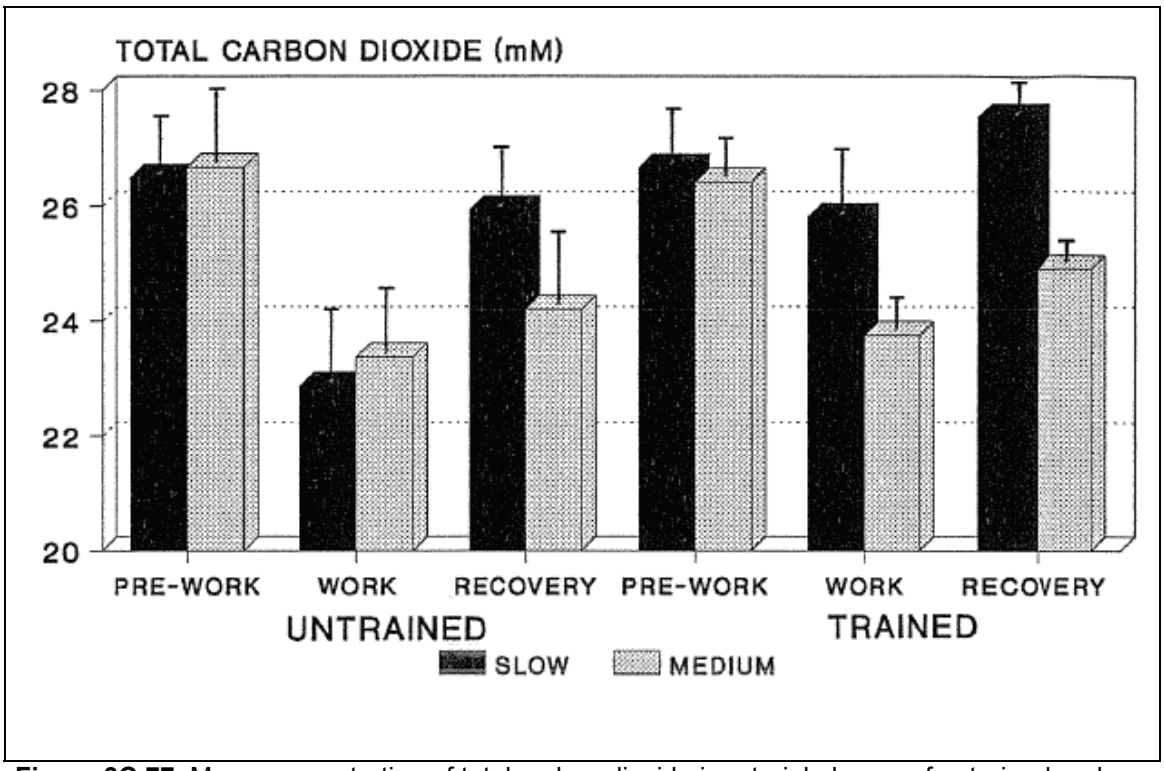


Figure 3C.77 Mean concentration of total carbon dioxide in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

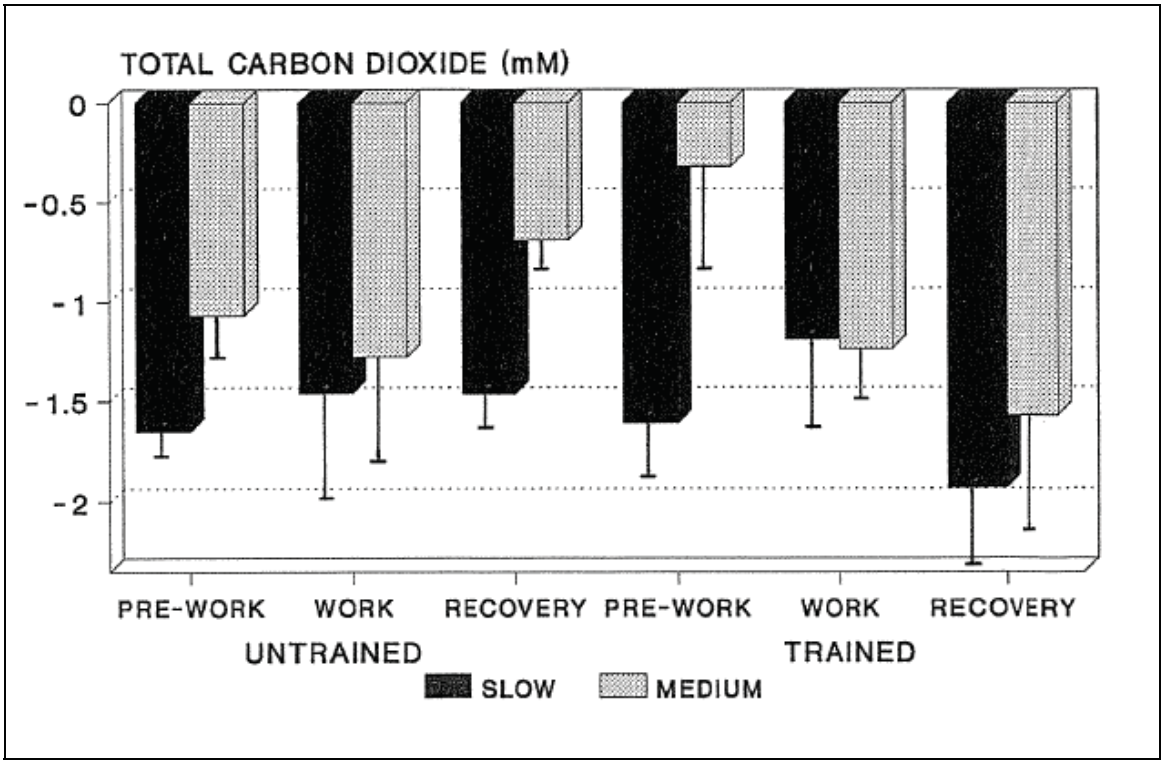


Figure 3C.78 Mean arterio-venous concentration difference of total carbon dioxide in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Table 3C.23 Comparison of arterial concentrations (A) and arterio-venous concentration differences (A-V) of total carbon dioxide (TCO_2) in blood of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
TCO_2							
A: Mean	24.82	25.81	25.80	24.69	26.56	23.94	25.87
± SE	0.45	0.33	1.02	0.87	0.63	0.67	0.68
Significance	P=0.1562		P=0.1133		P=0.0029		
A–V: Mean	-1.31	-1.34	-1.54	-1.06	-1.16	-1.29	-1.47
± SE	0.12	0.22	0.41	0.40	0.25	0.30	0.34
Significance	P=0.9281		P=0.1645		P=0.7860		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

All treatments, *viz* physiological states, walking speeds and work status had no significant effect on the net movement of TCO_2 concentration across the hind-limb muscles (table 3C.23). Values indicate a net output from the ind-limb muscles across work status. There is a trend of increasing net output during the *Work* period at the medium speed as compared to a decreasing net output at the slow walking speed (figure 3C.78).

Data on TCO_2 concentration in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.79. Corresponding data for A-V concentration differences across the hind-limb muscle of the animals are presented in figure 3C.80.

Walking speed had no significant effect (P=0.1204) on TCO_2 concentration in arterial blood, however, the concentration was affected by work status (P=0.0124). The concentration of TCO_2 in arterial blood decreased during the *Work* period and increased during the *Recovery* period (figure 3C.79). There was a trend for decreasing in TCO_2 concentration during *Work* with increasing walking speed (figure 3C.79).

The net movement of TCO_2 across the hind-limb muscle was not significantly affected by walking speed (P=0.7707) or work status (P=0.6858; figure 3C.80). There was a trend for TCO_2 output to increase with increasing walking speed during the *Work* period (figure 3C.80).

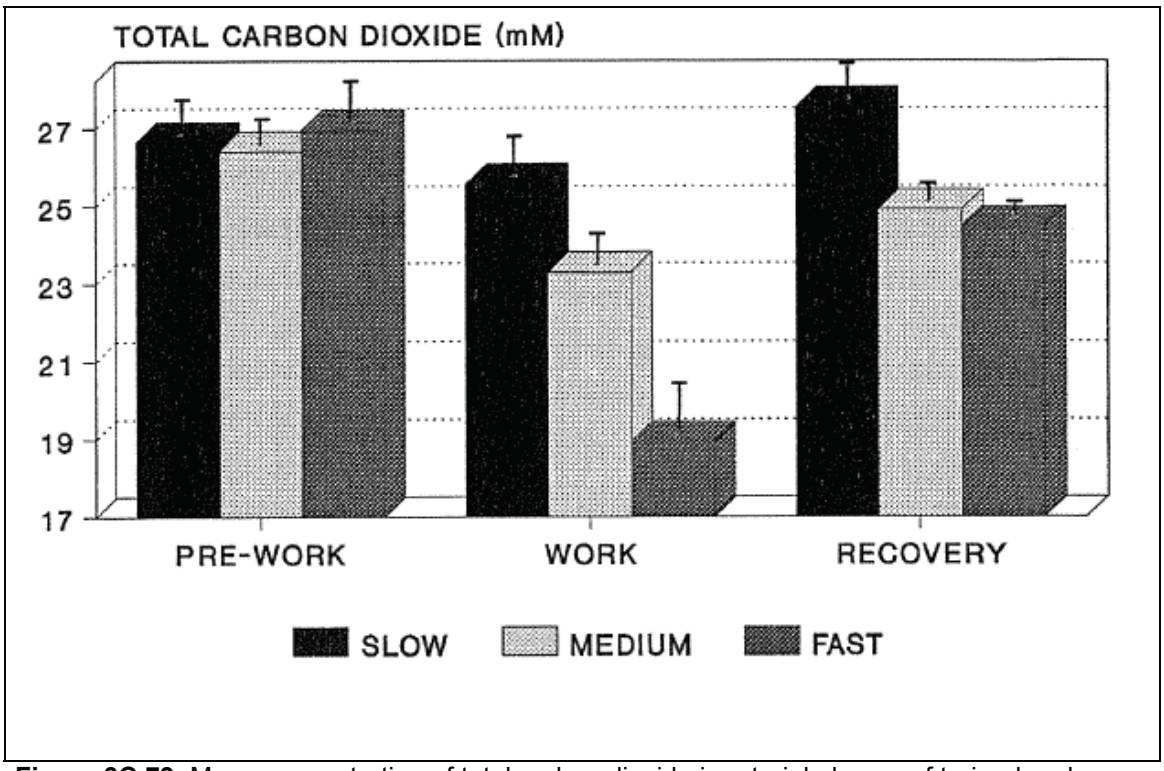


Figure 3C.79 Mean concentration of total carbon dioxide in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

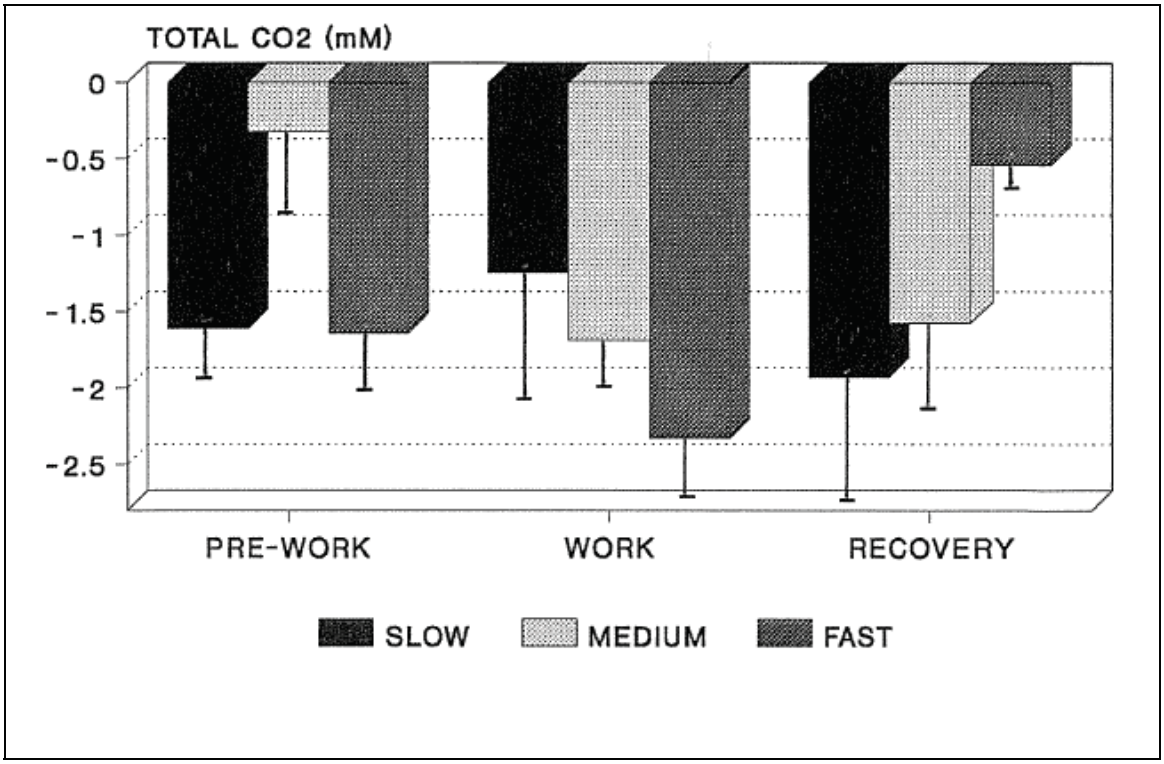


Figure 3C.80 Mean arterio-venous concentration difference of total carbon dioxide in plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at slow, medium and fast walking speeds. Vertical bars above lines represent standard errors.

The changes in the pattern of TCO_2 concentration in arterial blood and A-V concentration differences in untrained and trained sheep over work time across work status and walking speeds are presented in figures 3C.85 to 3C.87.

Bicarbonate

Data for HCO_3 concentrations in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.81. Corresponding data for A-V concentration differences of HCO_3 across the hind-limb muscle of the animals are presented in figure 3C.82.

Comparisons of mean arterial concentration and A-V concentration differences in sheep between physiological states, walking speeds and work status are presented in table 3C.24.

Table 3C.24 Comparison of arterial concentrations (A) and arterio-venous concentration differences (A-V) of bicarbonate in blood of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
Bicarbonate (mM)							
A: Mean	23.77	24.73	24.71	23.65	35.40	22.96	24.78
± SE	1.02	0.84	0.44	0.32	0.62	0.65	0.67
Significance	P=0.1537		P=0.1188		P=0.0045		
A–V: Mean	-1.14	-1.16	-1.35	-0.91	-1.01	-1.09	-1.32
± SE	0.38	0.39	0.12	0.20	0.23	0.29	0.33
Significance	P=0.9422		P=0.1719		P=0.7419		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

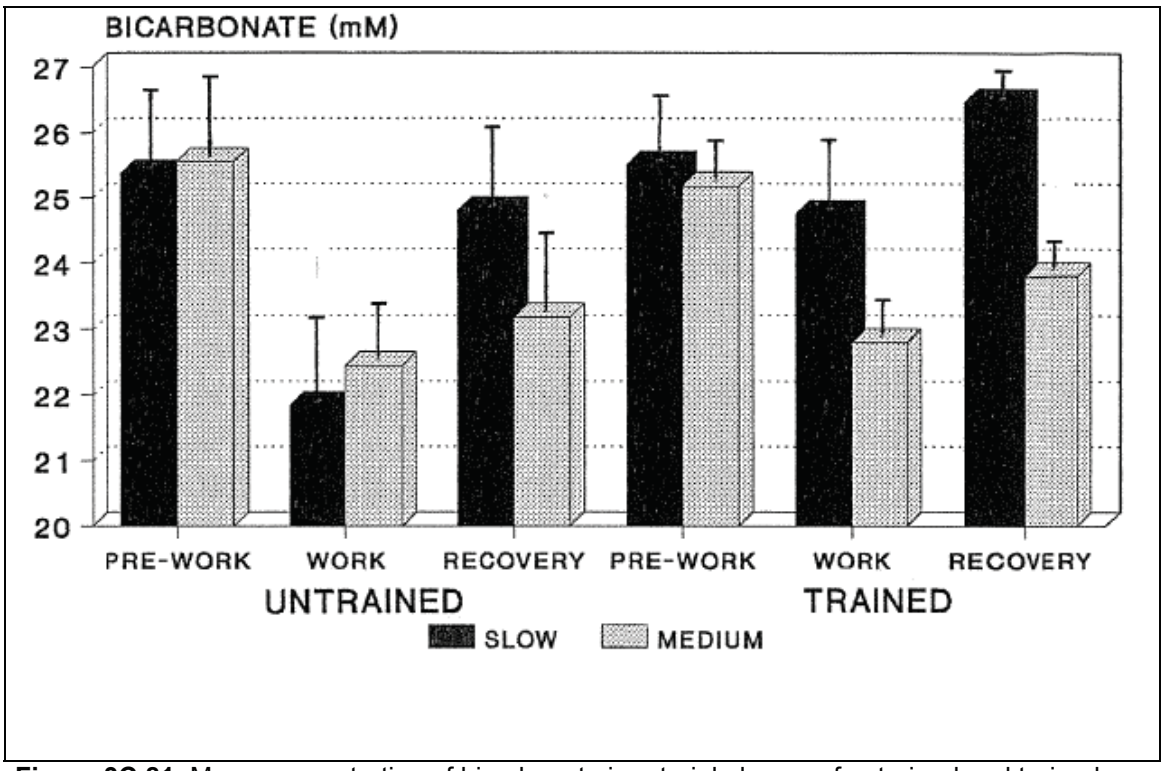


Figure 3C.81 Mean concentration of bicarbonate in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

