EFFECTS OF HYPOCALORIC FEEDING AND HIGH CARBOHYDRATE REFEEDING ON IN SITU MUSCLE FUNCTION, GLYCOLYSIS AND BODY COMPOSITION IN ADULT RATS

BY

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A thesis submitted in conformity with the requirements for the Degree of Doctor of Philosophy
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University of Toronto

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ABSTRACT

EFFECTS OF HYPOCALORIC FEEDING AND HIGH CARBOHYDRATE REFEEDING ON IN SITU MUSCLE FUNCTION, GLYCOLYSIS AND BODY COMPOSITION IN ADULT RATS

DOCTOR OF PHILOSOPHY, 1996
JOSEPH DAVID JEAN ANDRE BISSONNETTE
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Traditionally, the effects of hypocaloric intake and refeeding have been defined in terms of the amount of wasting and restoration of total lean body mass. However, changes in muscle performance appear to occur earlier, and correlate better with the risk of postoperative complications than changes in body composition. The studies in this thesis, in contrast to many studies on muscle and malnutrition, have been controlled for muscle wasting, muscle-type and electrolyte-micronutrient intakes during hypocaloric feeding and refeeding. In addition, glycogen levels, net glycogenolysis and glycolysis have been considered in this study.

The hypothesis of this research was that during malnutrition and the very early refeeding period, changes in muscle performance are: (i) independent of muscle size; (ii) muscle type-specific; (iii) related to altered glycolytic and glycogenolytic pathways; (iv) independent of substrate availability.

Isolated slow-twitch soleus (oxidative) and fast-twitch EDL (glycolytic) muscles were studied in situ, in adult, male, Wistar rats. Following one week of hypocaloric (HYPO) feeding and after being refed for 4 consecutive days, the muscles of each group were measured for fatiguability (% force loss over 40 tetani), maximal relaxation rate (MRR-%force loss/10 ms) and single tetanus Po(N). The MRR and the Po(N) of both muscles were altered by HYPO feeding. However, the Po(N) and MRR of
only the soleus normalized after four days of refeeding, despite an unresponsive muscle mass, yet serum glucose and muscle glycogen were not critical in avoiding fatigue. In the HYPO rats, a fall in net glycogenolysis was associated with an increased fatiguability of the soleus. In the EDL, by contrast, net glycogenolysis increased and fatigue was unchanged (p<0.01). With refeeding, fatigue increased in the EDL over 4 days despite normal glycogen levels, net glycogenolysis and glycolysis by day 4. Refeeding did not normalize soleus fatigue; glycogen levels and net glycolytic flux were sub-optimal, but net glycogenolysis had normalized.

In conclusion, malnutrition caused abnormal net glycogenolysis in both muscle types and subnormal glycolysis in the slow-twitch muscles only, confirming the third hypothesis. The performances of the muscles were dissimilar and changed independently from mass, protein and glycogen, confirming the first, second and fourth hypotheses.
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The earlier studies of fatiguability as they relate to muscular myopathies involved a structural investigation which necessitated muscle biopsies, histochemistry, electron microscopy, or electromyograms etc (116). The need to explore the function of muscle soon ensued as a logical progression to explore the contractile machinery of the muscle, and its recovery with treatment. Measurements of muscle function were initially conducted by Merton using the hand-held dynamometer (291). Work on muscle fatigue was pursued afterwards by Edwards et al. (110-123), Bergstrom et al. (29-31), Sahlin et al. (360b-363), Hultman et al. (201-205), Bigland-Ritchie et al. (34-37), Newsholme (310), Gollnick et al. (145-148) and others, using the Hammersmith Myometer (117) and electrically stimulated muscles. Their main interests evolved around either muscle pathology or exercise physiology (116).

More recently, however, muscle function has been used as an early assessor of nutritional status in experimental rats and in hospitalized patients. In these situations, muscle responds to undernutrition and to nutritional rehabilitation. Whittaker et al. (419), Nishio & Jeejeebhoy (315-316), Fong et al. (130), Russell et al. (354-359), Pichard et al. (336), Christie & Hill (73), and Windsor & Hill (424) have shown muscle function to be a significant clinical assessment tool by: First, documenting that function responded faster than traditional body composition parameters such as serum proteins and anthropometric measures. Second, showing muscle function to have an elevated specificity and sensitivity (Brough et al.) (52). Third, indicating that function appears to be independent of disease process and trauma (Brough et al.) (52). Fourth, using muscle function as a reliable predictor of post-surgical complications (Windsor & Hill) (424); similar findings were also reported by Zeiderman & McMahon (435). Fifth, showing the muscle behavior during nutritional depletion to be tied to anomalies in muscle metabolic pathway.
activities and to a diminished sarcoplasmic reticulum calcium uptake. Changes in oxidative phosphorylation and glycolysis have previously been documented (357, 316, 295, 338) using NMR technology. Defects in calcium flux have been previously described as a likely mechanism in skeletal muscle of malnourished rats (214) and have been reported in the heart muscle of rats hypocalorically fed (319). And while there is sufficient evidence to suggest that undernutrition produces important changes in both calcium flux and muscle metabolic activity it is still unclear if such flux anomalies are in fact occurring in skeletal muscle or if metabolic irregularities result from insufficient glucose, glycogen or free fatty acids. Furthermore, it is also unclear how fast-twitch and slow-twitch muscle types respond to nutritional deprivation or to early refeeding. Consequently, in this thesis, I have studied the biochemical metabolites of fast and slow-twitch muscles concurrently with key parameters of muscle function in hypocalorically fed and refed rats. In doing so I have described key glycolytic metabolite concentrations and fluxes in the two muscle types and presented data pertaining to net glycogenolysis. This has not been done before in the undernourished and refed states. Moreover, the control, hypocaloric and refed diets consist of liquid elemental formulas that have identical micronutrient contents (vitamins, minerals, and trace elements). This is a novel approach that ensures that the undernourished model produces protein energy malnutrition and not a form of vitamin-derived or trace-element-derived malnutrition.

This thesis provides relevant biochemical data measured concurrently with maximal relaxation rate, fatigue and tetanic tension and it completes previous NMR work (295, 338). The functional and biochemical data is pertinent to the prolonged hypocalorically fed rats and the first 4 consecutive days of refeeding. Fatigue is looked at from the perspective
of net glycogenolysis, glycolysis and muscle glycogen concentrations. These findings, although from an animal model, provide further insight into the functional mechanisms of rehabilitation of the malnourished patient and is but one step in understanding how to maximize recovery.
ACKNOWLEDGEMENTS

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GLOSSARY

Actin........................Protein found in thin filament of muscle.
Adrenal medulla..............Endocrine gland, that releases 80% epinephrine and 20% norepinephrine.
Aerobic......................Requiring oxygen.
β-cells of pancreas.........Insulin secreting cells of the pancreas.
Anaerobic.....................Not requiring oxygen.
Calmodulin....................Calcium transport carrier in most cells.
Calsequestrin..................Calcium binding protein in the S.R.
Central fatigue..............Fatigue cause by a defect of the C.N.S.
Cortisol.......................Glucocorticoid produced by the adrenal medulla.
Creatine kinase.............Enzyme that catalyses the reaction:
P Cr + ADP + H+ -------> ATP + Cr.
Endurance.....................The ability to resist fatigue.
Energetics....................The ability to convert energy into function.
Epinephrine...................Catecholamine secreted by adrenal medulla.
Excitation-contraction
Coupling.......................Muscle action potential depolarizes the T-Tubules, causing Ca²⁺ release from S.R. and binding of Ca²⁺ on troponin followed by cross-bridge activity and relaxation of muscle.
Fatigue.......................The inability to sustain tension during repeated contractions (% force lost over time).
Force frequency curve.....Tension generated at various frequency rates of stimulation expressed in hertz. The curve displays the increasing force with rising stimulation frequency.

Glucagon...............Hormone produced by the α-cells of the pancreas in response to catecholamine release.

Gluconeogenesis...........Biochemical process whereby glucose is produced from amino acids, glycerol, lactate, and pyruvate,

Glycogenolysis............Biochemical process which describes the breakdown of glycogen→glucose-6-phosphate

Glycolysis................An anaerobic biochemical process which transforms glucose to lactate/pyruvate for the production of ATP.

High-frequency fatigue.....Loss of force at high frequency stimulations, related to an impaired neuromuscular transmission.

β-hydroxyacyl CoA dehydrogenase.............Series of enzymes involved in the oxidation of fatty acids with more than 4 carbons (310)

Hyperglycemia.............High serum glucose.

In situ.....................In an unaltered biological system.

In vitro.....................In an artificial environment.

In vivo.....................In biologically active tissue.

Isometric contractions.....Force production with no shortening of muscle fibres.

Isotonic contractions.....Shortening of muscle fibres to generate a tension greater than the load.

Lipolysis.................Oxidative breakdown of fat.
Low-frequency fatigue..... Loss of force at low frequency stimulation, due to impaired excitation-contraction coupling.

Maximal relaxation rate....% Force loss/10 ms; % loss of height of tension/ms & equal to 0.693/t^2.

Maximal tetanic force.....Tetanus produced at maximal stimulation frequency.

Metabolic acidosis........Low pH (high acidity) in blood and body fluids.

Myosin ...................Thick contractile protein of muscle.

Oxidative Phosphorylation.. Electrons moving down the respiratory chain of the mitochondria, flow to oxygen and release energy, causing the phosphorylation of ADP to ATP; known as the chemiosmotic theory.

Peripheral Fatigue.........Fatigue related to an impairment at the neuromuscular junction; failure in the muscle action potential; & failure of excitation-contraction coupling.

Phosphocreatine..........High phosphate reserve in the muscle.

Phosphorylase a & b........Active & inactive enzymes used in glycogen breakdown.

Phosphofructokinase........Enzyme that catalyses the phosphorylation of fructose-6-phosphate to fructose, 1,6-diPhosphate (310)

Phosphorylation..........The transfer of a phosphate group from a high-energy phosphate donor to ADP; a process known as substrate-level phosphorylation. A phosphate transfer, forming a phosphate ester; process catalyzed by a kinase enzyme.

