THE EFFECT OF CYCLOSERINE ON SKELETAL MUSCLE METABOLISM AND CONTRACTILE FUNCTION

THE EFFECT OF CYCLOSERINE ON METABOLISM AND CONTRACTILE FUNCTION IN RODENT SKELETAL MUSCLE.

By

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ABSTRACT

We hypothesized that acute inhibition of the contraction-induced expansion of the muscle TCA cycle intermediate (TCAI) pool via would not adversely effect metabolism or contractile function. Forty rats were anaesthetized and the gastrocnemius muscle (GAS) from one leg was vascularly isolated and perfused with saline (CON) or a red cell media containing DL-cycloserine (CYCLO; Sigma C-7005; dose=0.05 mg/g), an inhibitor of alanine aminotransferase (AAT). After 1h of perfusion, the GAS muscle was either snap frozen (CON-Rest, n=11; CYCLO-Rest, n=9) or stimulated to contract for 10 min (1 Hz, 0.3 ms, 2 V) with blood flow fixed at 30 ml min^{-1} $100g^{-1}$ and then snap frozen (CON-Stim, n=10; CYCLO-Stim, n=10). The maximal activity of AAT was lower ($P \leq 0.05$) at both CYCLO-Rest (0.61±0.02 mmol·kg^{-1}w.w./min; mean ± SEM) and CYCLO-Stim (0.63±0.01 mmol·kg^{-1}w.w./min) vs CON-Rest (3.56±0.16 mmol·kg^{-1}w.w./min) and CON-Stim (3.92±0.29 mmol·kg^{-1}w.w./min). Consistent with lower net flux through AAT, muscle [alanine] was lower ($P \leq 0.05$) after CYCLO-Stim (6.97±0.26 mmol·kg^{-1} dw) compared to CON-Stim (8.55±0.56 mmol·kg^{-1} dw) and not different vs CON-Rest (6.79±0.41 mmol·kg^{-1} dw). The sum of five measured TCAI (malate, fumarate, citrate, isocitrate, and 2-oxoglutarate) was higher ($P \leq 0.05$) at both CON-Rest (2.10± 0.09 mmol·kg^{-1} dw) and CON-Stim (2.48± 0.11 mmol·kg^{-1} dw) vs CYCLO-Rest (1.56± 0.11 mmol·kg^{-1} dw) and CYCLO-Stim (1.88± 0.15 mmol·kg^{-1} dw) respectively. Despite the reduction in [TCAI] following CYCLO treatment, there was no difference between conditions in muscle lactate accumulation or phosphocreatine degradation after

10 min of stimulation. Contractile function was not different ($P \leq 0.05$) between conditions at either rest or stimulation and the decline in force production over ten minutes of stimulation was identical (~60%) between CON-Stim and CYCLO-Stim respectively. We conclude that flux through AAT was reduced after cycloserine treatment, however the acute inhibition of TCAI expansion did not compromise aerobic energy provision. These data support the hypothesis that the contraction-induced increase in muscle [TCAI] is not causally linked to oxidative energy delivery.

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LIST OF ABBREVIATIONS

AAT	alanine aminotransferase
AdSuc	adenylosuccinate
AMPD	AMP deaminase
BCAA	branched-chain amino acids
DCA	dichloroacetate
GDH	glutamate dehydrogenase
ME	malic enzyme
PC	pyruvate carboxylase
PCC	propionyl-CoA carboxylase
PDC	pyruvate dehydrogenase complex
PDH	pyruvate dehydrogenase
PEPCK	phosphoenolpyruvate carboxykinase
PLP	pyridoxal 5'-phosphate
PNC	purine nucleotide cycle
TCA cycle	tricarboxylic acid cycle
TCAI	tricarboxylic acid cycle intermediate(s)

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

At the onset of exercise, there is as much as a 100-fold increase in the rate of ATP turnover in skeletal muscle (Hochachka and Matheson, 1992). To satisfy this increased demand, an observed increase in the oxidative pathways, including those of the tricarboxylic acid (TCA) cycle, experience an increase in flux (Gibala et al, 1997; Gibala et al, 1998; Gibala et al, 1997; Aragon et al, 1980). Associated with this increase in TCA cycle flux is an increase in the total concentration of intermediates of the TCA cycle (TCAI) (Gibala et al, 1997; Gibala et al, 1997; Gibala et al, 1998; Sahlin et al, 1990; Aragon et al, 1980). As the largest increases in the total concentration of TCAI occurs within the first few minutes of exercise, the physiological significance of this anaplerotic expansion has been the subject of much debate (Gibala et al, 1998; Sahlin et al, 1990; Graham and Gibala, 1998). There are those who would suggest that anaplerotic expansion is both temporally and causally related to mitochondrial respiration, such that an increase in TCAI is essential for the up-regulation of aerobic energy provision in response to contraction (Sahlin et al, 1995; Sahlin et al, 1990; Wagenmakers et al, 1998). Alternatively, there are others who believe that anaplerosis is simply the result of a mismatch between rates of pyruvate production and oxidation (Graham and Gibala, 1998; Constantin-Teodosiu et al, 1999). This 'mass action effect' involves the shuttling of

excess carbon units into the TCA cycle to be housed in a 'sink' until they can be subsequently oxidized.

In order to elucidate the physiological significance of anaplerosis in relation to aerobic energy provision, it is necessary to manipulate the expansion of the TCAI pool. While a number of dietary and training manipulations have successfully altered muscle [TCAI] during exercise, no study has acutely inhibited anaplerosis to examine the effect on mitochondrial respiration. Inhibition of alanine aminotransferase (AAT), a key anaplerotic pathway in skeletal muscle, presents a potential site for acute attenuation of TCAI expansion in order to investigate how oxidative energy provision might be effected.

The following review of literature focuses on TCA cycle metabolism in mammalian skeletal muscle and in particular, the role of the intermediates of the cycle in relation to oxidative energy provision. Previous investigations that have examined the physiological significance of anaplerosis are summarized, and consideration is given to the potential use of DL-cycloserine, an inhibitor of alanine aminotransferase, as a tool in metabolic research studies. Finally, the review summarizes the utility and evolution of the rat hindlimb model for the purpose of metabolic research studies.

1.1 OVERVIEW OF MUSCLE TCA CYCLE METABOLISM

1.2.1. Function of the TCA cycle

The tricarboxylic acid (TCA) cycle consists of a series of eight biochemical reactions that take place in the mitochondria. The cycle accepts the substrate acetyl-coenzyme A (acetyl-CoA) which can be derived from all three potential energy sources: glucose, fatty acids, and amino acids. While the TCA cycle intermediates (TCAI) are neither created nor destroyed by the operation of the larger cycle, the reactions of the

cycle serve to oxidize acetyl-CoA thereby forming GTP via substrate phosphorylation and reduced coenzymes (NADH and $FADH_2$) that are ultimately oxidized in the electron transport chain forming ATP via oxidative phosphorylation (Newsholme et al, 1983).

1.2.2. Regulation of TCA cycle flux in muscle

Flux through the TCA cycle is primarily regulated by three enzymes: isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase (also known as α-ketoglutarate dehydrogenase), and citrate synthase (Newsholme et al, 1983). Isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase are non-equilibrium, allosterically modulated enzymes with activities lower than the other enzymes of the cycle (Spriet et al, 1999). The activity of these enzymes increases with exercise in response to contraction-stimulated modulation (Spriet et al, 1999). Mitochondrial [Ca^{2+}] accumulation, increasing NAD:NADH and ADP:ATP ratios, and cations (e.g. Mg^{2+}) all stimulate isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase activity. In addition, increasing [succinyl CoA] inhibits 2-oxoglutarate dehydrogenase. The flux-generating reaction citrate synthase is activated by its substrates acetyl-CoA and oxaloacetate and inhibited by its product citrate (Spriet et al, 1999). However, citrate synthase is saturated by acetyl-CoA and not oxaloacetate (Newsholme et al, 1983) and it appears that inhibition by citrate is overridden. This override on inhibition is evident by the large increases in cycle flux in response to contraction (Aragon et al, 1980; Putnam et al, 1995) despite large increases in [citrate] (Spriet et al, 1999). Citrate synthase is also inhibited by ATP (Groff et al, 2000).

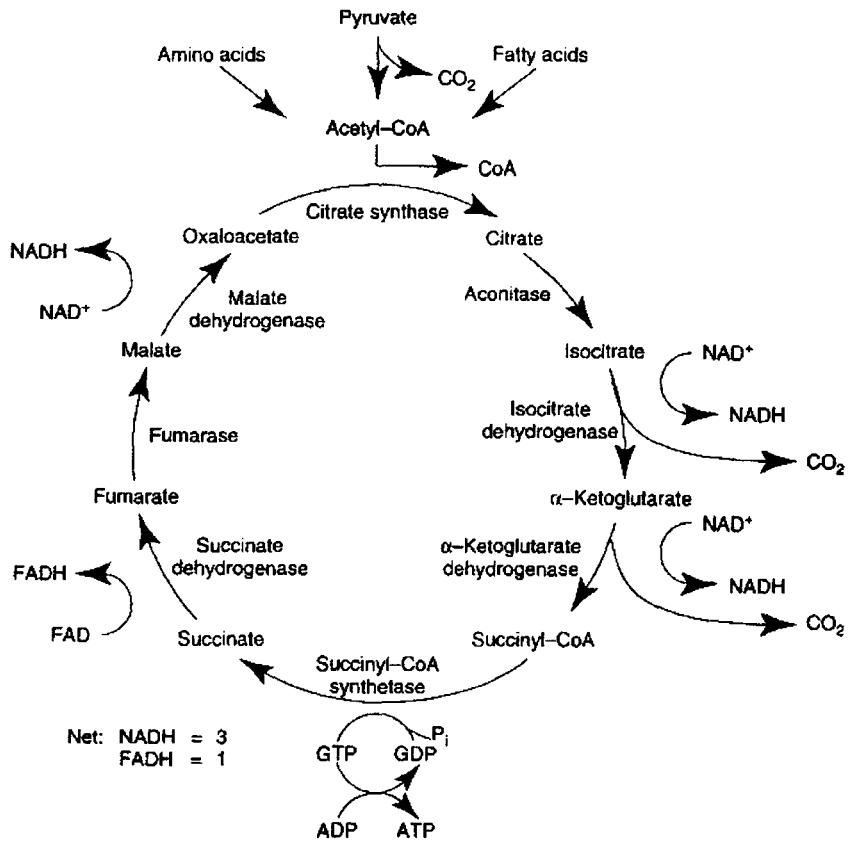


Fig. 1.1. Overview of the TCA cycle (Powers and Howley, 1998)

1.3 EFFECT OF MUSCLE CONTRACTION ON TCAI METABOLISM

1.3.1. The concept of "anaplerosis"

Although the intermediates of the TCA cycle are neither formed nor destroyed through the net operation of the cycle, there is a continuous flux of carbon units into and out of the cycle through various ancillary reactions in which TCAI take part (Newsholme et al, 1983; Gibala et al, 2000). Those reactions which lead to the replenishment of a metabolic cycle are termed anaplerotic (Kornberg, 1966) and in this case, defend against the loss of carbon from the TCA cycle due to its many near-equilibrium side reactions (Gibala et al, 2000). The ongoing delicate balance between anaplerotic and cataplerotic pathways associated with the TCA cycle, particularly in response to various metabolic environments, determines the net concentration of TCAI at any point.

1.3.2. Changes in TCAI during contraction

Early work that examined the response of TCAI to contraction primarily involved the electrical stimulation of isolated rodent muscle preparations. Perhaps the most concise study was that conducted by Aragon et al (1980). Using an in situ rat hindlimb preparation, the concentrations of seven of the TCAI were measured at both rest and during electrical stimulation. The sum of the seven measured TCAI (ΣTCAI) increased from 1.44 at rest to 1.93, 2.82, and 3.0 $mmol \cdot kg^{-1}$ d.w. after 5, 10, and 15 min of stimulation, respectively. The 4-fold increase above rest in malate and fumarate concentrations were the largest and earliest changes observed of all of the intermediates. Similar increases in TCAI were observed in other investigations that involved stimulation and hypoxia (Flanagan et al, 1986; Pastoris et al, 1994). Perfusion studies have also demonstrated increases in TCAI in response to excess pyruvate availability (Lee et al,

1979), though the individual changes in intermediates were somewhat divergent to those trends observed by Aragon et al (1980) suggesting differential influences of stimulation versus perfusion models (Graham and Gibala, 1998).

Recent attempts to examine the response of TCAI to contraction in human skeletal muscle demonstrated a similar contraction-induced increase in [TCAI] as previously observed in rodent skeletal muscle, though individual intermediate responses were somewhat divergent (Graham and Gibala, 1998; Gibala et al, 1997; Sahlin et al, 1990; Gibala et al, 1999; Gibala et al, 1997b). In fact, Gibala et al (1997) found a 7-fold greater increase in the expansion of the sum of seven TCAI than that previously observed in rodents (Aragon et al, 1980). Despite this apparent species difference in the rate and magnitude of TCAI expansion, it would appear as though malate, fumarate, and succinate dominate the total expansion by approximately 90% in human skeletal muscle (Graham and Gibala, 1998; Gibala et al, 1997). Of interest is the realization that all three of these intermediates reside in the second half of the cycle.

Anaplerosis of the TCA metabolic pathways demonstrates a peak expansion within the initial few minutes of moderate to intense exercise (Gibala et al, 1998; Sahlin et al, 1990; Graham and Gibala, 1998; Dawson et al, 2003). In fact, approximately 60% of this net pool expansion occurs within the first minute alone (Gibala et al, 1997). It appears as though expansion of the TCAI pool is dependent upon not only exercise intensity, with greater expansion associated with increasing intensity (Gibala et al, 1998; Sahlin et al, 1995), but also upon the duration of exercise (Gibala et al, 1997a; Gibala et al, 1997b; Sahlin et al, 1990). As previously noted, peak expansion occurs within the first few minutes of exercise with no greater expansion following 15 min of contraction. However, this 3-4 fold higher increase in concentration over resting values decreases with

prolonged exercise to exhaustion, though still remaining 2-fold higher than rest at the actual point of exhaustion (Gibala et al, 1997b, Sahlin et al, 1990).

Table 1.1. Concentrations of TCAI in human and rat skeletal muscle (adapted from Graham and Gibala, 1998)

Time	Intensity	Ref	Cit.	Isocit.	2OG	Suc.	Fum.	Mal.	OAΣ	TCAI
Rest		Aragon (1980)	0.56	0.03	0.15	0.31	0.07	0.32	0.001	1.44
Rest		Gibala (1997)	0.40	0.05	0.04	0.32	0.06	0.36	0.010	1.23
Rest		Gibala (1997)	0.30	0.07	0.04	0.21	0.06	0.39	0.017	1.10
Rest		Sahlin (1990)	0.30	-----	-----	-----	0.10	-----	------	-----
1 min	80%	Gibala (1997)	0.41	0.11	0.05	0.73	0.23	1.59	0.027	3.12
3 min	80%	Gibala (1997)	0.43	0.19	0.05	0.88	0.36	1.94	0.030	3.86
5 min	70%	Gibala (1997)	0.56	0.15	0.03	1.49	0.37	2.20	0.021	4.80
5 min	75%	Sahlin (1990)	0.60	-----	-----	-----	0.80	3.10	------	-----
5 min	80%	Gibala (1997)	0.52	0.22	0.03	1.01	0.38	2.17	0.034	4.37
5 min	95%	Spencer (1990)	0.56	-----	-----	-----	1.00	4.05	-----	
5 min	Stim	Aragaon (1980)	0.57	0.03	0.16	0.38	0.15	0.65	0.003	1.93
15 min	70%	Gibala (1997)	0.68	0.14	0.03	1.31	0.39	2.31	0.021	4.88
15 min	Stim	Aragon (1980)	0.94	0.05	0.20	0.57	0.25	1.07	0.008	3.09
Exh	70%	Gibala (1997)	0.60	0.09	0.01	0.78	0.22	1.40	0.009	3.08
Exh	70%	Spencer (1991)	0.43	-----	-----	-----	0.24	1.01	-----	-----
Exh	75%	Spencer (1991)	0.70	-----	-----	-----	050	1.80	-----	-----

All values are mmol·kg^{-1} dry muscle. For human studies, mode of exercise was two-legged cycling (Gibala et al, 1997; Sahlin et al, 1990; Spencer et al, 1991; Spencer et al, 1991) or dynamic knee extensor exercise (Gibala et al, 1997). Intensity refers to percentage of VO_2max or percentage of knee extensor maximal work capacity. For rodent study (Aragon et al, 1980), contractions were induced via electrical stimulation (Stim) of hinquarters using a frequency, duration and intensity of 5 Hz, 10ms and 0.5-5.0 V, respectively. Exhaustion (Exh) data is from human studies in which subjects began with "normal" pre-exercise muscle glycogen levels (i.e., no dietary manipulation) and cycled to volitional fatigue. Mean cycling time to exhaustion was 96, 75, and 135 min for Gibala et al, 1997, Sahlin et al, 1990, and Spencer et al, 1991, respectively. For Aragon et al, 1980 and Sahlin et al, 1990 TCAI values were not reported in text of original publications (except for OA data in Ref.), and therefore have been derived from figures. Abbreviations: Cit., citrate; Iso., isocitrate; 2OG, 2-oxoglutarate; Suc., succinate; Fum., fumarate; Mal., malate; OA; oxaloacetate; ΣTCAI, sum of all TCAI except succinyl CoA.

1.3.3. Mechanisms for change in muscle TCAI during contraction

A number of mechanisms have been proposed to explain the observed increase in TCAI with the initiation of contraction. As highlighted by Graham and Gibala (1998), there exists six possible reactions that could potentially result in the anaplerotic entrance of carbon units into the TCA cycle. Briefly, these mechanisms include the purine nucleotide cycle (PNC; net-reaction, Eqn. 1), and those reactions catalyzed by glutamate dehydrogenase (GDH; Eqn. 2), alanine aminotransferase (AAT; Eqn. 3), pyruvate carboxylase (PC; Eqn. 4), phosphoenolpyruvate carboxykinase (PEPCK; Eqn. 5), and malic enzyme (ME; Eqn. 6).

$$\text{aspartate} + \text{GTP} \rightarrow \text{fumarate} + \text{ammonia} + \text{GDP} + P_i \quad (1)$$

$$\text{glutamate} + \text{NAD+} \leftrightarrow \text{2-oxoglutarate} + \text{ammonia} + \text{NADH} \quad (2)$$

$$\text{glutamate} + \text{pyruvate} \leftrightarrow \text{2-oxoglutarate} + \text{alanine} \quad (3)$$

$$\text{pyruvate} + CO_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + P_i \quad (4)$$

$$\text{phosphoenolpyruvate} + CO_2 + \text{IDP} + P_i \leftrightarrow \text{oxaloacetate} + \text{ITP} \quad (5)$$

$$\text{pyruvate} + CO_2 + \text{NAD(P)H} \leftrightarrow \text{malate} + \text{NAD(P)}^+ \quad (6)$$

Ultimately, with the bulk of anaplerotic expansion occurring within the first few minutes of contraction, it is apparent that the key mechanism of expansion must have a minimum capacity of at least several $mmol \cdot kg^{-1} \cdot min^{-1}$ d.w., adequate substrate availability, and appropriate enzymatic activation (Graham and Gibala, 1998). Due to the low maximal activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in mammalian skeletal muscle and the kinetics of malic enzyme, it is not believed that the

reactions catalyzed by these three enzymes play a key role in anaplerotic expansion (Brodal et al, 1990; Crabtree et al, 1972; Aragon et al, 1980).

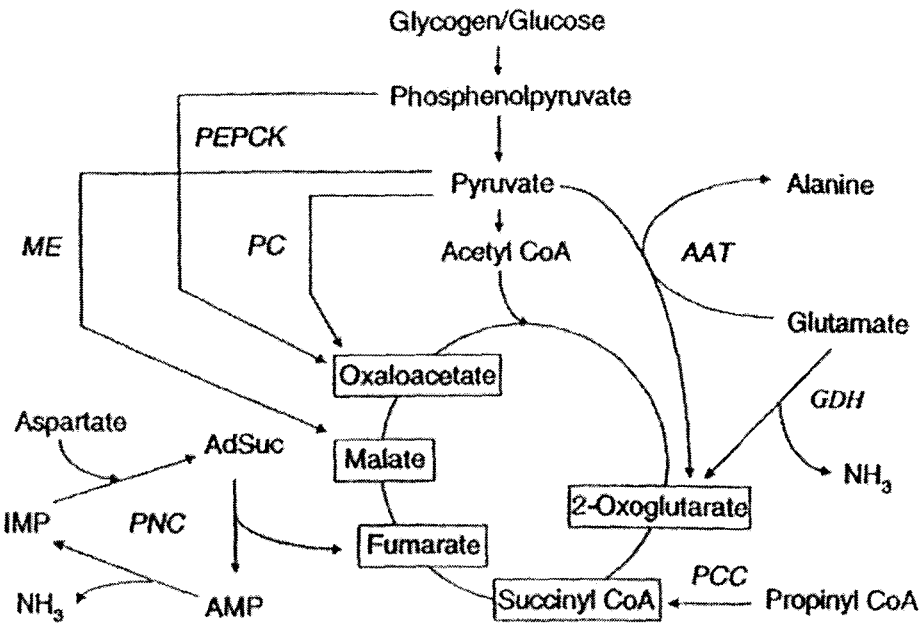


Fig. 1.2. Anaplerotic pathways in cardiac and skeletal muscle. AAT, alanine aminotransferase; PCC, propionyl-CoA carboxylase; GDH, glutamate dehydrogenase; PNC, purine nucleotide cycle; AdSuc, adenylosuccinate; ME, malic enzyme; PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase (from Gibala et al, 2000).