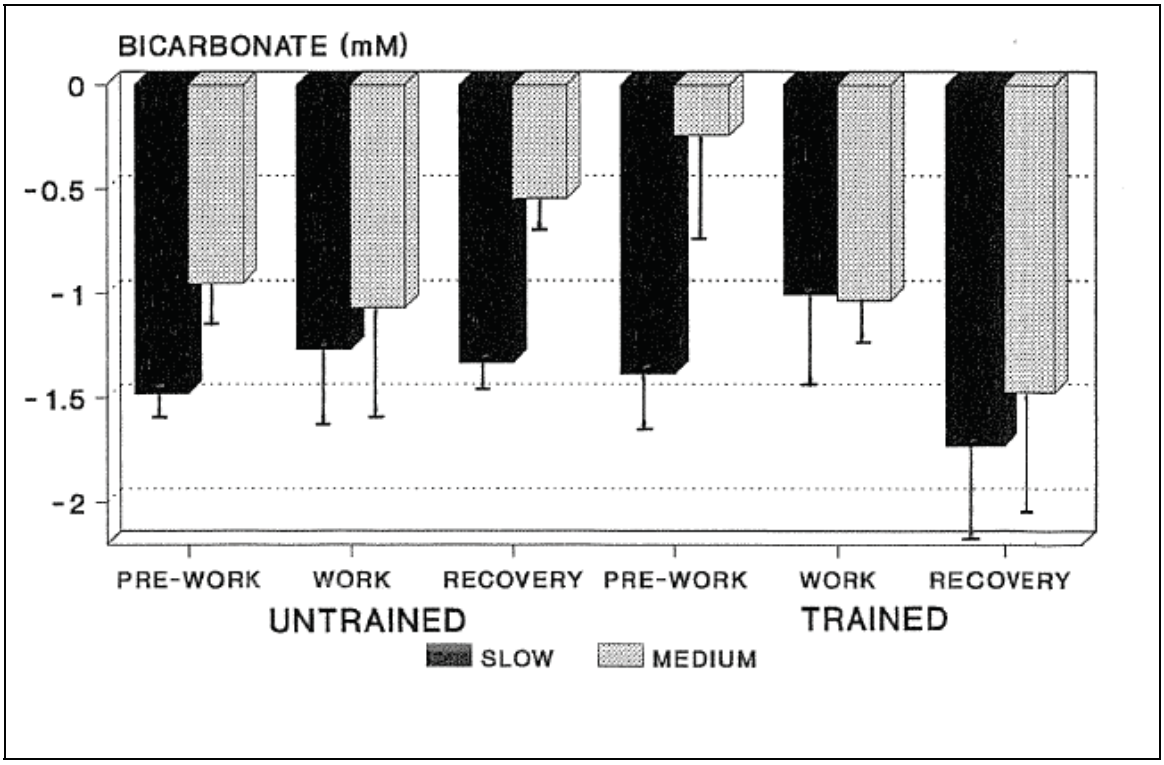


Figure 3C.82 Mean arterio-venous concentration difference of bicarbonate in plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Physiological states and walking speed had no significant effect on HCO_3 concentration in arterial blood (table 3C.24). In trained animals HCO_3 concentration was higher compared to untrained animals, however, this difference was due to the higher concentration in the *Work* period. The HCO_3 concentration was significantly affected by work status (table 3C.24). The concentrations in arterial blood decreased during the *Work* period and increased to remain below *Pre-work* values in the *Recovery* period. The exception was in the trained animals at slow speed (figure 3C.81).

All treatments, *viz* physiological states, walking speed and work status had no significant effects on the net movement of HCO_3 across the hind-limb muscle (table 3C.24). Values indicate a net output across work status (figure 3C.82). Net output decreased during the *Work* period in sheep walking at the slow speed and increased during the *Work* period in sheep walking at the medium speed (figure 3C.82).

Data on HCO_3 concentration in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.83. Corresponding data for A-V concentration differences across the hind-limb muscle of the animals are presented in figure 3C.84.

Walking speed had no significant effect (P=0.1103) on the arterial concentration of HCO_3, however, work status did have a significant effect (P=0.0155). The concentration of HCO_3 decreased during the *Work* period and there was a trend of decreasing concentration with increasing speed during this period.

The net output of HCO_3 across the hind-limb muscle was not significantly affected by walking speed (P=0.7936) or work status (P=0.7358). The HCO_3 output across the hind-limb muscle decreased during the *Work* period, in sheep walking at the slow speed, compared to sheep walking at medium and fast speeds where the HCO_3 output increased (figure 3C.84).

The changes in the pattern of HCO_3 concentration and A-V concentration differences in untrained and trained sheep over work time across work status and walking speeds are presented in figures 3C.85 to 3C.87.

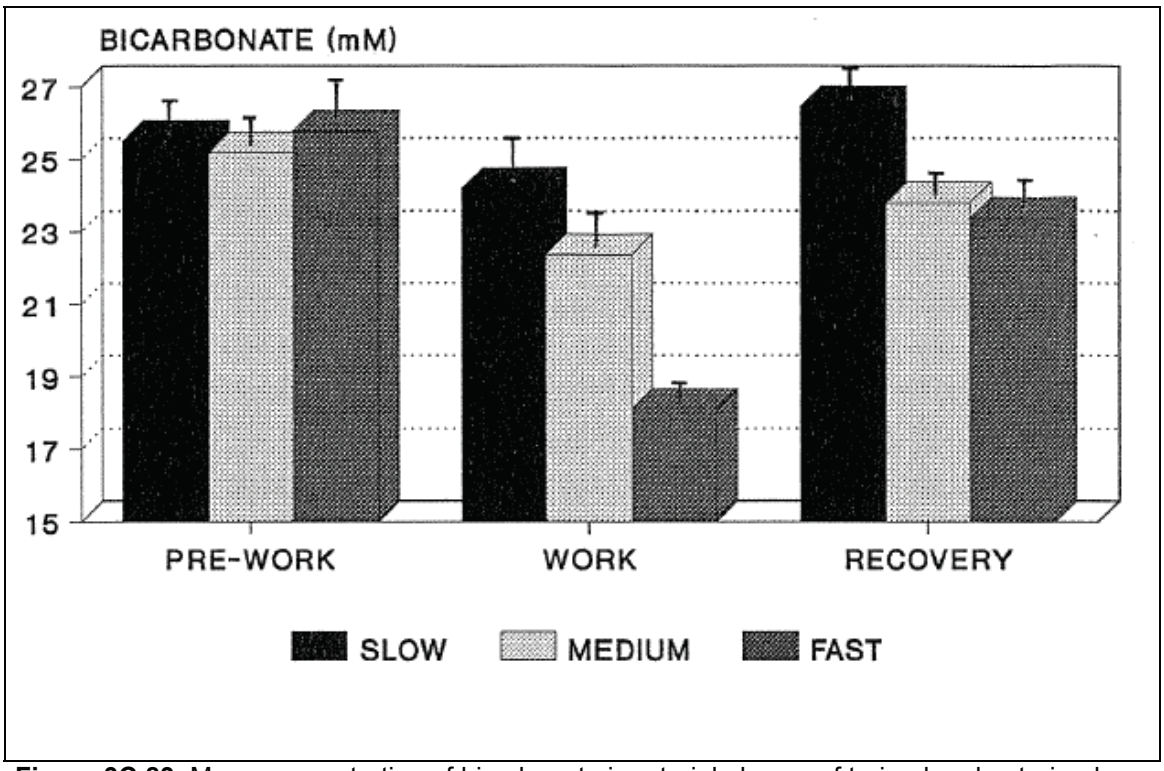


Figure 3C.83 Mean concentration of bicarbonate in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

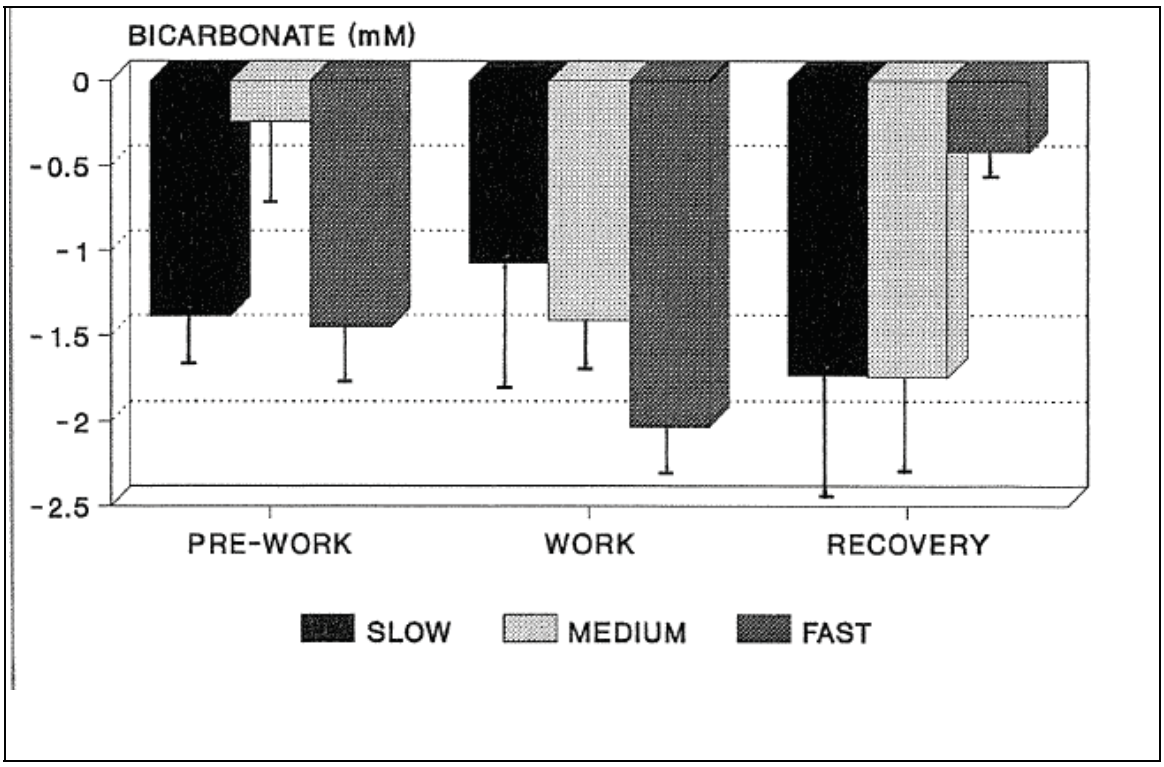


Figure 3C.84 Mean arterio-venous concentration difference of bicarbonate in plasma of trained and untrained sheep during the *Pre-work, Work* and *Recovery* periods and at slow, medium and fast walking speeds. Vertical bars above lines represent standard errors.

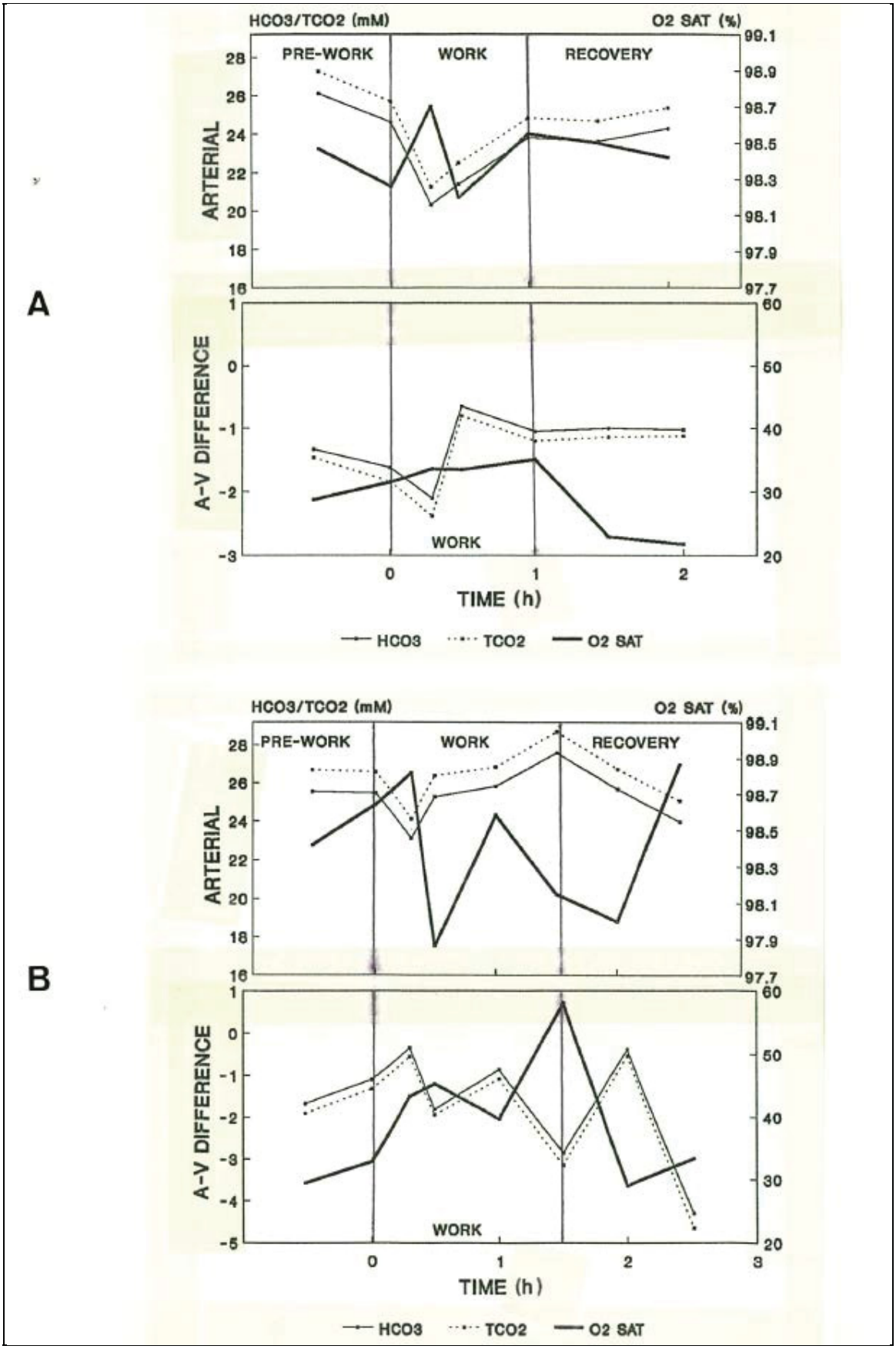


Figure 3C.85 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of oxygen saturation (O_2SAT), total carbon dioxide (TCO_3) and bicarbonate (HCO_3) in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work and Recovery*) at the slow walking speed.

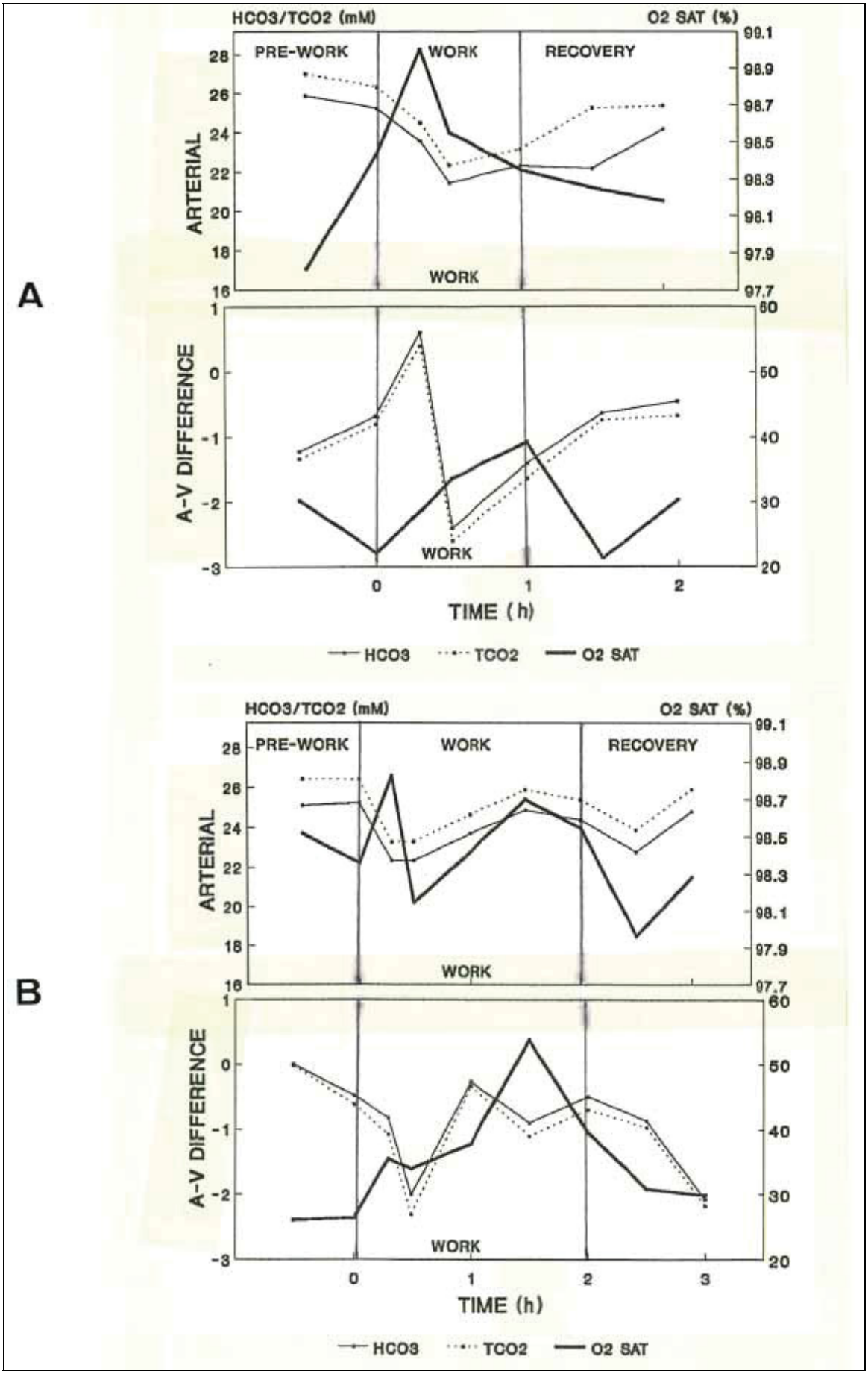


Figure 3C.86 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of oxygen saturation (O_2SAT), total carbon dioxide (TCO_3) and bicarbonate (HCO_3) in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) at the medium walking speed.