Relaxation time ............ Time for peak tension to fall to 0.

Reliability ................ Ability to repeat measurement and get the same result.

Sensitivity ................ Ability of a test to detect true positives.

Specificity ................. Ability of a test to detect false negatives.

Succinate dehydrogenase (SDH). Enzyme that catalyses the oxidation of succinate to fumarate (310).

Tetanic contraction ......... Tetanus produced by a single stimulation at a maximal frequency.

Tropomyosin ................. Strand of regulatory protein associated with the thin actin protein filament.

Troponin ..................... Globular regulatory protein associated with the thin actin protein filament.

Twitch ....................... Single contraction at a frequency of 1 Hz.
ABBREVIATIONS

α-Ketog.........................Alpha-ketoglutarate
A.C.D.H........................Acyl-CoA dehydrogenase
B.C.M............................Body cell mass
C.H.I......................Creatine height index
C.K..............................Creatine kinase
CN-----------------Control well fed
CSA................Cross-sectional area
Cr................Creatine
E.C.W...............................Extracellular water
EDL..............................Extensor digitorum longus
F1,6-diP .......................Fructose, 1,6 di-Phosphate
F10/F50 ......................Ratio of tension at 10 Hertz
to tension at 50 Hertz
F10/F100 ......................Ratio of tension at 10 Hertz
to tension at 100 Hertz
F.F.M............................Fat free mass
F.F.S............................Fat free solids
F,6-P.............................Fructose, 6-Phosphate
F_{max}.......................Maximal tetanic tension
G,6-P.............................Glucose, 6-Phosphate
HCD............................High carbohydrate diet
HYPO..............................Hypocaloric
I.C.W............................Intracellular water
Lo(cm)..............................Length of muscle for maximal twitch
MRR.............................Maximal relaxation rate
NMR..............................Nuclear magnetic resonance
P.C.M.............................Protein calorie malnutrition
P.E.M.............................Protein energy malnutrition
PFK.........................Phosphofructokinase
PCr..........................Phosphocreatine
Pi.............................Inorganic phosphate
Po(N)........................Maximal tension in Newtons
Po(N)/g.....................Maximal tension corrected for wet weight of muscle
Pt............................Twitch tension produced at 1 Hz
PUFA........................Polyunsaturated fatty acid
RE-1.........................First day refed
RE-4.........................Fourth day refed
SDH...........................Succinate dehydrogenase
S.E.M........................Standard error of mean
S.R..........................Sarcoplasmic reticulum
T.B.N........................Total body nitrogen
T.B.S........................Total body solids
T.B.W........................Total body water
T.P.N........................Total parenteral nutrition
\Delta G_{ATP}..................Free energy change for ATP hydrolysis.
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CHAPTER 1 INTRODUCTION

1.1 GENERAL BACKGROUND

Malnutrition is associated with significant weight loss (234, 246), increased risk of infections, postoperative complication, and death (289, 223, 304, 271). It is also associated with markedly reduced levels of serum proteins (39, 40, 368), and with abnormal parameters of immunocompetence (265b, 304b). None of the biochemical and immunocompetence measurements are specific to reduced nutrient intakes, but also occur as a result of disease and trauma (213, 263, 286, 368, 373, 430). Previous work by Russell et al., (356, 354, 355, 357, 358, 359), Pichard et al. (338, 336), Christie and Hill (73), Windsor and Hill (424), Mijan de la Torre et al. (295), Zeiderman and McMahon (435), and Nishio & Jeejeebhoy (315, 316) showed that muscle function was profoundly influenced by nutrition, more so than body composition parameters such as weight, anthropometry and blood proteins.

It has been clear for some time that significant weight loss can compromise outcome. In fact, in the 1930's, Studley (391) showed weight loss in postoperative patients and in patients with injuries to be associated with overall complications and a decreased chance of survival. The ability to predict individual outcome remained, however, limited as weight alone is not a useful detector of nutritional status (39), nor is it a good predictor of outcome (57b). More recently, it has been shown that muscle function is impaired by malnutrition and is improved by refeeding over time (73, 295, 338). Furthermore, abnormal muscle function has been shown to predict the occurrence of complications (424, 435) in surgical patients with significant sensitivity and specificity. This predictability is not possible using the loss of body protein alone (424), nor anthropometry and liver function (424). Also, the size of the muscle does not accurately reflect muscle protein and energy stores (185). Yet, muscle function predicts complications and responds rapidly to refeeding; hence, the earliest
changes in muscle function are studied here, after the initiation of refeeding following a period of hypocaloric intake in adult rats. The intent is to investigate the effect of changes in glycogen concentration, net glycogenolysis and glycolysis on muscle function. The rat is chosen as the experimental animal, as it allows an exhaustive exploration of two distinct muscle types, their metabolic processes, in addition to changes in body composition. The fatiguability (% force loss over 40 tetani) will be measured, as well as maximal relaxation rate (MRR = 0.693/t), and the single tetanus (Po(N)) (tension produced) of an in situ rat muscle after 7 days of hypocaloric feeding, followed by a study of randomized rats refeed over the first 4 days following underfeeding. Well fed control rats will be studied will be compared. This early refeeding period has been shown by Christie and Hill (73) to be highly responsive to nutritional support, therefore, the mechanisms for the early and rapid response will be studied. Furthermore, previous investigators did not control for the intake of electrolytes and macronutrients, which could have influenced muscle performance. For example, K⁺, Mg²⁺, P intakes can profoundly alter muscle function. The effects of nutrition on the performance of muscles, composed of different fiber types had not been previously differentiated. In addition, the importance of restricted protein-calorie intake and high carbohydrate feeding on the glycogen levels, net glycogenolysis and glycolytic cycle in relation to muscle performance had not been evaluated. In this thesis I propose to focus on these particular areas of the interaction of nutrition and muscle.

1.2 PURPOSE OF STUDY
The questions that frame this study are: (1) Is muscle performance altered by protein-calorie malnutrition and restored by refeeding independently of muscle size?; (2) what are the differences in performance between fast and slow-twitch muscles within the contexts of
undernutrition and early refeeding?; (3) how do alterations in muscle function relate to net glycogenolysis and an altered glycolytic cycle?; and (4) how do the availability of muscle glycogen and glucose influence the function of muscle?.

Finally, the early response of muscle to refeeding concurrently with changes in body composition will be examined, and will show that neither muscle size or weight, nor substrate availability are limiting factors in the development of muscle fatigue and early recovery. The findings will suggest that fatiguability may be associated with anomalies in metabolic activity.

1.3 ORGANIZATION OF THESIS

To facilitate the logical flow of ideas and concepts, culminating in a description of relevant findings, understanding the effects of malnutrition on muscle becomes essential and pivotal to this thesis. To acquire such an understanding necessitates a stepwise literature review that explores first, the etiology of fatigue from a non-metabolic perspective, looking specifically at membrane potential, and the role of glycogen. Second, the impact of undernutrition on muscle is reviewed, looking again at membrane excitability, and other physiological parameters. Third, the metabolic influences of muscle fatigue are reviewed with a specific focus placed on the influence of undernutrition. Fourth, the body composition and metabolic repercussion of refeeding on muscle are reviewed.

Following the literature review, the parameters and methodologies to be used during the experimental work are described in Chapter 3. Next, the effect of changes in diet on the eating patterns of the rats are briefly explored. This is followed by chapter 5, a study of the changes
in body composition taking place with malnutrition and refeeding.

Chapter 6 is a study of stimulated muscles and of the metabolic activity occurring in the muscles. This latter chapter is primarily a mechanical and biochemical investigation of muscle fatigue.

Chapter 7 is a general discussion of the major finding of the thesis; it is composed of sections that describe the changes in body composition, the influence of diet, and the biochemical findings within the context of fatigue. And finally, the main experimental findings are summarized in chapter 8.
CHAPTER 2 REVIEW OF THE LITERATURE

This literature review is organized in such a way, that it provides a theoretical framework for the experimental research of this thesis. The two main headings of this framework are: (a) the influence of undernutrition on muscle fatigue; and (b) the effect of refeeding on fatigue and body composition. These sections are developed in a manner that relevant issues of muscle physiology and function can be reviewed within the context of hypocaloric feeding and refeeding.

2.1 UNDERNUTRITION AND MUSCLE FATIGUE

Prolonged caloric deprivation produces debilitating physical wasting with which is associated important losses of muscle and visceral protein, loss of liver (183) and muscle glycogen (440), and breakdown of muscle structure (358). These losses may produce a muscular fatigue that originates from metabolic and non-metabolic etiologies (158). Metabolic causes include decreases in oxidative phosphorylation, glycogenolysis or glycolysis, and reductions in glycogen concentrations. Non-metabolic causes include a failure in the contractile machinery that does not stem from a loss in metabolic integrity. These failures include an impairment in action potential generation, alteration in membrane potential, and changes in the excitability of the sarcolemma (121). In addition, glycogen concentration and distribution within the muscle is believed to provide a protective role which is not clearly understood (124, 329, 50), but which goes beyond its energy value as a substrate.

All of these factors are likely causes of fatigue and can affect the performance of muscle in a singular or additive manner. By the 1970’s, a significant body of knowledge pertaining to muscle fatigue (29-31, 145, 146, 177-179, 360, 360a) had already been generated from Sweden, where the initial initiators of the needle biopsy technique provided
information from samples of exercising muscle. This knowledge has been subsequently reviewed by Edwards (122). Overall, the data suggests that the causes of fatigue are found mostly in the muscle itself (378). This means that in investigating the performance of muscle in underfeeding, fatigue could be assigned to problems in membrane depolarization, to the reduced size and mass of the muscle, to the loss of glycogen from muscle, to changes in excitation-contraction coupling. All of these can arguably be used as causes of fatigue in a variety of settings like marathons, anaerobic exercise, malnutrition, 100 meter running and within a non-metabolic model of fatigue.