1.3.3.1. *The purine nucleotide cycle and glutamate dehydrogenase*

Early investigations suggested that perhaps the purine nucleotide cycle (PNC) played a key role in contraction-stimulated anaplerosis (Aragon et al, 1980; Swain et al, 1984; Flanagan et al, 1986). Flanagan et al (1986) observed that administration of AICAriboside, an inhibitor of adenylosuccinate lyase, in stimulated rat muscle resulted in

marked muscle dysfunction, leading them to conclude that the PNC plays a key anaplerotic role in producing TCA cycle intermediates to enhance aerobic energy production in contracting muscle. Similarly, Aragon et al (1980) noted that following the inhibition of adenylosuccinate synthetase, the concomitant increase in IMP would suggest that the operation of the PNC accounts for 72% of the expansion of the citric acid cycle pool during the first 10 min of exercise.

Recent studies suggest that the PNC is not the key anaplerotic mechanism in human muscle. Following five minutes of dynamic knee extensor exercise at ~80% of maximum workload, Gibala et al (1997) observed a significant increase in the sum of seven measured TCAIs. However, despite this increase in TCAIs, there was no measurable change in intramuscular aspartate concentration with negligible uptake during exercise. Combined with a small degree of ammonia accumulation, these data implied that the PNC played only a minimal role in anaplerotic expansion. Additionally, the small observable accumulation of ammonia would also appear to suggest that glutamate dehydrogenase and the PNC combined, account for less than 25% of anaplerosis (Graham and Gibala, 1998; Gibala et al, 1997). In a subsequent more definitive study, Tarnopolsky et al (2001) observed similar levels of anaplerotic expansion in AMP deaminase (AMPD) deficient patients and healthy controls. If in fact the PNC played a significant role in anaplerosis, then one would suspect that a deficiency in AMPD, the key enzyme of the PNC, would result in attenuated anaplerosis and this is apparently not the case.

1.3.3.2. *Alanine aminotransferase*

The reaction catalyzed by alanine aminotransferase is generally accepted as the primary anaplerotic mechanism (Sahlin et al, 1995; Sahlin et al, 1990; Gibala et al, 1997). It is believed that with contraction, an increased production of pyruvate in excess of that which can be oxidized in the pyruvate dehydrogenase (PDH) reaction, drives the near-equilibrium reaction catalyzed by AAT to the right, thereby forming 2-oxoglutarate, an intermediate of the cycle. Support for this mechanism comes in the observed decrease in glutamate and correlated increase in alanine and TCAI in association with contraction (Gibala et al, 1997; Sahlin et al, 1990; Gibala et al, 1997; Howarth et al, 2003; Dawson et al, 2003).

1.4 PHYSIOLOGICAL SIGNIFICANCE OF CHANGES IN MUSCLE TCAI DURING CONTRACTION

1.4.1. Overview of theory linking TCAI with mitochondrial respiration

The significance of the observed expansion of TCAI with contraction has spawned much debate. At present, there exists two theories surrounding the significance of anaplerosis in relation to aerobic energy provision. The initial theory put forward suggested that the concentration of the TCAI was symbiotically related to the capacity for TCA cycle flux and aerobic energy provision (Sahlin et al, 1990; Wagenmakers et al, 1990). Specifically, it was proposed that during prolonged exercise, a depletion in carbohydrate stores would result in the impairment of aerobic energy production by reducing the levels of carbon-skeletons available to the TCA cycle (Sahlin et al, 1990). A second proposed mechanism was that the excessive acceleration of branched-chain amino

acid (BCAA) metabolism, as observed during prolonged exercise, would result in the draining of 2-oxoglutarate (α-ketoglutarate) and hence carbon, from the TCA cycle especially if the glutamine formed was released from the muscle (Wagenmakers et al, 1990; Graham and Gibala, 1998). The loss of 2-oxoglutarate was further postulated to impede TCA cycle flux and ultimately the oxidation of glucose and fatty acids (Wagenmakers et al, 1990).

The alternate theory with regards to the physiological significance of changes in muscle TCAI during contraction asserts that the TCAI serve as a 'sink' for pyruvate produced in excess of that which can be oxidized in the TCA cycle at the onset of contraction (Graham and Gibala, 1998). Utilizing the anaplerotic pathway catalyzed by AAT, pyruvate in combination with glutamate, drives this near-equilibrium reaction to the right thereby producing 2-oxoglutarate and alanine. 2-oxoglutarate enters as an intermediate of the TCA cycle and suggestively accounts for the significant increases in the intermediates of the second span of the cycle (Gibala et al, 1997).

1.4.2. Studies which have attempted to manipulate muscle TCAI

1.4.2.1. *CHO availability*

Spencer et al (1991) observed that CHO ingestion attenuated the decline in TCAI during prolonged exercise. It was proposed that the CHO ingestion attenuated the decline in TCAI during prolonged exercise by increasing the concentration of hexose monophosphates resulting in the maintenance of pyruvate concentration for a greater duration and thereby sustaining a production in excess of its oxidation (Spencer et al, 1991). Results from this study however were not definitive due to the fact that measurements were taken only at rest and at the end of a prolonged ride to fatigue. In

addition, exercise was terminated prior to fatigue in the CHO supplemented trial. The largest expansion in TCAI has been demonstrated as occurring within the first few minutes of exercise and therefore requires measurement during that time point (Gibala et al, 1997; Gibala et al, 2002).

Gibala et al (2002) observed rather surprisingly that the contraction-induced expansion of the TCAI pool was in fact enhanced in low glycogen conditions. No significant difference in glycogenolytic rate or lactate accumulation was observed. In addition to the possibility that perhaps the low glycogen manipulation was not sufficient enough to impede pyruvate formation, it was postulated that pyruvate dehydrogenase complex flux was attenuated and/or that increased glutamate availability led to greater TCAI accumulation (Gibala et al, 2002). However, of great interest is the fact that regardless of this enhanced expansion of the TCAI pool, there was no concomitant improvement in aerobic energy provision suggesting that the two phenomenon are not causally related (Gibala et al, 2002).

Wagenmakers et al (1990) suggested that in response to decreasing CHO availability due to glycogen depletion during prolonged exercise, there is an increased reliance on BCAA oxidation much to the detriment of the larger functioning of the TCA cycle and aerobic energy provision. Wagenmakers and colleagues (1990) postulated that the drain of 2-oxoglutarate out of the TCA cycle to foster the oxidation of the BCAA would result in the loss of carbon skeletons out of the muscle in the form of glutamine and ultimately impede the flux of the TCA cycle. Follow-up investigations by Gibala and colleagues (1999, 2002) determined that not only is the decrease in muscle TCAI during prolonged exercise not compromising to aerobic energy provision or attributable to the

depletion of muscle glycogen concentration, but that ingestion of BCAA results in no measurable effect on TCAI.

1.4.2.2. *Manipulation of PDH*

Gibala et al (1999b) investigated the effect of PDH activation on TCAI expansion during exercise. Administering dichloroacetate (DCA), an activator of the pyruvate dehydrogenase complex (PDC), it was postulated that the resultant up-regulation of pyruvate oxidation would better match its contraction-stimulated production thereby attenuating the anaplerotic shuttling of excess pyruvate into its TCAI sink. While expansion was significantly attenuated at rest following DCA administration, a similar absolute increase in TCAI during exercise was still observed suggesting that perhaps there was an initial mismatch between glycolytic and PDC flux or that perhaps elevated acetyl-CoA:CoAsh ratios inhibited PDC flux (Gibala et al, 1999).

1.4.2.3. *Glutamine Supplementation*

Bruce et al (2001) examined the potential of glutamine supplementation to, independent of pyruvate availability, enhance TCAI expansion at the onset of moderate intensity exercise. While glutamine supplementation did in fact lead to an increase in TCAI pool size following 10 min of exercise, phosphocreatine utilization and lactate accumulation were similar between trials, suggesting no improvement in aerobic energy provision and certainly no causal link between TCAI pool size and oxidative energy production (Bruce et al, 2001).

1.4.2.4. *McArdle's Disease*

McArdle's patients suffer from a deficiency in muscle glycogen phosphorylase. As a result of this deficiency, these patients are not able to metabolize glycogen and hence have significantly attenuated productions of pyruvate as generated via glycogenolysis/glycolysis. Sahlin et al (1995) observed significantly less TCAI pool size expansion during contraction in McArdle's patients compared to controls. Peak levels of expansion of TCAI in McArdle's patients were in fact 22% that of controls at the same relative intensities, however no observable difference was seen between groups at the same absolute intensity. There was no change in alanine and increases in glutamate and glutamine concentrations in McArdle's patients suggested that the PNC served as an alternative anaplerotic pathway to AAT. Though it was suggested that the lower work rates and maximal oxygen consumption (VO_2 max) in McArdle's patients may have been related to the attenuated TCAI pool size and that there was potentially increased flux through the PNC, ultimately the findings of the study are far from definitive. The morphology of McArdle's disease leads to the potential for limited substrate availability for the TCA cycle and hence a potential reduction in aerobic energy production.

1.4.2.5. *Training Studies*

Two recent training studies (Dawson et al, 2003; Howarth et al, 2002) have attempted to manipulate the expansion of the TCAI pool. Understanding that the pyruvate 'sink' is the result of a mismatch between pyruvate production and oxidation, it was thought that training would elicit a tighter metabolic coupling of the glycolytic pathway and minimizing excess pyruvate production and attenuating anaplerosis. Briefly, both short-term training (Dawson et al, 2003) and long-term training (Howarth et al,

2002) protocols did in fact lead to a significant attenuation in TCAI pool expansion. In addition, the increased sparing of phosphocreatine and reduced accumulation of lactate would further suggest that despite this training induced attenuation, aerobic energy provision was not impeded. However, these studies are not definitive in that there are multiple metabolic adaptations to training and extraneous variables related to the use of human subjects such as dietary control, individual training responses, etc.

To date, a number of experimental manipulations have been undertaken in an attempt to elicit the physiological significance of anaplerosis in relation to aerobic energy provision. Due to the fact that a number of these manipulations involve multiple extraneous variables such as metabolic deficiencies and whole body training effects, perhaps the acute inhibition of the key anaplerotic pathway (AAT) would serve to definitely determine the role of anaplerosis in aerobic energy provision. It can be hypothesized that inhibiting AAT would result in the decreased production of 2-oxoglutarate and hence an attenuation in its flux into the second span of the cycle with the potential effect on aerobic energy provision during contraction elucidated.

1.5 BACKGROUND REGARDING DEVELOPMENT OF PRESENT STUDY

1.5.1. The rat hindlimb model

The rat hindlimb perfusion model is one of the most widely used preparations employed in the study of skeletal muscle metabolism (Bonen et al, 1994). First described by Ruderman et al (1971), the preparation has evolved over the years, however its principle remains the same: experimentally prepared perfusate is introduced into the arterial side of the rat's vasculature at a controlled rate of flow with the venous efflux

collected (Bonen et al, 1994). This preparation has proven quite useful in studying metabolism in that it allows investigators to exercise an extreme amount of control over the metabolic environment which they are interested in (Graham et al, 1998) and perhaps resembles most closely in vivo environments (Bonen et al, 1994). Manipulation of the metabolic milieu can occur at the level of substrate, ion or hormonal introduction and/or omission; muscle stimulation protocols; the selection of fibre types; and in the overall state of health of the animal (Bonen et al, 1994; Graham et al, 1998).

The original preparation described by Ruderman et al (1971) involved the perfusion of a medium containing aged human erythrocytes. Perfusion was cannually introduced into the aorta with its exit via the vena cava. Surgical preparation was extensive and involved the evisceration and excision of most of the pelvic anatomy of the rat followed by the ligation of the vasculature of the hindquarter so as to ensure its isolation. Modifications to this model have come at both the levels of surgical preparation and perfusate composition and delivery. In terms of preparation, the most significant refinement has been the isolation of specific limbs and muscle groups in the rat. Walker et al (1982) found that in cannulating the femoral artery and femoral vein in the groin, as opposed to the aorta and vena cava, contralateral limbs could be used as controls. This lower point of cannulation resulted in the restriction of 95% of inflow to the hindlimb; ensured that those muscles being perfused represented a large portion of the total tissue; allowed for muscle specific stimulation; and resulted in venous effluents reflective of the metabolic response to contraction (Walker et al, 1982; Gorski et al, 1986; Brault et al, 2001). Further support for the lower cannulation comes in the observed stability in the concentrations of key metabolites like ATP, PCr, glycogen, and lactate (Walker et al, 1982; Reimer et al, 1975; Karlsson et al, 1976) and the response of glucose

transport to insulin (Richter et al, 1976) both prior to and following surgical preparation. This maintenance of the metabolic milieu throughout the duration of preparation is key for the extrapolation of analyses to larger metabolic processes.

In addition to surgical modifications, a number of refinements have occurred in relation to the perfusate. While blood bank time-expired human blood was originally a common perfusate, concerns revolving around potential contamination and the time involved in rejuvenating erythrocytes plagued investigators (Bonen et al, 1994). The rejuvenation of aged human erythrocytes ensures adequate oxygen saturation (Rennie et al, 1977) though the use of bovine erythrocytes as an alternative results in a closer match to rat erythrocyte volume than human blood does (Spriet et al, 1985). A less time consuming perfusate is the use of albumin and cell-free mediums which eliminate the potential influence of erythrocyte metabolism (Shiota et al, 1986; McDermott et al, 1989; Bonen et al, 1994). Fluorocarbon emulsions offer a blood substitute for isolated perfusions however the literature on it remains somewhat limited (Shiota et al, 1984; Kubo et al, 1989; Bonen et al, 1994). Perfusion is commonly controlled by a roller pump with more and more investigators opting for a single-pass system of circulation (Spriet et al, 1985) as opposed to the reservoir recycling system (Ruderman et al, 1971) which can affect the constituents of the perfusate.

To date, no preparation has been able to eliminate two remaining limitations of the isolated hindlimb perfusion model that are related to the specificity of the both muscle group contraction and perfusate delivery. It has been well established that in as much as blood flow is unique to both fibre type and location within the hindquarter of the rat, so to is the recruitment pattern (Armstrong et al, 1984; Laughlin et al, 1982; Armstong et al, 1983). In perfusing the hindquarter as a group of muscles, it is impossible to discriminate

whether or not muscles which have received the medium are the same as those which are contracting. Recently, Baker and colleagues (2002) have successfully isolated the gastrocnemius muscle of the rat to control not only the muscle which is contracting, but to also ensure a constant delivery of perfusate to that same muscle. This exquisite surgical isolation will allow investigators to clearly elucidate a mechanistic understanding of a specific muscle rather than examining the entire hindquarter metabolic response.

1.5.2. Effect of cycloserine on muscle metabolism

Cycloserine (4-amino-3-isoxazolidinone), as a structural analogue of alanine, is a naturally occurring, irreversible inhibitor of pyridoxal 5'-phosphate (PLP)-dependent enzymes (Fenn et al, 2003). Existing in both natural D- and synthetic L-isomer forms, it has been therapeutically administered for the treatment of tuberculosis, Alzheimer's disease, schizophrenia and most recently, spinocerebellar ataxia (for specific references, see Ogawa et al, 2003). With regards to muscle metabolism, the D- and L-enantiomorphs have been identified for their capacity to inhibit aminotransferase enzymes (Cornell et al, 1984, Barbieri et al, 1960). L-cycloserine alone has the ability to inhibit six different aminotransferases (Azarkh et al, 1960; Wong et al, 1973). A number of metabolic studies, particularly those concerned with amino acid metabolism have used cycloserine because of its ability to inhibit alanine aminotransferase in particular (Blackshear et al, 1975; Cornell et al, 1984; Barbieri et al, 1960; Shiota et al, 1984; Perez-Sala et al, 1986).

The elucidation of the specificity of action of cycloserine has been an important step towards understanding the employability of the compound in muscle metabolism research. As there exists a great potential for enzyme inhibitors to have multiple sites of action when administered to intact metabolic systems, due caution must be exercised in

extrapolating results from investigations involving isolated hindquarters and incubated mediums (Perez-Sala et al, 1986; Cornell et al, 1984). Barbieri et al (1960) determined in rat liver homogenate that the isomers of cycloserine behave differently in terms of their inhibition of transamination reactions and specificity towards substrates. L-cycloserine was determined to have no effect on the formation of oxaloacetate from L-aspartate and 2-oxoglutarate at the same concentration which strongly inhibits the formation of pyruvate from 2-oxoglutarate and alanine (Barbieri et al, 1960). D-cycloserine on the other hand, requires a concentration equal to fifty times that required of L-cycloserine for the complete inhibition of the alanine aminotransferase (Barbieri et al, 1960). Follow-up investigations by Cornell et al (1984) with incubated hepatocytes demonstrated more specifically that L-cycloserine, administered at a dose (50 μM) resulting in 90% inhibition of alanine aminotransferase, only decreases aspartate aminotransferase activity by a metabolically insignificant 10%.

Blackshear et al (1975) reported that L-cycloserine administration decreased blood alanine release by 80%. In that study, male Ash/Wistar rats received either a single bolus injection of L-cycloserine 1h prior to or immediately following functional hepatectomization. L-cycloserine administration immediately after functional hepatectomy significantly attenuated the rise in blood [alanine] seen in control animals due to the removal of the glucose-alanine cycle. This rise in blood [alanine] was further attenuated with pre-treatment of the inhibitor, suggesting that the combined effect of alanine aminotransferase inhibition and hepatectomy resulted in a decrease in the production and release of muscle alanine. The removal of liver gluconeogenesis increased blood [lactate] and [pyruvate] although blood [glucose] was not significantly different between conditions. This study clearly demonstrated that consistent with the

findings of Ruderman et al (1974) approximately 80% of the alanine released from extrasplanchnic tissues is formed via the alanine aminotransferase reaction, and this enzyme can be inhibited by the administration of L-cycloserine.

1.2 SUMMARY

The physiological significance of changes in the TCAI in response to contraction, is an area of study that remains highly elusive. A number of investigators have previously attempted to alter anaplerotic expansion of the TCAI through manipulation of diet and contraction stimulus in order to determine its relationship to mitochondrial respiration (Spencer et al, 1991; Gibala et al, 2002; Wagenmakers et al, 1990; Gibala et al, 1999; Gibala et al, 1999b; Bruce et al, 2001; Dawson et al 2003; Howarth et al, 2002). While some of these manipulations have been successful in altering expansion, they have at the same time introduced multiple co-variables that make their findings far from definitive. It can therefore be argued that perhaps the best way by which to examine the effect of anaplerosis on aerobic energy provision is to acutely inhibit the expansion of the TCAI pool. Administration of DL-cyloserine appears to be a viable strategy by which to acutely inhibit anaplerosis due to its ability to inhibit alanine aminotransferase, the enzyme which catalyzes the key anaplerotic pathway in cardiac and skeletal muscle. For pragmatic reasons related primarily to the unsuitability of drug administration in humans and the unspecific delivery of that drug to certain muscle groups, the rat hindlimb model involving vascular isolation and the deliverance of perfusate prior to stimulation, is the best model to employ for the inhibition of alanine aminotransferase through administration of DL-cyloserine. Ultimately, if anaplerosis is in fact not causally linked to aerobic energy provision and solely the result of a mismatch between pyruvate

production and oxidation, then the inhibition of alanine aminotransferase, although attenuating the mass action effect of TCAI expansion, should not adversely affect contractile function or metabolism.

CHAPTER 2

THE EFFECT OF CYCLOSERINE ON METABOLISM AND CONTRACTILE FUNCTION IN RODENT SKELETAL MUSCLE

2.1 INTRODUCTION

The TCA cycle, as a key component of oxidative metabolism, experiences an increase in flux by up to 100-fold at the onset of skeletal muscle contraction (Hochachka and Matheson, 1992). Concomitantly, there is an observed contraction-induced increase in the concentration of intermediates of the TCA cycle (TCAI) (Gibala et al, 1998; Gibala et al, 1997; Gibala et al, 1997; Graham and Gibala, 1998; Sahlin et al, 1990; Dawson et al, 2003). The physiological significance of this increase in TCAI during the initial minutes of an acute bout of exercise has been the subject of much debate. There are those proponents who believe that the observed increase in intermediates is crucial for the initiation and up-regulation of aerobic energy provision and ultimately causally related to mitochondrial respiration (Wagenmakers et al, 1990; Sahlin et al, 1990). Alternatively, others propose that the increase in total concentration of TCAI is simply the result of a mismatch between pyruvate production and oxidation and so in fact, the intermediates serve as a pool or sink for excess carbon units (Gibala et al, 1998; Constantin-Teodosiu et al, 1999).

A number of investigations have attempted to manipulate the expansion of the TCAI during contraction. Previous studies involving glutamine ingestion (Bruce et al, 2001) and low glycogen availability (Gibala et al, 2002) suggested that regardless of enhancing TCAI expansion, there was no concomitant improvement in aerobic energy provision as evidenced by similar levels of PCr degradation and lactate accumulation

compared to the control state. Recent investigations (Dawson et al, 2003; Howarth et al, 2003) have employed both short and long-term training protocols in an attempt to attenuate TCAI expansion by eliciting a better match between pyruvate production and its oxidation. Following training, TCAI expansion was attenuated with no impairment of aerobic energy provision as judged by changes in maximal oxygen consumption (VO_2 max), PCr degradation, lactate accumulation, and glycogen sparing (Dawson et al, 2003; Howarth et al, 2003). The difficulty with training studies however is that a number of metabolic variables are manipulated in response to the training. Ultimately, the acute inhibition of anaplerotic expansion is warranted in order to test the hypothesis that a decline in total TCAI concentration is causally related to contraction-induced mitochondrial respiration.

The purpose of the present study was to examine the effect of acutely inhibiting muscle TCAI expansion and determining the effect on muscle metabolism and contractile function. We hypothesized that the inhibition of alanine aminotransferase, a key anaplerotic pathway, would lead to an attenuated expansion of the TCAI pool without compromising aerobic energy provision.

2.2 METHODS

2.2.1. Animals

Forty healthy male Wistar rats with a mean total body weight of ~400 g and hindlimb (gastrocnemius) weight of ~2.5 g were used for this study. There were four experimental conditions: Control-Rest (n=11), Control-Stimulation (n=10), Cycloserine-Rest (n=9), Cycloserine-Stimulation (n=10). The experimental protocol was approved by

University of Nottingham, Queen's Medical Centre, United Kingdom Home Office with all experiments conducted at the University of Nottingham.