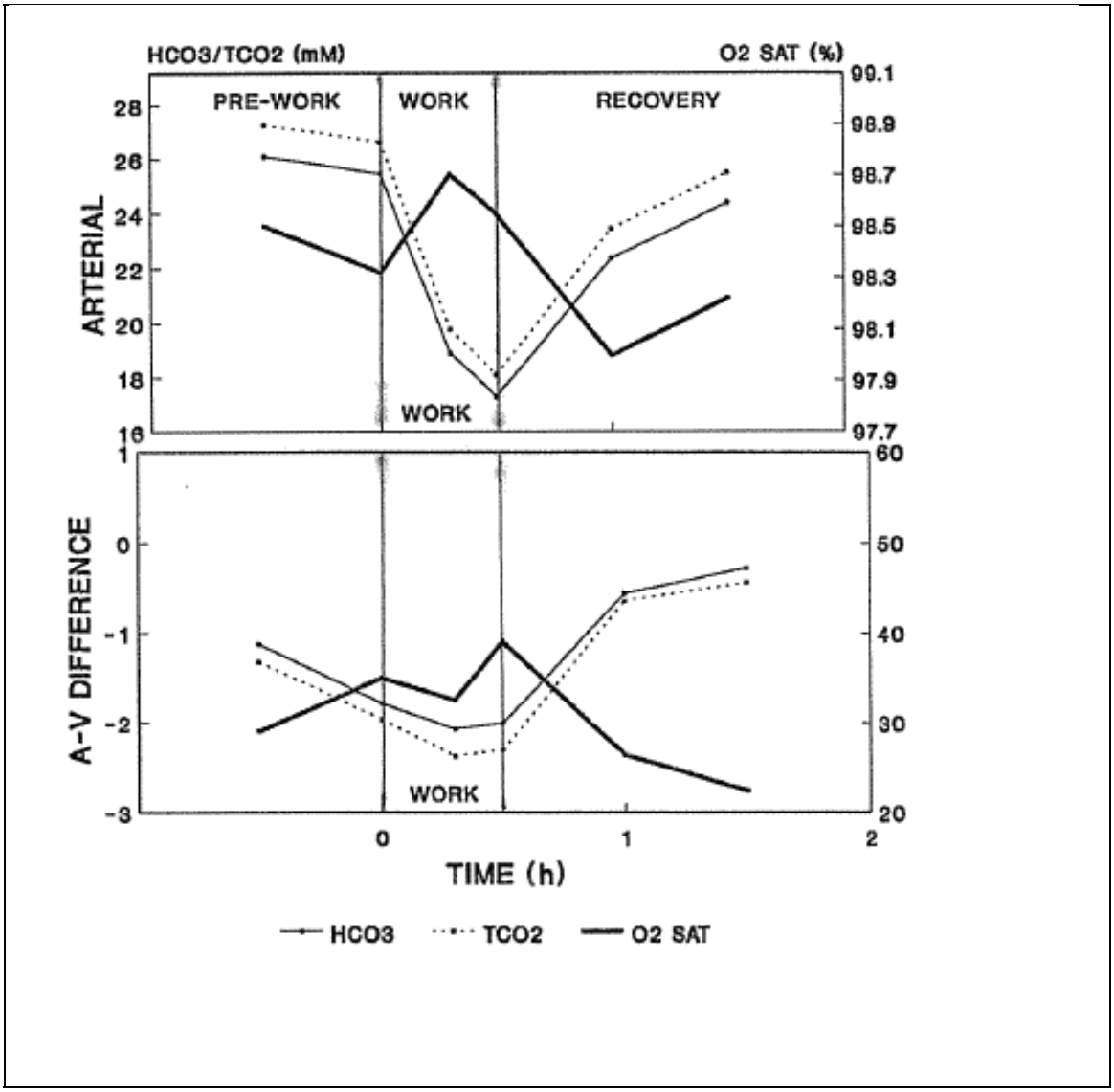


Figure 3C.87 The concentration in arterial plasma and the arterio-venous (A-V) concentration difference of oxygen saturation (O_2SAT), total carbon dioxide (TCO_3) and bicarbonate (HCO_3) in trained sheep over time across work status (*Pre-work, Work* and *Recovery*) at the fast walking speed.

Partial pressure of carbon dioxide

Data on pCO_2 in arterial blood of untrained and trained sheep across work status and at slow and medium walking speeds are presented in figure 3C.88.

Comparison of mean pCO_2 in sheep between physiological sates, walking speeds and work status are presented in table 3C.25.

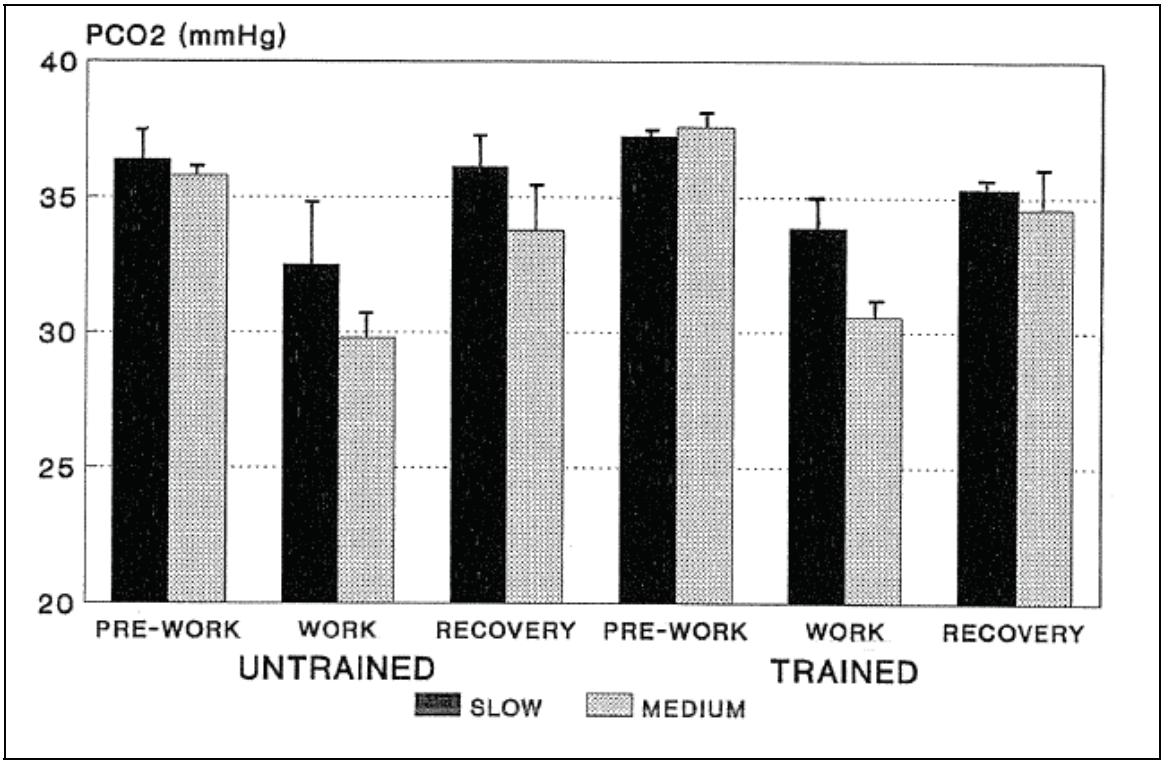


Figure 3C.88 Mean concentration of partial pressure of carbon dioxide (pCO_2) in arterial plasma of untrained and trained sheep during the *Pre-work, Work* (1 hour) and *Recovery* periods and at the slow and medium walking speeds. **Vertical lines above bars represent standard errors.**

Table 3C.25 Comparison of arterial partial pressure of carbon dioxide (pCO_2) in blood of sheep, between physiological states, walking speeds and work status
Values are means ± standard errors (SE).

	Treatments						
	Physiological states[1]		Walking speeds		Work status[2]		
	UNT	T	Slow	Medium	Pre-W	W	REC
pCO_2 (mm Hg)							
A: Mean	33.95	34.57	35.10	33.22	36.76	31.67	35.25
± SE	0.78	0.69	1.02	0.90	0.24	1.06	0.92
Significance	P=0.5415		P=0.0605		P=0.0000		

[1] UNT = untrained; T = trained
[2] Pre-W = pre-work period; W = work period; REC = recovery period

Physiological states had no significant effect on pCO_2 (table 3C.25). The differences between walking speeds approaches significance (P=0.0605). The pCO_2 was higher at the slow speed as compared to the medium walking speed (table 3C.25). Work status significantly affected pCO_2 (table 3C.25). The pCO_2 in arterial blood decreased during the

Work period and increased to remain below *Pre-work* values in the *Recovery* period (table 3C.25).

Data on pCO_2 in arterial blood of trained sheep at three different work status and walking speeds are presented in figure 3C.89.

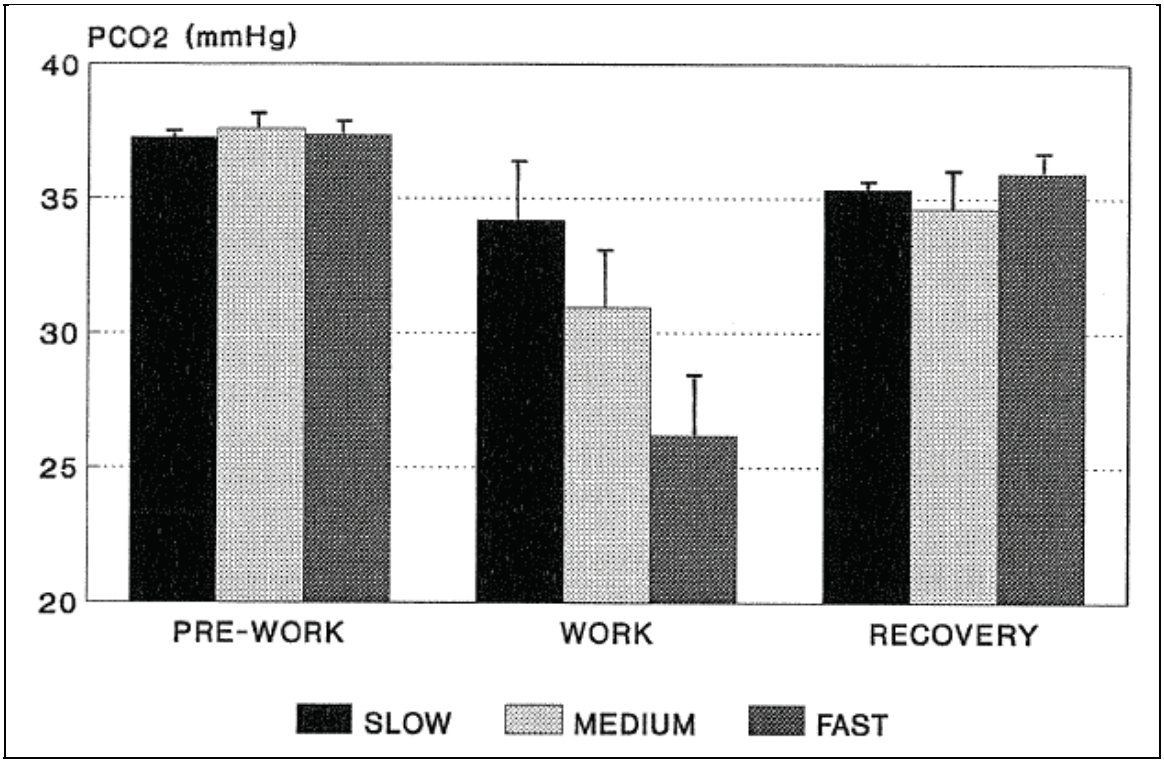


Figure 3C.89 Mean concentrations of partial pressure of carbon dioxide (pCO_2) in arterial plasma of trained and untrained sheep during the *Pre-work, Work* (30 minutes) and *Recovery* periods and at the slow, medium and fast walking speeds. **Vertical lines above bars represent standard errors.**

Walking speed had no significant effect (P=0.2898) on pCO_2, however, work status affected pCO_2 (P=0.0004). The pCO_2 decreased during the *Work* period across all walking speeds with a trend of decreased pCO_2 with increasing walking speed.

The pattern of changes of pCO_2 in arterial blood in untrained and trained sheep over time across work status and walking speeds are presented in figure 3C.90.

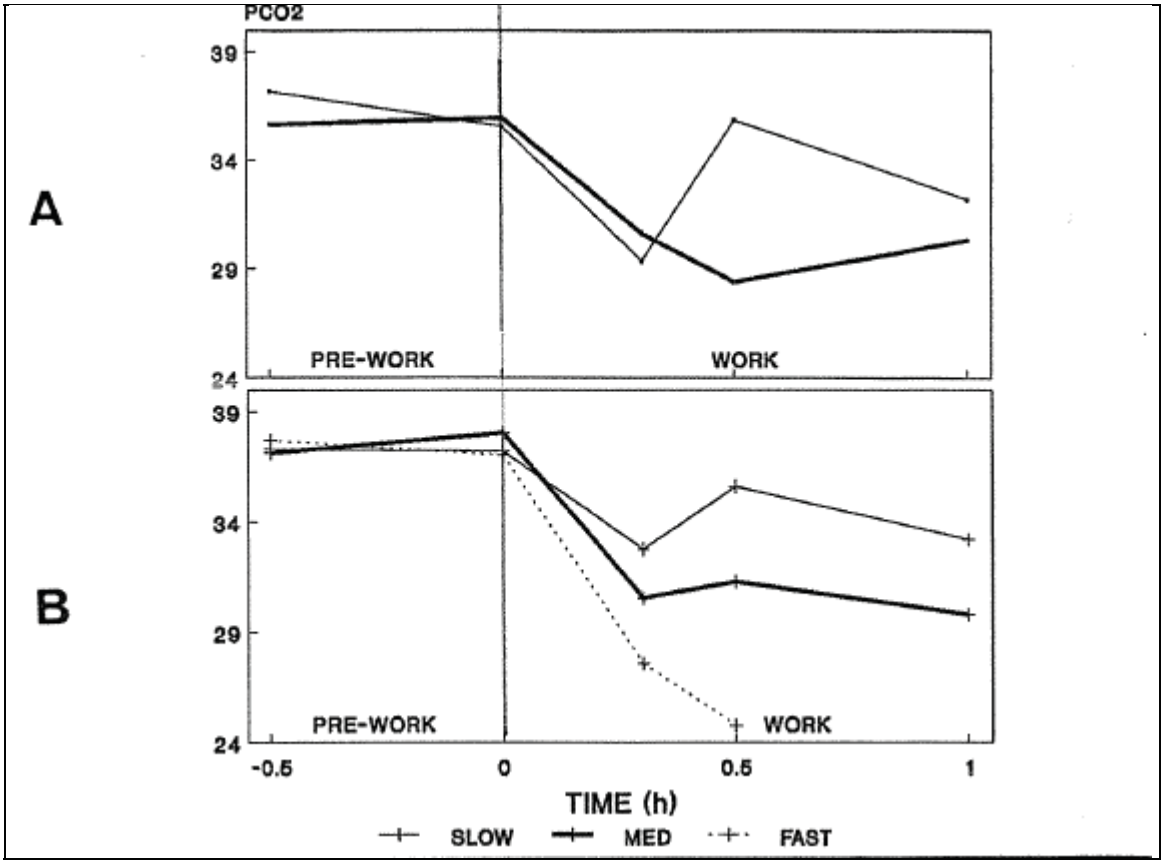


Figure 3C.90 Changes in partial pressure of carbon dioxide (pCO_2) in arterial blood in untrained (A) and trained (B) sheep over time across work status (*Pre-work, Work* and *Recovery*) periods at slow, medium and fast walking speeds.