2.1.1 Non-Metabolic Causes of Fatigue

2.1.1.1 Membrane depolarization. In the hypocalorically fed rat, a significant depolarization of the soleus' sarcolemma occurs (130), but no change is seen the soleus of the two day fasted rat or in the gastrocnemius of the hypocalorically fed and fasted rat. This disparity could not be explained by the level of available energy or free Mg\(^{2+}\), because in a later study (338), these were found to be the same in controls, fasted, hypocalorically fed and refed rats. Therefore, the activity of the Na\(^+-\)K\(^-\) pump was likely not disturbed by important changes in free Mg\(^{2+}\), or free available energy (337). The reasons why one muscle-type depolarized and another did not, and why the hypocaloric diet produced a depolarization and fasting did not, may be due: (a) to the hypothalamic regulation of the sympathetic nervous system and of the endocrine organs causing a specific loss of K\(^-\) in the slow-twitch muscle (130); (b) to the peripheral nerve regulation causing an altered Na\(^+-\)K\(^-\) pump; c) to a dissociation between the membrane potential and intracellular potassium activity (aK\(_i\)) (130). It can be argued that such incongruity may be related to an increased Na\(^+\) influx through the membrane of the cell or to variations in the electrogenecity of the Na\(^+-\)K\(^-\) pump, ensuring that an elevated Na\(^+\) concentration gradient is
maintained across the membrane. Electrolyte abnormalities occur in hypocaloric and fasted rats (357), and are especially related to an increase in total water content, mostly as extracellular water in the muscle of rats hypocalorically fed for 21 days. No differences in water content were observed between fasted and well fed control rats. Also reported was an intracellular build up of muscle calcium in rats, while extracellular K\(^+\), Mg\(^{2+}\), Cl\(^-\) and Na\(^+\) levels rose significantly. A build-up in intracellular calcium will likely result in a mitochondrial influx (214) and muscle fatigue.

Intracellular potassium plays a role in muscle fatigue (336) within the context of malnutrition. [K\(^+\)]\(_i\) levels fall in both the soleus and EDL during malnutrition. However, only a decline in free ionic potassium [Ak\(^+\)]\(_i\), was found to affect the membrane potential of the soleus negatively. Changes in intracellular Mg\(^+\) and P\(^-\) concentrations did not parallel those of K\(^+\), thereby confirming that the intracellular changes were not due to a nonspecific fall in intracellular electrolytes, nor to a dilution effect caused by an increase in intracellular water. In contrast, Russell et al. (357) were unable to observe any fall in [K\(^+\)]\(_i\) in rats hypocalorically fed for 21 days. Furthermore, Mehta et al. (290) correlated the fall in [K\(^+\)]\(_i\) content in malnutrition with deficits of glycolytic and TCA enzymes activity and with deficits in available energy. The idea that intracellular K\(^+\) concentrations would fall in a state of undernutrition suggests a slow-down of the energy dependent Na\(^+\)-K\(^+\) pump (75, 293). This pump has been shown to be dependent on energy derived from glycolysis and oxidative phosphorylation. Therefore, it is likely that the reported fall in a\_G\(_{ATP}\) during malnutrition, reported by Pichard et al. (338) in an earlier study, could slow down the pump. Moreover, refeeding did normalize membrane potential and [Ak\(^+\)]\(_i\) in the soleus; this result strengthens the association between a\_G\(_{ATP}\) and intracellular free K\(^+\) because refeeding has been shown to normalize a\_G\(_{ATP}\) (338). The type of fatigue caused by a loss of membrane
potential in muscle is referred to as peripheral fatigue (121) and has received support (291, 36, 37, 220). Peripheral fatigue can arise from impaired neuromuscular transmission, failure of the muscle action potential or from impaired excitation-contraction coupling (121). The loss of action potential (depolarization) of the muscle membrane arises from the accumulation of extracellular K⁺ and a Na⁺ depletion (121). The role played by K⁺ in muscle fatigue has received much attention (219, 121, 377), primarily because of the association with decreased membrane excitability (158). Hence, the basis of fatigue appears to be linked to a significant potassium flux from the exercising muscle (377). Potassium efflux at the level of the sarcolemma has been linked to a number of mechanisms, notably the ATP-sensitive K⁺ channel, the Ca²⁺ sensitive K⁺ channel, the inward rectifier K⁺ channel associated with membrane permeability, and the delayed rectifier K⁺ channel that functions at the level of repolarization following an action potential (378). There is, however, increasing evidence suggesting that primarily the ATP-sensitive K⁺ channels and the Ca²⁺ sensitive K⁺ channels may be involved in the onset of fatigue (378). An action potential failure of the sarcolemma (222) would in fact result from an increase in K⁺ efflux through these channels (34, 377). Potassium ion flux from the cell has been observed during maximal exercise in man (75). The loss of this potassium can be devastating since K⁺ has been implicated in the transport of Ca²⁺ across the S.R. (396), thereby directly impacting on the process of excitation-contraction coupling (396). Further consideration should also be given to the hypokalemic action of insulin (438), which results in a K⁺ retention in muscle. In vitro studies have shown insulin to cause an increase in EDL (437), and soleus [K⁺]; (128) and to cause a rise in membrane potential (Eₘ) (438). In contrast, in vivo muscles subjected to varying insulin and glucose concentrations, produced a hyperpolarization of the soleus membrane in the rat (128), a phenomenon that may be related to a diminished Na⁺ -K⁺ permeability.
ratio (75). It can also be related with an insulin stimulated efflux of Na⁺ out of the cell (75). The mechanism is explained by insulin converting the Na⁺-K⁺-ATPase into an active form with greater affinity for Na⁺. A new steady state is created with a much larger transmembrane gradient (75). It is generally agreed that the activity of the Na⁺-K⁺ pump is sensitive to acute changes in insulin, epinephrine, and norepinephrine (75). In muscles that are already K⁺ deficient, insulin produces a depolarizing effect on the muscle which results in a fall in twitch tension (321, 325). The mechanism, in this case, can relate to either a rise in Na⁺ influx (325) or to a general decrease in membrane conductance (75). Within the malnourished rat, only the soleus appears to suffer a significant intracellular K⁺ loss (336). There is, however, very little known about K⁺ flux in malnutrition and nothing known about K⁺ flux during the process of early refeeding. In addition to membrane depolarization, one would also have to question the repercussions of undernutrition on fiber size, and weight, and determine whether there is significant protein erosion and fiber atrophy in the contractile machinery. These important determinants as well as the influence of metabolic changes will be reviewed in the following sections.

2.1.1.2 Influence of fiber type, size and weight. Patients with muscle diseases, immobilization, traumatic cord lesions, and endocrine abnormalities, have shown decreases in muscle force contractions, which appear to result from slow-twitch (159, 285, 106) and fast-twitch fiber atrophies (109, 367, 165, 166). In addition, the recovery of function in some instances (osteomalacic myopathy) parallels the growth of muscle fibers (434). Fiber size and weight are also altered by caloric deprivation, causing an erosion of muscle tissue (185) which decrease the force of contraction (258). Hence, a depression in tension arising from the loss of body cell mass (374) is implicated in the process of malnutrition-generated fatigue, as it relates to a loss of PCr and
possibly nitrogen. These losses are likely to cause a decrease in actomyosin cross-bridge cycling; however, no correlation has been found between the loss of exercise capacity and the nitrogen loss. In addition, the recovery of muscle function, does not occur concurrently with the recovery of muscle mass. Wood et al. (426) found, in fact, that nitrogen accretion in muscle was not necessarily associated with a normal return of muscle function. Similarly, no correlation could be found between the loss of exercise capacity and the nitrogen loss (356, 426). Nitrogen, in fact, is a variable of body composition which can be used only to a limited degree as an assessor of fatigue. Nitrogen accretion, is not a useful indicator during the refeeding process because refeeding does not fully reconstitute lost body proteins within a short period, nor concomitantly with improvement in function (356).

Similarly, others have found (216, 215, 130, 356, 354) that the loss and restoration of muscle mass does not fully correlate with contraction, relaxation, force, and endurance in muscle. Factors, other than fiber size and the number of contractile proteins influence the performance of the muscle and, in so doing, generate fatigue. For example, despite similar weight loss and protein indices, some patients had abnormal muscle function and others did not (424). Those with a depressed function also had the greater post-operative risks and the longer hospital stay. Weight loss and estimations of body protein stores, alone, had poor predictive capabilities for outcome.

There was a maximal early improvement of all physiological measurements in 4 days of refeeding (73), showing that function responds earlier than body composition. This finding confirmed earlier work (356), that demonstrated an early restoration of function following 2 to 4 weeks of nutritional repletion in fasted obese patients and anorectics, respectively. More recently, others (188) have demonstrated, in postoperative patients that suffered weight loss, significant improvements in muscle function within 30 days and full recovery of
function by 90 days, even though body composition parameters were still sub-optimal. Moreover, functional improvements occurred equally well in patients fed either by peripheral parenteral nutrition or by total parenteral nutrition (389). Although it was clear that the restoration of protein stores over an extended period of therapy usually coincided with total improved physiological function, the reason for the early rapid changes remained unclear. Further evidence of a dissociation of function from structure during hypocaloric feeding is presented as histochemical evidence of fast-twitch fiber atrophy in the gastrocnemius with functional losses that were comparable to those observed in muscles made up of different fiber types (358). Such a dissociation of force-frequency characteristics from fiber-types is also supported by Moxham et al. (301). The preferential loss of fast twitch fibers during malnutrition was also documented by Gardiner et al. (137) and Golberg et al. (143), although there is evidence to the contrary (374, 411).