2.2.2. Isolated Rat Gastrocnemius Muscle Perfusion Model

2.2.2.1 Equipment

Surgical mask and gloves

Heated surgical table maintained at ~ 40 ° C

Rectal thermometer (Omron, Hamburg, Germany)

Electric hair clippers

Gauze and cotton tips

Scalpel and blade number 11

Curved forceps

Spring bow scissors

Spencer Well clamps

Small bulldog clip

Small and large curved blunt scissors

1 ml and 5 ml disposable syringes

21G, 23G and 25G disposable needles

Electric cautery or diathermy (type 474, VDE, Germany)

Tissue glue (Histoacryl or tissue adhesive (3M,UK))

Polypropylene tubing (Portex. Refs: 800 / 100 / 420 / 100 (tracheotomy); 800 / 100 / 200 / 100 (Femoral artery and vein)).

Sterile saline (0.9 % NaCl) and heparinized sterile saline (10 U ml^{-1})

2.2.2.2 Surgical Procedures

A rat was anaesthetised with a long acting barbiturate (Inactin; Sigma T-133): 120 mg kg^{-1} body mass, i.p), which was dissolved in sterile saline and delivered using a 1 ml disposable syringe and 25G needle. Surgical intervention was not performed until a depth of anaesthesia was reached that inhibited pedal and corneal reflex. The hair from the left hindquarter and throat region was then shaved using electric hair clippers. A tracheotomy was then performed.

2.2.2.3. Tracheotomy

An incision (~ 2 cm in length) was made along the skin in front of the throat region, in the direction of head to tail, which exposed two overlapping muscle blocks that are situated in front of the trachea. Using blunt forceps, the two muscle blocks were dissected apart to enable visualisation of the trachea. An incision was made through approximately three-quarters of the tracheal lumen, in between the tracheal cartilaginous rings, and a ~ 5 cm length of polypropylene tubing (Portex. Ref: 800 / 100 / 420 / 100) was inserted 1 – 1.5 cm into the trachea towards the lungs. A surgical thread was tied and knotted around the cannulated trachea securing the tubing in place. This allowed the rat to breath freely without obstruction from the tongue and mouth region for the remaining duration of the surgical procedure. Using tissue glue, the initial incision in the skin was sealed, providing additional support for the tracheal tubing. The rat maintained respiration of its own accord.

2.2.2.4. Vascular isolation of the Gastrocnemius muscle

The skin behind the left Achilles tendon of the anaesthetised rat was pinched and a cut made through the skin without damaging the underlying musculature. To separate the skin away from the hind limb musculature, blunt scissor blades (closed) were inserted into the incision between the skin and muscle, the blades were then carefully opened to prise the skin away from the musculature. This process was repeated, working upward from the foot towards to back of the knee, and then applied to the whole hindquarter. The loosened skin was then removed from the entire hindquarter using a cauteriser and scissors, which allowed the Femoral artery and Femoral vein to be visualised. All the branches of the Femoral artery and Femoral vein from the point where they originate in the left hind quarter, to where they innervate the back of the Gastrocnemius muscle, were carefully tied off and / or cauterised, leaving only the Femoral artery and Femoral vein intact. Therefore these vessels provided the sole supply of blood to and from the Gastrocnemius muscle and both vessels were separated and dissected from one another to facilitate cannulation and perfusion of the muscle via these vessels.

2.2.2.5. Exposing the Gastrocnemius muscle

In order for the Gastrocnemius muscle to be exposed, the overlaying musculature (Biceps Femoris) was removed by cauterisation without damaging the Gastrocnemius itself, or the sciatic nerve. This was performed by gentle elevation of the left foot, which revealed the outline of the Gastrocnemius muscle. Using this as a visual guide, a small puncture was carefully made through the overlaying musculature, parallel to the tibia, from the Achilles tendon towards the back of the knee using forceps. Once punctured, the point of entry was carefully teased apart enabling visualisation of the sciatic nerve and

the back of the Gastrocnemius muscle. Curved forceps were then placed down inside the puncture, between the back of the Gastrocnemius and the inner-side of the overlaying musculature, and the tips pushed through the overlaying musculature in the direction of the groin. Once pierced, the muscle was cauterised along the forceps, protecting the nerve and Gastrocnemius muscle from the cauterisation process, leaving the overlaying musculature from the inner thigh region only attached along the front of the tibia. This muscle was then peeled back and cauterised along the tibia until the entire inner-side of overlaying musculature was removed. Blunt curved scissors were then used to cut the connective tissue in between the outer-side of the Gastrocnemius muscle and the inner-side of the outer facing overlaying musculature. Using the same technique as previously described, the muscle overlying the Gastrocnemius muscle was removed. At this stage the whole Gastrocnemius was in tact and attached to the heel by the Achilles tendon and at the back of the knee. Using blunt curved scissors, the Gastrocnemius muscle was teased away from the back of the tibia in between the Achilles tendon and the back of the knee. Surgical thread (grade 1 / 0) ~ 45 cm was placed in between the tibia and the Gastrocnemius muscle and a double knot tied around the Achilles tendon, leaving one end of thread long enough for later attachment to the isometric force transducer. The Gastrocnemius muscle was then detached from the heel by cutting the Achilles tendon in between the knot and the heel, leaving the Gastrocnemius muscle attached at the back of the knee only.

2.2.2.6. Cannulating the Femoral artery and vein

Cannulas for both the Femoral artery and the Femoral vein were prepared by inserting a blunt needle (size 23G) into ~ 25 cm lengths of polypropylene tubing (800 /

100 / 200 / 100), and a 1 ml disposable syringe containing heparinised saline (10 U / ml) was attached to the needle. A bevel was made in each piece of tubing at the opposite end to the needle, to facilitate the cannulation of the vessels. The filled syringes were then plunged slowly in order to pass heparinised saline into the cannulas, this allowed a clear perfusion route through the cannulas to be checked. Two lengths of surgical thread (2 / 0, ~ 30 cm in length) were passed underneath the Femoral artery and each were tied in a loose loop around the vessel at either end of the artery. The same was done for the Femoral vein. Starting with the Femoral vein, the loop nearest to the aorta was tied tightly around the vein as far way from the Gastrocnemius as possible. Spencer Wells were used to clamp the end of the thread where the knot had been tied, creating a small amount of tension on the thread so that the vein was tort. A small bulldog clip was placed onto the vein as near to the Gastrocnemius as possible. Using springbow scissors, an incision was made approximately three-quarters of the way through the lumen of vein, and using fine curved forceps the beveled end of one of the pre-made cannulas was inserted into the lumen of the vessel. The cannula was passed down inside the vessel until there were only a few millimetres between the end of the cannula and the edge of the Gastrocnemius muscle. The second loosely tied thread around the vein, was then tied to secure the venous cannula at the point where it was inserted. This process was repeated on the Femoral artery, however the use of the bulldog clip for the artery was not required. Once cannulation of both vessels was complete, the syringe on the venous cannula was removed, and heparinized saline was very slowly pushed through the syringe on the arterial cannula. The entire content of the arterial syringe was used to flush through the muscle; this prevented any blood clots during the subsequent perfusion. After flushing, the syringe was placed back onto the venous cannula needle and the rat was carefully

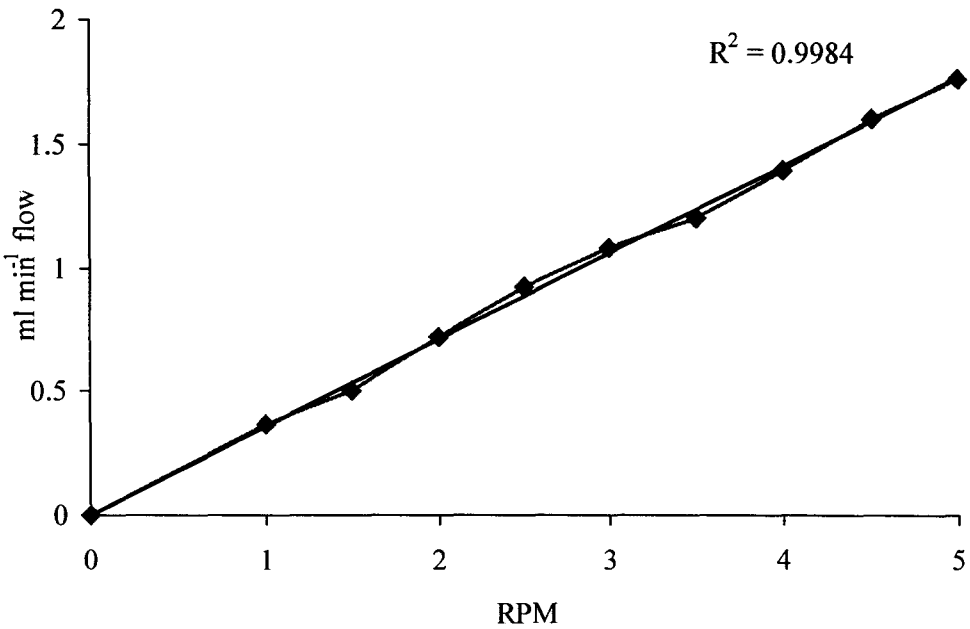
moved to the perfusion apparatus and connected via the arterial cannula to an already primed perfusion delivery system. At this stage the anaesthetised rat was sacrificed, using a direct injection of Sagatal (Pentobarbital Sodium, ~ 5 ml) into the heart.

2.2.2.7. Perfusion and contraction of the Gastrocnemius muscle

Once the arterial cannula was connected to the perfusate delivery system (See section 2.2.2.8.), the blood pressure transducer (SensoNor, Horten, Norway) on the arterial line recorded the perfusion pressure across the muscle. The flow rate was then increased in a stepwise fashion until a rate of approximately 15 ml min^{-1} $100g^{-1}$ of wet muscle was reached. This was determined from an estimation of muscle weight achieved by using the isometric force transducer as a strain gauge, and a calibrated peristaltic pump (model 205U, Watson and Marlow, Cornwall, UK) (See Figure 2.1.1.). The exposed left knee was then clamped into a ridged position so that muscle force could be measured without inertia from the movement of the rat carcass during stimulation. After securing the knee in place, the surgical thread attached to the Achilles tendon was then tied to a calibrated isometric force transducer (Grass Instruments, Warwick, USA). The hook electrode (Harvard Instruments, USA) was then placed around the sciatic nerve near to where it innervated the Gastrocnemius muscle, and held in position by a clamp attached to the framework of the perfusion model apparatus (See section 2.2.2.8.). The electrode wires were then connected to the electrode and the stimulator (model S88, Grass Instruments, Quincy Mass, USA). Using a series of single electrically evoked twitches and alteration of the resting length of the muscle, achieved by moving the adjustable knee clamp, the resting length that evoked the maximal peak twitch was determined. Blood flow was increased two fold at the start of stimulation to simulate contraction induced

muscle vasodilatation. Stimulation lasted for a period of 10 min and involved trains of 1Hz, 0.3msec at 2V. The muscle was immediately excised at the end of the contraction duration and frozen in liquid nitrogen. The muscles were stored under these conditions until required for further analysis.

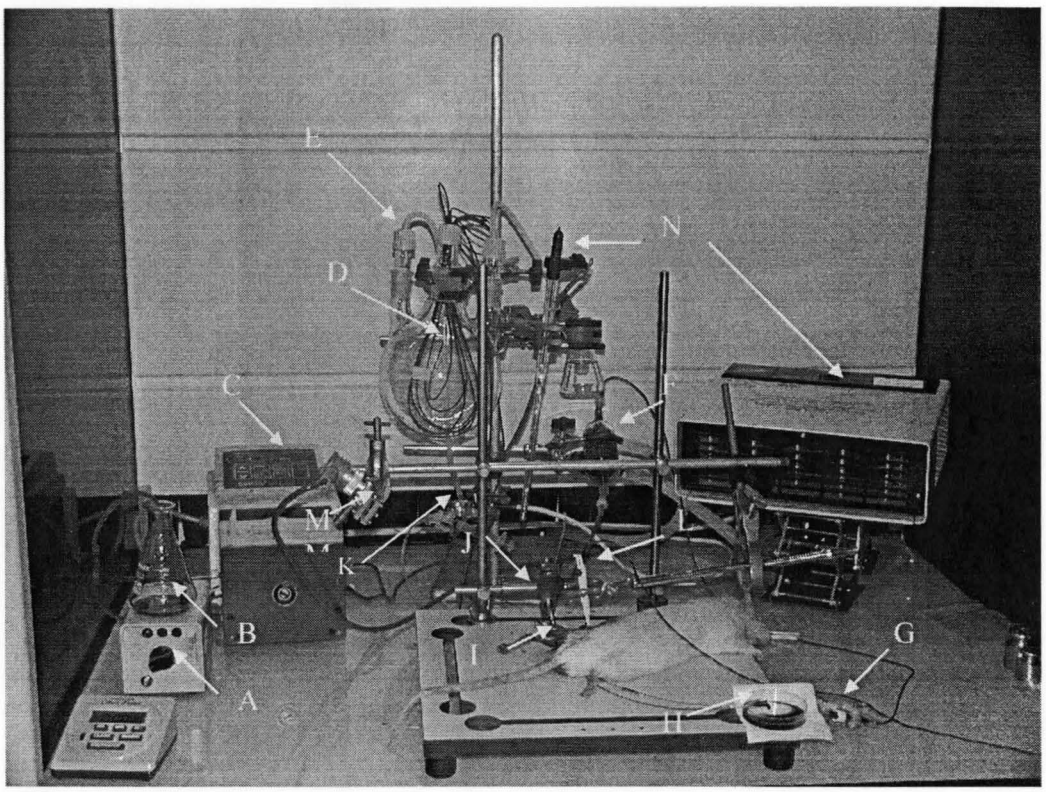
Figure 2.1.1 Calibration graph for Watson and Marlow peristaltic pump model 205U



2.2.2.8. Perfusion Model Apparatus

Figures 2.1.2 and 2.1.3 show the apparatus, and the set-up for measuring perfusion and contraction of the rat Gastrocnemius muscle.

Figure 2.1.2 Perfusion Model Apparatus (1)



KEY

A : Heated stirrer

B : Perfusate reservoir

C : Peristaltic pump

D : Silastic lung / gas permeable tubing

E : 95 / 5 % O_2 / CO_2 supply

F: Water heated jacket

G: Femoral arterial cannula

H : Femoral venous cannula

I : Gastrocnemius muscle

J : Knee clamp

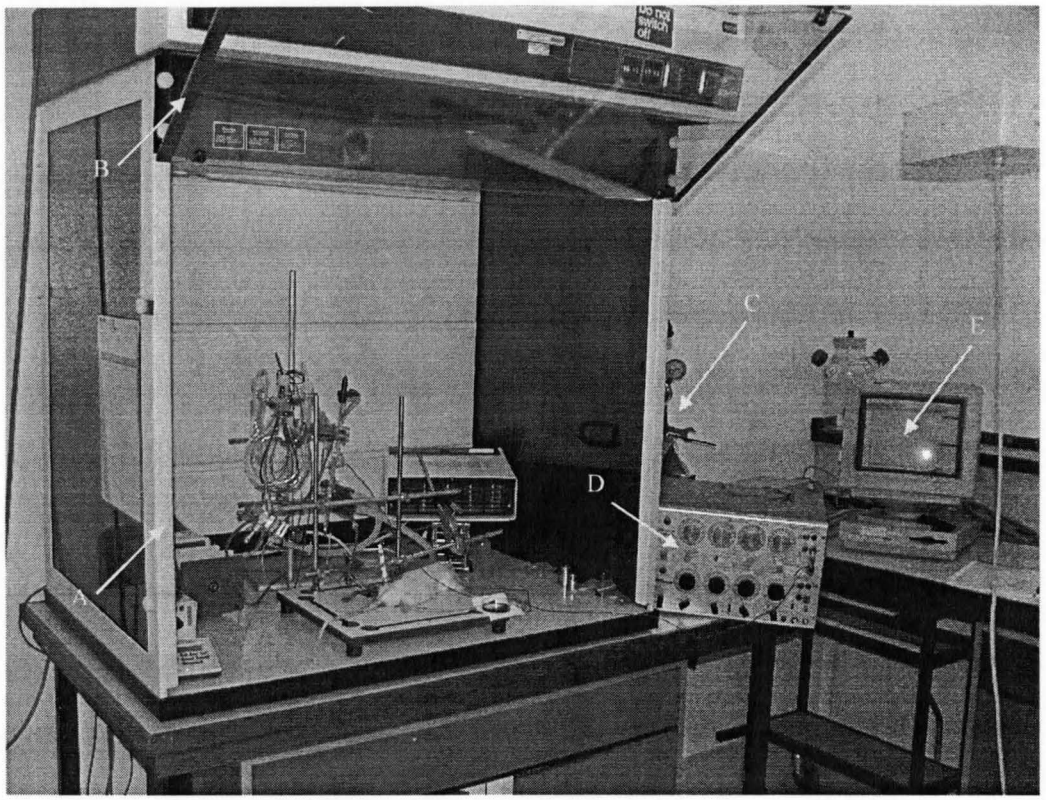
K : Blood pressure transducer

L : Sciatic nerve hook electrode

M : Grass isometric force transducer

N : Thermocouple heater

Figure 2.1.3 Perfusion Model Apparatus (2)



Key

A : Temperature controlled cabinet

B : Fold down cabinet screen

C : O_2 / CO_2 gas cylinder

D : Grass S88 Stimulator

E : Mac Lab data acquisition software

2.2.2.9. Isolation of Red Cells From Whole Blood

Whole blood from pigs was collected from a Nottingham abattoir in collecting vessels containing citrate (3 % citrate (Sigma-Aldridge), mixed with whole blood in a ratio of 1 part citrate : 9 parts blood).

Fifty millilitres (ml) of 6 % Dextran (Sigma-Aldrich) dissolved in distilled water was placed into 200 ml conical centrifuge tubes (Nalgene; 1 tube provides perfusate for 1 perfusion experiment) to which 150 ml of whole blood was added. The tube was gently inverted to mix and the red blood cells (RBC's) were left to sediment at room temperature for 1 hour. The supernatant above the sedimented RBC was carefully aspirated and discarded. The remaining RBC pellet was resuspended in 200 ml of 0.9 % sterile saline using a rolling mixer, for approximately 10 minutes. The resuspended RBC's were then centrifuged for 15 minutes at 2000 g at 4 °C, and the aspiration, resuspension and centrifugation procedure repeated a further three times. After the final wash, the RBC pellet was resuspended in filtered modified Krebs buffer (See section 2.2.2.10) to a final volume of 200 ml, and using the rolling mixer allowed to resuspend for 10 minutes. The RBC's were then centrifuged for 15 minutes at 2000 g at 4 °C. This resupension process was repeated and the resulting suspension was stored at 4 °C until required (kept for up to 5 days).

Prior to use, the perfusate was placed on a rolling mixer for 10 minutes and then centrifuge for 15 minutes at 2000 g at 4 °C and the RBC pellet resuspended in 30 ml of filtered modified Krebs buffer. Haematocrit (Hct), pH and glucose concentration levels in the perfusate were then recorded using a blood gas analyser (ABL 70 or 600, Radiometer Copenhagen Ltd) and glucose analyser (Yellowsprings). A glucose concentration of 6 mM, Hct of 47 % and a pH 7.4, were obtained by addition of glucose

(500 mM stock), $NaHCO_3$ (500 mM stock) and /or Krebs buffer respectively, as required. Control conditions had a saline-containing perfusate while cycloserine conditions had a total dose of 0.05 mg/g of DL-cycloserine (Sigma C-7005) added to the perfusate prior to perfusion.

2.2.2.10. Krebs Buffer for Suspension of Isolated Red Cells

Krebs buffer containing 118 mM NaCl (BDH), 4.7 mM KCl (BDH), 1.2 mM KH_2PO_4 (BDH), 1.2 mM $MgSO_4$ (BDH), 25 mM $NaHCO_3$ (BDH), 6 mM glucose (Sigma-Aldrich), 1.27 mM $CaCl_2$ (BDH), 5 % BSA (Sigma-Aldrich), 100 μ units ml^{-1} Insulin (TCS Biologicals) and 0.15 mM Na pyruvate (Boehringer Mannheim) was made and the pH was adjusted to 7.4 using 1M $NaHCO_3$. The buffer was filtered through 0.44 μm filter paper prior to use.

2.2.2.11. Muscle Function Data Analysis

The force production from electrically stimulated Gastrocnemius muscle was recorded via an isometric force transducer (Grass Instruments, Warwick, USA), which was directly connected to a Mac Lab recording system (Mac Lab 400, AD Instruments, Australia). The software packages (Chart v.3.1) recorded the force output via the isometric force transducer, where measurements of resting tension, peak tension, and area under the time-tension curve could be obtained following completion of each experiment. Data was subsequently expressed as force (Kg. 100 g^{-1} of wet muscle), or as a percentage of peak tension, or as area under the time-tension curve (integral of force over time (grams·$second^{-1}$)).

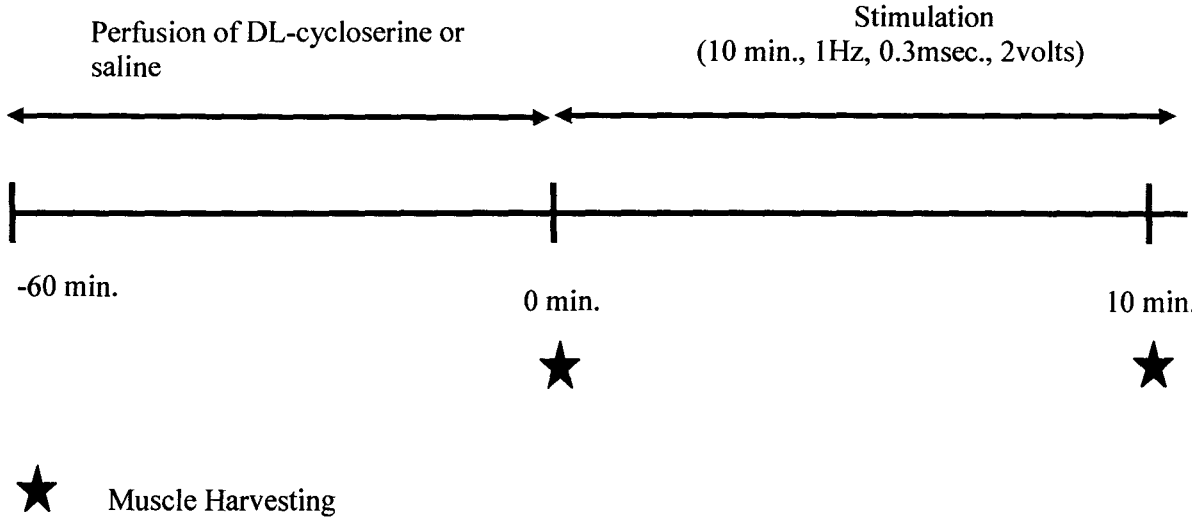


Fig. 2.1. Schematic of experimental protocol.