3C.4 Discussion

Work capacity

In this experiment it is clear that training enhanced work capacity; the duration of work by sheep being increased by 54, 139 and 369% for slow (0.665 m/sec), medium (1.036 m/sec) and fast (1.384 m/sec) speeds respectively. The advantage of training was most clearly demonstrated at a higher level of work.

The EE of the working sheep in this experiment was calculated using the coefficient for efficiency for doing mechanical work derived for buffalo by Lawrence and Stibbards (1985). Sheep expend much more energy for walking (2.5 J/kg/m; Clapperton 1964) than do Brahman cattle/buffalo (2.09 J/kg/m; Lawrence 1985) and cattle (2 J/kg/m; ARC 1980). A coefficient for doing work by sheep/goats/dogs could not be found in the literature but it might be expected to be higher than that for Brahman cattle/buffalo. The EE values calculated for sheep in this experiment are higher than the value of 1.67x maintenance energy, determined for oxen by Lawrence (1985) but were only slightly greater than values (2.3–2.7x

maintenance) reported by Teleni et al. (1991) for buffalo walking at the same slow speed (0.69 m/sec) on a treadmill.

Acid-base balance

Mild respiratory alkalosis occurred in walking speeds across both physiological states except that with the untrained animal at slow speed there was a slight decrease in blood pH.

Respiratory alkalosis is indicated by increases in circulating blood pH and decreases in pCO_2 during work. Although the pH of the blood leaving the hind-limb muscle was lower than that entering (table 3C.21) hyperventilation and excessive elimination of CO_2 resulting in a reduction in H_2CO_3 (reflected by reduced pCO_2) caused the wholebody blood pH to increase. There was metabolic alkalosis due to the fact that HCO_3^- levels did not increase significantly above *Pre-work* values during the *Work* period. Respiratory alkalosis was also observed in exercising sheep by Pethick et al. (1991).

Respiratory alkalosis occurred in the second phase of respiration where there was slower deeper breathing after an initial stage of rapid shallow breaths. An example of this is shown for sheep #193 in figure 3C.91 where RR towards the end of work sharply decreased although PR and RT were still increasing.

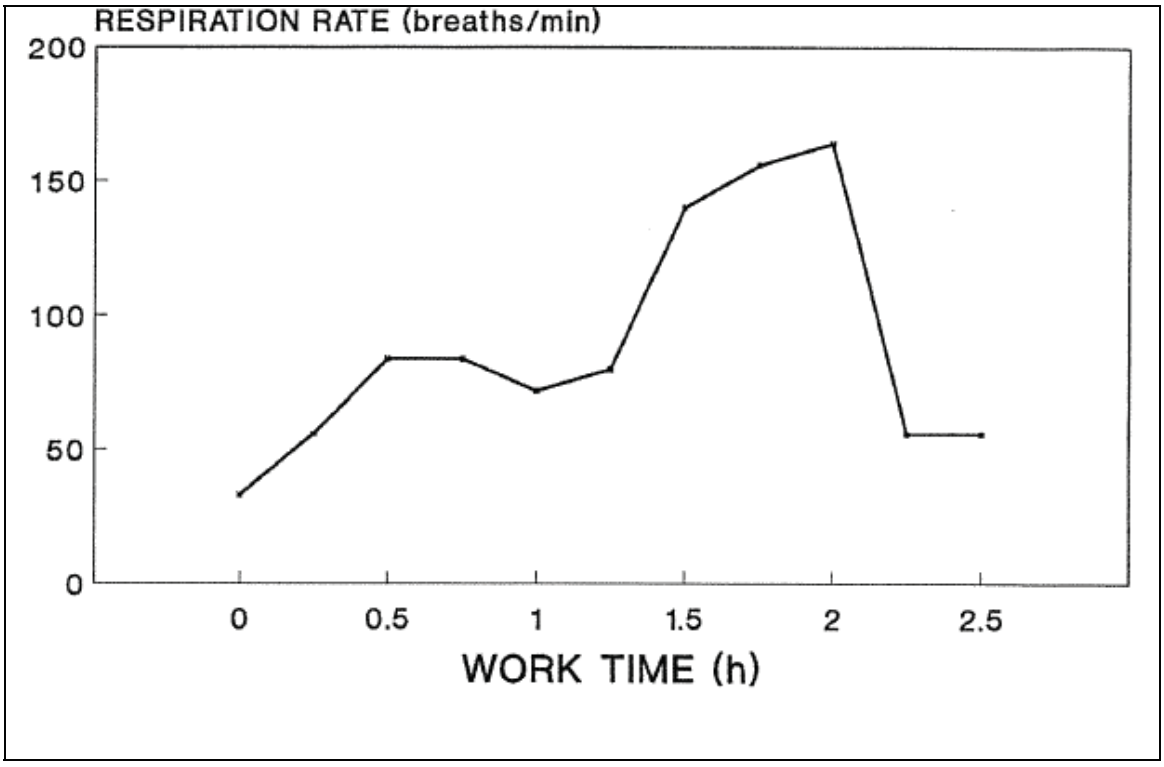


Figure 3C.91 Changes in respiration rate (breaths/min) during work for trained sheep #193 walking at the slow speed.

Although lactate in the circulation increased in concentration during the *Work* period, the rate of increase was not large enough to effect a reduction in blood pH. The efficient buffering systems of the body were able to cope with the increases in lactic acid production.

Substrate utilisation

The arterial concentrations of glucose, FFA, lactate and N increased during the *Work* period (see figures 3C.32–34; 3C.42 and 3C.49). This is in agreement with results of other studies using sheep (Biswas et al. 1991; Pethick et al. 1991). The pattern of utilisation of these substrates as fuels for contracting muscles varied with physiological states and speeds of walking (or workload).

The untrained sheep appeared initially to be utilising glycogen during work as there was an initial reduction in uptake of glucose and FFA by the hind-limb muscle. However, it was of interest that lactate was being taken up and probably oxidised by the hind-limb muscle during work. For sheep walking at medium speed, Pcr was most probably utilised in the first 30 minutes of work to regenerate ATP from ADP. This is consistent with the increased release of creatinine from the hind-limb muscle (see figure 3C.40) during the *Work* period. In the last 30 minutes of work for untrained sheep, the uptake of FFA by muscle increased [see figure 3C.32(A)–34(A)] indicating an increasing reliance on fat metabolism for ATP generation. However, lactate was also taken up by muscle during this period (see figure 3C.32–3C.34). The uptake of lactate might have been an indication that muscle glycogen was depleted.

In the trained sheep, there appeared to be no FFA utilisation by the hind-limb muscle during the *Work* period although there was a general increase in arterial concentration of FFA. Rennie et al. (1974) suggested that fat utilisation would be higher in trained than in untrained humans. They did not undertake an A-V concentration difference study of the working muscle of their subjects but the respiratory quotient of 1 which they recorded in the trained humans would indicate that carbohydrate was the predominant fuel during work.

In this experiment, glucose uptake by the hind-limb muscle increased during the *Work* period. In addition to this, lactate was taken up by muscles of the trained animal while there was little or no uptake of FFA. This might appear somewhat contrary to expectation (see e.g. Henriksson 1977) but it should be noted that comparisons between physiological states have been made for one hour of work only at the slow and medium speed of walking. It is probable that beyond the one hour of work the dominance of FFA as a fuel for the contracting muscle would become more evident.

In this study there was a tendency for arterial concentrations of FFA to be higher in the trained than in the untrained animal. Also, the RT during work was higher in the trained sheep; this being consistent with increased fat utilisation. The increased and higher O_2 uptake by muscles of the trained sheep, would suggest an enhanced oxidative capacity in these animals. The A-V concentration data would not show definitively any usage of FFA from the muscle fat depot by the contracting muscle. Such a usage is a possibility and would need to be quantified in future studies.

The arterial glucose concentrations (3.8–4.4 mM) in this experiment are higher than that reported by Biswas et al. (1991) for sheep pulling a cart (2.6 mM) and that reported by Pethick et al. (1991) for sheep walking up an incline of 9☜. Glucose and lactate concentrations in the artery were positively related (r=0.8864; see figure 3C.92) suggesting that anaerobic glycolysis in tissues of the working animal was an important metabolic process.

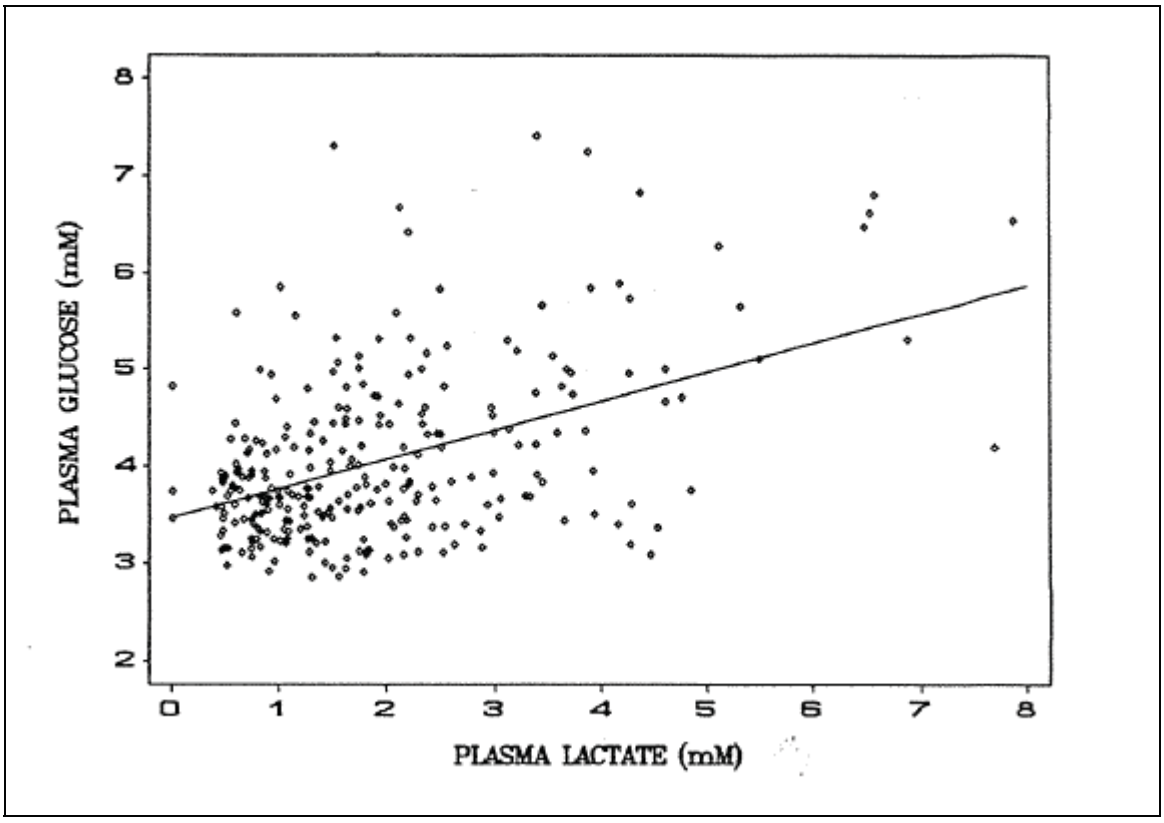


Figure 3C.92 The relationship between plasma lactate and glucose concentration (mM). **(y=5.39x + 62.60; r=0.89; P=0.0000)**

The uptake of protein/AA–N and NH_3–N by the working muscle would suggest that there was probably catabolism of AA in muscle during the *Work* period. The uptake of NH_3–N,

particularly is of interest as the metabolite might be utilised in the glutamine synthetase reaction to form glutamine in muscle (see Teleni 1993). This AA could then have a significant role in the neutralisation of acid urine during work.

Lactate

The observed general increases in lactate concentrations in arterial plasma of the trained and untrained sheep during the *Work* period, reflected the greater rate of production of the metabolite relative to its removal from the bloodstream. The lower concentrations of lactate in the circulation of trained sheep are probably due to the enhanced removal rates of the metabolite as a consequence of training. MacRae et al. (1992), Donovan and Brooks (1983) and Oyono-Enguelle et al. (1990) found this to be so in humans after endurance training. There are reports which suggest that lactic acid production is reduced in the trained individual (Holloszy & Booth 1976). In this experiment it is likely that the rate of production of lactate was not diminished (and glycogen not spared) as it appears that FFA was not significantly utilised by the hind-limb muscle of the trained animal.
Although the concentrations of circulating lactate increased during work, data on A-V concentration difference show clearly that the hind-limb muscles do not always product this metabolite and in fact do actually take it up, probably for use as a fuel. That lactate might be a significant energy-yielding substrate during exercise is also evident from results of studies by other workers (e.g. Stanley et al. 1986; Gladden 1989).

Since there was no net release of lactate from the hind-limb muscles, the increase in the concentration of circulating lactate must have been due to net releases of lactate from other tissues, e.g. the liver and respiratory muscles. Pethick et al. (1991) showed that the gut of sheep could release and take up lactic acid during exercise. Wasserman et al. (1973, 1987) reported that in dogs, the liver produced lactate at the onset of exercise and for an extended time during exercise. They concluded that extrahepatic sites can be completely responsible for the removal of lactate from the circulation during exercise. Davis et al. (1985) and Wasserman et al. (1987) suggested that the liver is segregated into periportal and perivenous sections enabling production of lactic acid in an effort to maintain substrate supply. Consistent with the results of this experiment, there is evidence from other studies (Stanley et al. 1986) which suggest that contracting skeletal muscles, not the liver, are major sites of lactic acid uptake from the circulation. During exercise, the proportion of total blood flowing to the hind-limb muscles is likely to be greater than that to the liver (Bell & Hales 1985). It would be reasonable to suggest therefore that the oxidation of lactic acid in working muscles would be the preferred pathway to oxidation or gluconeogenesis in the liver.

Welch and Stainsby (1967) showed that lactate was taken up by contracting skeletal muscle in the dog. Stanley et al. (1986) and Jorfeldt (1970) using radioactive lactate tracers in humans calculated that half the lactate produced was oxidised in contracting skeletal muscles. Similar conclusions were reached by Corsi et al. (1972) and Granata et al. (1976) who also used radioactive-labelled lactic acid.

The amount of lactic acid taken up by muscles is related to O_2 consumption in dogs (Issekutz et al. 1976), rats (Donovan & Brooks 1983) and humans (Stanley et al. 1986). In the current experiment, O_2 and lactate uptake were positively related (r=0.5104; P=0.0000). The pattern of lactate movement across the hind-limb muscles of sheep in this experiment during the first 20 minutes of work is in agreement with the reports by Welch and Stainsby (1967) and Flock et al. (1939).

It appears that the concentration of circulating lactate is a balance between release from and uptake by diverse tissues which themselves can change from producers to consumers and vice versa depending on conditions. This supports the theory of Brooks (1985) about a "lactate shuttle" in which lactic acid transported to the liver for gluconeogenesis is not from blood-bornw glucose from the diet (see section 2.3.4).

Using data from the current experiment it might be suggested that the OBLA in sheep walking on a treadmill and pulling a draught load equivalent to 11% of its live weight would occur at a walking speed of approximately 1.04 m/sec (figure 3C.94).

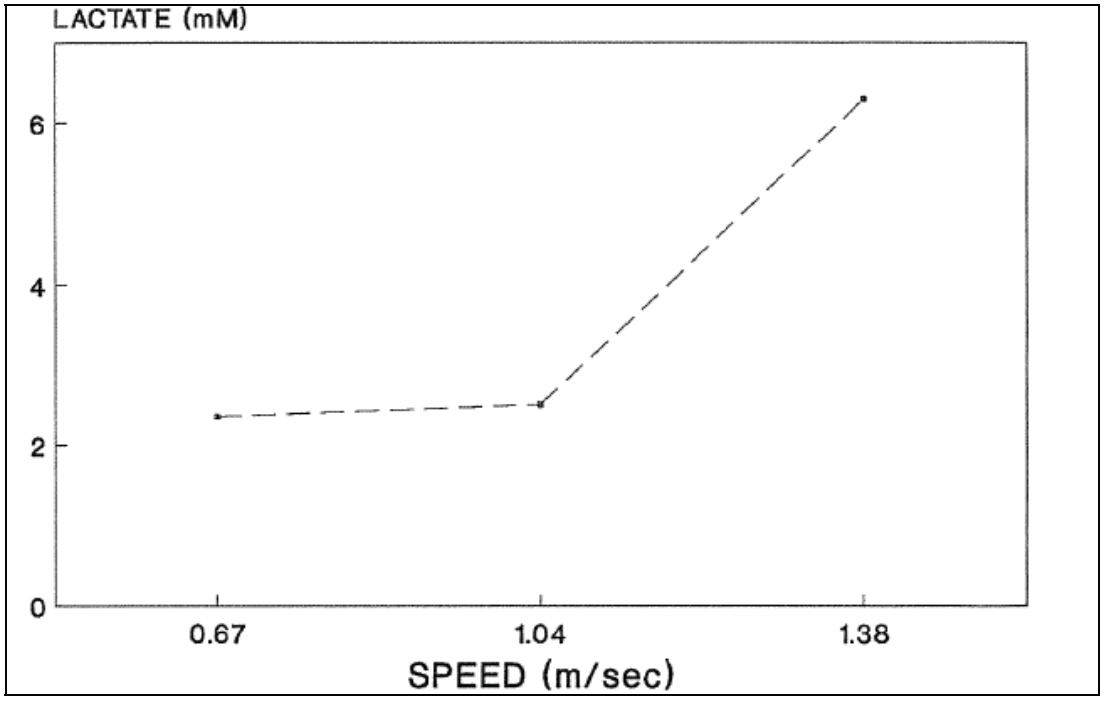


Figure 3C.93 The relationship between lactate concentration in arterial plasma and speed of walking during the first 30 minutes of the *Work* period.