Another index of the integrity of the contractile machinery is the size of the muscle, represented by the cross-sectional area. In keeping with this structural model of fatigue, Burke (54) showed that muscle fiber diameter was directly related to the force generation potential. However, abnormal muscle function still occurs when muscle integrity remains intact in instances of psychogenic weakness, effort syndrome, and impaired neural motor control (146). The way in which this function can be returned to normal, in these situations, consists of enhancing membrane permeability as well as intracellular energetics. At present, there is some evidence (258) that undernutrition causes a significant decline in muscle cross-sectional area of both fast-twitch and slow-twitch fibers. Lewis et al. (258) point out the greater susceptibility of fast-twitch fibers to cross-sectional loss in the diaphragm. It is not known, however, how the cross-sectional area of muscle compares to the function of muscle in the leg or rat hindlimb during undernutrition and recovery. It is difficult to ascertain whether
the fall in force, observed during undernutrition (357), is due to structural loss only or to a defect in energetics. Consequently, an accepted approach is to normalize the force with various parameters. In starvation, varied results in tension normalization have been reported in the literature, which have featured either an increase in tension (72), a decrease in tension (144), or simply no changes (258, 229) in tension. Nishio et al. (314) found that the muscle of hypocaloric rats exhibited increases in tension once corrected for muscle wet, dry weight, and total protein. In contrast, soleus and EDL tension in HYPO and fasted rats, once corrected for µmole creatine, did not vary from controls, whereas tension corrected for grams of myofibrillar protein showed significant change in the EDL, but no changes in the soleus. They were both unable to explain these differences, nor able to determine an optimal method for tension normalization. Furthermore, it becomes intriguing that despite a loss of muscle protein, wet weight, dry weight and cross-sectional area during starvation, tension development (not adjusted for any of the aforementioned parameters) can still be maintained (314).

It could be questioned whether muscle can, in fact, serve as a sensitive indicator of nutritional status because of this independence from muscle structure and body composition (374). In other instances, the function and structure of muscle are strongly related; clinical observations of anomalous muscle contractions are seen with the shrinkage of the muscle fibers in cases of disuse atrophy, myopathy, or osteomalacia (109, 367, 165, 166). In malnutrition, the issue seems to revolve around muscle glycogen content. Muscle glycogen has been proposed as a critical substrate that may protect sarcomere integrity of the muscle and ensure tension development, or that may be somehow involved in excitation-contraction coupling (158).
2.1.1.3 Protective role of muscle glycogen. A protective role for glycogen, which puts into doubt its exclusive role as a critical energy substrate reserve, has been suggested.

The protective role of glycogen against fatigue involves the preservation of cellular and myofibrillar integrity. Several models of fatigue have been proposed in the literature, which are linked with the depletion of glycogen during exercise. The loss of glycogen would likely affect: (a) the integrity of the sarcoplasmic reticulum-glycogenolytic complex (124) -- in this instance, the Ca\(^{2+}\) released from the S.R. cannot affect the early activation of glycogenolysis -- (b) myofibrillar integrity (329), particularly at the level of the cytoskeletal proteins found in the Z line (25); (c) the Ca\(^{2+}\) reentry into the S.R. during the relaxation of the muscle -- this interference takes place because of the presence of glycogenolytic enzymes, which are highly active during the contraction processes of the muscles (50); (d) the hydration of the muscle (372), which therefore would precipitate fatigue -- the hydrophillic property of glycogen would cause a dehydration of muscle concurrently with glycogen depletion; and (e) the intracellular concentration of K\(^+\) because of the possible buffering role of glycogen on K\(^+\) efflux from the cell (201). In fact, K\(^+\) losses parallel glycogen losses. The loss of glycogen does not necessarily always generate muscle fatigue; fatigue still occurs despite the persistence of some glycogen concentrations in muscle (83, 147). No specific model of fatigue has received full support in the literature. This is probably related to the fact that motor unit recruitment varies during exercise of various intensities (147). Sub-maximal exercise calls for the recruitment of slow twitch (ST) and fast-twitch(a) (FTa) fibers (145, 413) initially. However, after continued sub-maximal intensity exercise, or with maximal and supra-maximal exercise, recruitment of mostly fast twitch fibers takes place (145). When the effects of specific fiber-types are considered, exhaustion was found to occur when
close to 90% of the ST and PTa fibers were completely depleted of glycogen (83). Hence, glycogen depletion is critical in the onset of muscle fatigue depending on the fiber types recruited. Therefore, muscle groups that are mostly composed of one type of fiber should ideally be used when studying muscle fatigue.

2.1.2 Metabolic Causes of Fatigue
Metabolic alterations, refers to either glycogenolytic, glycolytic flux, and/or TCA cycle anomalies, causing a decrease in ATP generation and a cellular ATP deficit (51). The ability of the muscle cells to meet the energy demands of the excitation and contraction processes is compromised (310).

Fatigue defined as the percent force loss over 40 tetanic contractions, implies that there is a modified metabolism that either utilizes less substrate or that is dependent on the quantity of substrate available and the kind of exercise performed. The reason for the modified metabolism is not clear; however, there are four constructs, popular with physiologists, that tie metabolism to fatigue: (i) a decrease in substrate availability and/or a decline in substrate utilization including phosphocreatine (Pcr) (266, 337, 360b), free fatty acids (310, 341, 344, 365) glycogen and glucose (29, 88, 146, 158, 186, 344); (ii) a change in pH and altered enzyme activity (44, 70, 114, 167, 207, 363, 403); (iii) a change in muscle energy status (96, 105, 115, 116, 227, 278), which could possibly reduce the recycling of inositol to PIP2 and thereby compromise excitation-contraction coupling (105, 234b); and (iv) a change in calcium kinetics (112, 278). These constructs are modified by undernutrition. Undernutrition introduces new variables (muscle wasting and endocrine alterations) typically not encountered by the exercise physiologist.

The link between muscle fatigue and malnutrition was made as far back as 1950 by Keys et al. (230), who inferred that undernutrition, and
therefore a low energy status, were behind the fall in hand-grip strength following repeated contractile effort.

More recent functional data obtained during undernutrition appears, however, to support the notion of either a compromised energy status or a diminished energy demand. Specific abnormalities in skeletal muscle function occur in patients with malnutrition (266). After feeding for 4 weeks with TPN, improvements in muscle performance occurred. In 1983, Russell et al. (354) demonstrated that in the fasting state, the electrically stimulated force declined, although the voluntary force did not. Within 4 weeks of refeeding, fatiguability, maximal relaxation rate (MRR), and F10/Fmax all returned to normal. This recovered normalcy was seen at a time when body weight was still 71% of ideal body weight, creatinine height index was only 65% of normal, and total body nitrogen was 78% of normal. Church et al. in 1984 (74) found that a return to normal muscle function could be obtained within 48 hours when intravenous nutritional support was given with glucose and potassium. This suggests that the revival of cellular ATP or a replenishment of muscle glycogen reserves as a critical determinant of function.

In the clinical setting, pathological factors also could be important confounders in muscle function. In identifying the extent to which such factors affected muscle function, Fraser et al. in 1984 (134) and Berkelhammer et al. in 1985 (32) could not identify any significant influence of chronic obstructive lung disease, peritoneal, or hemodialysis on F10/F50 and MRR. Brough et al. in 1986 (52) concluded that neither steroid administration, nor major surgical procedures, altered normal muscle function. They also found no significant impact of trauma without sepsis on function, despite marked negative nitrogen balance that could not be reversed by increased nutrient support. Sepsis in well nourished patients (those eating more than 90% of their resting metabolic rate) had minimal influence on muscle function parameters.
compared to malnourished septic patients. Malnourished patients suffered falls in both maximal relaxation rate and the ratio of low frequency stimulation to high frequency stimulation (F10/F50).

The sensitivity and specificity of muscle function measurements were verified by Brough et al. (52) by establishing a Receiver Operator Characteristic (R.O.C.) curve. The well nourished without malabsorption and the malnourished with malabsorption could be distinguished with a sensitivity of 83% and a specificity of 98%. This indicates a very acceptable probability of detecting true positives and a very high probability of detecting true negatives.

The relationship between muscle, malnutrition, and refeeding describes the performance of muscle as dependent on energy, (or at least on the muscle’s ability to generate energy) rather than on structure. Work by Whittaker et al. (419) showed that, after refeeding, the muscle function tests of force at 10 Hz (expressed as a percentage of the maximum force at 100 Hz -- F10/F100), and of relaxation, were reversed, compared to underfed rats, and were no longer different from the control group; thereby confirming the involvement of nutrition in the recovery process of muscle energetics. The recovery, with refeeding, of similar functional parameters (also reflective of energetics) in addition to fatigue, were also reported in rats (354, 358).

Muscle fatigue observed during malnutrition and refeeding is not solely dependent on muscle size or protein (216, 356, 215). This suggests that muscle energetics may be disrupted. Early on, changes in glycolytic and oxidative enzymes in malnutrition, were reported, although fiber type specificity were not determined (74, 357, 358). Recently, Nishio & Jeejeebhoy (316) pointed out a significant increase in the in situ relaxation half-times, in both the soleus and EDL muscles of the hypocaloric rats, with a significant fall in PFK activity in both muscles as well. These findings reinforced the idea that a disturbance
in metabolism was in fact occurring in both fast-twitch and slow-twitch muscles in the underfed state. Furthermore, deterioration of relaxation time and tension \([Po(N)]\) in the EDL and soleus of in situ controls were generated by creating an anaerobic milieu using cyanide. The fall in performance was related to a diminished oxidative phosphorylation. In the EDL of hypocaloric rats, the anaerobic milieu did not worsen the relaxation half-time, the \(Fs/Fmax\) or the \(Po/g(N/g)\) compared to controls, thereby suggesting that oxygenation of the muscle during malnutrition is not critical, in the EDL, in sustaining the performance because of the very active glycolytic pathway. In the case of the soleus, however, these same parameters of function declined in the hypocaloric state because of the soleus' decreased glycolytic capacity (316).