2.2.3. Muscle Analyses

After the gastrocnemius muscle was snipped away from its attachment posterior to the knee, the muscle was immediately plunged into liquid nitrogen. All samples were divided into two pieces. One piece was kept in liquid nitrogen for subsequent analysis of muscle enzyme activities, while the other piece was freeze dried, powdered and dissected free of blood and connective tissue. All samples were stored at -86°C until they were ready to be analyzed.

A ~10 mg portion of freeze-dried muscle was extracted on ice using 0.5 M PCA containing 1 mM EDTA (volume in µl = mg freeze dried tissue x 80) for 10 min. Samples were then centrifuged and the supernatant was collected and weighed, with 2.2 M $KHCO_3$

being added to the supernatant to neutralize the extract (volume in μl = supernatant weight (mg)/4.1). Subsequently samples were vortexed and centrifuged and the resulting supernatant frozen and stored for the subsequent analyses of most enzymes and metabolites, except glycogen, pyruvate, and 2-oxoglutarate. Pyruvate and 2-oxoglutarate were analyzed using fresh extract (volume in μl = mg freeze dried tissue x 40), but otherwise the extract was stored at -86°C until each metabolite assay was performed. The extraction procedure to measure glycogen concentration was adapted from Harris and colleagues (1974) and used 500 μl of 2.0 N HCL added to ~ 2 mg freeze dried muscle. Samples were incubated at 100°C for 2 hrs, and then 500 μl of 2.0 NaOH was added. The extract was stored at -86°C until ready for analysis. Pyruvate, malate, fumarate, 2-oxoglutarate, citrate, isocitrate, alanine, glutamate, creatine (Cr), phosphocreatine (PCr), ATP, muscle lactate (La) and glycogen (glucose assay) concentrations were determined with a Hitachi F-2500 fluorescence spectrophotometer, using fluorometric assay procedures described by Passoneau and Lowry (1993).

For determination of alanine aminotransferase (AAT) and citrate synthase (CS) maximal activities, muscle samples were homogenized using methods described by Henriksson and colleagues (1986) to a 50 times dilution. AAT activity was determined on a Hitachi F-2500 fluorometer using a protocol described by Passoneau and Lowry (1993) and adapted for the assay of human muscle samples (see Appendix III). The main change was the use of 10 μl of homogenate which had been diluted to 800 times using 20mM imidazole buffer, pH 7.0, containing 0.02% BSA (Henriksson et al, 1986). CS activity was determined on an Ultrospec 3000 pro UV/Vis Spectrophotometer using a method described by Carter and colleagues (2001). An extraction dilution of 50 times was used and the results were expressed in $mmol.kg^{-1}$ $ww.min^{-1}$. The intra-assay

reproducibility for the various metabolite and enzyme measurements are summarized in Appendix III.

2.2.4. Statistics

All muscle metabolites were analyzed using a one-way analysis of variance (ANOVA) owing to the four independent groups of rats. When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey HSD post hoc test. Significance for all analysis was set at $P \leq 0.05$. All values are presented as mean ± standard error of the mean (SEM).

2.3 RESULTS

2.3.1. Intramuscular metabolites

Muscle [glycogen] was not different ($P > 0.05$) between control and cycloserine conditions at rest or following stimulation (Table 2.1, Fig. 2.2). Stimulation resulted in a ~25% ($P \leq 0.05$) reduction in [glycogen] in both conditions. Muscle [pyruvate] and [lactate] increased following contraction, though there was no difference between conditions (Table 2.1, Fig. 2.3).

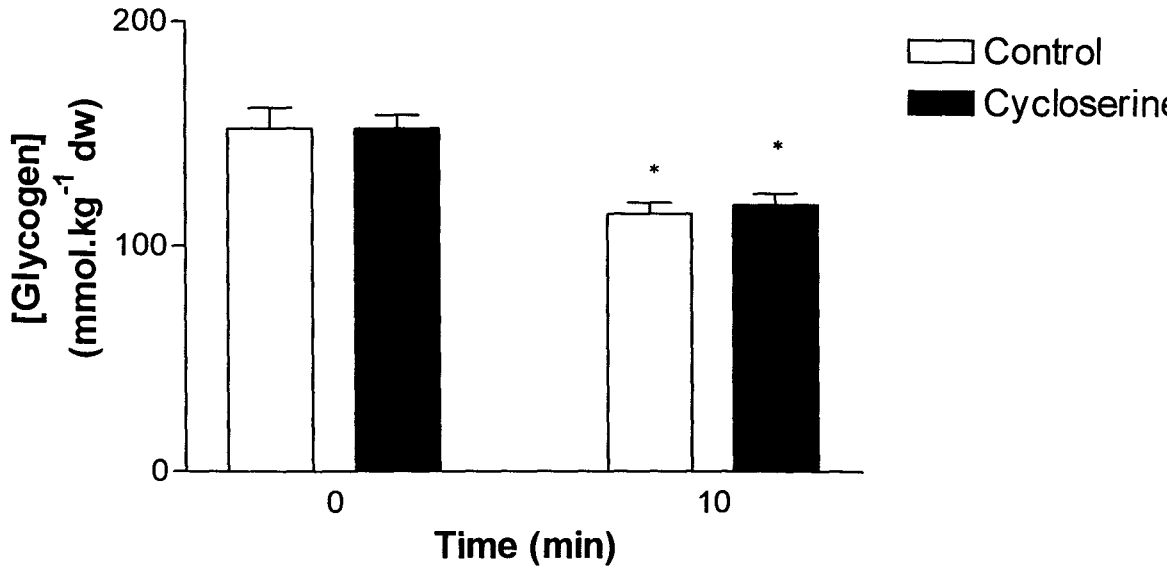


Fig. 2.2. Concentration of muscle glycogen at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min).

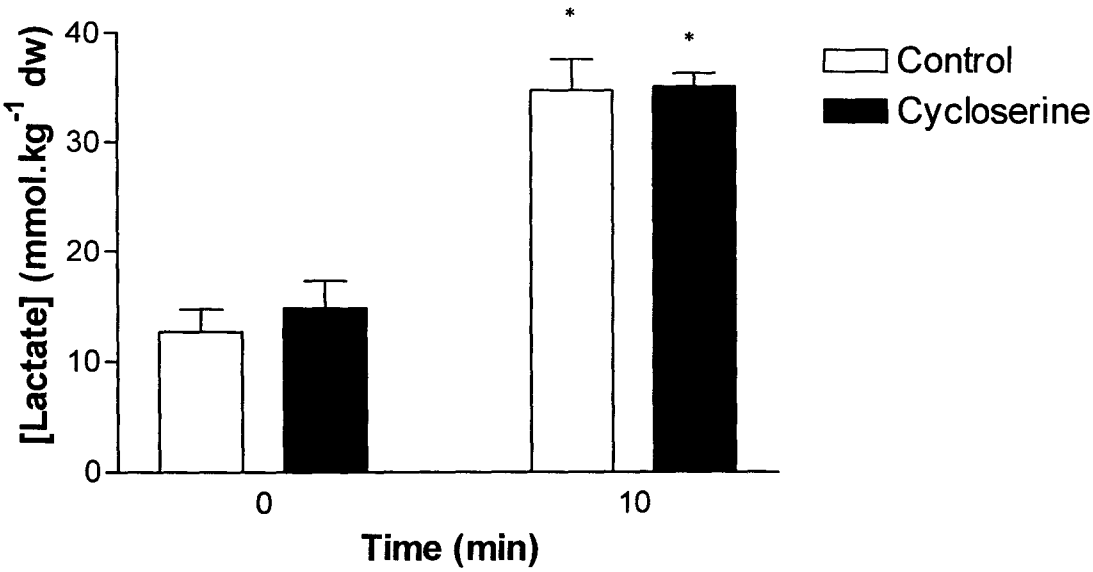


Fig. 2.3. Concentration of muscle lactate at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min).

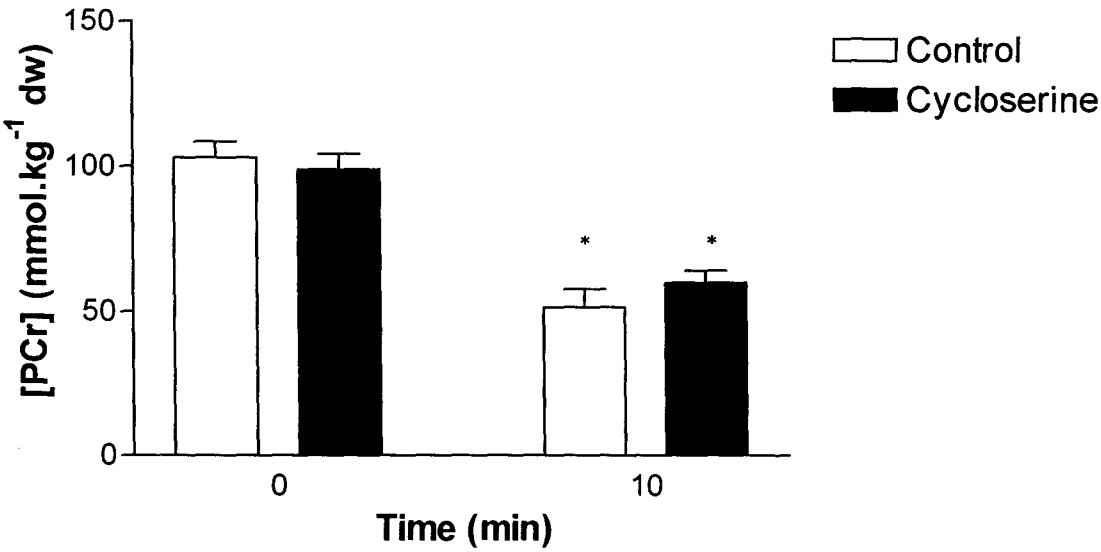


Fig. 2.4. Concentration of muscle phosphocreatine at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min).

Table 2.1. ***Muscle metabolite concentrations at rest and following stimulation***

	Control		Cycloserine	
	Rest	**Stimulation**	**Rest**	**Stimulation**
Cr	51.9 ± 4.3	88.6 ± 5.6*	54.9 ± 4.4	87.4 ± 2.8*
PCr	102.9 ± 5.6	51.4 ± 6.3*	98.9 ± 5.3	59.8 ± 4.3*
ATP	25.6 ± 0.5	22.9 ± 0.6*	25.4 ± 0.4	22.9 ± 0.4*
Lactate	12.7 ± 2.0	34.6 ± 2.9*	14.8 ± 2.5	35.0 ± 1.2*
Pyruvate	0.36 ± 0.06	0.72 ± 0.03*	0.26 ± 0.03	0.60 ± 0.04*
Glycogen	152 ± 9	114 ± 5*	152 ± 6	118 ± 5*

Values are means ± SEM in mmol.kg $^{-1}$dw. * Significantly different from rest. $P \leq 0.05$.

[PCr] decreased following contraction in both control and cycloserine conditions (Table 2.1, Fig. 2.4) but there was no difference between conditions. Similarly, ATP was lower ($P \leq 0.05$) during contraction compared to rest, however there was no difference between conditions (Table 2.1, Fig. 2.5).

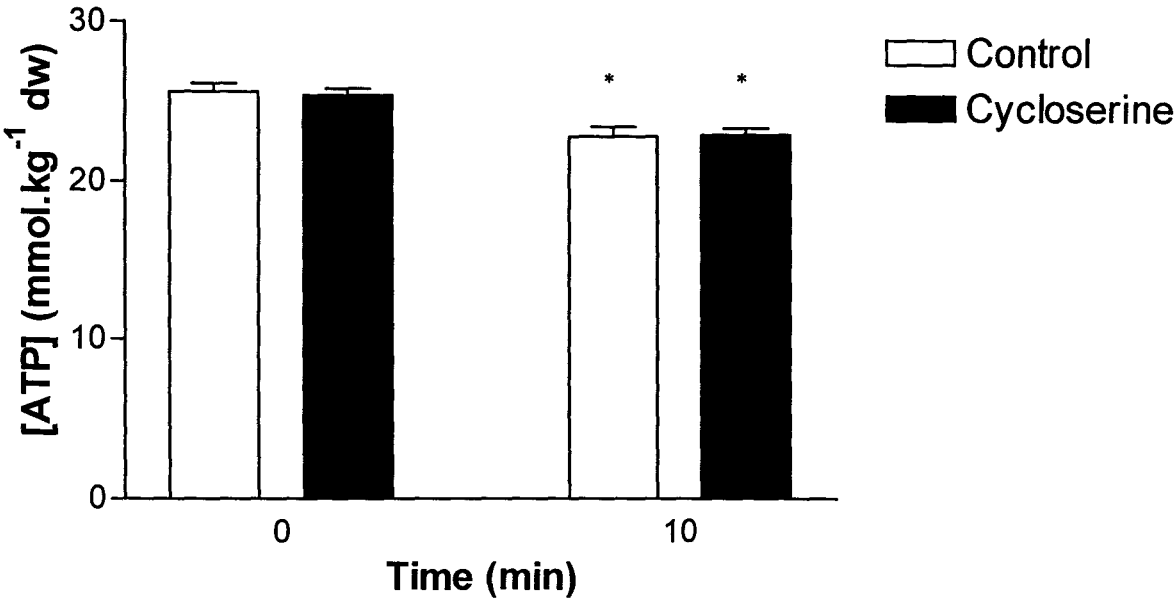


Fig. 2.5. Concentration of muscle ATP at rest and stimulation following pre-treatment with saline or cycloserine, control and cycloserine. $^{*}P \leq 0.05$ vs Rest (0 min).

2.3.2. TCAI

There was no difference in the total concentration of the 5 measured TCAI (ΣTCAI) at rest, however, the contraction-stimulated expansion of the TCAI pool was attenuated ($P \leq 0.05$) in the cycloserine compared to control condition. Importantly, the

[TCAI] during cycloserine contraction was not different ($P \leq 0.05$) from control rest ΣTCAI (Fig. 2.6). Values for the five individual TCAI are summarized in Table 2.2. The contraction-induced increase in the ΣTCAI was dominated by changes in malate which accounted for ~36-47% of the overall pool expansion in both conditions respectively (Fig. 2.7.). [Citrate] did not increase with contraction in either condition (Fig. 2.8.).

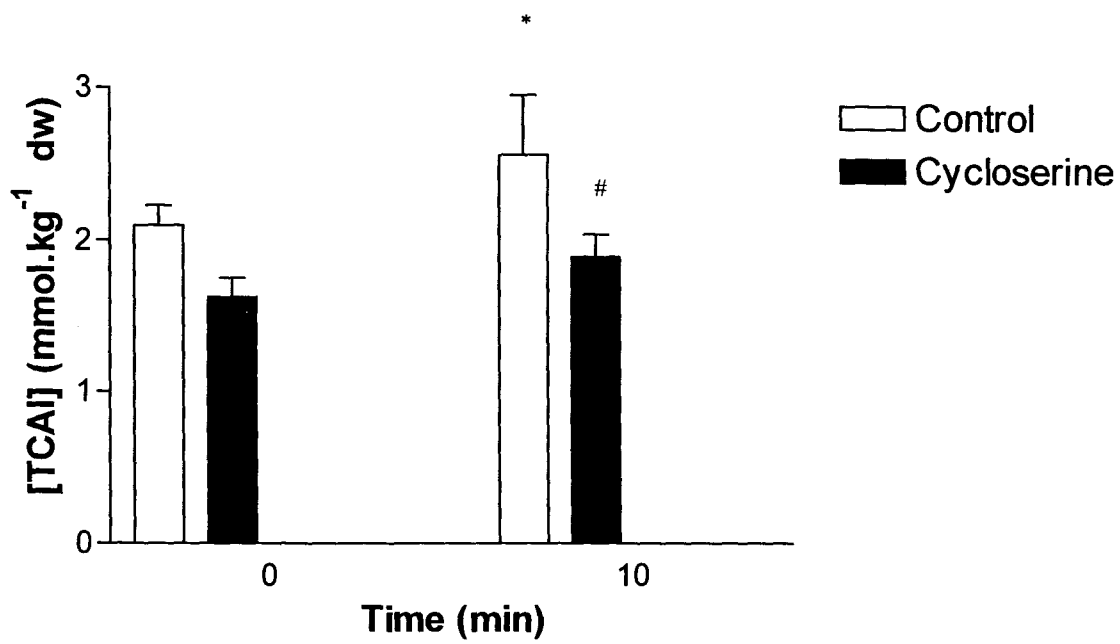


Fig. 2.6. Sum of muscle TCAI at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min). # $P \leq 0.05$ vs control at the same time point.

Table 2.2. ***TCAI concentrations in muscle at rest and following stimulation***

	Control		Cycloserine	
	Rest	**Stimulation**	**Rest**	**Stimulation**
Malate	0.67 ± 0.08	1.21 ± 0.07*	0.43 ± 0.03	0.68 ± 0.04*#
Fumarate	0.10 ± 0.02	0.20 ± 0.02*	0.06 ± 0.01	0.13 ± 0.01*#
2-oxoglutarate	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.09 ± 0.00
Citrate	1.07 ± 0.12	0.89 ± 0.09	0.99 ± 0.11	0.96 ± 0.12
Isocitrate	0.17 ± 0.01	0.18 ± 0.01	0.06 ± 0.01#	0.04 ± 0.01*#
Sum of TCAI	2.10 ± 0.13	2.56 ± 0.12	1.62 ± 0.13	1.89 ± 0.15#

Values are means ± SEM in mmol.kg^{-1} d.w. * Significantly different from rest. $P \leq 0.05$. #Significantly different from control at same time point. $P \leq 0.05$.

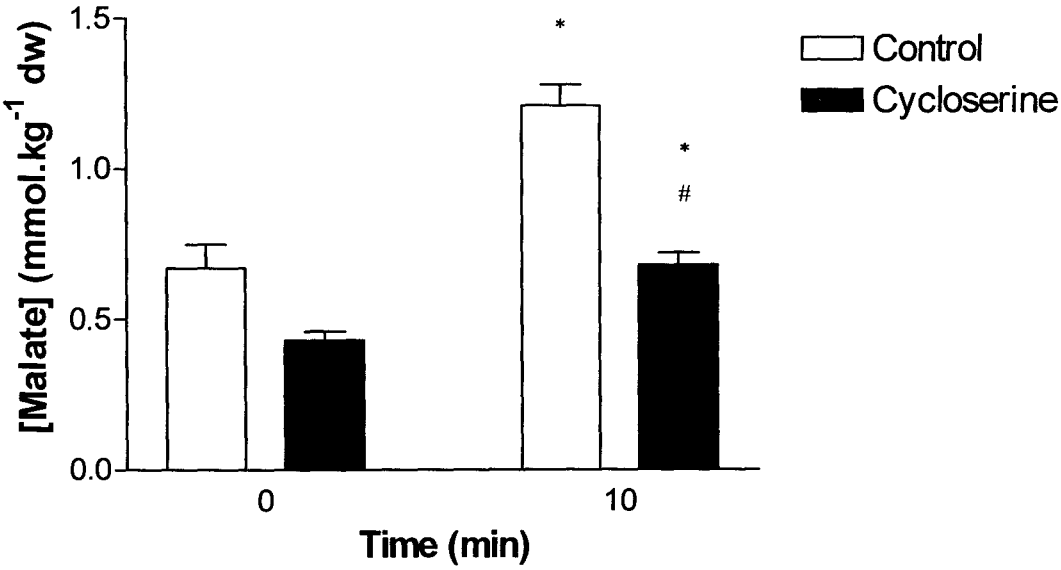


Fig. 2.7. Concentration of muscle malate at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min). # $P \leq 0.05$ vs control at the same time point.

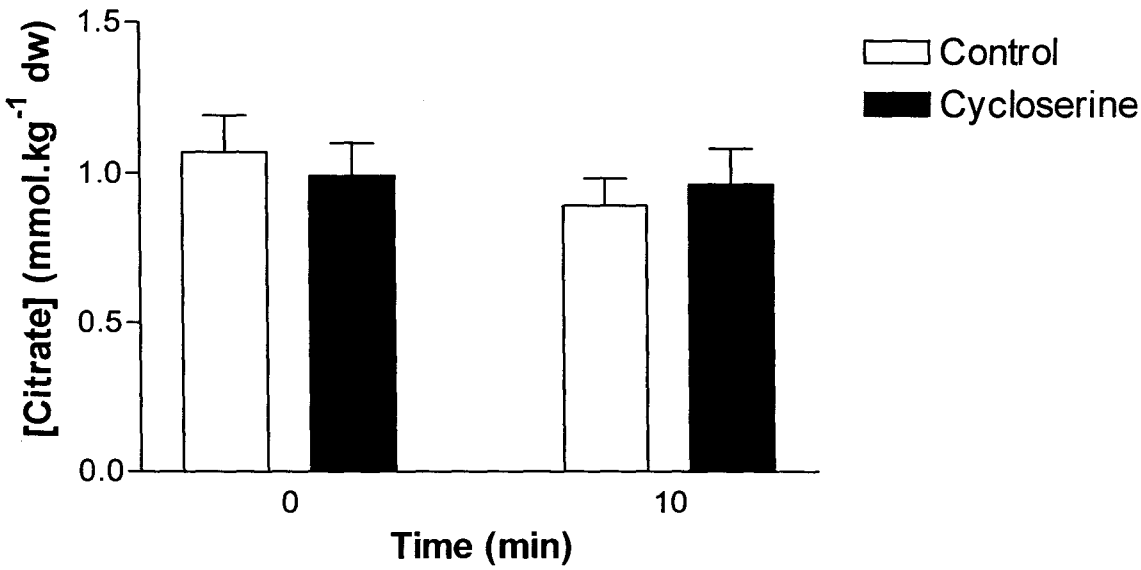


Fig. 2.8. Concentration of muscle citrate at rest and stimulation following pre-treatment with saline or cycloserine.