Hyperthermia

As the heat load of the body (measured by RT) of the working animal increased, so also did the thermoregulatory responses in RR and PR. The relationship between these variables are shown in figures 3C.94 and 3C.95 using pooled data from the experiment. For each 1☜C increase in RT there was an increase of 62 breaths/minute in RR. Thus for the critical body temperature of 41☜C, the animal would be expected to be respiring at 218 breaths/minute; a rate which the animal would marginally be able to cope with.

It would appear that the more limiting factors to work capacity of the animals would be the earlier attainment of critical RT and/or RR resulting in a state of hyperthermia and the onset of fatigue. An association between increased body temperature and increased rate of glycolysis has been demonstrated by Kozlowski et al. (1985) in dogs. It is possible that the onset of fatigue in hyperthermia is due to the depletion of glycogen stores.

The increases in ST matched those of RT (r=0.9423). This relationship reflects the increased transfer of heat through blood to the skin for dissipation. Bell and Hales (1985) reported that in resting sheep during heat stress, bloodflow decreased from 50 to 34% to the hind limbs but increased by 1 and 3% to the skin and respiratory muscles respectively.

Cardiovascular system

Training increased the O_2-carrying capacity of the blood. This is evident in the higher concentration of haemoglobin observed in the blood of the trained sheep during the *Pre-work* and *Work* periods. Also there was increased O_2 uptake by the hind-limb muscles, of the same animals, indicating a higher oxidative capacity of the tissue.

Additional evidence of an improved cardiovascular system in the trained animals was their lower mean PR at rest compared to that of the untrained animals. This is in agreement with results reported by Rennie et al. (1974) and Henriksson (1977) for humans. Consistent with this was the more rapid decline in PR in the trained than in the untrained sheep after work ceased (see figure 3C.14)

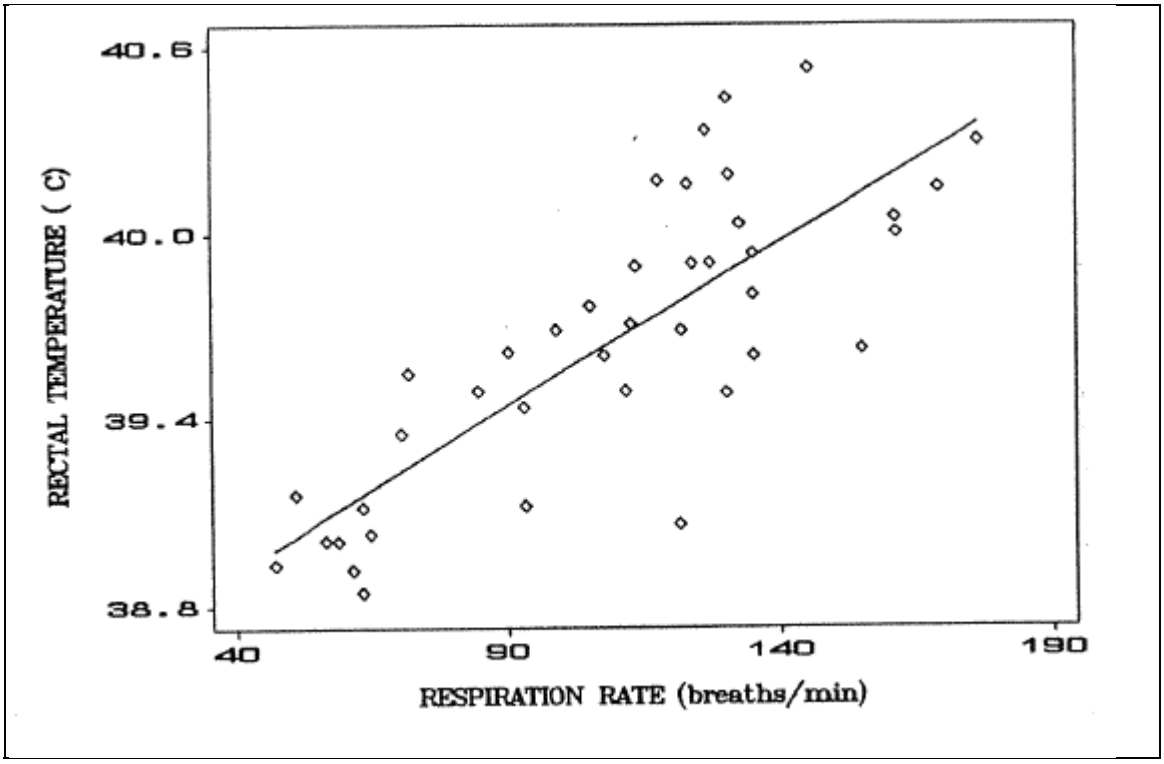


Figure 3C.94 The relationship between rectal temperature and respiration rate in working sheep. **(y=0.01x + 38.82; r=0.82; P=0.0000)**

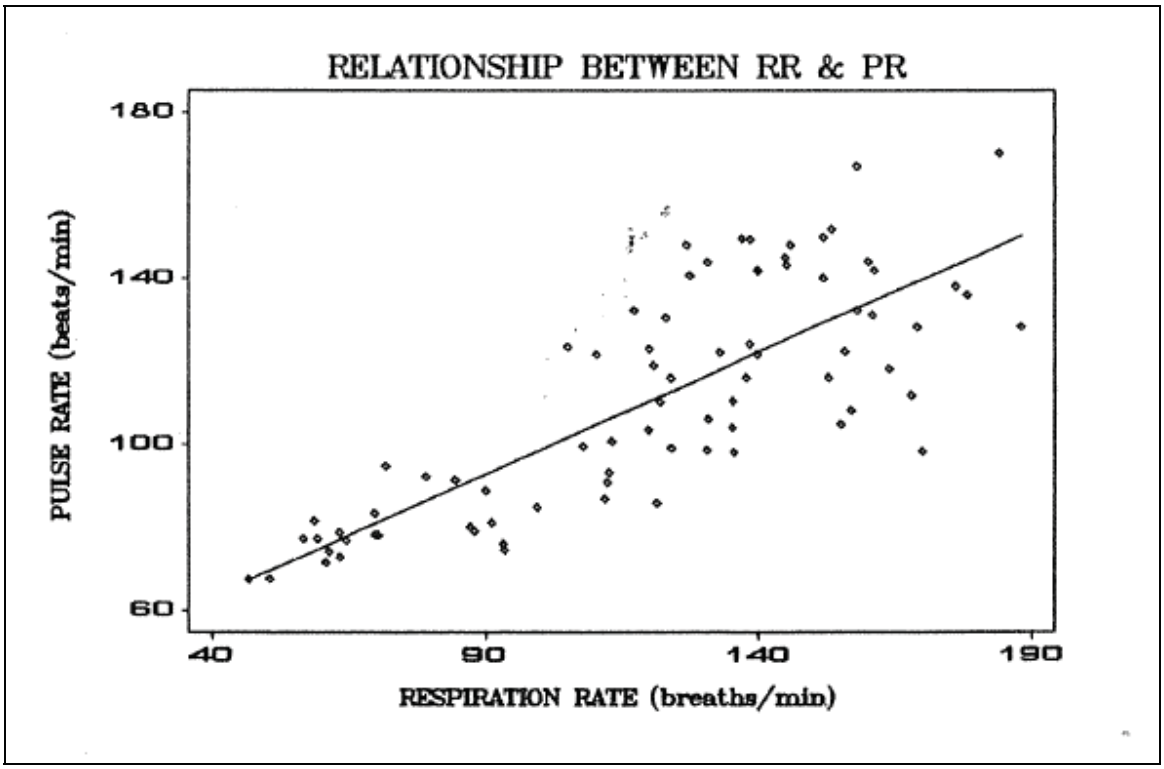


Figure 3C.95 The relationship between rectal temperature and pulse rate in working sheep. **(y=0.44x + 57.24; r=0.90; P=0.0000)**

Other factors that may have limited work capacity

The observed increased concentration of K^+ in plasma of sheep during the *Work* period is in agreement with results of studies in humans (Wilkerson et al. 1982; Neilson et al. 1984; Castellino et al. 1987). The increased K^+ concentration could adversely affect the force of muscle contraction by causing a depolarisation of the cell membrane (Lindinger & Sjogaard 1991) which would result in a decreased amplitude of the action potential (Sandercock et al. 1985) thereby reducing Ca^{2+} release from the SR and hence the force of muscle contraction. The increased concentration of K^+ also stimulates RR and PR as well as vasodilation within the muscle which results in increased bloodflow (Lindinger & Sjogaard 1991).

Wholebody O_2SAT was decreased at fatigue which may have been due to the high RT causing a reduced affinity of haemoglobin for O_2.

Conclusion

It might be concluded from the results of this experiment that a relatively short period of intensive training would increase the work capacity of animals. This is due mainly to the resulting improvement in the cardiovascular system and oxidative capacity of the animals.

It would appear that in the working sheep, hyperthermia rather than acidosis is the main contributor to the onset of fatigue. Acidosis may become significant at a higher work rate than was examined in this experiment. An additional consideration is the increase in concentration of K^+ in circulating plasma and this could contribute to fatigue.

In the work rate examined, it would appear that lactate was produced by tissues other than the hind-limb muscles which, contrary to expectation, took up lactate presumably to use as an oxidative substrate. This probably explains why acidosis did not appear to play a significant role in the onset of fatigue in the animals studied.

SECTION 4

GENERAL DISCUSSION

Work Capacity

It is clear from the series of experiments conducted in this study that short periods (10-15 days) of intensive training could significantly increase the work capacity of ruminant animals. Such improvements could range from 20% to over 100%, depending on the work load to which the animal is subjected. It appears from the observations made that within the environmental conditions normally experienced in the tropics, draught cattle and buffalo could be expected to be able to pull a maximum draught load equivalent to 11-12% of their live weight.

Under field conditions, draught loads in land cultivation (eg ploughing) are relatively constant. It can therefore be reasonably assumed that the live weight of the animals which are required to work would be of utmost importance. This is particularly so if animals to be used for draught work are breeds of smaller sizes, eg the Bali and Madura. For those breeds to be able to cope with heavy work such as ploughing, it might be necessary that they undergo a short period of training before they can undertake the task. Of the three cattle breeds observed, it was clearly demonstrated that the Ongole was superior, in its work capacity, to the Bali and Madura. It appears that the superiority of the Ongole is due to its white coat colour which gives it advantages in the area of body heat load, ie the animal, comparatively, would have a lower rate of absorption of radiant heat from the environment. In addition to this, it appears also that the Ongole has a different pattern of utilisation of energy-yielding substrates. There is no clear reason for this and the area certainly requires further investigation.

Training improves the efficiency of the cardiovascular system with an enhanced capacity to distribute O_2 and other metabolites to relevant tissues. The lower capacity for O_2 transport in the untrained animals, might be a reason for the apparent dominance of anaerobic metabolism in the muscle of these animals.

Acid-Base Balance

Mild respiratory alkalosis occurred in all sheep (except the untrained sheep walking at the slow speed) during work. Contrary to what was anticipated, there was no evidence of lactic acidosis in fatigued animals.

It is not clear whether respiratory alkalosis would increase the work capacity of an animal by delaying the onset of fatigue. There are conflicting reports on this from studies using rats and humans (Jones et al. 1977; Sutton et al. 1981; Spriet et al. 1986). Alkalosis results in an increased concentration of plasma lactate by facilitating release of the latter from working muscles (Mainwood et al. 1972; McCartney et al. 1983). Increased concentration of plasma lactate, however, may be due to an increased rate of release of lactate in tissues, or to an enhanced rate of glycolysis which could possibly lead to glycogen depletion.

Although lactate in the circulation of animals in this study increased in concentration during the work period, the rate of increase was not large enough to effect a reduction in blood pH. The efficient buffering systems of the body were able to cope with the increases in lactate production.

Substrate Utilisation

The reduction in the concentration of glucose in plasma of buffalo and cattle during the work period, indicated net uptakes of glucose by the muscles of these animals. This was confirmed by the A-V study of sheep. Glucose concentrations in plasma were reduced in cattle in the last 30 minutes of work, suggesting that the liver glycogen stores were depleted (Brzezinska 1987). In the Ongole cows, there was an increase in glucose concentration at 1.5 - 2.0 hours of work. It is likely that this indicated the point in time during the *Work* period when there was a major changeover from glucose to FFA oxidation in these animals. Thus there appeared to be a different pattern of utilisation of glucose by this breed of cattle compared with the Bali, for example, which appeared to depend largely on FFA for ATP generation. That the Ongole cows might have been in better physical condition than the other cows, was suggested by the fact that the hind-limb muscle of sheep in the trained state did not take up FFA to any significant degree during the *Work* period. However, the better physical condition of the Ongole cows is only a possibility as there was no direct evidence to suggest that they were any fitter than the Bali or Madura cows. Further studies to evaluate any species-based differences in the pattern of nutrient utilisation by muscles of Ongole, Bali, Madura and Buffalo cows are needed.

In untrained cattle it is possible that amino acids were used directly as energy substrates or as gluconeogenic precursors. In the buffalo the urea profile indicated that in trained animals on the heavy work load, AA were being utilised. The uptake of protein/AA-N and NH3-N by the working muscle of sheep would suggest that there was probably AA catabolism in the muscle during the work period. The uptake of NH3-N particularly is of interest as the metabolite might be utilised in the glutamine synthetase reaction to form glutamine in muscle

(see Teleni 1993). This AA could then have a significant role in the neutralisation of acid urine during work.

Lactate

The general picture which emerged from the series of experiments undertaken in this study is that the OBLA in the untrained working animals early in the *Work* period, particularly if the animals were subjected to heavy work loads. Of the three breeds of cattle studied, the Bali and Madura cows showed an earlier OBLA compared with the Ongole cows. Whether this is a real breed difference is unclear and warrants further investigation.

Although the concentration of lactate in the animals studied increased during the *Work* period, it did not appear to have a major role in the onset of fatigue. Training appeared to cause a general reduction in the concentration of lactate in plasma as well as lower increases in the concentration of the metabolite during work. Such a pattern is due, in the main, to the increased clearance rate of lactate after training (Donovan & Brooks 1983; Oyono-Enguelle et al. 1990; MacRae et al. 1992) and partially due to its decreased rate of production (Holloszy & Booth 1976).

An interesting observation is that although the concentration of lactate might increase in the circulation, the working hind-limb muscle does actually take up the metabolite presumably for use as fuel (see section 3C). Since there was no net release of lactate from the hind-limb muscle the increases in circulating lactate can only be from other tissues, eg liver and respiratory muscles.

Hyperthermia

Hyperthermia appears to be the major factor causing fatigue in the species studied. Hyperthermia has been shown (Edwards et al. 1972; Kozlowski et al. 1985) to cause increased rates of glycolysis which may result in the depletion of glycogen stores. There is also a reduced affinity of haemoglobin for O_2 at high body temperatures resulting in reduced O_2 saturation.

As expected, RR and PR increased in all animals observed during work. These are thermoregulatory responses to increases in body heat load.

If 41☜C is considered to be the critical temperature at which work can no longer continue, then a predicted work duration at which hyperthermia might cause fatigue, can be calculated from the rate of rise in RT observed in the animals studied. In the untrained Buffalo pulling loads 3 and 4, the predicted work duration to hyperthermia (41☜C) was 32 and 40 minutes

beyond the actual mean work duration of these animals respectively. In this case, it can only be assumed that factors other then hyperthermia were responsible for the onset of fatigue. On the other hand, untrained Madura and trained sheep walking at slow and medium speed worked for 33, 73 and 90 minutes, respectively, longer than the predicted work duration. In the trained sheep, although the rate of rise in RT was greater than in the untrained sheep, the actual work duration was much greater. Respiration rate was higher in the untrained animals, suggesting that the panting mechanism of cooling was responsible for the lower RT in the untrained sheep as compared to the trained sheep. Sheep have been found to be more tolerant to heat stress then other species (Lee 1950). Therefore, the critical RT in sheep at which hyperthermia would cause fatigue might be greater than 41☜C. This may explain why the observed work duration was greater than that predicted in the trained sheep walking at the slow and medium speeds. If this is the case, then, as consistent with the observation in the Buffalo, other factors may have been responsible for the onset of fatigue in the untrained sheep.

In all other situations than those discussed above, the predicted duration of work was similar to the actual work time and in some cases was exact to the minute. In all of these cases it is reasonable to conclude that hyperthermia was the main cause of fatigue.