The impact of malnutrition on the slowing relaxation rate, however, has not received widespread support (137, 258, 282). Various indices of relaxation rate were studied by Nishio et al. (315) in an attempt at assigning the controversy to methodological discrepancies, such as the type of fibers stimulated (fast-twitch versus slow-twitch), the extent of deprivation (hypocaloric versus fasted), the extent of muscle stimulation (twitch versus tetanus) and whether the half-relaxation time or half-time for exponential decay of tension were measured as relaxation indices. They found a significant fall in muscle relaxation of both the slow-twitch and fast-twitch muscles of hypocalorically fed (HYPO) rats only, regardless of the relaxation index used and regardless of whether relaxation was measured in a tetanic or twitch contraction. The dissimilar reaction between the fasted and the hypocaloric states has not been well documented and the reason for such incongruity is not known. Nevertheless, these findings confirm that the differences in relaxation of muscle is not dependent on the indices of measurement used and suggest that the extent and duration of nutritional deprivation may be an important consideration. In keeping with this hypothesis, two possibilities surface: first, the severity of the protein erosion
(149b), with the erosion being greater in the HYPO rats compared to the 2 day fasted rats; and second, a significant fall in the free energy change of ATP hydrolysis (96) may be influential in decreasing the relaxation rate of the muscles. The authors also concede that further study is necessary in order to fully understand the varying impact of nutritional deprivation on muscle performance. There are in fact many factors involved in the development of fatigue. One of the most attractive, in the study of malnutrition, is the tissue concentrations of ATP and the turnover rate of ATP. These have been shown to play a key role in generating an expected force as well as in initiating the relaxation process (234b). In examining a possible link between ATP generation and fatigue, within the context of undernutrition, it is important to understand this relationship within the following scenarios: (i) a decrease in substrate availability; (ii) the decreased activity of enzyme pathways; (iii) a fall in pH; (iv) a fall in $\Delta G_{\text{ATP}}$; and (v), a disturbance in calcium kinetics. These scenarios are reviewed in the remainder of this subsection.

2.1.2.1 Decreased substrate availability/utilization. Key substrates around which muscle is known to be dependent are phosphocreatine (PCr), free fatty acids (FFA), glycogen, and plasma glucose (234b). The availability of these substrates are dependent on the levels of vascularization, oxygen availability, stimulation protocol being used, and nutritional status. The amount of substrates utilized is dependent on the kind of stimulation that is taking place and the extent of food deprivation; their transformation into utilizable high energy phosphates takes place through the pathways of glycogenolysis, glycolysis, oxidative phosphorylation or creatine kinase reaction kinetics.
(a) **Phosphocreatine.** Phosphocreatine (PCr) concentrations in the muscle are essential for contractions lasting less than 10 seconds in humans (266) and less than 2 seconds in rats (337). PCr acts as a buffer that helps maintain ATP levels constant. The creatine is formed extra-muscularly in a transamination reaction between arginine and glycine followed by a methylation reaction that ultimately forms creatine (305). The latter is then picked up by the muscle. Undernutrition causes PCr concentrations to fall in muscle (338, 357), and further decreases the muscle’s ability to rephosphorylate Cr (295) following stimulation. This fall in PCr, while it may likely cause a fall in the tetanic contraction (357), does not in itself cause fatigue since cellular ATP concentrations generally remain constant in the malnourished state (295, 338, 358). In addition, in vitro work by Sahlin et al. (360b) demonstrated that during high frequency stimulations in normal muscle, PCr dropped rapidly, initially, but remained constant afterwards, despite significant and progressive declines in muscle tension. They also observed an equimolar increase in creatine (Cr) with the decline in PCr as well as a heightened release of Pi, following the simultaneous stoichiometric balance described in the reaction:

\[
\text{PCr} + \text{ADP} + H^+ \rightarrow \text{ATP} + \text{Cr}.
\]

This reaction fosters the accepted view that within the first 2 seconds of a tetanic contraction, in rats, the ATP is preferentially coming from PCr reserves in the muscle (337, 214) and that PCr is not a determinant of muscle fatigue (180, 97).

The ability of the muscle to synthesize PCr is a reflection of the aerobic nature of the exercise being performed, since it has been shown by Harris et al. (171) that resynthesis of PCr does not take place until blood flow to the muscle resumes following repetitive tetanic contractions to fatigue. In addition, resynthesis remains subnormal in the malnourished state (295).
(b) Glucose, glycogen and free fatty acids (FFA). Protein-energy malnutrition implies a diminished macronutrient supply to the biological system; the energy deficit that ensues precipitates a fall in insulin, and a rise in glucagon and epinephrine. The glucagon is liver specific and catalyses an hepatic glycogenolysis. The epinephrine acts on both the muscle and liver and also degrades glycogen (64). Low muscle glycogen has been reported by Zorzano et al. (440) in fasted rats and by Rossel et al. (352) in starved individuals. Sakaida et al. (364) suggest that muscle glycogen is used following the depletion of hepatic glycogen, for the maintenance of serum glucose concentrations in starved mice. Reduced muscle glycogen concentrations seem to occur with low cellular ATP (185). This depressed energy status, typically featured by a low ATP to ADP ratio, normally favours a higher energy state by encouraging glycogenesis (81). Because substrate is lacking in the malnourished state, recent evidence points to gluconeogenic precursors as being able to foster the synthesis of glycogen (207). It has been suggested, however, that gluconeogenesis is likely occurring in white muscle only, and that lactate and pyruvate cannot be considered as gluconeogenic precursors (324). In addition, it is not known whether hypocaloric feeding affects the glycogen content of fast-twitch and slow-twitch fibers equally or whether the expected decline in glycogenolysis would be observed in both of these muscle fibers.

What is known is that the hormonal milieu creates a shift in substrate preferences that follows the glucose-fatty acid cycle, depending on the degree of caloric restriction; hence, when glucose is unavailable for metabolism, free fatty acids are preferentially used. Conversely, when glucose is abundant, lipolysis is inhibited and the use of free fatty acids are restricted (251). While the glucose fatty acid cycle has been observed in perfused heart and diaphragm, it has not been consistently observed in skeletal muscle (23).
In an undernourished state -- typified by lower serum glucose, a greater reliance on fatty acids for energy, and significantly lower insulin levels -- there is a decreased glucose utilization by the muscle (207), which leads to an associated fall in force (203) and a decrease in relaxation rate (315). Goodman et al. (149) however, in working with the perfused unstimulated rat hindquarter of starved rats, found that glucose metabolism was not inhibited by the presence of exogenous fatty acids. This observation was also supported by others (23, 217) and seems to suggest that the exercised muscle creates an internal environment that is conducive to the operation of the glucose-fatty acid cycle (330b).

It is also suspected that the operation of the glucose-fatty-acid cycle may be restricted to certain types of muscle fibers. Rennie et al. (344) conclude that the cycle operates only in slow-twitch muscle fibers, as are found in the soleus, during periods of greater FFA availability. The cycle was not found by others (440) in resting muscle, but, but was quite visible in exercised soleus of 48 hours starved rats. Furthermore, in states of starvation, there is little evidence that glycogenolysis is reduced during exercise (440).

By contrast, fast-twitch fibers (found in the EDL) would solely depend on glycogen regardless of whether insulin levels are high or low. Mackie and Terjung (270) propose that blood flow is not altered with repeated tetany in fast-twitch muscles. They point out that the blood flow difference in the white gastrocnemius is close to zero. Fast-twitch fibers, therefore, would become exclusively reliant on internal glycogen reserves, and protected from external substrate and hormonal changes. This suggests that the influence of the glucose-fatty acid cycle is minimal in fast-twitch white muscles (344), leaving anaerobic metabolism (glycolysis and glycogenolysis) relatively undisturbed by malnutrition and consequently able to ensure a guaranteed supply of ATP regardless of blood flow. In contrast to this interpretation, Russell et al. (357)
reports a fall in PFK activity during a 2 and 5 day fast, while SDH activity remained similar to controls and ACDH levels were significantly higher than controls. Russell’s study was done on rat gastrocnemius, known to contain mostly fast-twitch fibers (9), therefore clearly in opposition to Rennie’s conclusion (334). However, when rats were hypocalorically fed for 21 days, there was a fall in both SDH and PFK even though FFA concentrations were elevated, indicating a loss of the compensatory role of oxidative phosphorylation in fast-twitch muscles during prolonged hypocaloric feeding. This was also found by others (316). Again the glycogenolytic capacity of the fast-twitch muscle has not been demonstrated during malnutrition and recovery.

In the absence of glycogen, a critical energy supply is missing, thereby making a sustained glycolytic flux near impossible (273, 310). Edwards et al. (115), using biopsies of fatigued muscles, were able to link observed fatigue with a metabolic decline in the glycolytic cycle. This decline would limit ADP rephosphorylation and would impact on the muscle’s ability to generate and maintain force, because glycolysis provides TCA cycle intermediates.

These intermediates are ultimately responsible for the formation of high-energy phosphates arising from the electron transport chain in the inner membrane of the mitochondria (410). Because oxidative phosphorylation does not generate ATP fast enough to sustain heavy prolonged work, the role of glycolysis becomes invaluable in supplementing additional ATP at a much faster rate (158), therefore responding to the needs of the fast-twitch muscles.

Minimizing the loss of glycogen from muscle, through the ingestion of dietary glucose, is a practice that has successfully retarded fatigue in both humans and rats (186, 347, 429). It also has been shown that the recovery and maintenance of euglycemia, at a time of low glycogen reserves, allows muscle recovery and favours a continuation of exercise (10, 78) by continuing to promote glycolysis. The ability, on the other
hand, to extend the work capacity by sparing glycogen reserves of the muscle with FFA has only been shown in rats (186). Furthermore, there are physiological and biochemical limitations to relying on FFA as an energy substrate. First, FFAs are not able to supply enough citric acid cycle intermediates, such as Acetyl CoA, to meet the heightened energy demand of heavy work (310). Rather, these intermediates can be supplied directly through the glycolytic cycle, and as such glycogenolysis and glycolysis supplement the ATP generated from the TCA cycle (14). Second, the mechanism of cellular uptake of FFA must also be considered. Recent evidence points to a carrier-mediated transport system (341) for FFA which could limit the rate of cellular uptake and, therefore, its rate of oxidation to Acetyl CoA. Consequently, the energy requirement of high intensity dynamic exercise cannot be met by this uptake model of FFA (365).

2.1.2.2 Subnormal enzyme activity. Russell and Atwood et al. (357) and Russell and Walker et al. (358) studied the muscle enzyme system during starvation. They found that the enzyme activity of PFK, SDH and Acetyl CoA dehydrogenase fell significantly, following prolonged hypocaloric feedings, a finding which suggested a reduced glycolysis and oxidative phosphorylation. Subsequent studies by the same group (295, 338), using NMR technology, also found a diminished oxidative phosphorylation with underfeeding. These changes were specifically associated with the gastrocnemius (primarily made up of fast-twitch fibers).