2.3.3. Muscle amino acids

In comparing control vs. cycloserine conditions, there was no difference in the resting concentrations of alanine or glutamate (Table 2.3, Fig. 2.9, Fig. 2.10). The contraction- induced net change in glutamate and alanine was significantly attenuated in the cycloserine condition by ~50% and ~37% respectively ($P \leq 0.05$)(Fig. 2.9). Contraction resulted in a significant ($P \leq 0.05$) decrease in glutamate and increase in alanine in the control condition only.

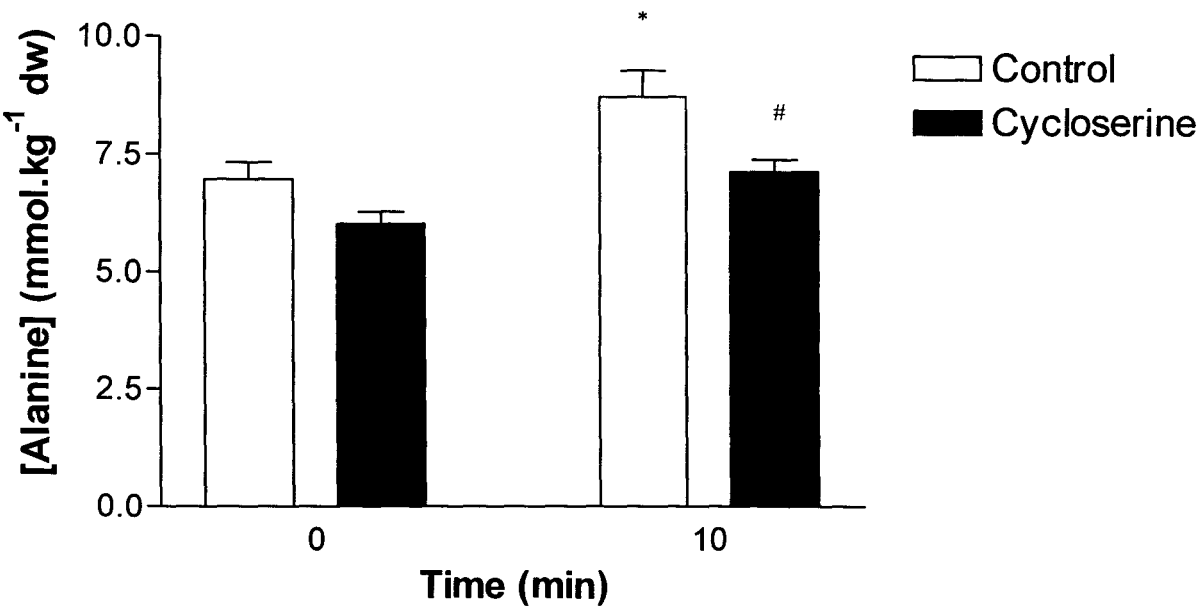


Fig. 2.9. Concentration of muscle alanine at rest and stimulation following pre-treatment with saline or cycloserine. * $P \leq 0.05$ vs Rest (0 min). # $P \leq 0.05$ vs control at the same time point.

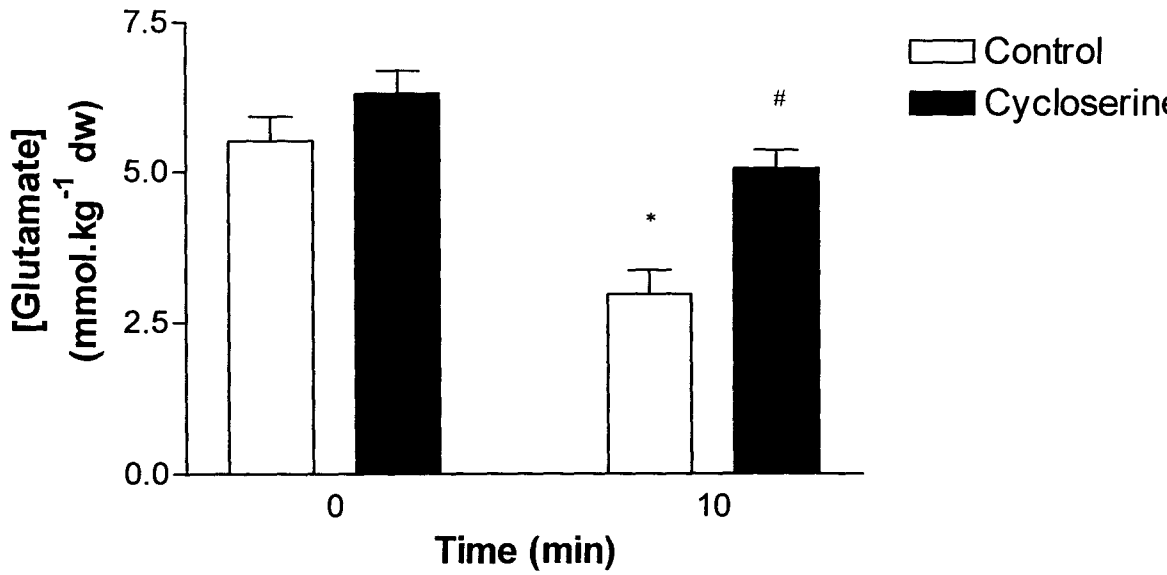


Fig. 2.10. Concentration of muscle glutamate at rest and stimulation following pre-treatment with saline or cycloserine.* $P \leq 0.05$ vs Rest (0 min). # $P \leq 0.05$ vs control at the same time point.

2.3.4. Muscle enzymes

The maximal enzyme activity of AAT was ~83% lower ($P \leq 0.05$) in the cycloserine condition at both rest and following stimulation compared to control (Table 2.4, Figure 2.11). Citrate synthase maximal activity was similar between conditions at rest with a slightly less ($P \leq 0.05$) maximal activity following stimulation in the cycloserine compared to control group (Table 2.5).

Table 2.3. ***Amino acid concentrations in muscle at rest and following stimulation***

	Control		Cycloserine	
	Rest	**Stimulation**	**Rest**	**Stimulation**
Alanine	6.96 ± 0.37	8.71 ± 0.56*	6.02 ± 0.25	7.12 ± 0.26#
Glutamate	5.52 ± 0.41	2.97 ± 0.40*	6.31 ± 0.38	5.05 ± 0.30#

Values are means ± SEM in mmol.kg^{-1} d.w. * Significantly different from rest. $P \leq 0.05$. # Significantly different from control at same time point. $P \leq 0.05$.

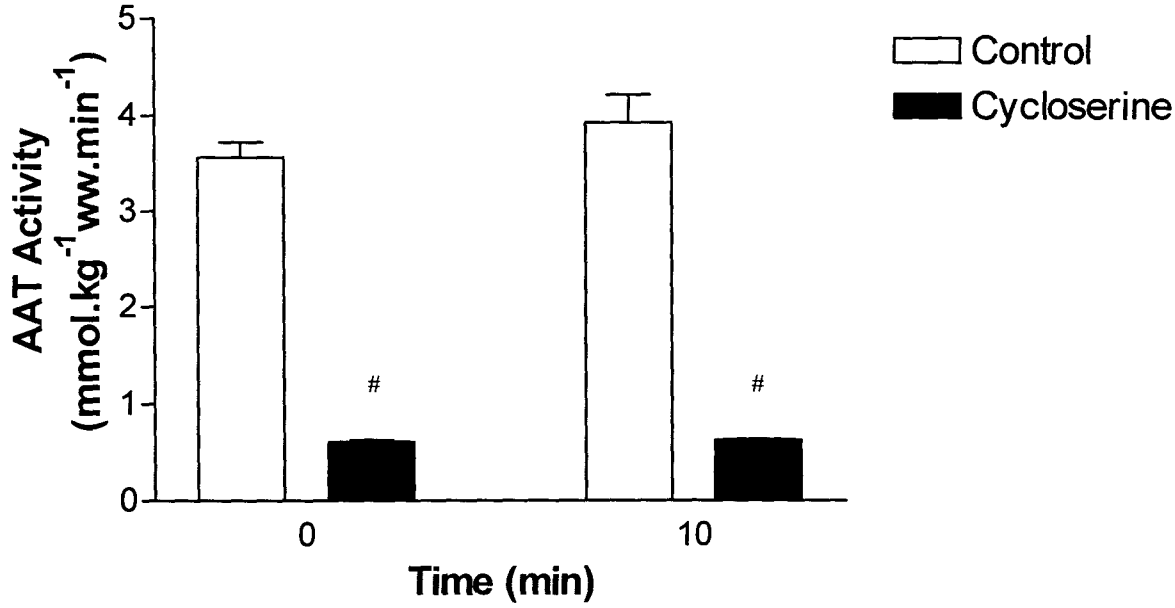


Fig. 2.11. Enzyme activity of muscle alanine aminotransferase at rest and stimulation following pre-treatment with saline or cycloserine. control and cycloserine. $^{\#}P \leq 0.05$ vs control at the same time point.

2.3.3. Contractile Function

There was no significant difference in peak tension generated between control and cycloserine conditions ($P \leq 0.05$). Over the course of 10 min of stimulation, the percent decline in force production in both absolute and relative terms was not significantly different between conditions with both groups fatigued by ~ 60% at 10 min (Table 2.5).

Table 2.4. ***Muscle enzyme activities at rest and following stimulation***

	Control		Cycloserine	
	Rest	Stimulation	Rest	Stimulation
Citrate Synthase	27.44 ± 1.03	28.63 ± 0.93	24.62 ± 1.11	24.80 ± 0.59[#]
Alanine Aminotransferase	3.56 ± 0.16	3.92 ± 0.29	0.61 ± 0.02[#]	0.63 ± 0.01[#]

Values are means ± SEM in $mmol.kg^{-1}$ d.w . [+] Significantly different from cycloserine rest. $P \leq 0.05$. [*] Significantly different from control at same time point. $P \leq 0.05$. [φ] Significantly different from control rest. $P \leq 0.05$.

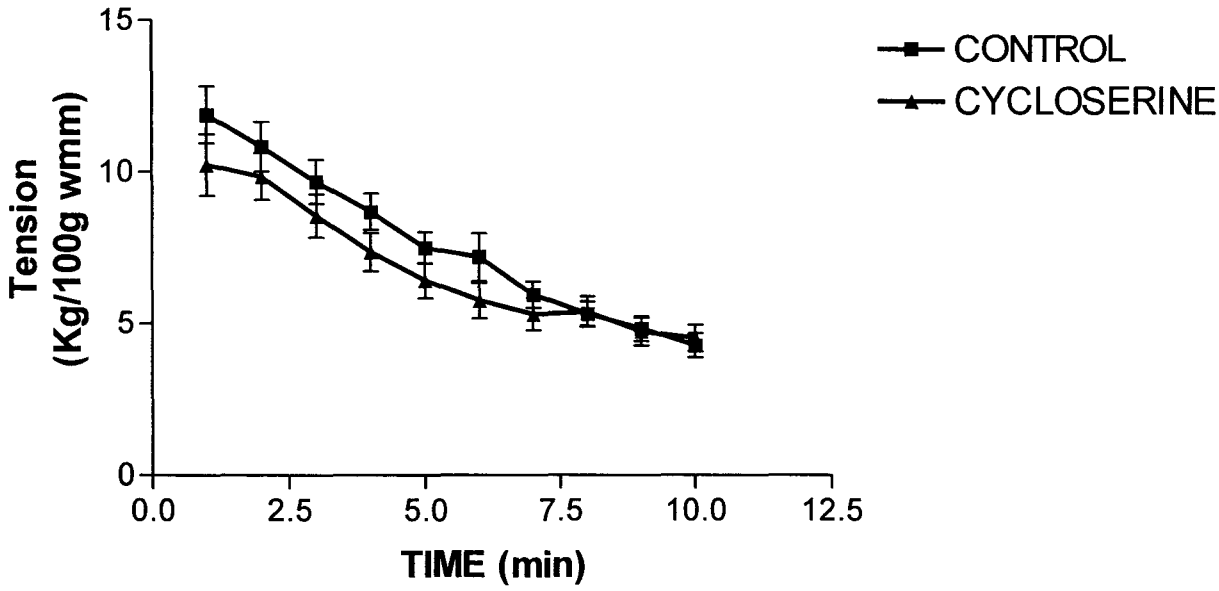


Fig. 2.13. Force production over 10 min of stimulation following pre-treatment with saline or cycloserine.

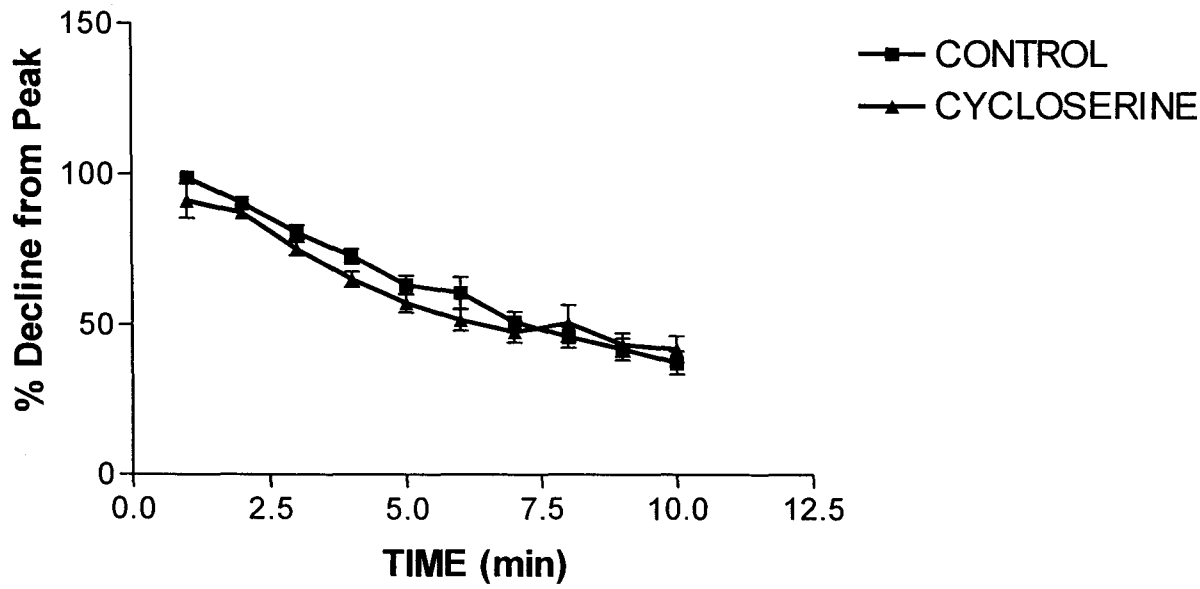


Fig. 2.14. Decline in force production over 10 min of stimulation, following pre-treatment with saline or cycloserine.

Table 2.5. ***Percent decline in tension during 10 minutes of stimulation***

	1min	2min	3min	4min	5min	6min	7min	8min	9min	10min
Control	99 ± 1	91 ± 2	81 ± 2	73 ± 3	63 ± 3	61 ± 5	51 ± 3	46 ± 4	42 ± 4	37 ± 4
Cycloserine	91 ± 6	87 ± 2	76 ± 2	65 ± 3	57 ± 3	52 ± 4	48 ± 4	50 ± 6	43 ± 4	42 ± 4

Values are means ± SEM.

2.4 DISCUSSION

The main finding of the present study was that acute inhibition of alanine aminotransferase attenuated the contraction-induced expansion of the TCAI pool with no concomitant affect on contractile performance or metabolism. The lack of a causal relationship between the [TCAI] and indicators of mitochondrial respiration provides support for the "mass action" theory whereby the expansion of the TCAI pool, in response to contraction stimulus, occurs as a result of a mismatch between rates of pyruvate production and oxidation, independent of aerobic energy provision.

Effectiveness of cycloserine as an inhibitor of alanine aminotransferase

Perfusion with cycloserine for 1hr acutely inhibited alanine amiotransferase (AAT) activity at rest and following the 10 min stimulation protocol. Alanine aminotransferase activity was attenuated by ~83% at both rest and following stimulation in the cycloserine conditions. It is clear that not only is the degree of inhibition on the part of cycloserine consistent with previous findings, but furthermore, that a constant level of inhibition was maintained following 1hr of perfusion (Cornell et al, 1984). Recognized as a key anaplerotic pathway, it would be expected that the acute inhibition of the AAT pathway would result in the significant attenuation of the expansion of the [TCAI] in response to contraction. Indeed, the inhibition of AAT via cycloserine perfusion though not altering resting [TCAI], did result in less of an increase in [TCAI] following stimulation. While the fluorometric analysis of AAT in the present study only assesses the potential maximal activity of the enzyme and does not represent actual flux

through the pathway, analysis of the net change in muscle [glutamate] and [alanine] does provide an insight into the effects of inhibition on anaplerotic expansion. In response to contraction, flux through the AAT pathway while forming the TCA cycle intermediate 2-oxoglutarate, also results in a decrease in muscle [glutamate] and an increase in [alanine]. Pre-treatment with cycloserine resulted in a decrease in the net change in the concentration of muscle glutamate and alanine from rest to 10 min of stimulation. This attenuation in net change affirms not only the successful inhibition of AAT via the action of cycloserine, but also reaffirms the crucial role of this pathway in anaplerosis such that its inhibition resulted in less TCAI expansion.

Effect of attenuated of [TCAI] on contractile function and metabolism

Despite a significant decrease in the [TCAI] following cycloserine perfusion, there was no effect on contractile function or metabolism. While it has been previously suggested that the concentration of TCAI is causally related to the capacity for TCA cycle flux and aerobic energy provision (Sahlin et al, 1990; Wagenmakers et al, 1990), the present results illustrate this not to be the case. Attenuation of the [TCAI] occurred independently of any effect on oxidative metabolism as supported by the lack of a significant difference in key metabolic markers between control and cycloserine conditions. Despite a decrease in [TCAI], there was no increase in lactate accumulation or phosphocreatine degradation. Furthermore, glycogen breakdown was not different from control. The lack of a change in the concentrations of these metabolites suggests that the muscle was not in a state of compromised oxidative energy provision as

previously theorized would be the case (Sahlin et al, 1990; Wagenmakers et al, 1990). With no significant difference in muscle [lactate] and [pyruvate] between control and cycloserine conditions, the fate of the excess carbon skeletons normally housed in the pool of TCAI may be questioned. It is worthy to note that changes in TCAI concentrations in response to contraction are in the range of $mmol \cdot kg^{-1}$ dry muscle (Graham and Gibala, 1998). Ultimately, an ~83% suppression of AAT as presently was the case, could potentially translate into as little as ~1.62 $mmol \cdot kg^{-1}$ dry muscle excess carbon based on previous findings (Gibala et al, 1997). Accumulation of excess carbon units within this range would certainly not have a significant impact on the measurable concentrations of pyruvate or lactacte. Furthermore, this small concentration falls within the range of intra-assay variability (Appendix III) and technical limitations in terms of sensitivity of measurement. In addition to the metabolite data, the contractile function of the isolated gastrocnemius muscle itself supports a lack of a compromise in skeletal muscle metabolism following AAT inhibition and a resultant decrease in [TCAI]. Peak tension generated was not significantly different between control and cycloserine conditions. The rate or percent of decline in force production over the 10 min of stimulation was identical regardless of pre-treatment with saline or cycloserine.

Comparison of TCAI response with previous investigations

Changes in the concentrations of individual intermediates of the TCA cycle as well as the sum of the five TCAI measured (ΣTCAI), are somewhat similar to trends previously observed in human and rodent skeletal muscle (Gibala et al, 1998; Gibala et

al, 1997; Sahlin et al, 1990; Aragon et al, 1980). In response to contraction, there was an increase in most TCAI above resting concentrations, with malate accounting for ~36-47% of the total expansion of the pool. As previously observed in human and rodent studies alike, the concentrations of malate, fumarate, and citrate comprised the largest portion of the total TCAI pool (Sahlin et al, 1990; Aragon et al, 1980). The time course for peak expansion has previously been determined to be within the first few minutes of exercise, however due to the limitations of the present model, analysis was only conducted at rest and at the end of 10 min of stimulation (Gibala et al, 1997). Changes in the concentrations of citrate and 2-oxoglutarate were significantly less then those of the other intermediates, however as has been suggested elsewhere, it is possible that there is a rapid conversion of these intermediates into those which follow in the cycle (Newsholme et al, 1983). Despite the observed changes in individual intermediates in the control conditions, the relative changes in concentration in the cycloserine conditions were significantly attenuated when compared to those previously reported in rat hindlimb stimulation and perfusion trials (Aragon et al, 1980; Lee and Davis, 1979). The cycloserine stimulation condition experienced an overall ~26% reduction in expansion of total TCAI concentration such that its concentration following contraction (1.89 ± 0.15 mmol·kg^{-1} dw) was not significantly different from that of control rest (2.56 ± 0.12 mmol·kg^{-1} dw) and there was no measurable difference in oxidative metabolism or contractile function. As previously mentioned, it would appear that despite the significant attenuation in TCAI expansion, there was no impairment of aerobic energy provision.