It is possible that the onset of fatigue in the state of hyperthermia is due to the depletion of glycogen stores.

Plasma Potassium

The observed increases in plasma potassium concentrations in the sheep during the work period could adversely affect the force of muscle contraction. Increased potassium causes a depolarisation of the cell membrane (Lindinger & Sjogaard 1991) which would result in a decreased amplitude of the action potential (Sandercock et al. 1985) thereby reducing Ca^{2+} release from the SR and hence the force of muscle contraction. The increased concentration of potassium could also stimulate PR and RR as well as vasodilation within the muscle which would result in increased blood flow (Lindinger & Sjogaard 1991).

Conclusion

It is concluded from the results of this study that intense training of short duration could increase the work capacity of ruminant animals by improving their cardiovascular system and increasing their oxidative capacity.

It is likely, with the range of work loads imposed on animals in this study, that hyperthermia, and not acidosis, would be the major factor causing the onset of fatigue. At higher work loads

than were used, acidosis may become a more important factor in the onset of fatigue. Hyperthermia, and possibly alkalosis, may facilitate the depletion of glycogen stores; a condition which could result in the onset of fatigue.

The most interesting observation was the uptake of lactate by the hind-limb muscles of working sheep while other tissues, possibly the liver, gut and respiratory muscles contributed to the production of the metabolite. It is suggested that the utilisation of lactate by the hind-limb muscles probably reduced the risk of acidosis in these animals.

Another factor which could be important in the onset of fatigue is the K^+ which could increase significantly in its concentration in plasma and therefore reduces the force of muscle contraction.

REFERENCES

Abernethy PJ, Thayer R and Taylor AW (1990) Acute and chronic response of skeletal muscle to endurance and sprint exercise. A review. *Sports Med* **10:** 365–389

Adelstein RS and Eisenberg E (1980) Regulation and kinetics of actin-myosin-ATP interaction. *Ann Rev Biochem* **49:** 921–956

Agricultural Research Council (1980) The nutrient requirements of ruminant livestock. Commonwealth Bureaux, Slough.

Ahlborg B, Bergstrom J, Ekelund L-G and Hultman E (1967) Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiol Scand* **70:** 129–142

Ahlborg G (1985) Mechanism of glycogenolysis in non-exercising human muscle during and after exercise. *Am J Physiol* **248:** E540–E545

Ahlborg G, Felig P, Hagenfeldt L Hendler R and Wahren J (1974) Substrate turnover during prolonged exercise in man: Splanchnic and leg metabolism of glucose, free fatty acids and amino acids. *J Clin Invest* **53:** 1080–1090

Allen DG, Lee JE and Westerblad H (1989) Intracellular calcium and tension during fatigue in isolated single muscle fibres from *Xenopus Laevis*. *J Physiol* **415:** 433–458

Allen DG, Westerblad H, Lee JA and Lannergren J (1992) Role of excitation-contraction coupling in muscle fatigue. *Sports Med* **13:** 116–126

Anderson P and Henriksson J (1977) Training induced changes in the subgroups of human type II skeletal muscle Fibres. *Acta Physiol Scand* **99:** 123–125

Anon (1972) The employment of draught animals in agriculture. FAO, Rome, Italy.

Asano Y, Liberman VA and Edelman IS (1976) Thyroid thermogenesis relationships between Na+, K+-ATPase activity in rat skeletal muscle. *J Clin Invest* **57:** 368–379

Asmussen E (1979) Muscle fatigue. *Med and Sci in Sports* **11:** 313–321

Astrand PO (1967) Diet and athletic performances. *Fed Proc Am Soc Exp Biol* **26:** 1772–177

Baker MA and Chapman LW (1977) Rapid brain cooling in exercising dogs. *Science* **195:** 781–783

Baker MA and Hayward JN (1968) The influence of nasal mucosa and the carotid rete upon hypothalmic temperature in sheep. *J Physiol* **198:** 561–579

Bakrie B, Komarudin-Ma'sum and Teleni E (1991) Measurement of draught force. *Draught Animal Bulletin* **2:** 34–39

Ballard FJ, (1972) Supply and utilization of acetate in mammals. *Am J Clin Nutr* **25:** 773–777

Barwell I and Ayre M (1982) The harnessing of draught Animals. Intermediate technology publications. U.K.

Battle DC, Peces R, LaPointe MS and Daugirdas JT (1993) Cytosolic free calcium regulation in response to acute changes in intracellular pH in vascular smooth muscle. *Am J Physiol* **264:** C932–C942

Bayly WM (1989) The energetic basis of exercise. In: Jones *Equine Sports Med* 11–17

Beaver WL, Wasserman K and Whipp BJ (1986a) A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* **60:** 2020–2027

Beaver WL, Wasserman K and Whipp BJ (1986b) Bicarbonate buffering of lactic acid generated during exercise. *J Appl Physiol* **60:** 472–478

Bell AW and Hales JRS (1985) Conflicting requirements of exercise and heat stress for blood flow distribution in domestic animals. In: *Draught Animal Power for Production*. ed JW Copland, ACIAR Proceedings Series No.10, ACIAR, Canberra, pp 37–45

Bell AW, Hales JRS, King RB and Fawcett AA (1983) Influence of heat stress on exercise-induced changes in regional blood flow in sheep. *J Appl Physiol* **55:** 1916–1923

Bergman EN and Heitman RN (1978) Metabolism of amino acids by the gut, liver, kidneys and peripheral tissues. *Fed Proc* **37:** 1228–12232

Bergstrom G (1974) Blood pressure, cardiac output and blood gas tension in the horse at rest and during exercise. *Acta Vet Scand* **48:** 1–88

Bergstrom J (1962) Muscle electrolytes in man. Determined by neuron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients and patients with chronic diarrhoea. *Scand J Clin Lab Invest* **14 (suppl 68):** 7–11

Bergstrom J and Hultman E (1966) The effect of exercise on muscle glycogen and electrolytes in Humans. *Scand J Clin Lab Invest* **18:** 16–20

Bertocci LA, Haller RG, Lewis SF, Fleckenstein JL and Nunnally RN (1991) Abnormal high-energy phosphate metabolism in human muscle phosphofructokinase deficiency. *J Appl Physiol* **70:** 1201–1207

Bigland-Richie (1981) EMG and fatigue of human voluntary and stimulated contraction. In: *Human Muscle Fatigue: physiological mechanisms.* Pitman Medical, London (Ciba Foudation Symposium 82) pp130–156

Bird AR, Chandler KD and Bell AW (1981) Effects of exercise and plane of nutrition on nutrient utilization by the hind limb of the sheep. *Aust J Biol Sci* **34:** 541–550

Biron R, Burger A, Chinet A, Clausen T and Duois-Ferriere R (1979) Thyroid hormones and the energetics of active sodium-potassium transport in mammalian skeletal muscles. *J Physiol (Lond)* **297:** 47–60

Biswas D, De Sarkar MK and Bhattacharyya B (1991) Study of work capacity of large ruminants with sheep as Model animal. *Indian Vet J* **68:** 421–425

Bittner DL and Manning J (1967) Automated neocuprine method: critical factors and normal values. In: *Automation in Analytical Chemistry, Technicon Symposia 1966* Vol 1, Mediac Inc, White Plains, NY, pp33–36

Bjorntorp P (1991) Importance of fat as a support nutrient for energy: metabolism of athletes. *J Sports Sci* **9:** 71–76

Blanchard EM and Solaro RJ (1984) Inhibition of actomyosin and troponin calcium binding of dog cardiac myofibrils by acidic ph. *Circ Res* **55:** 382–391

Bligh J (1973) *Temperature Regulation in Mammals and Other Vertebrates.* eds A Neuberger and EL Tatum, Horth-Holland Publ Co, Amsterdam, p91

Blomstrand E, Celsing F and Newsholme EA (1988) Changes in plasma concentrations of aromatic and branched-chain amino acids during exercise in man and their possible role in fatigue.*Acta Physiol Scand* **133:** 115–121

Bockman E and McKenzie J (1983) Tissue adenosine content in active soleus and gracilis muscles of cat. *Am J Physiol* **244:** H522–H559

Bonen A, McDermott JC and Hutber CA (1989) Carbohydrate metabolism in skeletal muscle: An update of current concepts. *Int J Sports Med* **10:** 385–400

Brinnel H, Cabanac M and Hales JRS (1987) Critical upper levels of body temperature, tissue thermosensitivity and selective brain cooling in hyperthermia. In: *Heat Stress: Physical Exertion and Environment.* eds JRS Hales and DAB Richards, Elsevier, Amsterdam, pp209–240

Broberg S and Sahlin K (1989) Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *J Appl Physiol* **67:** 116–122

Brooke MH and Kaiser KK (1970) Muscle fibre types: How many and what kind? *Arch Neurol* **23:** 369–379

Brookes GA (1985) Lactate, glycolytic end product and oxidative substrate during exercise in mammals: "The Lactate Shuttle". In: *Comparative Physiology and Biochemistry: Current*

Topics and Trends, Vol A, Respiratiom and Metabolism - Circulation. ed R Gilles, Springer-Verlag, NY

Brzezinska Z (1987) Muscle metabolism during prolonged physical exercise in dogs. *Arch Inter Pyhsiol Biochim* **95:** 305–312

Bunyavejchewin P, Veerasit P, Chaidirek P and Chantalakhana C (1985) Changes in body temperature and working efficiency of Thai swamp buffalo. In: *Draught Animal Power for Production*, ACIAR Proceedings Series No.10, ACIAR, Canberra, pp46–50

Castellino P, Simonson DC and DeFronzo RA (1987) Adrenergic odulation of potassium metabolism during exercise in normal and diabetic humans. *Am J Physiol* **252:** E68–E76

Chesy G, Caputa M, Kadziela W, Kozak W and Lachowski A (1985) Selective brain cooling in the ox (Bos Taurus) during heavy work. *J Therm Biol* **10:** 57–61

Chinet A, Clausen T and Girardier L (1977) Microcalorimetric determination of energy expenditure due to active sodium-potassium transport in the soleus muscle and brown adipose tissue of the rat. *J Physiol* **265:** 43–61

Clapperton JL (1964) The energy metabolism of sheep walking on the level and on gradients.*Br J Nutr* **18:** 47–54

Clausen T, Everts ME and Kjeldsen K (1987) Quantification of the maximum capacity for active sodium-potassium transport in rat skeletal muscle. *J Physiol (Lond)* **338:** 163–181

Coakley JH, Wagenmakers AJM and Edwards RHT (1989) Excessive ammonia production during cycle ergometry in McArdles disease. *Clin Sci* **76:** 49

Coggan AR, Kohrt WM, Spina RJ, Bier DM and Holloszy JD (1990) Endurance training decreases plasma glucose turnover and oxidation during moderate intensity exercise in man. *J Appl Physiol* **68:** 990–996

Connett RJ, Gayeski TEJ and Honing CR (1984) Lactate accumulation in fully aerobic, working dog gracillis muscle. *Am J Physiol* **246:** H120–H128

Cooke R and Pate E (1985) The effect of ADP and phosphate on the contraction of muscle fibres. *Biophys J* **48:** 789–798

Cooke R, Franks GB, Luciani GB and Pate E (1988) The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J Physiol (Lond)* **395:** 77–97

Cori CF (1931) Mammalian carbohydrate metabolism. *Physiol Rev* **11:** 143–275

Corsi A, Midrio M, Granata AL, Corgnati A and Wolf D (1972) Lactate oxidation by skeletal muscle *in vivo* after denervation. *Am J Physiol* **223:** 219–222

Costill DL (1986) Muscle metabolism and electrolyte balance during heat acclimation. *Acta Physiol Scand* **128:** 111–118

Costill DL, Coyle EF, Delsky G, Evans W and Hoopes D (1977) Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J Appl Physiol* **43:** 695–699

Crampes F, Beauville M, Reviere D and Garrigues M (1986) Effect of physical training in humans on the response of isolated fat cells to epinephrine. *J Appl Physiol* **61:** 25–29

Dagenais GR, Tancredi RG and Zieler KL (1976) Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the forearm.*J Clin Invest* **58:** 421–431

Danforth WH (1965) Activation of glycolytic pathway in muscle. In: *Control of Energy Metabolism.* eds B Chance, RW Estabrooke and JR Williamson, Academic Press, NY, pp287–297

Davies NW, Standen NB and Stanfield PR (1992) The effect of intracellular pH on ATP-dependent K+ channels of frog skeletal muscle. *J Physiol* **445:** 549–568

Davis MA, Williams PE and Cherrington AD (1984) Effect of a mixed meal on hepatic lactate and glucogenic precursor metabolism in dogs. *Am J Physiol* **247:** E362–E369

Davis MA, Williams PE and Cherrington AD (1985) Effect of glucagon on hepatic lactate metabolism in the conscious dog. *Am J Physiol* **248:** E463–E470

Di Prampero PE (1981) Energetics of muscular exercise. *Rev Physiol Biochem Pharmacol* **11:** 142–222

Dohm GL, Kasperek GJ, Tapscott EB and Beecher GR (1980) Effect of exercise on synthesis and degradation of muscle protein. *Biochem J* **188:** 255–262

Dole VP (1956) A relationship between non-esterified fatty acids in plasma and the metabolism of glucose. *J Clin Invest* **35:** 150–154

Donovan CM and Brookes GA (1983) Endurance training affects lactate clearance, not lactate production. *Am J Physiol* **244** (Endocrinol Metab 7)**:** E83–E92

Dorup I and Clausen T (1993) Correlation between magnesium and potassium contents in muscle: role of Na+-K+ pump. *Am J Physiol* **264:** C457–C463

Du Boulay G, Kendall BE, Crockard A, Sage M and Belloni G (1975) The autoregulatory capacity of Galen's rete cerebri and its connections. *Neuroradiography* **9:** 171–181

Dudley GA and Terjung RL (1985) Influence of acidosis on AMP deaminase activity in contracting fast-twitch muscle. *Am J Physiol* **248:** C43–C50

Early RJ, McBride BW and Ball RO (1988) Phenylalanine metabolism in sheep infused with glucose plus insulin. II. Effects on *in vivo* and *in vitro* protein synthesis and related energy expenditures. *Can J Anim Sci* **68:** 721–730

Eberstein A and Sandow A (1963) Fatigue mechanisms in muscle fibres. In: *The Effect of Use and Disuse on Neuromuscular Function*. eds E Gutman and P Itnik. Elsevier, Amsterdam, pp515–526

Edstrom L and Nystrom B (1969) Histochemical types and sizes of fibres in normal human muscles. *Acta Neural Scand* **45:** 257–269

Edwards RHT (1981) Human muscle function and fatigue. In: *Human Muscle Fatigue: Physiological Mechanisms.* Pitman Medical, London (Ciba Foundation Symposium 82), pp1–19

Edwards RHT, Harris RC, Hultman E, Kaijser L, Koh D and Nordesjo L-O (1972) *J Physiol* **220:** 335–352

Engelhardt WV (1977) Cardiovascular effects of exercise and training in horses. *Adv Vet Sci* **21:** 173–205

Farber SJ, Pellegrino D, Cokan NJ and Earle DP (1951) Observations on the plasma potassium level of man. *Am J Med Sci* **221:** 678–687

Fenn WO (1936) Electrolytes in muscle. *Physiol Rev* **16:** 450–487

Fiestcorn G, Nagel A and Jessen C (1982) Effects of body temperature on cardiovascular function in the exercising goat. *Pflugers Arch* **394:** R38

Findlay JD and Ingram DL (1961) Brain temperature as a factor in the control of thermal polypnoea in the ox (*Bos taurus*). *J Physiol (Lond)* **155:** 72–81

Fitts RH and Metzer JM (1988) Mechanisms of muscular fatigue. In: *Principles of Exercise Biochemistry*. ed JR Poortmans, Karger, Basel, pp212–229

Fitzsimons RB and Hoh JFY (1981) Isomyosins in human type 1 and 2 skeletal muscle fibres. *Biochem J* **193:** 229–233

Fletcher WH and Hopkins FG (1907) Lactic acid in amphibian muscle. *J Physiol* **35:** 247–309

Flock EV, Ingle DJ and Bollman JL (1939) Formation of lactic acid, an initial process in working muscle. *J Biol Chem* **129:** 99–110

Foster DW (1984) From glycogen to ketones - and back. *Diabetes* **33:** 1188–1199

Galbo H (1985) The hormonal response to exercise.*Roceedings of the Nutrition Society* **44:** 257–265

Gandevia SC (1992) Some central and peripheral factors affecting human motorneural output in neuromuscular fatigue. *Sport Med* **13:** 93–98

Gertz EW, Wisneski JA, Stanley WC and Neese RA (1988) Myocardial substrate utilisation during exercise in humans: dual carbon-labelled carbohydrate isotope experiments. *J Clin Invest* **82:** 2017–2025

Gladden LB (1989) Blood flow and A-V lactate difference during lactate uptake by *in situ* canine skeletal muscle. *Med Sci Sports Exerc* **21:** S35

Gochman N, and Schmitz JM (1972) Application of a new peroxide indicator reaction to a specific, automated determination of glucose with glucose oxidase. *Clin Chem* **18:** 943–950

Godt RE and Nosek TM (1989) Changes of extracellular millieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *J Physiol (Lond)* **412:** 155–180