Fast-twitch and slow-twitch muscles were differentiated by Nishio & Jeejeebhoy (316). They studied the soleus (slow twitch) and EDL (fast twitch) muscles of malnourished rats within an aerobic and an anaerobic milieu. Since the soleus has mostly slow twitch fibers and therefore less glycolytic activity, an anaerobic milieu was expected to decrease
the force generated by this muscle. The force produced by the EDL (mostly glycolytic fast twitch fibers) was not expected to be reduced within the same anaerobic milieu. The published results complied with the expected model. They showed, in addition, that the hypocaloric diets and fasting, produced a decreased activity of the enzyme PFK in both the soleus and EDL, a finding that suggests that both fiber-types (unstimulated) are susceptible to glycolytic enzyme slow-down during underfeeding.

2.1.2.3 Lower muscle pH. A fall in muscle pH appears to affect the contractile machinery in terms of a heightened fatigue (97, 125), a depression in tetanic force (142) and an increased relaxation time (361). In malnutrition, however, the evidence does not indicate a low muscle pH environment of any significance (338, 295). Although there is little opposition to the etiological explanation that a subnormal pH induces fatigue, it has been suggested that other influences within the intracellular milieu are also contributing factors -- notably, rises in intracellular concentrations of Pi, ammonia and hypoxia (142, 234b).

Lunt et al. (268) showed with fasted humans that exercise did not cause any change in pH compared to controls. In light of the decreased glycolysis (previously discussed), this observation indicates that lactate levels were not likely to have risen significantly with stimulation. This seems to contrast with results by Pichard et al. (338) reporting a fall in unstimulated muscle pH during malnutrition. This fall was in fact quite small and had a minimal effect on the equilibrium constant of the creatine kinase reaction (Kck) and the $\Delta G^0_{\text{ATP}}$ of the equation. Furthermore, it was not likely to be of any clinical significance, since a subsequent set of experiments by the same group (295), involving stimulation of muscle, could not detect any difference in pH levels between controls, fasted rats, and hypocaloric rats. Even though lactate levels were significantly higher in HYPO compared to
control rats, there was very little difference between lactate levels at
the beginning and at the end of stimulation in HYPO animals, indicating
that glycolysis may have been limited.

2.1.2.4 Depressed $\Delta G_{ATP}$. The impact of malnutrition on free energy
has been explored in the unstimulated muscle of the malnourished rat by
Pichard et al. (338). They studied the unstimulated gastrocnemius in
situ using NMR. They reported falls in the ratios of CrP to Pi, of CrP
to ATP, in $\Delta G_{ATP}$, and a maintenance of ATP in hypocalorically fed and in
fasted rats. These changes have been attributed to the fall in CrP and
rise in free ADP (FADP) concentrations while Pi concentrations were
unchanged by feeding protocols. Similar findings were reported by Mijan
de la Torre et al. (295) in stimulated rats. In repeated in situ
tetanized muscle contractions, separated by 1 minute intervals, Mijan de
la Torre et al. (295) found higher Pi and FADP concentrations in
stimulated HYPO rat muscles, and lower PCr levels compared to the well-
fed rats. In addition, they reported a lower PCr:Pi ratio in HYPO rats
-- representative of a poor transfer of chemical concentrations in
muscle to work capacity or function (68) -- and a diminished $\Delta G_{ATP}$ at the
end of stimulation.

The reasons for these changes are substrate dependent, and in that
sense creatine kinase reaction kinetics would drive the reaction (PCr +
ADP + H$^-$ ---->ATP + Cr) towards the right in conditions where greater
hydrogen ions are present, signifying a lower pH. A similar
effect would occur when FADP concentrations are also elevated (360b).
Moreover, the rate of PCr recovery after contraction was significantly
slower in the fasted and HYPO group. They hypothesized that under-
nutrition caused this slower recovery, thereby creating a lower PCr:Pi
ratio as well as lower $\Delta G_{ATP}$ (less negative). They attributed these
changes to an impaired rephosphorylation of ADP, because of the
likelihood that mitochondrial activity was altered by malnutrition.
Ruderman et al. (353) have suggested that such an impairment could be
taking place by ketones inhibiting pyruvate dehydrogenase and perhaps by a deficient redox (150).

In addition, Mijan et al. (295) reported a rise in gastrocnemius lactate levels in HYPO rats, a finding supported by Jacob et al. (210) in starved rats. Put within the context of a diminished glycolysis, it was assumed that this build-up was associated with a slowdown in oxidative capability. Muscle analysis confirmed (295, 237) again that the PCr to ATP ratio fell. More importantly, the rate of change of the PCr:Pi ratio, in the post-exercising period, was significantly lower in underfed rats. The Pi:PCr ratio is used as an indirect representation of the level of FADP (66) and, within this capacity, a rise in the ratio supports the notion of a diminished energetic state. The implication is that, since Cr is not being rephosphorylated as quickly, the buffering role of PCr in the phosphorylation of FADP is also not occurring as efficiently, resulting in an elevated FADP. This gives further credence to the notion that a diminished oxidative phosphorylation may be taking place during malnutrition (204).

It is also possible that the fall in $\Delta G_{\text{ATP}}$ associated with hypocaloric feedings could be indicative of a possible slow-down in energy dependent cellular transport mechanisms, such as the S.R. Ca$^{2+}$ ATPase and the sarcolema based Ca$^{2+}$Mg$^{2+}$ ATPase. This has been proposed on the basis of higher concentrations of cytosolic calcium observed in malnourished man and animal (291, 419, 358) compared to levels found in the sarcoplasmic reticulum.

2.1.2.5 Abnormal muscle calcium kinetics. Changes in calcium kinetics during malnutrition has not been the subject of any extensive study. Noteworthy, however, are the observations of Russell & Whittaker (357), Russell & Walker (358) and Whittaker & Desai (419), in both humans and rats. They reported a higher intracellular calcium by comparison to controls in the malnourished muscle. In reference to this finding, Jeejeebhoy (214) discussed the possible mechanisms in the 1988
Rhoads lecture. He concluded that because there is a fall in $G^\circ_{\text{ATP}}$, there would be an expected decline in sarcoplasmic-free calcium. It is uncertain, however, how malnutrition affects the S.R. calcium uptake, the calcium-release channel, and the S.R Ca-ATPase. Furthermore, it is not known if the fall in free energy is also related to a decline in myofibrillar-ATPase (278) during malnutrition.

The rise in cytosolic calcium would result in a mitochondrial calcium influx (311). While the presence of $\text{Ca}^{2+}$ has been shown to play a significant role in oxidative phosphorylation by activating the dehydrogenase enzymes (287), it is likely that excessive calcium, may in fact, poison the mitochondria. Cell homeostasis can be further unattainable, since the cellular efflux of calcium by means of the $\text{Ca}^{2+}$-$\text{Mg}^{2+}$ ATPase system and the $\text{Na}^+$ exchange system could be compromised within the context of an energy deficient cell.

2.2 REFEEDING, MUSCLE FATIGUE AND BODY COMPOSITION
The previous section was dedicated to reviewing the impact of undernutrition on the performance of muscle. The main focus of this section will be placed on reviewing how muscle reacts when it is refeed. Several aspects must be considered within this paradigm. First, the impact of nutrition on compositional changes, meaning the extent of protein and fat deposition, and of fluid accumulation. Second, the issue of metabolic changes which influence the efficiency in energy utilization needs to be reviewed. This will be done within the construct of compensatory growth; clinical repercussions are examined. Third, functional changes in muscle are reviewed within the recovery phase of hospitalized patients. Fourth, the impact of the macronutrient composition of the diet on muscle function, is reviewed.
2.2.1 Compositional changes. Body compositional changes during refeeding were studied by Vaisman et al. (409) in teenage girls with anorexia nervosa. They concluded that during the first week of refeeding most of the changes in fat-free body mass were attributed to a notable increase in extracellular water. Intracellular water (ICW) gradually increased, but at a much slower rate. Russell et al. (356) reported very little changes in body composition over a short refeeding period. They attributed improvements in contraction-relaxation characteristics to ICW and recovery of electrolytes. Heymsfield and Casper (184) correlated early rapid weight gain during recovery from semi-starvation to mostly sodium retention and extracellular water accretion, within the context of continuous nasoenteric feeding. Any alacritous gain in lean tissue was mostly visceral during early refeeding. Accretion of lean mass during the later phase of recovery took place mostly in skeletal muscle. They concluded that the retained protein energy was maximal with a kilocalorie to nitrogen ratio that varied between 1.3-1.5. Over time, the relative proportion of deposited protein to non-protein energy progressively declined. Hence, during the later phase of recovery, the rate of weight gain declined by 50%, and the most important compositional changes were attributed to the accretion of active cell mass and fat. This is in agreement with Russell et al. (356), who found a 13% gain in nitrogen and a 46% gain in fat in patients with anorexia nervosa.

Although these human data suggest that there are few compositional changes other than extracellular fluid accumulation during early refeeding, animal data indicate that there is an early, rapid response to the initiation of feeding, implicating some kind of compensatory growth. There is however some controversy over what tissue is primarily being synthesized. Farrell and Williams (126), using rats, demonstrated a high net availability of metabolizable energy for weight gain, indicating a high efficiency of fat deposition. Their slaughter data
confirm that most of the retained energy is in the form of adipose tissue; they also recognised that most of the weight gain was essentially in the form of lean tissue (126). They explain this on the basis that lean tissue weighs more than fat. Their results agree with those of Thompson et al. (397), who reported a high deposition efficiency of lean tissue in sheep. A diminished rate of protein turnover has been proposed as an acceptable mechanism, because it is normally considered a very energy demanding process (55). This reduced turnover is supported by Ogata et al. (322) who found a decrease in protein degradation and an increase in synthesis during the catch-up growth phase. It has been suggested that the differences in body responses to weight gain may be related to the previous dietary treatment (plane of nutrition), the chemical composition of the diet, feeding frequency, and age of the animal (127). Providing diet, refeeding protocol, and age of animals are consistent, the severity of the dietary restriction that induces malnutrition becomes a key determinant in how the body will respond to refeeding. Harris et al. (169) reported similar compositional losses between starved and underfed rats; yet, upon refeeding, they observed an accelerated deposition of fat during the first 6 days in the underfed rats, with regular increments in body protein over the 17 day refeeding period. These results contrast with the starved-refed rats in which body protein levels were restored back to normal before fat stores recovered. Despite the delay in fat deposition, the starved rats did fully restore their adipose reserves.