Limitations of experimental model

Many of the limitations of the present study are related to the rat hindlimb model employed and the inability to use human subjects in the administration of cycloserine. While we are confident that the present model involving the vascular isolation of the gastrocnemius muscle allows for the exquisite control of perfusion delivery and muscle stimulation, Home Office approval and guidelines in the United Kingdom limited the ability to exercise some levels of experimental control. Perhaps in the future the continued refinement of the model may allow for the use of the contralateral gastrocnemius as a control thereby eliminating the need for four independent groups of animals. Furthermore, it would be of great interest to evaluate the effect of DL-cycloserine perfusion on force generating capacity by measuring peak tension generated after 1h of perfusion and following this capacity over a period of time in contrast to the stimulation protocol. Appreciating that rat skeletal muscle is certainly more homogenous than that of humans, it still varies greatly within a single muscle, gastrocnemius included. These variations stem from differences in fibre type composition, recruitment patterns, and force generating capabilities (Armstrong et al, 1984; De Ruiter et al, 1995; De Ruiter et al, 1995; De Ruiter et al, 1996). The employment of isolated muscle incubations may allow for the better selection of muscle fiber composition and the refinement of the metabolic milieu being manipulated. However, it has been suggested elsewhere (Bonen et al, 1994) that despite the expense and complexity of the rat hindlimb model, it is still perhaps the model which most closely resembles the in vivo environment. An additional limitation of the present study and therefore a future consideration, stems from the fact

that a number of the metabolites analyzed exist in both mitochondrial and cytosolic fractions. In the future, the isolation of mitochondria would perhaps best address the issue of compartmentalization. The employment of radioactive-labeled carbon in future studies into the mechanisms of oxidative energy provision, in particular at the level of the TCA cycle, could best combat the longstanding issue of measuring TCA cycle flux and allow for the in-depth analysis of the fate of carbon skeletons entering the pyruvate dehydrogenase complex en route to oxidation in the TCA cycle.

Cycloserine as an inhibitor

Cycloserine as an inhibitor of alanine aminotransferase (AAT) has been investigated for the treatment of a number of clinical conditions. In relation to skeletal muscle metabolism specifically, cycloserine has been identified for its ability to inhibit aminotransferases, in particular alanine aminotransferase. A number of investigations have looked at the concentrations of cycloserine required to inhibit AAT while limiting its potential action on other enzymes such as aspartate aminotransferase (Barbieri et al, 1960; Cornell et al, 1984). For example, there has been some concern related to the potential of cycloserine to act as a decarboxylating agent on pyruvate (Perez-Sala et al, 1986) however, there is little in the way of research to warrant such concern, in particular due to the fact that the maximal activity of pyruvate carboxylase in skeletal muscle is so low (Brodal et al, 1990; Crabtree et al, 1972; Aragon et al, 1980). Cycloserine as an inhibitor does not have a specificity to muscle alanine aminotransferase alone, with its L-enantiomorph alone expressing the capacity to inhibit six different aminotransferases

(Azarkh et al, 1960; Wong et al, 1973). However the use of the vascularly isolated gastrocnemius model eliminates the potential of its acting on additional aminotransferases in the liver for example. Furthermore, previous studies have determined that [cycloserine] sufficient enough to inhibit AAT (~90%) is not adequate enough to inhibit at a level of metabolic significance other aminotransferases like aspartase aminotransferase (Cornell et al, 1984).

Summary and future directions

Ultimately, we remain confident that the present study definitively acknowledges a lack of a causal or temporal relationship between TCAI pool expansion and mitochondrial respiration. Cycloserine acutely inhibited alanine aminotransferase activity resulting in the significant attenuation of [TCAI] in response to contraction. This attenuated expansion did not result in an increase in lactate accumulation, phosphocreatine utilization or glycogen degradation and in combination with the maintenance of contractile function, indicates that the muscle's oxidative capacity was not compromised. It is clear, that the expansion of the TCAI pool occurs as a result of a mass action effect of pyruvate fluxing through the AAT pathway and exists independent of the up-regulation of aerobic energy provision in response to contraction stimulus. Considered perhaps the definitive study with regards to the elucidation of the physiological significance or lack of significance of the concentration of TCAI in relation to contractile function and oxidative metabolism, much of the future direction in this area of research revolves around methodological considerations which have been previously

outlined. Perhaps the one remaining area for future investigation is related to the deeper understanding of the relationship between the oxaloacetate, citrate, and TCA cycle flux and anaplerosis. While only the latter metabolite saturates the enzyme citrate synthase, the extremenly low concentration of oxaloacetate has made it difficult to measure and therefore its influence and the influence of the other pathways it is linked to on anaplerosis for example, remains elusive. The continued manipulation of the pathways associated with the TCA cycle through dietary manipulations, stimulation protocols and/or metabolic conditions will allow for the interplay of the complex network of reactions and their significance in relation to mitochondrial respiration to be elucidated.

2.5 CONCLUSIONS

The main finding of the present study was that the acute inhibition of alanine aminotransferase via DL-cycloserine perfusion results in a significant attenuation in the expansion of the TCAI pool in rodent skeletal muscle. The decrease in flux through the alanine aminotransferase reaction is further evidenced by the attenuated net change in glutamate and alanine. Despite the significant attenuation in the total [TCAI] following cycloserine administration, there was no impairment of contractile function suggesting that the maintenance of force generating capacity combined with similar levels of muscle lactate, PCr, glycogen, and ATP between conditions, would indicate that mitochondrial respiration and total muscle [TCAI] are not causally related.

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APPENDIX I

RAW DATA

RAW DATA: MUSCLE GLYCOGEN

CONTROL

GLYCOGEN (mmol.kg^{-1} dw)			
Animal			**REST**
16/10	**CON(1)**	**P**	211.41
20/09	**CON**	**P**	184.99
22/08	**CON**	**P**	141.99
9/10	**CON(3)**	**P**	168.78
5/9	**CON(3)**	**P**	113.26
24/09	**CON(3)**	**P**	132.76
13/09	**CON(3)**	**P**	129.59
17/10	**CON(3)**	**P**	142.70
4/10	**CON(3)**	**P**	139.18
17/09	**CON(1)**	**P**	171.63
11/10	**CON(3)**	**P**	131.00
MEAN			**152**
SD			29
SEM			9

GLYCOGEN (mmol.kg^{-1} dw)			
Animal			**STIMULATED**
3/10	**CON(1)**	**10**	112.69
20/09	**CON**	**10**	117.36
18/09	**CON**	**10**	117.74
15/10	**CON(4)**	**10**	101.40
11/10	**CON(1)**	**10**	124.14
18/10	**CON(2)**	**10**	106.18
19/09	**CON**	**10**	125.44
17/10	**CON(1)**	**10**	148.15
25/09	**CON(3)**	**10**	96.15
3/10	**CON(3)**	**10**	91.12
MEAN			**114**
SD			17
SEM			5

CYCLOSERINE

GLYCOGEN (mmol.kg^{-1} dw)			
Animal			**REST**
9/10	**CYCLO(1)**	**P**	151.53
2/10	**CYCLO(1)**	**P**	146.60
25/09	**CYCLO(2)**	**P**	129.28
25/09	**CYCLO(1)**	**P**	185.52
4/10	**CYCLO(2)**	**P**	149.45
4/10	**CYCLO(1)**	**P**	152.98
9/10	**CYCLO(2)**	**P**	168.60
24/09	**CYCLO(1)**	**P**	134.95
1/10	**CYCLO(1)**	**P**	145.99
MEAN			**152**
SD			17
SEM			6

GLYCOGEN (mmol.kg^{-1} dw)			
Animal			**STIMULATED**
24/09	**CYCLO(2)**	**10**	111.53
15/10	**CYCLO(1)**	**10**	139.41
15/10	**CYCLO(2)**	**10**	137.20
16/10	**CYCLO(2)**	**10**	119.27
26/09	**CYCLO(1)**	**10**	130.37
26/09	**CYCLO(2)**	**10**	117.14
17/10	**CYCLO(4)**	**10**	105.66
1/10	**CYCLO(2)**	**10**	101.58
17/10	**CYCLO(2)**	**10**	95.47
16/10	**CYCLO(3)**	**10**	125.09
MEAN			**118**
SD			15
SEM			5

RAW DATA: MUSCLE LACTATE

CONTROL

MUSCLE LACTATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	5.49
20/09	CON	P	7.94
22/08	CON	P	6.77
9/10	CON(3)	P	4.73
5/9	CON(3)	P	24.20
24/09	CON(3)	P	20.74
13/09	CON(3)	P	17.32
17/10	CON(3)	P	20.17
4/10	CON(3)	P	11.07
17/09	CON(1)	P	11.39
11/10	CON(3)	P	10.05
MEAN			12.72
SD			6.77
SEM			2.04

CYCLOSERINE

MUSCLE LACTATE (mmol.kg^{-1} dw)		
Animal		REST
9/10	CYCLO(1) P	21.49
2/10	CYCLO(1) P	9.39
25/09	CYCLO(2) P	17.81
25/09	CYCLO(1) P	29.11
4/10	CYCLO(2) P	12.82
4/10	CYCLO(1) P	11.09
9/10	CYCLO(2) P	10.15
24/09	CYCLO(1) P	17.00
1/10	CYCLO(1) P	4.63
MEAN		14.83
SD		7.38
SEM		2.46

MUSCLE LACTATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	38.36
20/09	CON	10	27.13
18/09	CON	10	45.25
15/10	CON(4)	10	48.19
11/10	CON(1)	10	23.31
18/10	CON(2)	10	29.76
19/09	CON	10	39.19
17/10	CON(1)	10	21.26
25/09	CON(3)	10	38.99
3/10	CON(3)	10	34.67
MEAN			34.61
SD			9.05
SEM			2.86

MUSCLE LACTATE (mmol.kg^{-1} dw)		
Animal		STIMULATED
24/09	CYCLO(2) 10	40.09
15/10	CYCLO(1) 10	37.08
15/10	CYCLO(2) 10	32.19
16/10	CYCLO(2) 10	34.98
26/09	CYCLO(1) 10	34.48
26/09	CYCLO(2) 10	37.17
17/10	CYCLO(4) 10	31.69
1/10	CYCLO(2) 10	26.98
17/10	CYCLO(2) 10	37.75
16/10	CYCLO(3) 10	37.44
MEAN		34.99
SD		3.83
SEM		1.21

RAW DATA: PYRUVATE

CONTROL

PYRUVATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	0.33
20/09	CON	P	0.38
22/08	CON	P	0.86
9/10	CON(3)	P	0.26
5/9	CON(3)	P	0.30
24/09	CON(3)	P	0.17
13/09	CON(3)	P	0.35
17/10	CON(3)	P	0.42
4/10	CON(3)	P	0.36
17/09	CON(1)	P	0.15
11/10	CON(3)	P	0.37
MEAN			0.36
SD			0.19
SEM			0.06

CYCLOSERINE

PYRUVATE (mmol.kg^{-1} dw)		
Animal		REST
9/10	CYCLO(1) P	0.27
2/10	CYCLO(1) P	0.15
25/09	CYCLO(2) P	0.29
25/09	CYCLO(1) P	0.24
4/10	CYCLO(2) P	0.39
4/10	CYCLO(1) P	0.27
9/10	CYCLO(2) P	0.34
24/09	CYCLO(1) P	0.24
1/10	CYCLO(1) P	0.16
MEAN		0.26
SD		0.08
SEM		0.03

PYRUVATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	0.70
20/09	CON	10	0.77
18/09	CON	10	0.89
15/10	CON(4)	10	0.79
11/10	CON(1)	10	0.64
18/10	CON(2)	10	0.55
19/09	CON	10	0.70
17/10	CON(1)	10	0.59
25/09	CON(3)	10	0.71
3/10	CON(3)	10	0.83
MEAN			0.72
SD			0.11
SEM			0.03

PYRUVATE (mmol.kg^{-1} dw)		
Animal		STIMULATED
24/09	CYCLO(2) 10	0.47
15/10	CYCLO(1) 10	0.48
15/10	CYCLO(2) 10	0.77
16/10	CYCLO(2) 10	0.57
26/09	CYCLO(1) 10	0.68
26/09	CYCLO(2) 10	0.51
17/10	CYCLO(4) 10	0.60
1/10	CYCLO(2) 10	0.48
17/10	CYCLO(2) 10	0.64
16/10	CYCLO(3) 10	0.76
MEAN		0.60
SD		0.11
SEM		0.04

RAW DATA: PHOSPHOCREATINE

CONTROL

PHOSPHOCREATINE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	129.62
20/09	CON	P	108.73
22/08	CON	P	133.70
9/10	CON(3)	P	117.37
5/9	CON(3)	P	77.09
24/09	CON(3)	P	77.09
13/09	CON(3)	P	98.01
17/10	CON(3)	P	104.09
4/10	CON(3)	P	98.05
17/09	CON(1)	P	89.77
11/10	CON(3)	P	98.30
MEAN			102.9
SD			18.7
SEM			5.6

CYCLOSERINE

PHOSPHOCREATINE (mmol.kg^{-1} dw)		
Animal		REST
9/10	CYCLO(1) P	77.70
2/10	CYCLO(1) P	97.53
25/09	CYCLO(2) P	87.22
25/09	CYCLO(1) P	89.63
4/10	CYCLO(2) P	117.61
4/10	CYCLO(1) P	98.26
9/10	CYCLO(2) P	123.97
24/09	CYCLO(1) P	85.06
1/10	CYCLO(1) P	113.44
MEAN		98.9
SD		16.0
SEM		5.3

PHOSPHOCREATINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	43.48
20/09	CON	10	56.02
18/09	CON	10	43.99
15/10	CON(4)	10	33.20
11/10	CON(1)	10	69.87
18/10	CON(2)	10	58.37
19/09	CON	10	48.96
17/10	CON(1)	10	94.56
25/09	CON(3)	10	26.02
3/10	CON(3)	10	39.61
MEAN			51.4
SD			19.8
SEM			6.2

PHOSPHOCREATINE (mmol.kg^{-1} dw)		
Animal		STIMULATED
24/09	CYCLO(2) 10	50.82
15/10	CYCLO(1) 10	65.70
15/10	CYCLO(2) 10	84.91
16/10	CYCLO(2) 10	69.97
26/09	CYCLO(1) 10	48.94
26/09	CYCLO(2) 10	68.81
17/10	CYCLO(4) 10	43.07
1/10	CYCLO(2) 10	51.34
17/10	CYCLO(2) 10	45.20
16/10	CYCLO(3) 10	68.82
MEAN		59.8
SD		13.7
SEM		4.3

RAW DATA: CREATINE

CONTROL

CREATINE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	36.60
20/09	CON	P	37.90
22/08	CON	P	43.00
9/10	CON(3)	P	35.90
5/9	CON(3)	P	75.70
24/09	CON(3)	P	77.70
13/09	CON(3)	P	57.20
17/10	CON(3)	P	54.50
4/10	CON(3)	P	54.80
17/09	CON(1)	P	48.00
11/10	CON(3)	P	49.10
MEAN			51.9
SD			14.4
SEM			4.3

CREATINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	109.3
20/09	CON	10	74.3
18/09	CON	10	95.4
15/10	CON(4)	10	103.8
11/10	CON(1)	10	70.2
18/10	CON(2)	10	89.4
19/09	CON	10	97.9
17/10	CON(1)	10	61.9
25/09	CON(3)	10	110.9
3/10	CON(3)	10	72.7
MEAN			88.6
SD			17.6
SEM			5.6

CYCLOSERINE

CREATINE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	64.00
2/10	CYCLO(1)	P	59.50
25/09	CYCLO(2)	P	70.80
25/09	CYCLO(1)	P	64.60
4/10	CYCLO(2)	P	51.90
4/10	CYCLO(1)	P	44.80
9/10	CYCLO(2)	P	36.30
24/09	CYCLO(1)	P	66.40
1/10	CYCLO(1)	P	36.20
MEAN			54.9
SD			13.2
SEM			4.4

CREATINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	96.00
15/10	CYCLO(1)	10	82.30
15/10	CYCLO(2)	10	77.50
16/10	CYCLO(2)	10	76.10
26/09	CYCLO(1)	10	95.00
26/09	CYCLO(2)	10	78.80
17/10	CYCLO(4)	10	101.40
1/10	CYCLO(2)	10	94.00
17/10	CYCLO(2)	10	86.30
16/10	CYCLO(3)	10	86.90
MEAN			87.4
SD			8.8
SEM			2.8

RAW DATA: TOTAL CREATINE

CONTROL

TOTAL CREATINE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	166.22
20/09	CON	P	146.63
22/08	CON	P	176.70
9/10	CON(3)	P	153.27
5/9	CON(3)	P	152.79
24/09	CON(3)	P	154.79
13/09	CON(3)	P	155.21
17/10	CON(3)	P	158.59
4/10	CON(3)	P	152.85
17/09	CON(1)	P	137.77
11/10	CON(3)	P	147.40
MEAN			154.7
SD			10.2
SEM			3.1

TOTAL CREATINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	152.78
20/09	CON	10	130.32
18/09	CON	10	139.39
15/10	CON(4)	10	137.00
11/10	CON(1)	10	140.07
18/10	CON(2)	10	147.77
19/09	CON	10	146.86
17/10	CON(1)	10	156.46
25/09	CON(3)	10	136.92
3/10	CON(3)	10	112.31
MEAN			140.0
SD			12.6
SEM			4.0

CYCLOSERINE

TOTAL CREATINE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	141.70
2/10	CYCLO(1)	P	157.03
25/09	CYCLO(2)	P	158.02
25/09	CYCLO(1)	P	154.23
4/10	CYCLO(2)	P	169.51
4/10	CYCLO(1)	P	143.06
9/10	CYCLO(2)	P	160.27
24/09	CYCLO(1)	P	151.46
1/10	CYCLO(1)	P	149.64
MEAN			153.9
SD			8.7
SEM			2.9

TOTAL CREATINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	146.82
15/10	CYCLO(1)	10	148.00
15/10	CYCLO(2)	10	162.41
16/10	CYCLO(2)	10	146.07
26/09	CYCLO(1)	10	143.94
26/09	CYCLO(2)	10	147.61
17/10	CYCLO(4)	10	144.47
1/10	CYCLO(2)	10	145.34
17/10	CYCLO(2)	10	131.50
16/10	CYCLO(3)	10	155.72
MEAN			147.2
SD			8.0
SEM			2.5

RAW DATA: ATP

CONTROL

ATP (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	25.54
20/09	CON	P	24.90
22/08	CON	P	27.04
9/10	CON(3)	P	25.88
5/9	CON(3)	P	27.22
24/09	CON(3)	P	26.55
13/09	CON(3)	P	25.58
17/10	CON(3)	P	26.61
4/10	CON(3)	P	25.99
17/09	CON(1)	P	21.69
11/10	CON(3)	P	24.99
MEAN			25.6
SD			1.5
SEM			0.5

CYCLOSERINE

ATP (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	24.08
2/10	CYCLO(1)	P	23.90
25/09	CYCLO(2)	P	26.35
25/09	CYCLO(1)	P	26.03
4/10	CYCLO(2)	P	27.34
4/10	CYCLO(1)	P	25.50
9/10	CYCLO(2)	P	25.98
24/09	CYCLO(1)	P	25.52
1/10	CYCLO(1)	P	23.57
MEAN			25.4
SD			1.3
SEM			0.4

ATP (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	24.01
20/09	CON	10	23.81
18/09	CON	10	23.08
15/10	CON(4)	10	19.75
11/10	CON(1)	10	24.21
18/10	CON(2)	10	23.81
19/09	CON	10	24.04
17/10	CON(1)	10	24.18
25/09	CON(3)	10	22.59
3/10	CON(3)	10	18.99
MEAN			22.8
SD			1.9
SEM			0.6

ATP (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	24.09
15/10	CYCLO(1)	10	22.95
15/10	CYCLO(2)	10	24.63
16/10	CYCLO(2)	10	22.83
26/09	CYCLO(1)	10	21.83
26/09	CYCLO(2)	10	24.14
17/10	CYCLO(4)	10	22.40
1/10	CYCLO(2)	10	23.33
17/10	CYCLO(2)	10	20.15
16/10	CYCLO(3)	10	22.98
MEAN			22.9
SD			1.3
SEM			0.4

RAW DATA: SUM OF TCAI

CONTROL

SUM OF TCAI (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	2.40
20/09	CON	P	1.96
22/08	CON	P	2.74
9/10	CON(3)	P	1.42
5/9	CON(3)	P	2.47
24/09	CON(3)	P	2.10
13/09	CON(3)	P	2.56
17/10	CON(3)	P	1.53
4/10	CON(3)	P	1.89
17/09	CON(1)	P	2.26
11/10	CON(3)	P	1.77
MEAN			2.10
SD			0.43
SEM			0.13

SUM OF TCAI (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	2.42
20/09	CON	10	2.52
18/09	CON	10	3.13
15/10	CON(4)	10	2.13
11/10	CON(1)	10	2.37
18/10	CON(2)	10	2.32
19/09	CON	10	3.00
17/10	CON(1)	10	2.55
25/09	CON(3)	10	3.12
3/10	CON(3)	10	2.08
MEAN			2.56
SD			0.39
SEM			0.12

CYCLOSERINE

SUM OF TCAI (mmol.kg^{-1} dw)		
Animal		REST
9/10	CYCLO(1) P	1.61
2/10	CYCLO(1) P	1.83
25/09	CYCLO(2) P	1.60
25/09	CYCLO(1) P	1.81
4/10	CYCLO(2) P	1.15
4/10	CYCLO(1) P	1.34
9/10	CYCLO(2) P	2.30
24/09	CYCLO(1) P	1.06
1/10	CYCLO(1) P	1.89
MEAN		1.62
SD		0.39
SEM		0.13

SUM OF TCAI (mmol.kg^{-1} dw)		
Animal		STIMULATED
24/09	CYCLO(2) 10	1.78
15/10	CYCLO(1) 10	2.19
15/10	CYCLO(2) 10	2.98
16/10	CYCLO(2) 10	1.56
26/09	CYCLO(1) 10	1.95
26/09	CYCLO(2) 10	2.15
17/10	CYCLO(4) 10	1.33
1/10	CYCLO(2) 10	1.80
17/10	CYCLO(2) 10	1.29
16/10	CYCLO(3) 10	1.89
MEAN		1.89
SD		0.49
SEM		0.15