Goe MR (1983) Current status of research on animal traction. *World Anim Rev* **45:** 2–17

Gollnick PD and Hermanson L (1973) Biochemical adaptations to exercise: Anaerobic metabolism. *Exercise Sport Sci Rev* **1:** 1–43

Gollnick PD and Saltin B (1982) Significance of skeletal muscle oxidative enzyme enhancement and endurance training. *Clin Physiol* **2:** 1–12

Gollnick PD, Struck PJ and Bogyo TP (1967) Lactic dehydrogenase activities of rat heart and skeletal muscle after exercise and training. *J Appl Physiol* **22:** 623–627

Gollnick PD, Soule RG, Taylor AW, Williams C and Ianuzzo CD (1970) Exercise-induced glycogenolysis in the rat: Hormonal influence. *Am J Physiol* **219:** 729–733

Gollnick PD, Armstrong RB, Saltin B, Saubert IV CW, Sembrowich WL and Sheperd RE (1973) Effect of training on enzyme activity and fibre composition of human skeletal muscle. *J Appl Physiol* **34:** 107–111

Gollnick PD, Piehh K and Saltin B (1974) Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol* **241:** 45–57

Gollnick PD, Riedy M, Quintinskie JJ and Bertocci LA (1985) Differences in metabolic potential of skeletal muscle fibres and their significance for metabolic control. *J Exp Biol* **115:** 191–199

Gonzalez-Serratos H, Somlyo A, McClellan G, Shuman H, Borrero LM, and Somlyo AP (1978) Composition of vacuoles and sarcoplasmic reticulum in fatigued muscle: Electron probe analysis. *Proc National Acad Sci USA* **75:** 1329–1333

Graham TE, Bangsbo J, Gollnick PD, Juel C and Saltin B (1990) Ammonia metabolism during intense dynamic exercise and recovery in humans. *Am J Physiol* **259:** E170–E176

Graham TE, Kiens B, Hargreaves M and Richter EA (1991) Influence of fatty acids on ammonia and amino acid flux from active human muscle. *Am J Physiol* **261:** E168–E176

Granata AL, Midrio M and Corsi A (1976) Lactate oxidation by skeletal muscle during contractions *in vivo*. *Pflugers Arch* **366:** 247–250

Green HJ, Reidman H and Pette D (1983) Fibre type specific transformations in the enzyme activity pattern of rat vastus lateralis muscle by prolonged endurance exercise. *Pfluger Arch Eur J Physiol* **399:** 216–222

Gregg SG, Mazzeo RS, Budinger TF and Brookes GA (1989) Acute anaemia increases lactate production and decreases clearance during exercise. *J Appl Physiol* **67:** 756–764

Gregg VA and Milligan LP (1980) Inhibition of Na+, K+ ATPase of intact mouse soleus muscle by Mg+. *Biochem Biophys Res Commun* **95:** 608–611

Gregg VA and Milligan LP (1982a) *In vitro* costs of Na+, K+ ATPase activity and protein synthesis in muscle and calves of differing age and breed. *Br J Nutr* **48:** 65–71

Gregg VA and Milligan LP (1982b) O_2 consumption and Na+, K+-ATPase-dependent respiration in muscle of lambs and lactating and non-lactating ewes. In: *Energy Metabolism of Farm Animals.* eds A Ekern and F Sundstol, Eur Assoc Prod Publ 29. Informasjonsteknikk A/S, Ski, Norway, pp66–69

Guernsey DL and Stevens ED (1977) The Cell membrane sodium pump as a mechanism for increasing thermogenesis during cold acclimation in rats. *Science* **196:** 908–910

Hales JRS (1983) Circulatory consequences of hyperthermia: an animal model for studies of heat stroke. In: *Heat Stroke and Temperature Regulation.* eds M Khogali and JRS Hales, Academic Press, Sydney, p223

Hales JRS and Dampney RAL (1975) The redistribution of cardiac output in the dog during heat stress. *J Therm Biol* **1:** 29–34

Hales JRS and Findlay JD (1968) Respiration of the ox: normal values and the effect of exposure to hot environments. *Respir Physiol* **4:** 333–362

Hales JRS, Findlay JD and Mabon RM (1967) Tissue hypoxia in oxen exposed to severe heat. *Respir Physiol* **3:** 43–46

Hammel HT (1968) Regulation of internal body temperature. *Physiol Rev* **41:** 521–606

Hargreaves M, Kiens B and Richter EA (1988) Effect of fatty acids on glucose uptake during exercise. *Can J Sports Sci* **13:** 14P

Hargreaves M, Kiens B and Richter EA (1991) Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. *J Appl Physiol* **70(1):** 194–201

Harris P, Bateman M and Gloster J (1962) The regional metabolism of lactate and pyruvate during exercise in patients with rheumatic heart disease. *Clin Sci* **23:** 545–560

Harris RC, Bergstrom J and Hultman E (1971) In: *Muscle Metabolism During Exercise.* eds B Pernow and B Saltin. Plenum Press, NY, pp301–305

Havel RJ (1971) Influence of intensity and duration of exercise on supply and use of fuels. In: *Muscle Metabolism During Exercise.* eds B Pernow and B Saltin, Plenum Press, NY, pp315–325

Heisler N (1975) Intracellular pH of isolated rat diaphragm muscle with metabolic and respiratory changes of extracellular ph. *Physiol* **23:** 243–255

Henderson SA, Black AL and Brookes GA (1985) Leucine turnover and oxidation in trained and untrained rats during rest and exercise. *Am J Physiol* **249:** 137–144

Henriksson J (1977) Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J Physiol* **270:** 661–675

Hermansen L (1971) In: *Muscle Metabolism During Exercise.* eds B Pernow and B Saltin, Plenum Press, NY, pp401–407

Hermansen L (1973) Biochemical adaptations to exercise: Anaerobic metabolism. *Exercise and Sports Sci Rev* **1:** 1–43

Hermansen L (1981) Effect of metabolic changes on force generation in skeletal muscle during maximal exercise. In: *Human Muscle Fatigue: Physiological Mechanisms.* Pitman Medical, London (Ciba Foundation Symposium 82) pp75–88

Hermansen L and Osnes J-B (1972) Blood and muscle pH after maximal exercise in man. *J Appl Physiol* **32:** 304–308

Hermansen L, Ptuett ED, Osnes JB, and Giere FA (1970) Blood glucose and plasma insulin in response to maximal exercise and glucose infusion. *J Appl Physiol* **29:** 13–16

Hildman B, Storelli C, Haase W, Barac-Nieto M and Murer H (1980) Sodium ion/lactate co-transport in rabbit small intestinal brush-border-membrane vesicles. *Biochem J* **186:** 169–176

Hill A, Gorman REL and Kerr WD (1972) Circulatory transport of L-Glutamine in fasted mammals: cellular sources of urine ammonia. *Am J Physiol* **223:** 1470–1476

Hill AV (1955) Influence of the external medium on the internal pH of muscle. *Proc Roy Soc Lond B Sci* **144:** 1–22

Hill AV and Lupton H (1923) Muscular exercise, lactic acid and the supply and utilization of oxygen. *Quart J Med* **16:** 135–171

Himwich HE, Koskoff YD and Nahlum LH (1930) Studies in carbohydrate metabolism. I. A glucose-lactic acid cycle involving muscle and liver. *J Biol Chem* **85:** 571–584

Hodgkin AL and Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* **117:** 500–544

Hodgson DR, McCutcheon LJ, Byrd SK, Brown WS, Bayly WM, Brengelmann GL and Gollnick PD (1993) Dissipation of metabolic heat in the horse during exercise. *J Appl Physiol* **74:** 1161–1170

Hohorst HJ (1965) In: *Methods of Enzymatic Analysis*. ed HU Bergmeyer, Verlag Chemie, Weinheim, pp266–270

Holloszy JO (1967) Biochemical adaptation in muscle. Effect of exercise on mitochondrial oxygen uptake and respiratory enzyme activity on skeletal muscle. *J Biol Chem* **242:** 2278–2282

Hoppeler H, Luthi P, Claussen H, Weibel ER and Howald H (1973) The ultrastructure of the normal human skeletal muscle: a morphometric analysis on untrained men, women and well-trained orienteers. *Pflugers Arch Eur J Physiol* **344:** 217–232

Howitz BA and Eaton M (1977) Ouabain-sensitive liver and diaphragm respiration in cold-acclimated hamster. *J Appl Physiol* **42:** 150–153

Hultman E and Bergstrom J (1962) Plasma potassium determination. *Scand J Clin Lab Invest* **64:** 87–93

Hultman E, Sjoholm H, Sahlin K and Edstrom L (1981) Glycolytic and oxidative energy metabolism and contraction characteristics of intact human muscle. In: *Human Muscle Fatigue: Physiological Mechanisms.* Pitman Medical, London (Ciba Foundation Symposium 82) pp19–40

Huxley HE (1976) The structural basis of contraction and regulation in skeletal muscle. In: *Molecular Basis of Motility.* eds LMG Heilmeyer Jr, JC Ruegg and TH Wieland. Springer-Verlag, Berlin

Huxley HE and Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature (Lond)* **173:** 973–976

Huxley HE and Niedergerke R (1954) Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature (Lond)* **173:** 971–973

Ingram DL and Whittow G (1962) The effect of heating the hypothalamus on respiration in the ox (*Bos taurus*). *J Physiol (Lond)* **163:** 200–212

Ismail-Beigi F and Edelman IS (1971) The mechanism of the calorigenic action of thyroid hormone: stimulation of Na+, K+-activated adenosine triphosphate activity. *J Gen Physiol* **40:** 710–722

Issekutz B, Shaw WAS and Issekutz AC (1976) Lactate metabolism in resting and exercising dogs. *J Appl Physiol* **40:** 312–319

Issekutz Jr B, Miller HI and Rodahl K (1966) Lipid and carbohydrate metabolism during exercise. *Fed Proc* **25:** 1415–1420

Jacob R, Kissling G, Ebrecht G, Holubarsch C, Medugorac I and Rupp H (1983) Adaptive and Pathological alterations in experimental cardiac hypertrophy. *Adv Myocardiol* **4:** 55

Jaffe M (1886) Ueber den Niederschlag, welchen Pikrinsaure in normalem Har erzeugt and ueber eine neue Reaction des Kreatinins. *Zeitschrift Fuer Physiologische Chemie* **10:** 391-400 (GER)

Jansson E (1980) Diet and muscle metabolism in man with reference to fat and carbohydrate utilization and its regulation. *Acta Physiol Scand* **487:** 1–24

Jansson E and Kaijser L (1977) Muscle adaptation to extreme endurance training in man. *Acta Physiol Scand* **100:** 315–324

Jansson E, Sjodin B and Tesch P (1978) Changes in muscle fibre type distribution in man after physical training (A sign of fibre type transportation?) *Acta Physiol Scand* **104:** 235–237

Jenkins AB, Storlien LH and Gooney GJ (1989) Effects of blockade on fatty acid oxidation on whole body and muscle glucose metabolism *in vivo*: Relationship with pyruvate dehydrogenase activity. *Diabetes* **38:** 23A

Jobis FF and Stainsby WN (1968) Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir Physiol* **4:** 292–300

Johnson A, Argyraky M and Throw J (1989) Effect of elevated free fatty acids on glucose metabolism in human skeletal muscle. *Diabetes* **38:** 66A

Johnson JA and Furarso RM (1972) The role of skin in carbohydrate metabolism. *Adv Metab Disord* **6:** 1–55

Jones NL, Sutton JR Taylor R and Tuews CJ (1977) Effect of pH on cardiorespiratory and metabolic responses to exercise. *J Appl Physiol* **43:** 959–964

Juel C (1991) Muscle lactate transport studied in sarcolemmal giant vesicles. *Biochim Biophys Acta* **1065:** 15–20

Karlsson J (1971) Lactate and phosphagen concentrations in working muscle of man. With special reference to oxygen deficit at the onset of work. *Acta Physiol Scand* **358:** 7–72

Kartiarso and Teleni E (1988) Aterio-venous concentration differences of plasma free fatty acids and 3-hydroxybutyrate across the hind-limb muscles of the working ruminant. *Proc Nutr Soc Aust* **13:** 104

Kelly F (1965) Improved method for microtitration of fatty acids. *Annalyt Chem* **37:** 1078–1079

Keynes RD and Aidley DJ (1981) *Nerve and Muscle.* 2nd edition. Cambridge University Press. p122, 146, 157

Kinderman W, Simon G and Kuel J (1979) The significance of aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Physiol* **49:** 13–23

Knochel JP, Dotin LN and Hamburger RJ (1972) Pathophysiology of intense conditioning in a hot climate. I. Mechanisms of potassium depletion. *J Clin Invest* **51:** 242–255

Koike A, Weiler-Ravell D, McKenzie DK, Zanconato S and Wasserman K (1990) Evidence that the metabolic acidosis threshold is the anaerobic threshold. *J Appl Physiol* **68:** 2521–2526

Kolb HA (1990) Potassium channels in excitable and non-excitable cells. *Rev Physiol Biochem Pharm* **115:** 51–91

Kozlowski S, Brzezinska Z, Kruk B,Kaciuba-Uscilko H, Greenleaf JE and Nazar K (1985) Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle. *J Appl Physiol* **59(3):** 766–773

Kruk B, Nazar K, Kacicuba-Uscilko H and Kozlowski S (1987) Enhanced glucose availability for working muscles reduces exercise hyperthermia in dogs. *Eur J Appl Physiol* **56:** 577–582

Lannergren J and Westerblad H (1991) Force decline due to fatigue and intracellular acidification in isolated fibres from mouse skeletal muscle. *J Physiol (Lond)* **434:** 307–322

Lawrence PR (1985) A review of nutrient requirements of draught oxen. In: *Draught Animal Power for Production*. ed JW Copland, ACIAR Proceedings Series No. 10, ACIAR, Canberra, pp59–63

Layzer RB (1990) Muscle metabolism during fatigue and work. *Baillieres Clin Endocrin Metab* **4:** 441–459

Lee DH (1950) Studies of heat regulation in the sheep, with special reference to the Merino. *Aust J Agric Res* **1:** 200–216

Lee JA, Westerblad H and Allen DG (1991) Changes in tetanic and resting [ca2+]i during fatigue and recovery of single muscle fibres from *Xenopus laevis*. *J Physiol (Lond)* **433:** 307–326

Lindholm A and Saltin B (1974) The physiological and biochemical response of standardbred horses to exercise of varying speed and duration. *Acta Physiol Scand* **15:** 1–19

Lindinger MI and Heigenhauser GJF (1988) Ion fluxes during tetonic stimulation in isolated perfused rat hindlimb. *Am J Physiol* **254:** R117–R126

Lindinger MI and Sjogaard G (1991) Potassium regulation during exercise and recovery. *Sports Med* **11:** 382–401

Lowenstein JH (1972) Ammonia production in muscle and other tissues: The purine nucleotide cycle. *Physiol Rev* **52:** 382–414

MacDougall JD, Reddan WG, Layton CR and Dempsey JA (1974) Effects of metabolic hyperthermia on performance during heavey exercise. *J Appl Physiol* **36:** 538–544

MacFarlane WV (1968) Adaptations of ruminants to tropics and deserts. In: *Adaptations of Domestic Animals.* ed ESE Hafez, Lea and Febiger, Philadelphia, p164

MacRae HSH, Dennis SC, Bosch AN and Noakes TD (1992) Effects of training on lactate production and removal during progressive exercise in humans. *J Appl Physiol* **72(5):** 1649–1656

Mainwood GWP, Worsley-Brown P and Paterson RA (1972) The metabolic changes in frog sartorius muscle during recovery from fatigue at different external bicarbonate concentrations. *Am J Physiol* **50:** 143–155

Mansour TE (1965) Studies on heart phosphofructokinase: active and inactive forms of the enzyme. *J Biol Chem* **240:** 2165–2171

Marsh WH, Fingerhut B, and Miller H (1965) Automated and manual direct methods for the determination of blood urea. *J Clin Chem* **11:** 624–627

Martin D, Kartiarso and Teleni E (1989) Muscle blood flows in draught animals. *DAP Project Bulletin* **9:** 17–18

Mbwambo K (1993) The effect of work on utilisation of sorghum hay by growing sheep. MSc Thesis, James Cook University

McBride BW (1986) Cellular energy expenditure on Na+, K+ transport and protein synthesis in hyperthyroid sheep. *J Dairy Sci* **69:** 175

McBride BW and Early RJ (1987) Effect of feeding frequency on tissue protein synthesis and related energy expenditures in sheep. *Can J Anim Sci* **67:** 1190

McCartney N, Heigenhauser GJF and Jones NL (1983) Effects of pH on maximal power output and fatigue during short-term dynamic exercise. *J Appl Physiol* **55:** 225–229

McFarland WL, Jacobs MS and Morgane PJ (1979) Blood supply to the brain of the dolphin, *Tursiops truncatus*, with comparative observations on special aspects of the cerebrovascular supply of other vertebrates. *Neuroscience and Biobehavioral Reviews*, Ankho Int Inc Fayetteville, New York, **3:** 1–93

McKinen DF (1983) An energetic basis of equine performance. *Equine Vet J* **15(2):** 123–1

Medbo IJ and Sejersted OM (1990) Plasma potassium changes with high intensity exercise. *J Physiol* **421:** 105–122

Meijer WCP (1962) Das Balirind (Balinese Cattle). Translated from Dutch by E Mohr. Die neue Brahm-Budherei No. 303, Wittenberg Luterstradt, A. Ziemsen Verlag

Metzger JM and Fitts RH (1987) Role of intracellular pH in muscle fatigue. *J Appl Physiol* **62:** 1392–1397

Miller RG, Boska MD, Moussavi RS, Carsen PJ and Weiner MW (1988) 31P NMR studies of high-energy phosphates and pH in human muscle fatigue: comparison of aerobic and anaerobic exercise. *J Clin Sci* **81:** 1190–1196

Ministry of Agriclture, Fisheries and Food (1975) Energy allowances and feeding systems for ruminants. Ministry of Agriculture. Fisheries and Food (HMSO): London

Ministry of Agriculture, Fisheries and Food (1984) Energy allowances and feeding systems for ruminants. *Reference Book 433*. London, Her Majesty's Stationary Office.