Complete food restriction, while frequently adopted in the literature, poses some difficulties with respect to possible physiological and endocrinological lag time arising with the sudden introduction of food. Hypocaloric feeding appears to be the preferred choice, because there is evidence (189) that total caloric restriction (starvation) decreases energy maintenance requirements proportionally
(342) and causes a slow reactivation of lipogenic enzymes upon refeeding (169, 255). Hypocaloric diets are, therefore, a more representative model of chronic malnutrition, which is typically found in the clinical setting. While the compositional changes taking place in early refeeding have been studied, it is not known how the early compositional changes relate to muscle performance.

2.2.2 Metabolic changes. There are two mechanisms that may explain the notion of compensatory growth observed in starved-refed rats: (a) a reduction in the energy cost of storage, and (b) energy conservation. Hill et al. (189), using 3 day fasted rats, detected a significant decline in maintenance energy (greater energy conservation) with caloric restrictions -- an adaptive process that allows a greater amount of ingested energy to be dedicated to the replenishment of energy reserves. There was no apparent decrease in the energy cost of storage, overall. Furthermore, they could not detect any significant increase in the carcass energy during the first 1-5 days. They attributed most of the weight gain to the replenishment of water and electrolytes. It was not until the 6th to 10th day that the efficiency of energy utilization increased in both the ad libitum and restricted refed rats (189). This may be a time-dependent phenomenon that is associated with a much slower hormonal response (insulin, lipoprotein lipase) during the early period of refeeding, followed by a hormonal recovery in a later phase (326, 135). The hormonal changes taking place with realimentation is descriptive of an adjustment phase, during which there is a slower insulin responsiveness (326) and lower lipoprotein lipase activity (135). Rats, however, have demonstrated a heightened hormonal response after 5-10 days of refeeding.

In humans, metabolic activity is considerably different from that of the rat. For instance, the energy requirement of a 300 g rat is between 250 and 300 kcal/Kg/day (156b), a requirement that is 10 times that of
the normal and modestly active adult male (265). The inter-species difference in metabolism is also visible in terms of how starvation impacts on the body. Hultman (201b) describes 40-50% glycogen loss in human muscle following 4 to 6 days of starvation, whereas similar declines in the rat are seen within 24 hours of starvation (81b). Furthermore, Nishio et al. (314), examined an experimental rat feeding protocol that causes a 20% loss of original weight over 7 days, while consuming 25% of required calories. A similar weight loss in humans subjected to an identical dietary restriction would take about 80 days (281b). Hence the rat's metabolic activity appears to be between 6 and 11 times faster than the human.

The metabolic changes with refeeding are dependent on whether the patient is semi-starved and suffering from chronic illness or whether he/she has a good nutritional status but is afflicted by an acute injury (182). Often, the clinician must respond to malnourished patients afflicted by a combination of chronic disease and acute injury, such as trauma (182), characterized by a complicated array of metabolic and clinical abnormalities. Aggressive refeeding protocols (which advocate continuous enteral feeding or total parenteral feeding) have been supported in such instances (181, 184); the objective is to increase the rate of lean tissue deposition.

The clinical implications of such protocols had to do with the negative repercussion for the kidneys, heart and respiratory system by the time-related load of the diet on these systems (184). Rapid refeeding generally is not advisable; the concern has to do more with rapidly refeeding critically ill malnourished patients, because of the possibility of death due to organ failure, hypokalemia and hypophosphatemia (235). A slow drip or infusion rate is preferable, because early refeeding is associated with a sub-maximal insulin response (368, 189). In addition, the thyroid and adrenergic endocrine system is stimulated (45b), giving rise to increased catecholamines (310) which
further raise glucose levels via glycogenolysis and gluconeogenesis. In addition the catecholamines stimulate lipolysis and the release of glucagon while decreasing insulin secretions (45b).

With the glucose-insulin response rapidly taking place with the initiation of refeeding (169), extracellular potassium moves into intracellular compartments, thereby causing an alarming fall in serum K+ levels. Similarly, serum phosphate and magnesium levels also decline, creating a life-threatening serum biochemical profile (368). These disturbances constitute what has been coined the "refeeding syndrome". Such disturbances were likely the cause of the sudden death that ensued from refeeding world war II prisoners. Although the autopsy of these soldiers revealed very little, aside from a marked decrease in organ sizes (370), Knochel et al. (235) later attributed the cause of death to hypophosphatemia and/or hypokalemia.

Other parameters that should be monitored with enteral nutritional support include serum glucose for hyperglycemia and hypoglycemia. The high circulating glucose can lead to an osmotic diuresis with ensuing dehydration, hypotension, cardiovascular and renal impairment (368, 182). Serum FFA or triglycerides levels should be monitored for hyperlipidemia.

Heymsfield and Casper (184) point out that it is not advantageous (from the stand-point of lean tissue deposition) to aim for more than a moderate level of repletion. The goal then, is to provide the patient with energy maintenance calories at an energy infusion rate of 1.2-1.8 kcal/Kg FFM·h (184). This conforms with findings by other investigators (1, 107).

In feeding with TPN, hepatic parenchymal abnormalities may occur. King (232), in feeding rats parenterally, reported fatty liver in 50% of cases, whereas intra-gastrically fed rats showed no such symptoms. Monitoring liver enzymes and bilirubin may assist in detecting periportal fatty infiltration (156). The rate of protein infusion or the
type of nutritional formula must be supervised carefully when the
parenteral route of administration is taken because of possible azotemia
(368). Moreover, in the practice of intravenous hyper-alimentation,
there may be an increased susceptibility to refeeding edema
(230), which occurs concomitantly with an increase in intravascular
volumes (181).

Hence, refeeding practices are of particular concern since it is clear
that infusion rates, especially with respect to TPN, can provoke
specific electrolyte alterations during the starve-refeed period, which
may ultimately affect survival if too rapid. Enteral feedings are also
limiting, in that they can promote hyperglycemia and adversely
compromise normal metabolic responses. These are important factors that
can abnormally alter muscle function, therefore justifying the use of
oral ad libitum food intake.

2.2.3 Improvement in function. The refeeding period is accompanied by
significant improvement in muscle function over time. In refeeding
patients with anorexia nervosa, Russell et al. (356) observed a
restoration of muscle relaxation and normal fatiguability within 4
weeks. A total recovery of all parameters of muscle function was
observed by 8 weeks, even though total body nitrogen remained 19.4%
below predicted values. During the recovery period, function correlated
more with muscle electrolytes than with total body nitrogen. Christie
and Hill (73), during intravenous refeeding of patients with
inflammatory bowel disease, observed an early maximal functional
recovery within 4 days of refeeding. This preceded body protein
restoration, which eventually ensued later on in the convalescence
period.

Functional recovery of rat muscle has not been studied within the
first 4 days of refeeding. This is a critical refeeding time during
which important tissue depositions are known not to take place (189).
2.2.4 Macronutrients and muscle function. The fuel provided by food is essentially found in three basic forms: carbohydrate, fat, and protein. The relative proportion of these macronutrients is suspected to influence the function of muscle in different ways, because they are digested, absorbed, transported, taken up by tissue, and metabolized dissimilarly.

2.2.4.1 Carbohydrate intake. There is little doubt that different proportions of macronutrients influence the insulin:glucagon ratio, and therefore, the accessibility of glucose to the cell (190, 274). Macdonald et al. (269) found, however, that dietary sucrose deposited more fat in rats compared to glucose, although no changes in weight gain were observed. The glucose, once it gains access to the muscle cell, is phosphorylated to glucose-6-phosphate (G,6-P) and undergoes further phosphorylation and hydrolysis as it flows through the glycolytic pathway, rapidly producing ATP. This ATP comes into play at the level of several energy dependent mechanisms -- notably the calcium ATPase pump, the Ca"Mg" ATPase, the myosin-ATPase, and the Na"-K" ATPase. These processes are likely to slow down in an environment with a low \( \Delta G_{\text{ATP}} \).

There is, however, some controversy over whether maximizing the carbohydrate content of the muscle can normalize performance during refeeding. This is an ambiguous area, as it has been shown that dietary composition did not alter glucose incorporation into muscle glycogen or fat (71). In contrast to these findings, Smith (369) found that a very high carbohydrate diet (in the area of 70% to 80% of calories) improved insulin sensitivity, while a lower carbohydrate content (40-60%) did not. In addition, regardless of the kind of carbohydrate, whether complex as in rice or potato, or relatively simple as in sucrose, it
produced similar declines in plasma glucose and insulin with 30 minutes of aerobic cycling (196). This suggests that the rate of utilization during exercise is not influenced by the structural complexity of the carbohydrates.

2.2.4.2 Fat intake. Several studies have reported a decreased number of insulin receptor sites on the muscle, liver, and adipose tissue following the administration of a high fat diet (162, 243, 274, 393). The mechanism may revolve around the impact of lipid composition of cellular membranes, which are known to be influenced by diet, on insulin binding (163, 141). The hindrance of glucose access to the cell implies less muscle glycogenesis (207). The impact of sparse quantities of glucose accessing the muscle should logically be felt at the level of muscle energetics (203, 315), if substrate availability plays a critical role.