RAW DATA: MALATE

CONTROL

MALATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	0.411
20/09	CON	P	0.530
22/08	CON	P	0.460
9/10	CON(3)	P	0.280
5/9	CON(3)	P	1.119
24/09	CON(3)	P	0.921
13/09	CON(3)	P	1.033
17/10	CON(3)	P	0.572
4/10	CON(3)	P	0.781
17/09	CON(1)	P	0.624
11/10	CON(3)	P	0.606
MEAN			0.67
SD			0.27
SEM			0.08

CYCLOSERINE

MALATE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	0.397
2/10	CYCLO(1)	P	0.458
25/09	CYCLO(2)	P	0.605
25/09	CYCLO(1)	P	0.568
4/10	CYCLO(2)	P	0.372
4/10	CYCLO(1)	P	0.376
9/10	CYCLO(2)	P	0.481
24/09	CYCLO(1)	P	0.350
1/10	CYCLO(1)	P	0.302
MEAN			0.43
SD			0.10
SEM			0.03

MALATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	1.175
20/09	CON	10	1.324
18/09	CON	10	1.429
15/10	CON(4)	10	1.100
11/10	CON(1)	10	0.959
18/10	CON(2)	10	1.254
19/09	CON	10	1.258
17/10	CON(1)	10	0.858
25/09	CON(3)	10	1.649
3/10	CON(3)	10	1.114
MEAN			1.21
SD			0.23
SEM			0.07

MALATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	0.934
15/10	CYCLO(1)	10	0.744
15/10	CYCLO(2)	10	0.795
16/10	CYCLO(2)	10	0.562
26/09	CYCLO(1)	10	0.636
26/09	CYCLO(2)	10	0.737
17/10	CYCLO(4)	10	0.552
1/10	CYCLO(2)	10	0.634
17/10	CYCLO(2)	10	0.533
16/10	CYCLO(3)	10	0.641
MEAN			0.68
SD			0.13
SEM			0.04

RAW DATA: FUMARATE

CONTROL

FUMARATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	0.035
20/09	CON	P	0.092
22/08	CON	P	0.109
9/10	CON(3)	P	0.085
5/9	CON(3)	P	0.196
24/09	CON(3)	P	0.129
13/09	CON(3)	P	0.081
17/10	CON(3)	P	0.101
4/10	CON(3)	P	0.146
17/09	CON(1)	P	0.001
11/10	CON(3)	P	0.118
MEAN			**0.10**
SD			0.05
SEM			0.02

FUMARATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	0.229
20/09	CON	10	0.166
18/09	CON	10	0.212
15/10	CON(4)	10	0.191
11/10	CON(1)	10	0.161
18/10	CON(2)	10	0.131
19/09	CON	10	0.244
17/10	CON(1)	10	0.124
25/09	CON(3)	10	0.346
3/10	CON(3)	10	0.163
MEAN			**0.20**
SD			0.07
SEM			0.02

CYCLOSERINE

FUMARATE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	0.070
2/10	CYCLO(1)	P	0.034
25/09	CYCLO(2)	P	0.081
25/09	CYCLO(1)	P	0.095
4/10	CYCLO(2)	P	0.043
4/10	CYCLO(1)	P	0.051
9/10	CYCLO(2)	P	0.133
24/09	CYCLO(1)	P	0.011
1/10	CYCLO(1)	P	0.019
MEAN			**0.06**
SD			0.04
SEM			0.01

FUMARATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	0.122
15/10	CYCLO(1)	10	0.223
15/10	CYCLO(2)	10	0.094
16/10	CYCLO(2)	10	0.133
26/09	CYCLO(1)	10	0.142
26/09	CYCLO(2)	10	0.138
17/10	CYCLO(4)	10	0.113
1/10	CYCLO(2)	10	0.128
17/10	CYCLO(2)	10	0.067
16/10	CYCLO(3)	10	0.118
MEAN			**0.13**
SD			0.04
SEM			0.01

RAW DATA: MALATE AND FUMARATE

CONTROL

MALATE AND FUMARATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	0.45
20/09	CON	P	0.62
22/08	CON	P	0.57
9/10	CON(3)	P	0.37
5/9	CON(3)	P	1.32
24/09	CON(3)	P	1.05
13/09	CON(3)	P	1.11
17/10	CON(3)	P	0.67
4/10	CON(3)	P	0.93
17/09	CON(1)	P	0.63
11/10	CON(3)	P	0.72
MEAN			0.77
SD			0.30
SEM			0.09

MALATE AND FUMARATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	1.40
20/09	CON	10	1.49
18/09	CON	10	1.64
15/10	CON(4)	10	1.29
11/10	CON(1)	10	1.12
18/10	CON(2)	10	1.39
19/09	CON	10	1.50
17/10	CON(1)	10	0.98
25/09	CON(3)	10	2.00
3/10	CON(3)	10	1.28
MEAN			1.41
SD			0.28
SEM			0.09

CYCLOSERINE

MALATE AND FUMARATE (mmol.kg^{-1}			
Animal			REST
9/10	CYCLO(1)	P	0.47
2/10	CYCLO(1)	P	0.49
25/09	CYCLO(2)	P	0.69
25/09	CYCLO(1)	P	0.66
4/10	CYCLO(2)	P	0.42
4/10	CYCLO(1)	P	0.43
9/10	CYCLO(2)	P	0.61
24/09	CYCLO(1)	P	0.36
1/10	CYCLO(1)	P	0.32
MEAN			0.49
SD			0.13
SEM			0.04

MALATE AND FUMARATE (mmol.kg^{-1}			
Animal			STIMULATED
24/09	CYCLO(2)	10	1.06
15/10	CYCLO(1)	10	0.97
15/10	CYCLO(2)	10	0.89
16/10	CYCLO(2)	10	0.70
26/09	CYCLO(1)	10	0.78
26/09	CYCLO(2)	10	0.88
17/10	CYCLO(4)	10	0.67
1/10	CYCLO(2)	10	0.76
17/10	CYCLO(2)	10	0.60
16/10	CYCLO(3)	10	0.76
MEAN			0.80
SD			0.14
SEM			0.04

RAW DATA: 2-OXOGLUTARATE

CONTROL

2-OXOGLUTARATE (mmol.kg^{-1} dw)

Animal			REST
16/10	CON(1)	P	0.086
20/09	CON	P	0.094
22/08	CON	P	0.093
9/10	CON(3)	P	0.086
5/9	CON(3)	P	0.082
24/09	CON(3)	P	0.067
13/09	CON(3)	P	0.074
17/10	CON(3)	P	0.094
4/10	CON(3)	P	0.086
17/09	CON(1)	P	0.089
11/10	CON(3)	P	0.099
MEAN			0.09
SD			0.01
SEM			0.00

CYCLOSERINE

2-OXOGLUTARATE (mmol.kg^{-1} dw)

Animal			REST
9/10	CYCLO(1)	P	0.055
2/10	CYCLO(1)	P	0.059
25/09	CYCLO(2)	P	0.077
25/09	CYCLO(1)	P	0.092
4/10	CYCLO(2)	P	0.085
4/10	CYCLO(1)	P	0.101
9/10	CYCLO(2)	P	0.088
24/09	CYCLO(1)	P	0.076
1/10	CYCLO(1)	P	0.105
MEAN			0.08
SD			0.02
SEM			0.01

2-OXOGLUTARATE (mmol.kg^{-1} dw)

Animal			STIMULATED
3/10	CON(1)	10	0.080
20/09	CON	10	0.074
18/09	CON	10	0.091
15/10	CON(4)	10	0.094
11/10	CON(1)	10	0.085
18/10	CON(2)	10	0.082
19/09	CON	10	0.081
17/10	CON(1)	10	0.073
25/09	CON(3)	10	0.080
3/10	CON(3)	10	0.084
MEAN			0.08
SD			0.01
SEM			0.00

2-OXOGLUTARATE (mmol.kg^{-1} dw)

Animal			STIMULATED
24/09	CYCLO(2)	10	0.094
15/10	CYCLO(1)	10	0.106
15/10	CYCLO(2)	10	0.080
16/10	CYCLO(2)	10	0.066
26/09	CYCLO(1)	10	0.083
26/09	CYCLO(2)	10	0.095
17/10	CYCLO(4)	10	0.093
1/10	CYCLO(2)	10	0.095
17/10	CYCLO(2)	10	0.095
16/10	CYCLO(3)	10	0.090
MEAN			0.09
SD			0.01
SEM			0.00

RAW DATA: ISOCITRATE

CONTROL

ISOCITRATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	0.21
20/09	CON	P	0.15
22/08	CON	P	0.22
9/10	CON(3)	P	0.16
5/9	CON(3)	P	0.24
24/09	CON(3)	P	0.18
13/09	CON(3)	P	0.20
17/10	CON(3)	P	0.15
4/10	CON(3)	P	0.15
17/09	CON(1)	P	0.12
11/10	CON(3)	P	0.13
MEAN			0.17
SD			0.04
SEM			0.01

ISOCITRATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	0.17
20/09	CON	10	0.21
18/09	CON	10	0.19
15/10	CON(4)	10	0.15
11/10	CON(1)	10	0.20
18/10	CON(2)	10	0.18
19/09	CON	10	0.20
17/10	CON(1)	10	0.18
25/09	CON(3)	10	0.21
3/10	CON(3)	10	0.16
MEAN			0.18
SD			0.02
SEM			0.01

CYCLOSERINE

ISOCITRATE (mmol.kg^{-1} dw)		
Animal		REST
9/10	CYCLO(1) P	0.07
2/10	CYCLO(1) P	0.09
25/09	CYCLO(2) P	0.05
25/09	CYCLO(1) P	0.09
4/10	CYCLO(2) P	0.03
4/10	CYCLO(1) P	0.03
9/10	CYCLO(2) P	0.06
24/09	CYCLO(1) P	0.04
1/10	CYCLO(1) P	0.06
MEAN		0.06
SD		0.02
SEM		0.01

ISOCITRATE (mmol.kg^{-1} dw)		
Animal		STIMULATED
24/09	CYCLO(2) 10	-0.02
15/10	CYCLO(1) 10	-0.02
15/10	CYCLO(2) 10	0.11
16/10	CYCLO(2) 10	0.06
26/09	CYCLO(1) 10	0.08
26/09	CYCLO(2) 10	0.05
17/10	CYCLO(4) 10	0.02
1/10	CYCLO(2) 10	0.01
17/10	CYCLO(2) 10	0.01
16/10	CYCLO(3) 10	0.09
MEAN		0.04
SD		0.05
SEM		0.01

RAW DATA: CITRATE

CONTROL

CITRATE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	1.66
20/09	CON	P	1.09
22/08	CON	P	1.85
9/10	CON(3)	P	0.81
5/9	CON(3)	P	0.83
24/09	CON(3)	P	0.80
13/09	CON(3)	P	1.17
17/10	CON(3)	P	0.61
4/10	CON(3)	P	0.73
17/09	CON(1)	P	1.43
11/10	CON(3)	P	0.81
MEAN			1.07
SD			0.41
SEM			0.12

CITRATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	0.76
20/09	CON	10	0.74
18/09	CON	10	1.21
15/10	CON(4)	10	0.60
11/10	CON(1)	10	0.97
18/10	CON(2)	10	0.67
19/09	CON	10	1.21
17/10	CON(1)	10	1.31
25/09	CON(3)	10	0.84
3/10	CON(3)	10	0.56
MEAN			0.89
SD			0.27
SEM			0.09

CYCLOSERINE

CITRATE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	1.01
2/10	CYCLO(1)	P	1.19
25/09	CYCLO(2)	P	0.79
25/09	CYCLO(1)	P	0.96
4/10	CYCLO(2)	P	0.62
4/10	CYCLO(1)	P	0.78
9/10	CYCLO(2)	P	1.54
24/09	CYCLO(1)	P	0.58
1/10	CYCLO(1)	P	1.40
MEAN			0.99
SD			0.33
SEM			0.11

CITRATE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	0.65
15/10	CYCLO(1)	10	1.14
15/10	CYCLO(2)	10	1.90
16/10	CYCLO(2)	10	0.74
26/09	CYCLO(1)	10	1.01
26/09	CYCLO(2)	10	1.13
17/10	CYCLO(4)	10	0.55
1/10	CYCLO(2)	10	0.94
17/10	CYCLO(2)	10	0.58
16/10	CYCLO(3)	10	0.95
MEAN			0.96
SD			0.40
SEM			0.12

RAW DATA: ALANINE

CONTROL

ALANINE (mmol.kg^{-1} dw)			
Animal			REST
16/10	CON(1)	P	7.21
20/09	CON	P	7.44
22/08	CON	P	4.99
9/10	CON(3)	P	5.14
5/9	CON(3)	P	9.02
24/09	CON(3)	P	7.70
13/09	CON(3)	P	7.25
17/10	CON(3)	P	5.64
4/10	CON(3)	P	6.83
17/09	CON(1)	P	7.28
11/10	CON(3)	P	8.05
MEAN			6.96
SD			1.24
SEM			0.37

ALANINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
3/10	CON(1)	10	9.74
20/09	CON	10	9.67
18/09	CON	10	8.6
15/10	CON(4)	10	6.85
11/10	CON(1)	10	7.09
18/10	CON(2)	10	12.17
19/09	CON	10	10.22
17/10	CON(1)	10	6.91
25/09	CON(3)	10	8.86
3/10	CON(3)	10	6.96
MEAN			8.71
SD			1.78
SEM			0.56

CYCLOSERINE

ALANINE (mmol.kg^{-1} dw)			
Animal			REST
9/10	CYCLO(1)	P	4.69
2/10	CYCLO(1)	P	5.84
25/09	CYCLO(2)	P	6.48
25/09	CYCLO(1)	P	5.93
4/10	CYCLO(2)	P	5.93
4/10	CYCLO(1)	P	7.52
9/10	CYCLO(2)	P	5.73
24/09	CYCLO(1)	P	5.91
1/10	CYCLO(1)	P	6.16
MEAN			6.02
SD			0.74
SEM			0.25

ALANINE (mmol.kg^{-1} dw)			
Animal			STIMULATED
24/09	CYCLO(2)	10	8.29
15/10	CYCLO(1)	10	6.32
15/10	CYCLO(2)	10	7.35
16/10	CYCLO(2)	10	5.58
26/09	CYCLO(1)	10	7.36
26/09	CYCLO(2)	10	7.96
17/10	CYCLO(4)	10	7.90
1/10	CYCLO(2)	10	7.01
17/10	CYCLO(2)	10	6.76
16/10	CYCLO(3)	10	6.67
MEAN			7.12
SD			0.83
SEM			0.26

RAW DATA: GLUTAMATE

CONTROL

GLUTAMATE (mmol.kg^{-1} dw)

Animal			REST
16/10	CON(1)	P	5.89
20/09	CON	P	7.23
22/08	CON	P	2.55
9/10	CON(3)	P	7.10
5/9	CON(3)	P	4.54
24/09	CON(3)	P	5.09
13/09	CON(3)	P	5.54
17/10	CON(3)	P	5.05
4/10	CON(3)	P	4.90
17/09	CON(1)	P	7.00
11/10	CON(3)	P	5.80
MEAN			5.52
SD			1.36
SEM			0.41

CYCLOSERINE

GLUTAMATE (mmol.kg^{-1} dw)

Animal			REST
9/10	CYCLO(1)	P	7.24
2/10	CYCLO(1)	P	8.33
25/09	CYCLO(2)	P	4.98
25/09	CYCLO(1)	P	5.45
4/10	CYCLO(2)	P	5.48
4/10	CYCLO(1)	P	6.38
9/10	CYCLO(2)	P	5.55
24/09	CYCLO(1)	P	5.77
1/10	CYCLO(1)	P	7.58
MEAN			6.31
SD			1.15
SEM			0.38

GLUTAMATE (mmol.kg^{-1} dw)

Animal			STIMULATED
3/10	CON(1)	10	3.44
20/09	CON	10	2.06
18/09	CON	10	1.68
15/10	CON(4)	10	1.48
11/10	CON(1)	10	2.98
18/10	CON(2)	10	3.37
19/09	CON	10	3.18
17/10	CON(1)	10	5.95
25/09	CON(3)	10	2.93
3/10	CON(3)	10	2.64
MEAN			2.97
SD			1.25
SEM			0.40

GLUTAMATE (mmol.kg^{-1} dw)

Animal			STIMULATED
24/09	CYCLO(2)	10	5.09
15/10	CYCLO(1)	10	4.85
15/10	CYCLO(2)	10	3.95
16/10	CYCLO(2)	10	6.08
26/09	CYCLO(1)	10	5.99
26/09	CYCLO(2)	10	4.70
17/10	CYCLO(4)	10	4.99
1/10	CYCLO(2)	10	4.44
17/10	CYCLO(2)	10	3.72
16/10	CYCLO(3)	10	6.68
MEAN			5.05
SD			0.95
SEM			0.30

RAW DATA: ALANINE AMINOTRANSFERASE

CONTROL

ALANINE AMINOTRANSFERASE
(mmol.kg^{-1} ww.min^{-1})

Animal			REST
16/10	CON(1)	P	3.45
20/09	CON	P	2.86
22/08	CON	P	3.20
9/10	CON(3)	P	3.14
5/9	CON(3)	P	3.83
24/09	CON(3)	P	3.18
13/09	CON(3)	P	3.58
17/10	CON(3)	P	3.47
4/10	CON(3)	P	3.43
17/09	CON(1)	P	4.67
11/10	CON(3)	P	4.35
MEAN			**3.56**
SD			0.54
SEM			0.16

ALANINE AMINOTRANSFERASE
(mmol.kg^{-1} ww.min^{-1})

Animal			STIMULATED
3/10	CON(1)	10	2.98
20/09	CON	10	4.17
18/09	CON	10	3.77
15/10	CON(4)	10	3.71
11/10	CON(1)	10	5.54
18/10	CON(2)	10	2.72
19/09	CON	10	4.86
17/10	CON(1)	10	3.96
25/09	CON(3)	10	4.59
3/10	CON(3)	10	2.94
MEAN			**3.92**
SD			0.91
SEM			0.29

CYCLOSERINE

ALANINE AMINOTRANSFERASE
(mmol.kg^{-1} ww.min^{-1})

Animal		REST
9/10	CYCLO(1) P	0.61
2/10	CYCLO(1) P	0.60
25/09	CYCLO(2) P	0.50
25/09	CYCLO(1) P	0.65
4/10	CYCLO(2) P	0.60
4/10	CYCLO(1) P	0.64
9/10	CYCLO(2) P	0.59
24/09	CYCLO(1) P	0.72
1/10	CYCLO(1) P	0.59
MEAN		**0.61**
SD		0.06
SEM		0.02

ALANINE AMINOTRANSFERASE
(mmol.kg^{-1} ww.min^{-1})

Animal			STIMULATED
24/09	CYCLO(2)	10	0.65
15/10	CYCLO(1)	10	0.61
15/10	CYCLO(2)	10	0.59
16/10	CYCLO(2)	10	0.67
26/09	CYCLO(1)	10	0.63
26/09	CYCLO(2)	10	0.61
17/10	CYCLO(4)	10	0.62
1/10	CYCLO(2)	10	0.70
17/10	CYCLO(2)	10	0.62
16/10	CYCLO(3)	10	0.63
MEAN			**0.63**
SD			0.03
SEM			0.01

RAW DATA: CITRATE SYNTHASE

CONTROL

CITRATE SYNTHASE (mmol.kg-1 ww.min-1)

Animal			REST
16/10	CON(1)	P	25.61
20/09	CON	P	29.84
22/08	CON	P	23.10
9/10	CON(3)	P	29.11
5/9	CON(3)	P	30.58
24/09	CON(3)	P	28.68
13/09	CON(3)	P	27.51
17/10	CON(3)	P	21.94
4/10	CON(3)	P	24.26
17/09	CON(1)	P	33.21
11/10	CON(3)	P	28.00
MEAN			27.44
SD			3.41
SEM			1.03

CITRATE SYNTHASE (mmol.kg-1 ww.min-1)

Animal			STIMULATED
3/10	CON(1)	10	26.41
20/09	CON	10	31.74
18/09	CON	10	29.84
15/10	CON(4)	10	24.45
11/10	CON(1)	10	30.15
18/10	CON(2)	10	24.82
19/09	CON	10	30.02
17/10	CON(1)	10	25.74
25/09	CON(3)	10	31.86
3/10	CON(3)	10	31.31
MEAN			28.63
SD			2.95
SEM			0.93

CYCLOSERINE

CITRATE SYNTHASE (mmol.kg-1 ww.min-1)

Animal			REST
9/10	CYCLO(1)	P	26.65
2/10	CYCLO(1)	P	27.39
25/09	CYCLO(2)	P	18.20
25/09	CYCLO(1)	P	29.60
4/10	CYCLO(2)	P	22.86
4/10	CYCLO(1)	P	24.94
9/10	CYCLO(2)	P	22.00
24/09	CYCLO(1)	P	24.88
1/10	CYCLO(1)	P	25.06
MEAN			24.62
SD			3.32
SEM			1.11

CITRATE SYNTHASE (mmol.kg-1 ww.min-1)

Animal			STIMULATED
24/09	CYCLO(2)	10	23.47
15/10	CYCLO(1)	10	26.35
15/10	CYCLO(2)	10	22.92
16/10	CYCLO(2)	10	27.57
26/09	CYCLO(1)	10	26.41
26/09	CYCLO(2)	10	22.92
17/10	CYCLO(4)	10	24.51
1/10	CYCLO(2)	10	27.14
17/10	CYCLO(2)	10	23.41
16/10	CYCLO(3)	10	23.28
MEAN			24.80
SD			1.87
SEM			0.59