Mole PA, Baldwin KM, Terjung RL and Holloszy JO (1973) Enzymatic pathways of pyruvate metabolism in skeletal muscle. *Am J Physiol* **244:** 50–54

Mutch BJ and Barister EW (1983) Ammonia metabolism in exercise and fatigue. *Med Sci Sports Exerc* **15:** 41–50

Nakamaru Y and Schwartz A (1972) The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. *J Gen Physiol* **59:** 22–32

Neilsen B, Sjogaard G and Bonde-Petersen F (1984) Cardiovascular, hormonal and body fluid changes during prolonged exercise. *Eur J Appl Physiol* **53:** 63–70

Neilsen B, Savard G, Richter EA, Hargreaves M and Saltin B (1990) Muscle blood flow and muscle metabolism during exercise and heat stress. *J Appl Physiol* **69:** 1040–1046

Newguard CB, Hirsch LJ, Foster DW and McGarry JM (1983) Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat: a direct or indirect pathway. *J Biol Chem* **258:** 1254–1256

Newsholme E and Start C (1973) Regulation of carbohydrate metabolism in muscle. In: *Regulation in Metabolism.* John Wiley and Sons, London, pp126–131

Noma A (1983) ATP-regulated K+ channels in cardiac muscle. *Nature (Lond)* **305:** 147–148

Nosek TM, Fender K and Godt RE (1987) It is diprotonated inorganic phosphate that depresses force in skinned muscle fibres. *Science* **236:** 191–192

Ohale LOC and Ghoshal NG (1982) Effect of reversible upper respiratory on the electroretinogram in sheep. *Am J Vet Res* **43:** 633–638

Oyono-Enguelle S, Marbach J, Heitz A, Ott C, Gartner M, Pape A, Vollmer JC and Freund H (1990) Lactate removal ability and graded exercise in humans. *J Appl Physiol* **68:** 905–911

Oyono-Enguelle S, Heitz A, Marbach J, Ott C, Pape A and Freund H (1993) Heat stress does not modify lactate exchange and removal abilities during recovery from short exercise. *J Appl Physiol* **74(3):** 1248–1255

Pethick DW (1984) Energy metabolism of skeletal muscle. In: *Ruminant Physiology: concepts and consequences.* Proc Symp at University of Western Australia. pp277–287

Pethick DW and Lindsay DB (1982) Acetate metabolism in lactating sheep. *Br J Nutr* **48:** 319–328

Pethick DW, Lindsay DB, Baker PJ and Northorp AJ (1983) The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. *Br J Nutr* **49:** 129–143

Pethick DW, Miller CB and Harman NG (1991) Exercise in merino sheep–the relationships between work intensity, endurance, anaerobic threshold and glucose metabolism. *Aust J Agric Res* **42:** 599–620

Piatti PM, Monti LD, Pacchioni M, Pontirli AE and Pozza G (1991) Forearm insulin- and non-insulin-mediated glucose uptake and muscle metabolism in man: Role of free fatty acids and blood glucose levels. *Metabolism* **40:** 926–933

Pilegaard H, Juel C and Wibrand F (1993) Lactate transport studied in sarcolemmal giant vesicles form rats: effect of training. *Am J Physiol* **264:** E156–E160

Ploug T, Stallknecht BM, Pedersen O, Kahn BB, Ohkuwa T, Vinten J and Galbo H (1990) Effect of endurance training on glucose transport capacity and glucose transporter expression in rat skeletal muscle. *Am J Physiol* **259:** E778–E786

Podlin DA, Munger PA amd Mazzeo RS (1991) Plasma catecholamine and lactate response during graded exercise with varied glycogen conditions. *J Appl Physiol* **71:** 1427–1433

Ramaswamy NS (1985) Draught animal power–socioeconomic factors. In: *Draught Animal Power for Production.* ed JW Copland, ACIAR Proceedings Series No.10, ACIAR, Canberra, pp20–25

Randle PJ, Newsholme EA and Garland PB (1964) Regulation of glucose uptake by muscle. Effect of fatty acids, ketone bodies and pyruvate, and alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm. *Biochem J* **93:** 652–687

Ravussin E, Bogardus C, Scheidegger K, LaGrange B, Horton ED and Horton ES (1986) Effect of elevated FFA on carbohydrate and lipid oxidation during prolonged exercise in humans. *J Appl Physiol* **60:** 893–900

Reinhart WH, Staubi M and Straub PW (1983) Impaired red cell filtrability with elimination of old red blood cells during a 100 km race. *J App Physiol* **54:** 827–830

Renaud JM, Allard Y amd Mainwood GW (1986) Is the change in intracellular pH during fatigue large enough to be the main cause of fatigue? *Can J Physiol Pharmacol* **64:** 764–767

Rennie MJ and Holloszy JO (1977) Inhibition of glucose uptake and gylcogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. *Biochem J* **168:** 161–170

Rennie MJ, Jennett S and Johnson RH (1974) The metabolic effects of strenuous exercise: A comparison between untrained subjects and racing cyclists. *Quart J Expt Physiol* **59:** 201–212

Rennie MJ, Winder WW and Holloszy JO (1976) a sparing effect of increased plasma fatty acids on muscle and liver glycogen content in the exercising rat. *Biochem J* **156:** 647–655

Robinson (1977) Livestock in Indonesia. *Research Report No. 1.* Centre for Animal Research and Development. Bogor, Indonesia.

Ronca-Testoni S, Raggi A and Ronca G (1970) Muscle AMP amino-hydrolase. III. A comparative study on the regulatory properties of skeletal muscle enzyme from various species. *Biochim Biophys Acta* **198:** 101–112

Rosler K, Hoppeler H, Conley KE, Claasen H, Gehr P and Howald H (1985) Transfer effects in endurance exercise. Adaptations in trained and untrained muscles. *Eur J Appl Physiol* **54:** 355–362

Rossignol B, Rossignol D, and PetitClerc C (1984) Improvement of creatinine measurement on RA-1000. *Clin Biochem* **17:** 203–204 (Abstract)

Rowell LB (1974) Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* **54:** 75–15

Ruderman NB and Berger M (1974) The formation of glutamine and alanine in skeletal muscle. *J Biol Chem* **249:** 5500–5506

Sahlin K (1992) Metabolic factors in fatigue. *Sports Med* **13:** 99–107

Sahlin K and Broberg S (1990) Adenine nucleotide depletion in human muscle during exercise: casualty and significance of AMP deamination. *Int J Sports Med* **2:** 562–567

Sahlin K, Broberg S and Ren JM (1989) Formation of IMP in human skeletal muscle during incremental dynamic exercise. *Acta Physiol Scand* **136:** 193–198

Sahlin K, Edstrom L, Sjoholm H and Hultman E (1981) Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *Am J Physiol* **240:** C121–C126

Sahlin K, Harris RC, Hultman E and Nylind B (1976) Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflugers Archiv* **367:** 143–149

Sahlin K, Palmskog G and Hultman E (1978) Adenine nucleotide and IMP content of the quadracepts muscle in man after exercise. *Pflugers Arch* **374:** 193–198

Saltin B (1981) Muscle fibre recruitment and metabolism in Prolonged exhaustive exercise. In: *Human Muscle Fatigue: Physiological Mechanisms.* Pitman Medical, London (Ciba Foundation Symposium 82) pp41–58

Saltin B (1990) Anaerobic capacity: past, present and prospective. In: *Seventh International Biochemistry of Exercise Symposium 1988*. ed AW Taylor, Campaign IL: Human kinetics, 1990. (Int Ser Sport Sci)

Saltin B, Henriksson J and Nygaard E (1977) *Annals New York Acad Sci* **301:** 3–29

Sandercock TG, Faulkner JA, Albers JW and Abbrecht PH (1985) Single motor unit and fibre action potentials during fatigue. *J Appl Physiol* **58:** 1073–1079

Schmid HA and Pierau FK (1993) Temperature sensitivity of neurons in slices of the rat PO/AH hypothalmic area: effect of calcium. *Am J Physiol* **264:** R440–R448

Sewell DA, Harris RC, Hanak J and Jahn P (1992) Muscle adenine nucleotide degradation in the thoroughbred horse as a consequence of racing. *Comp Biochem Physiol* **101B:** 375–381

Shantz PG, Sjoberg B and Svendenhag J (1986) Malate-aspartate and alpha-glycerphosphate shuttle enzyme level in human skeletal muscle: methodological considerations and effect of endurance training. *Acta Physiol Scand* **128:** 387–407

Simeons P, Lauwers H, De Geest JP and De Schaepdrijver L (1987) Functional morphology of the cranial Retia Mirabillia in the domestic mammals. *Scheiz Arch Tierheilk* **129:** 295–307

Sjodin B and Jacobs I (1981) Onset of blood lactate accumulation and marathon performance. *Int J Sports Med* **2:** 23–26

Sjogaard G and Saltin B (1982) Extra and intracellular water spaces in muscles of man at rest and with dynamic exercise. *Am J Physiol* **243:** R271–R280

Smadja C, Morin J, Ferre P and Girard J (1988) Metabolic fate of gastric glucose load in unrestrained rats bearing a portal vein catheter. *Am J Physiol* **254:** E407–E413

Smith JS Coronado R and Meissner G (1985) Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature (Lond)* **316:** 446–449

Snow DH, Baxter P and Rose RJ (1981) Muscle-fibre composition and glycogen depletion in horses competing in an endurance ride. *Vet Rec* **108:** 374–378

Spriet L, Lindinger MI, Heigenhauser GJF and Jones N (1986) Effects of alkalosis on skeletal muscle metabolism and performance during exercise. *Am J Physiol* **251:** R833–R839

Spriet L, Ren JM and Hultman E (1988) Epinephrine infusion enhances muscle glycogenolysis during prolonged electrical stimulation. *J Appl Physiol* **64:** 1439–1444

Stainsby WN and Brooks GA (1990) Control of lactic acid metabolism in contracting muscle and during exercise. *Exercise Sports Sci Rev* **18:** 29–63

Stainsby WN, Sumners C and Eitzman PD (1985) Effects of catecholamines on lactic acid output during progressive working contractions. *J Appl Physiol* **59:** 1809–1814

Stanley WC, Gertz EW, Wisneski JA, Neese RA, Morris and Brookes GA (1986) Lactate extraction during net lactate release by the exercising legs of man. *J Appl Physiol* **60:** 1116–1120

Stegman H, Kinderman W and Schnabel A (1981) Lactate kinetics and individual anaerobic threshold. *Int J Sports Med* **2:** 160–165

Storelli G, Corcelli A, Cassano G, Hildman B, Murer M and Lippe C (1980) Polar distribution of sodium-dependant and sodium-independent transport system for L-lactate in plasma membrane of rat enterocytes. *Pfluger Arch* **388:** 11–16

Stringer W, Casaburi R and Wasserman K (1992) Acid-base regulation during exercise and recovery in humans. *J Appl Physiol* **72:** 954–961

Sutton JR (1992) Limitations to maximal oxygen uptake. *Sports Med* **13:** 127–133

Sutton JR, Jones NL and Toews CJ (1981) Effect of pH on muscle glycolysis during exercise. *Clin Sci* **61:** 331–338

Taylor AW, Thayer R and Rao S (1972) Human skeletal muscle glycogen synthetase activities with exercise and training. *Cardiov J Physiol and Pharmacol* **50:** 411–415

Taylor CR (1966) The vascularity and possible thermoregulatory function of the horns in goats. *Physiol Zool* **39:** 127–129

Taylor CR (1977) Exercise and environmental heat loads: different mechanisms for solving different problems? In: *Enviromental Physiology II.* ed D Robertshaw, University Park Press, Baltimore, MD

Taylor DJ, Coppack SW, Cadoux-hudson TAD, Kemp GJ, Radda GK, Freyn KN and Ng LL (1991) Effect of insulin on intracellular pH and phosphate metabolism in human skeletal muscle *in vivo*. *Clin Sci* **81:** 123–128

Teleni E (1993) Catabolism and synthesis of amino acids in skeletal muscle: Their significance in monogastric mammals and ruminants. *Aust J Agric Res* **44:** 443–461

Teleni E and Annison EF (1986) Development of a hind-limb muscle preparation for metabolic studies. *Aust J Biol Sci* **39:** 271–281

Teleni E and Murray RM (1991) Nutrient Requirements of of Draft Cattle and Buffaloes. In: *Recent Advances on the Nutrition of Herbivores*. *Proc of the Third Int Symp on the Nutr Herbivores* Eds.YW Ho, HK Wong, N Abdullah and ZA Tajuddin, Malaysian Society of Animal Production, Malaysia, pp113–119

Teleni E, Pieterson R and De'ath G (1991) Feed utilization, energy expenditure and nitrogen balance in working female buffalo. *Aust J Agric Res* **42:** 1359–1372

Tesch PA and Karlsson J (1985) Muscle fibre types and size in trained and untrained muscles of elite athletes. *J Appl Physiol* **59:** 1716–1720

Tipton CM, Tcheng and Mergnes W (1971) Ligamentous strength measurements from hypophysectomized rats. *Am J Physiol* **221:** 1149–1150

Trivedi B and Danforth WH (1966) Effect of pH on the kinetics of frog muscle phosphofructokinase. *J Biol Chem* **241:** 4110–4112

Ui M (1966) A role of phosphofructokinase in ph-dependent regulation of glycolysis. *Biochim Biophys Acta* **124:** 310–322

Upadhyay RC and Madan ML (1985a) Draft performance of Haryana and crossbred bullocks in different seasons. *Indian J Anim Sci* **55:** 50–54

Upadhyay RC and Madan ML (1985b) Physiological responses to work in bullocks. *Indian J Comp Anim Physiol* **3:** 43–49

Upadhyay RC and Madan ML (1985c) Studies on blood acid-base status and muscle metabolism in working bullocks. *Anim Prod* **40:** 11–16

Upadhyay RC Madan ML and Rao MVN (1987) Draught animal power research in India. In: *World Review of Animal Production.* Vol XXIII No.1

Wasserman DH, Lacey DD, Green DR, Williams PE and Cherrington AD (1987) Dynamics of hepatic lactate and glucose balances during prolonged exercise and recovery in the dog. *Am J Physiol* **63:** 2411–2417

Wasserman K, Whipp BJ, Koyal SN and Beaver WL (1973) Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* **35:** 236–243

Webb MR, Hibberd MG, Goldman YE and Trentham DR (1986) Oxygen exchange between Pi in the medium and water during ATP hydrolysis mediated by skinned fibres from rabbit skeletal muscle. Evidence for Pi binding to a force generating state. *J Biol Chem* **261:** 15557–15564

Weiner MW, Moussavi RS, Baker AJ, Boska MD and Miller RG (1990) Constant relationships between force, phosphate concentrations, and pH in muscles with differential fatigability. *Neurology* **40:** 1888–1893

Welch HG and Stainsby WN (1967) Oxygen debt in contracting dog skeletal muscle *in situ. Resp Physiol* **3:** 229–242

Wendt I and Stephenson DG (1983) Effect of caffeine on the Ca-activated force production in skinned cardiac and skeletal muscle fibres of the rat. *Pflugers Arch* **398:** 210–216

Wheeler TJ and Lowenstein JM (1979) Adenylate deaminate from rat muscle: regulation by purine nucleotides and orthophosphate in the presence of 150mM KCL. *J Biol Chem* **254:** 8994–8999

Whittow G (1965) The effect of hyperthermia on the systemic and pulmonary circulation of the ox (*Bos taurus*). *Quart J Exp Physiol* **50:** 300–311

Wilkerson JE, Horvath SM, Gutin B, Molnar S and Diaz FJ (1982) Plasma electrolyte content and concentration during treadmill exercise in humans. *J Appl Physiol* **53:** 1529–1539

Williams CH and Twine JR (1967) CSIRO Div Plant Ind Technical Paper No. 24

Winder WW, Hickson RC, Hagberg JM and Ehsari AA (1979) Training-induced changes in hormonal and metabolic responses to submaximal exercise. *J Appl Physiol* **46:** 766–771

Yakovlev NN (1976) Biochemical mechanisms of adaptation of skeletal muscles to muscular activity. *Ukr Biokhim* **48:** 388–397

Zurovski Y, Eckstein L and Horowitz M (1991) Heat stress and thermal dehydration: lactacidemia and plasma volume regulation. *J Appl Physiol* **71:** 2434–2439