RAW DATA: CONTRACTILE FUNCTION

CONTROL

ID	Muscle Wet Weight (g)	Peak (g)	Rest (g)	Actual Peak (g)	Actual Peak Tension (kg/100g wmm)	1min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	2min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
18/09/02	2.68	551	211	340	12.69	548	210	338	12.61	99.4	488	192	296	11.04	87
19/09/02	2.94	558	167	391	13.30	548	173	375	12.76	95.9	520	157	363	12.35	93
20/09/02	2.66	384	168	216	8.12	378	161	217	8.16	100.5	349	147	202	7.59	94
25/09/02	2.89	513	73	440	15.22	505	64	441	15.26	100.2	447	58	389	13.46	88
3/10/2002	3.10	463	57	406	13.10	460	46	414	13.35	102	412	34	378	12.19	93
3/10/2002	2.89	618	289	329	11.38	590	261	329	11.38	100	563	232	331	11.45	101
11/10/2002	2.27	327	60	267	11.76	321	54	267	11.76	100	293	54	239	10.53	90
15/10/02	2.49	473	48	425	17.07	462	47	415	16.67	97.6	403	42	361	14.50	85
17/10/02	2.65	319	100	219	8.26	299	93	206	7.77	94.1	268	90	178	6.72	81
18/10/02	2.42	301	85	216	8.93	292	77	215	8.88	99.5	276	73	203	8.39	94
Mean	**2.70**	**451**	**126**	**325**	**11.98**	**440**	**119**	**322**	**11.86**	**98.9**	**402**	**108**	**294**	**10.82**	**91**
SEM	0.08	36	25	28	0.93	35	24	29	0.93	0.7	33	22	26	0.81	2

CYCLOSERINE

ID	Muscle Wet Weight (g)	Peak (g)	Rest (g)	Actual Peak (g)	Actual Peak Tension (kg/100g wmm)	1min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	2min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
9/24/2002	3.04	482	128	354	11.64	461	114	347	11.41	98	431	102	329	10.82	93
9/26/2002	2.88	462	128	334	11.60	431	70	361	12.53	108.1	384	64	320	11.11	96
9/26/2002	2.86	274	78	196	6.85	241	83	158	5.52	80.6	239	78	161	5.63	82
10/1/2002	3.07	493	130	363	11.82	480	114	366	11.92	100.8	447	101	346	11.27	95
10/15/2002	2.72	437	66	371	13.64	426	62	364	13.38	98.1	382	57	325	11.95	88
10/15/2002	3.08	432	76	356	11.56	412	73	339	11.01	95.2	367	70	297	9.64	83
10/16/2002	2.33	256	100	156	6.70	239	87	152	6.52	97.4	220	83	137	5.88	88
10/16/2002	2.39	326	70	256	10.71	302	66	236	9.87	92.2	261	63	198	8.28	77
10/17/2002	2.65	418	30	388	14.64	407	28	379	14.30	97.7	351	26	325	12.26	84
10/17/2002	2.83	382	17	365	12.90	179	16	163	5.76	44.7	339	15	324	11.45	89
Mean	**2.79**	**396**	**82**	**314**	**11.21**	**358**	**71**	**287**	**10.22**	**91**	**342**	**66**	**276**	**9.83**	**87**
SEM	0.08	27	13	26	0.82	34	10	31	1.01	6	25	9	25	0.77	2

RAW DATA: CONTRACTILE FUNCTION

CONTROL

ID	3min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	4min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
18/09/02	436	182	254	9.48	75	404	176	228	8.51	67
19/09/02	511	150	361	12.28	92	477	146	331	11.26	85
20/09/02	325	141	184	6.92	85	308	135	173	6.50	80
25/09/02	409	55	354	12.25	80	366	53	313	10.83	71
3/10/2002	328	19	309	9.97	76	302	19	283	9.13	70
3/10/2002	518	216	302	10.45	92	479	205	274	9.48	83
11/10/2002	253	50	203	8.94	76	224	48	176	7.75	66
15/10/02	354	41	313	12.57	74	301	40	261	10.48	61
17/10/02	250	88	162	6.11	74	233	86	147	5.55	67
18/10/02	256	71	185	7.64	86	241	69	172	7.11	80
Mean	**364**	**101**	**263**	**9.66**	**81**	**334**	**98**	**236**	**8.66**	**73**
SEM	32	21	24	0.73	2	30	20	21	0.61	3

CYCLOSERINE

ID	3min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	4min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
9/24/2002	376	94	282	9.28	80	332	91	241	7.93	68
9/26/2002	331	61	270	9.38	81	269	60	209	7.26	63
9/26/2002	212	78	134	4.69	68	206	76	130	4.55	66
10/1/2002	395	92	303	9.87	83	356	86	270	8.79	74
10/15/2002	355	56	299	10.99	81	314	54	260	9.56	70
10/15/2002	324	68	256	8.31	72	282	65	217	7.05	61
10/16/2002	200	80	120	5.15	77	185	77	108	4.64	69
10/16/2002	216	60	156	6.53	61	178	61	117	4.90	46
10/17/2002	306	25	281	10.60	72	272	25	247	9.32	64

RAW DATA: CONTRACTILE FUNCTION

CONTROL

ID	5min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	6min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
18/09/02	356	174	182	6.79	54	336	173	163	6.08	48
19/09/02	447	140	307	10.44	79	403	138	265	9.01	68
20/09/02	285	133	152	5.71	70	269	129	140	5.26	65
25/09/02	317	53	264	9.13	60	284	51	233	8.06	53
3/10/2002	275	19	256	8.26	63	421	16	405	13.06	100
3/10/2002	436	199	237	8.20	72	413	193	220	7.61	67
11/10/2002	192	49	143	6.30	54	166	48	118	5.20	44
15/10/02	242	40	202	8.11	48	204	40	164	6.59	39
17/10/02	222	85	137	5.17	63	211	83	128	4.83	58
18/10/02	225	68	157	6.49	73	210	68	142	5.87	66
Mean	**300**	**96**	**204**	**7.46**	**63**	**292**	**94**	**198**	**7.16**	**61**
SEM	28	19	19	0.52	3	30	19	28	0.78	5

CYCLOSERINE

ID	5min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	6min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
9/24/2002	288	90	198	6.51	56	252	89	163	5.36	46
9/26/2002	234	59	175	6.08	52	220	58	162	5.63	49
9/26/2002	194	75	119	4.16	61	180	74	106	3.71	54
10/1/2002	325	90	235	7.65	65	282	82	200	6.51	55
10/15/2002	298	53	245	9.01	66	291	52	239	8.79	64
10/15/2002	264	66	198	6.43	56	258	65	193	6.27	54
10/16/2002	184	77	107	4.59	69	178	76	102	4.38	65
10/16/2002	146	61	85	3.56	33	122	60	62	2.59	24
10/17/2002	237	25	212	8.00	55	216	25	191	7.21	49
10/17/2002	239	15	224	7.92	61	212	14	198	7.00	54

RAW DATA: CONTRACTILE FUNCTION

CONTROL

ID	7min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	8min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
18/09/02	336	171	165	6.16	49	331	168	163	6.08	48
19/09/02	394	137	257	8.74	66	373	135	238	8.10	61
20/09/02	256	129	127	4.77	59	238	126	112	4.21	52
25/09/02	250	50	200	6.92	45	208	50	158	5.47	36
3/10/2002	212	17	195	6.29	48	189	18	171	5.52	42
3/10/2002	387	190	197	6.82	60	363	187	176	6.09	53
11/10/2002	143	48	95	4.19	36	126	47	79	3.48	30
15/10/02	182	40	142	5.70	33	162	40	122	4.90	29
17/10/02	200	83	117	4.42	53	193	81	112	4.23	51
18/10/02	197	67	130	5.37	60	189	68	121	5.00	56
Mean	**256**	**93**	**163**	**5.94**	**51**	**237**	**92**	**145**	**5.31**	**46**
SEM	28	19	16	0.43	3	28	18	14	0.41	4

CYCLOSERINE

ID	7min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	8min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
9/24/2002	219	88	131	4.31	37	200	87	113	3.72	32
9/26/2002	212	57	155	5.38	46	209	57	152	5.28	46
9/26/2002	163	75	88	3.08	45	258	75	183	6.40	93
10/1/2002	269	90	179	5.83	49	245	87	158	5.15	44
10/15/2002	276	52	224	8.24	60	267	51	216	7.94	58
10/15/2002	267	65	202	6.56	57	279	64	215	6.98	60
10/16/2002	177	75	102	4.38	65	177	76	101	4.33	65
10/16/2002	123	60	63	2.64	25	120	59	61	2.55	24
10/17/2002	195	25	170	6.42	44	186	24	162	6.11	42
10/17/2002	187	14	173	6.11	47	162	14	148	5.23	41

RAW DATA: CONTRACTILE FUNCTION

CONTROL

ID	9min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	10min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
18/09/02	323	166	157	5.86	46	286	165	121	4.51	36
19/09/02	359	134	225	7.65	58	343	133	210	7.14	54
20/09/02	219	125	94	3.53	44	209	123	86	3.23	40
25/09/02	190	49	141	4.88	32	168	49	119	4.12	27
3/10/2002	175	19	156	5.03	38	163	19	144	4.65	35
3/10/2002	337	185	152	5.26	46	325	182	143	4.95	43
11/10/2002	116	47	69	3.04	26	104	46	58	2.56	22
15/10/02	140	39	101	4.06	24	118	39	79	3.17	19
17/10/02	191	81	110	4.15	50	187	80	107	4.04	49
18/10/02	181	66	115	4.75	53	169	66	103	4.26	48
Mean	**223**	**91**	**132**	**4.82**	**42**	**207**	**90**	**117**	**4.26**	**37**
SEM	27	18	14	0.41	4	26	18	13	0.40	4

CYCLOSERINE

ID	9min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak	10min Peak	Rest (g)	Actual (g)	Actual Peak Tension (kg/100g wmm)	% Decline from Peak
9/24/2002	188	87	101	3.32	29	174	87	87	2.86	25
9/26/2002	204	57	147	5.10	44	179	57	122	4.24	37
9/26/2002	172	74	98	3.43	50	174	72	102	3.57	52
10/1/2002	222	81	141	4.59	39	221	84	137	4.46	38
10/15/2002	246	51	195	7.17	53	243	51	192	7.06	52
10/15/2002	270	64	206	6.69	58	263	64	199	6.46	56
10/16/2002	172	75	97	4.16	62	175	73	102	4.38	65
10/16/2002	120	60	60	2.51	23	122	59	63	2..64	25
10/17/2002	168	24	144	5.43	37	160	24	136	5.13	35

APPENDIX II

STATISTICAL TABLES
1-WAY ANOVAS
TUKEY HSD POST HOC TESTS

MUSCLE GLYCOGEN (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	1667.30	152
CONTROL Stimulation	10	1140.38	114
CYCLOSERINE Rest	9	1364.90	152
CYCLOSERINE Stimulation	10	1182.72	118

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		9.983	0.000
Error (CONDITION)	4220.094	36	422.726		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	151.572	114.037	151.656	118.272
CONTROL REST		0.0011	1.0000	0.0039
CONTROL STIMULATION	0.0011		0.0018	0.9672
CYCLOSERINE REST	1.0000	0.0018		0.0061
CYCLOSERINE STIMULATION	0.0039	0.9672	0.0061	

MUSCLE LACTATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	139.87	12.72
CONTROL Stimulation	10	346.11	34.61
CYCLOSERINE Rest	9	133.49	14.83
CYCLOSERINE Stimulation	10	349.85	34.99

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		30.540	0.000
Error (CONDITION)	1495.88	36	48.981		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	12.715	34.611	14.832	34.985
CONTROL REST		0.0002	0.9067	0.0002
CONTROL STIMULATION	0.0002		0.0002	0.9994
CYCLOSERINE REST	0.9067	0.0002		0.0002
CYCLOSERINE STIMULATION	0.0002	0.9994	0.0002	

PYRUVATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	3.94	0.36
CONTROL Stimulation	10	7.18	0.72
CYCLOSERINE Rest	9	2.34	0.26
CYCLOSERINE Stimulation	10	5.95	0.60

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		25.126	0.000
Error (CONDITION)	0.429	36	0.017		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.359	0.717	0.261	0.596
CONTROL REST		0.0002	0.3550	0.0012
CONTROL STIMULATION	0.0002		0.0002	0.1822
CYCLOSERINE REST	0.3550	0.0002		0.0002
CYCLOSERINE STIMULATION	0.0012	0.1822	0.0002	

PHOSPHOCREATINE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	1131.82	102.90
CONTROL Stimulation	10	514.09	51.40
CYCLOSERINE Rest	9	890.42	98.90
CYCLOSERINE Stimulation	10	597.56	59.80

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		23.631	0.000
Error (CONDITION)	7051.675	36	298.405		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	102.893	51.408	98.936	59.785
CONTROL REST		0.0002	0.9563	0.0002
CONTROL STIMULATION	0.0002		0.0002	0.7033
CYCLOSERINE REST	0.9563	0.0002		0.0003
CYCLOSERINE STIMULATION	0.0002	0.7033	0.0003	

CREATINE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	570.40	51.90
CONTROL Stimulation	10	885.80	88.60
CYCLOSERINE Rest	9	549.44	54.90
CYCLOSERINE Stimulation	10	874.30	87.40

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		20.953	0.000
Error (CONDITION)	4045.483	36	193.077		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	51.855	88.580	54.944	87.430
CONTROL REST		0.0002	0.9598	0.0002
CONTROL STIMULATION	0.0002		0.0002	0.9978
CYCLOSERINE REST	0.9598	0.0002		0.0002
CYCLOSERINE STIMULATION	0.0002	0.9978	0.0002	

TOTAL CREATINE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	1702.22	154.70
CONTROL Stimulation	10	1399.88	140.00
CYCLOSERINE Rest	9	1384.92	153.90
CYCLOSERINE Stimulation	10	1471.88	147.20

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		4.703	0.007
Error (CONDITION)	475.369	36	101.074		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	154.774	139.988	153.88	147.188
CONTROL REST		0.0095	0.9973	0.3250
CONTROL STIMULATION	0.0095		0.0237	0.3907
CYCLOSERINE REST	0.9973	0.0237		0.4783
CYCLOSERINE STIMULATION	0.3250	0.3907	0.4783	

ATP (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	282.00	25.6
CONTROL Stimulation	10	228.48	22.8
CYCLOSERINE Rest	9	228.25	25.4
CYCLOSERINE Stimulation	10	229.33	22.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		9.923	0.000
Error (CONDITION)	23.068	36	2.325		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	25.635	22.847	25.363	22.933
CONTROL REST		0.0011	0.9785	0.0015
CONTROL STIMULATION	0.0011		0.0053	0.9993
CYCLOSERINE REST	0.9785	0.0053		0.0073
CYCLOSERINE STIMULATION	0.0015	0.9993	0.0073	

SUM OF TCAI (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	23.09	2.10
CONTROL Stimulation	10	25.63	2.56
CYCLOSERINE Rest	9	14.59	1.62
CYCLOSERINE Stimulation	10	18.93	1.89

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		8.344	0.000
Error (CONDITION)	1.524	36	0.183		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	2.100	2.564	1.621	1.892
CONTROL REST		0.0796	0.0782	0.6836
CONTROL STIMULATION	0.0796		0.0003	0.0064
CYCLOSERINE REST	0.0782	0.0003		0.5202
CYCLOSERINE STIMULATION	0.6836	0.0064	0.5202	

MALATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	7.34	0.67
CONTROL Stimulation	10	12.12	1.21
CYCLOSERINE Rest	9	3.91	0.43
CYCLOSERINE Stimulation	10	6.77	0.68

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		27.085	0.000
Error (CONDITION)	1.053	36	0.039		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.667	1.212	0.434	0.677
CONTROL REST		0.0002	0.0585	0.9995
CONTROL STIMULATION	0.0002		0.0002	0.0002
CYCLOSERINE REST	0.0585	0.0002		0.0521
CYCLOSERINE STIMULATION	0.9995	0.0002	0.0521	

FUMARATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	1.09	0.10
CONTROL Stimulation	10	1.97	0.20
CYCLOSERINE Rest	9	0.97	0.06
CYCLOSERINE Stimulation	10	1.28	0.13

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		12.257	0.000
Error (CONDITION)	0.033	36	0.003		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.099	0.197	0.060	0.135
CONTROL REST		0.0008	0.3329	0.4073
CONTROL STIMULATION	0.0008		0.0002	0.0514
CYCLOSERINE REST	0.3329	0.0002		0.0159
CYCLOSERINE STIMULATION	0.4073	0.0514	0.0159	

MALATE AND FUMARATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	8.43	0.77
CONTROL Stimulation	10	14.09	1.41
CYCLOSERINE Rest	9	4.94	0.49
CYCLOSERINE Stimulation	10	8.05	0.80

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		27.138	0.000
Error (CONDITION)	1.443	36	0.053		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.767	1.409	0.494	0.807
CONTROL REST		0.0002	0.0575	0.9790
CONTROL STIMULATION	0.0002		0.0002	0.0002
CYCLOSERINE REST	0.0575	0.0002		0.0273
CYCLOSERINE STIMULATION	0.9790	0.0002	0.0273	

2-OXOGLUTARATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	0.95	0.09
CONTROL Stimulation	10	0.82	0.08
CYCLOSERINE Rest	9	0.74	0.08
CYCLOSERINE Stimulation	10	0.90	0.09

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		0.991	0.408
Error (CONDITION)	0.000	36	0.000		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.086	0.0824	0.082	0.09
CONTROL REST		0.8568	0.8303	0.9084
CONTROL STIMULATION	0.8568		0.9999	0.4903
CYCLOSERINE REST	0.8303	0.9999		0.4676
CYCLOSERINE STIMULATION	0.9084	0.4903	0.4676	

ISOCITRATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	1.92	0.17
CONTROL Stimulation	10	1.85	0.18
CYCLOSERINE Rest	9	0.53	0.06
CYCLOSERINE Stimulation	10	0.40	0.04

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		49.517	0.000
Error (CONDITION)	0.058	36	0.001		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	0.174	0.185	0.058	0.039
CONTROL REST		0.8718	0.0002	0.0002
CONTROL STIMULATION	0.8718		0.0002	0.0002
CYCLOSERINE REST	0.0002	0.0002		0.6344
CYCLOSERINE STIMULATION	0.0002	0.0002	0.6344	

CITRATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	11.79	1.07
CONTROL Stimulation	10	8.86	0.89
CYCLOSERINE Rest	9	8.88	0.99
CYCLOSERINE Stimulation	10	9.58	0.96

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		0.473	0.703
Error (CONDITION)	0.061	36	0.130		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	1.072	0.887	0.986	0.959
CONTROL REST		0.6463	0.9505	0.8897
CONTROL STIMULATION	0.6463		0.9328	0.9698
CYCLOSERINE REST	0.9505	0.9328		0.9986
CYCLOSERINE STIMULATION	0.8897	0.9698	0.9986	

ALANINE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	76.55	6.96
CONTROL Stimulation	10	54.19	8.71
CYCLOSERINE Rest	9	87.07	6.02
CYCLOSERINE Stimulation	10	71.20	7.12

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		7.888	0.000
Error (CONDITION)	11.965	36	1.517		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	6.959	8.707	6.021	7.120
CONTROL REST		0.0129	0.3415	0.9906
CONTROL STIMULATION	0.0129		0.0003	0.0322
CYCLOSERINE REST	0.3415	0.0003		0.2292
CYCLOSERINE STIMULATION	0.9906	0.0322	0.2292	

GLUTAMATE (mmol.kg^{-1} dw)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	60.69	5.52
CONTROL Stimulation	10	29.71	2.97
CYCLOSERINE Rest	9	56.76	6.31
CYCLOSERINE Stimulation	10	50.49	5.05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		13.893	0.000
Error (CONDITION)	19.788	36	1.424		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	5.517	2.971	6.307	5.049
CONTROL REST		0.0003	0.4648	0.8060
CONTROL STIMULATION	0.0003		0.0002	0.0023
CYCLOSERINE REST	0.4648	0.0002		0.1186
CYCLOSERINE STIMULATION	0.8060	0.0023	0.1186	

ALANINE AMINOTRANSFERASE ($mmol.kg^{-1}$ $ww.min^{-1}$)

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	39.16	3.56
CONTROL Stimulation	10	39.24	3.92
CYCLOSERINE Rest	9	5.50	0.61
CYCLOSERINE Stimulation	10	6.33	0.63

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		113.094	0.000
Error (CONDITION)	32.406	36	0.287		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	3.560	3.924	0.611	0.633
CONTROL REST		0.4158	0.0002	0.0002
CONTROL STIMULATION	0.4158		0.0002	0.0002
CYCLOSERINE REST	0.0002	0.0002		0.9998
CYCLOSERINE STIMULATION	0.0002	0.0002	0.9998	

CITRATE SYNTHASE (mmol.kg-1 ww.min-1)
ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*
CONTROL Rest	11	301.84	27.44
CONTROL Stimulation	10	286.34	28.63
CYCLOSERINE Rest	9	221.58	24.62
CYCLOSERINE Stimulation	10	247.98	24.80

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
CONDITION		3		4.432	0.009
Error (CONDITION)	38.636	36	8.717		

Tukey HSD rest
Probabilities for Post Hoc Tests

	CON REST	**CON STIM**	**CYCLO REST**	**CYCLO STIM**
MEAN	27.440	28.634	24.620	24.798
CONTROL REST		0.7915	0.1647	0.1899
CONTROL STIMULATION	0.7915		0.0267	0.0304
CYCLOSERINE REST	0.1647	0.0267		0.9992
CYCLOSERINE STIMULATION	0.1899	0.0304	0.9992	

APPENDIX III

INTRA-ASSAY VARIABILITY

INTRA-ASSAY VARIABILITY

CV calculated based on mean/standard deviation (sd) of 6 repeats

CV = sd/mean x 100%

ASSAY	CV (%)
Alanine	1.8
Alanine aminotransferase	4.6
ATP	1.2
Citrate	2.9
Citrate Synthase	1.8
Creatine	2.8
Fumarate	18.6
Glutamate	2.1
Glucose (Glycogen)	1.4
Isocitrate	19.8
Lactate	1.8
Malate	9.0
Muscle glycogen	1.28
Phosphocreatine	1.6
Pyruvate	5.5