In contrast to these findings, however, Lavau & Susini (244) did not find glucose utilization by the soleus or the EDL to be affected by the composition of the diet. Horowitz and Coyle (196) could not detect any differences in the glycemic response to meals containing up to 35% fat. Suspecting the inconsistency in results to involve fat quality, Chisholm & O’Dea (71) controlled the polyunsaturated:saturated fat ratio (P:S) of both high fat and low fat diets. They found no difference in insulin receptor binding to the soleus, thereby demonstrating a similarity in response because of the identical P:S. A paradox does exist however in the literature: if fat quality in the diet does influence glucose utilization by the muscle, why have others found impaired glucose tolerance and insulin resistance (174, 274, 331) to be unchanged by variances in P:S ratios in high fat and low fat diets? The answer may well have been identified by Chisholm & O’Dea (71). Their results point in the direction of an in vivo reduction in hepatic glucose utilization while muscle is unaffected. Although the mechanism by which a high fat diet influences the plasma levels of glucose remains obscure, a worthy
consideration is the possibility of a diminished glucokinase activity. Chisholm and O'Dea (71) have certainly confirmed that rats on a high fat low carbohydrate diet exhibited a more elevated plasma glucose following a post-fasting glucose injection; on the other hand, the rats on the low fat high carbohydrate diet had lower serum glucose values. Their findings, however, do not support the need to manipulate macronutrient content of diet for maximal glucose uptake by the muscle; they showed that while fasting insulin levels tended to be lower in rats fed the high fat diet, the percentage insulin bound to soleus muscle strips was not different between diets. Furthermore, from in vitro work, by this same group found that glucose utilization in the soleus was smaller in the high fat diet, a finding that suggests a dissociation between glucose metabolism in the muscle and insulin binding to muscle. In contrast, Grundleger and Thenen (162) reported that feeding a high fat diet caused a decline in the number of insulin receptors on skeletal muscle. They conclude that diet composition does, in fact, influence glucose utilization by muscle. Their diet was elevated in saturated fat, a dietary trait that has been shown to decrease insulin binding by 80% (163). The high saturated fat diet is in contrast to the high P:S ratio diets used by Chisholm and O'Dea (71). Hence, they suggest that, providing the fat source is elevated in PUFAs, insulin insensitivity can be minimized. There is also some indication that diets with carbohydrate contents of at least 70% of calories, may maximize insulin sensitivity (369).

Horowitz and Coyle (196) approach the issue of fat in the diet strictly from the perspective of glycemic and insulinenic responses to food, carbohydrate oxidation and exercise. The foods that were ingested were classified as either high glycemic or moderate glycemic. They found that these two groups were independent of the simple or complex carbohydrate classification scheme. This group found that adding fat to rice or potato significantly blunted the post-exercise glycemic
response. In other words, blood sugars did not fall as sharply as controls. Adding fat also resulted in a lower peak blood glucose, a phenomenon that may relate to a slower gastric emptying. Moreover, they conclude that changing the glycemic index of the meal will not alter the sensation of fatigue (196).

2.2.4.3 Protein intake. Peret et al. (333), while studying rats on high and low protein diets, monitored the eating pattern of rats fed a solid chow-like diet over a 24-hour period. They found that the rats followed similar eating patterns, with specific peak amounts being consumed at around 2300 h and at 0700 h the next day. Overall, rats on the low protein diet consumed significantly more food, although the difference was only 2-3 grams. It is not known how the eating pattern would change with a liquid diet, nor is it known if hypocaloric feedings changes the eating pattern as well.

In addition, Peret et al. (333) have shown that the insulin:glucagon ratio is directly related to the liver pyruvate kinase enzyme and inversely associated with the phosphoenolpyruvate carboxykinase enzyme, during their study of the circadian rhythm in rats. Furthermore, they found that the amplitude of this ratio was greater during the daylight hours in rats eating the high protein diet (70%) and much higher in magnitude at night (in the dark) while consuming the low protein diet (10%). They conclude that 24 days are required before a steady state can be reached at which point pyruvate kinase activity was minimal in the high protein diet and phosphoenolpyruvate carboxykinase was significantly higher, but both insulin and glucagon values had normalized. This indicates the dissociation of these hormones from metabolic enzymes in the adaptive phase to the diet.

This same group, led by Robinson et al. (348), studied 8 week old rats for 24 days. They showed that the cytoplasmic redox state (NAD/NADH) in liver had similar diurnal changes in rats fed either diet. In the absorptive state (0300-900 h), cytosolic redox was increased in
rats fed both diets; however, the redox was clearly lower in rats fed the high protein diet. In contrast, the mitochondrial redox, measured in the liver of rats fed the high protein diet (70%), underwent a marked depression during the absorptive phases (2300 h and 0700 h), although diurnal changes were quite similar between the diets. This mitochondrial depression occurred concomitantly with maximum ketone body production, which signalled enhanced gluconeogenesis and ureogenesis during the absorptive phase. During the post-absorptive phase, both gluconeogenesis, and ureogenesis were sub-maximal (348). However, the group had previously noted in rats fed either high or low protein diets, that no correlation was possible between serum glucose and hormone levels over 24 hours (333), indicating that serum glucose in itself, is poorly representative of metabolic activity. Robinson et al. (348) used lactate, pyruvate and PEP as indices of glycolysis and gluconeogenesis, and malate, oxaloacetate and α-ketoglutarate as indices of oxidative phosphorylation. In rats consuming both the high and low protein diets, the α-ketoglutarate levels appeared to peak significantly in the absorptive phase, while significant troughs for malate and oxaloacetate were observed concurrently. There is no indication that diet composition consistently altered the diurnal cytosolic and mitochondrial redox, except at one or two peak times. In addition, throughout the day-light hours, rats on the high protein diet, consistently maintained lower lactate, pyruvate, malate, and oxaloacetate concentrations, compared to controls (10% protein), an indication of less glycolytic substrate. It is interesting that while α-ketoglutarate peaked, glutamine plummeted while on the high protein diet. A slowdown in mitochondrial redox implies a build-up of cytosolic metabolites because of a reduced oxidative capacity (64). Usually when α-ketoglutarate peaks so also do pyruvate and lactate (348). This suggests that α-ketoglutarate may be useful in assessing mitochondrial redox. Newsholme and Leech (310) describe α-ketoglutarate dehydrogenase as a "quantitative indicator of
the TCA cycle. It is not known if these diets alter metabolites in the same manner in muscle, and if similar patterns occur in adult rats.

The muscle's response to undernutrition and refeeding is an intriguing one, because its performance is dependent on the integrity of many mechanisms, notably: the central nervous system, neuromuscular transmission, excitation-contraction coupling (121), fiber size (54), substrate availability and utilization (344, 29, 360b, 347, 88), and the free energy status (105). In theory, should anyone of the aforementioned steps be compromised, fatiguability would likely increase. The integrity of the neurological system (34) and of the neuromuscular junction (35, 36, 37, 291) have been shown, however, not to be compromised during normal muscle fatigue. The reasons for fatigue appear, instead, to be located within the muscle (378).

Still unresolved is the extent to which substrate availability and utilization is critical to the energetics of the muscle, and ultimately to its performance during malnutrition and refeeding. In fact, there is some question whether, during malnutrition and early refeeding, the performance of the muscle (defined by the parameters of force, relaxation rate and fatiguability) is compromised by the diet composition, by a serious decline in the availability of energy or substrate, by the ability to use energy/substrate and/or to translate chemical energy into work. The substrate story is not a simple one, since it is not known if muscle glycogen concentrations or glycogenolysis are related to fatiguability within the context of undernutrition and refeeding. In other words: is the greater fatigue reported during malnutrition, the result of diminished muscle glycogen content, or the consequence of a suppressed net glycogenolysis, and/or a preferentially redirected dependency on FFA (186) or circulating glucose over glycogen (91)? There is, in addition, some suggestion of a heightened insulin insensitivity (343) resulting from circulating FFA or ketone bodies. Several studies suggest that there may be a slower
glycolysis and oxidative phosphorylation, but do not discriminate between muscle types during early refeeding, or support glycolytic deficits with substrate utilizations. Worthy of consideration in the analysis of muscle performance are important dietary confounders that can alter metabolic processes, notably, the degree of caloric restriction, the refeeding method, and the composition of the diet. These factors have in fact been considered in this thesis and will be outlined in chapter 3.
3.1 THE DEFINING PARAMETERS FOR THE EXPERIMENTAL WORK

There is a clear and observable erosion of muscle protein and a decrease in muscle size with malnutrition, a physiological impact that makes muscle an important indicator of nutritional status in instances of chronic semistarvation (185). The muscle mass does, however, erode slowly during starvation and recovers slowly as well during refeeding (183). There is logically some uncertainty over the extent to which protein erosion in the muscle can influence the function of muscle (197). This is a concern because, in early starvation, 50% of glycogen is lost from the muscle (185), a loss that can threaten muscle cell integrity (158).

It has also been suggested that muscle function is merely a reflection of a loss of muscle energy stores rather than an index of protein-calorie malnutrition (374), and that function can be improved simply by infusing glucose and potassium (65), similar to the practice observed in long distance runners (89). If this were so, then the impact of malnutrition on muscle function would be limited to the depletion of muscle energy reserves in the form of PCr and glycogen and to changes in circulating glucose. Consequently, this implies that without sufficient substrate, the muscle's performance would be greatly compromised. In maximally contracting slow-twitch muscle, the substrates involved are likely to be glycogen, glucose and FFA (49, 347, 381), whereas in fast-twitch muscle the preferred substrate is likely to be almost entirely the glycogen reserves (344).

Two questions can be asked with respect to substrate availability: first, given that the availability of substrate is associated with the macronutrient composition of the diet, can the function of muscle be maximized and normalized, using a high carbohydrate diet? Second, what
is the impact of muscle glycogen reserves on muscle fatigue, as well as
the influence of circulating glucose, insulin and FFA in the fatigu-
bility of muscle during underfeeding and early refeeding?

There are, in addition, several uncertainties with respect to the
importance of muscle glycogen reserves: it is unclear (a) whether the
pre-exercise muscle glycogen content is related (2, 29, 157) or
unrelated (357, 284, 84, 86, 87, 227) to fatigue; (b) whether pre-
exercise glycogen concentrations pre-determines the rate of net
glycogenolysis (347, 19); or (c) whether the role of glycogen in muscle
fatigue is mostly a non-metabolic one (157).

Current evidence suggests that metabolic activity, rather than simply
substrate availability, is compromised with underfeeding. Undisturbed
muscle glycogen concentrations have been reported with hypocaloric
feeding (357); in addition, biochemical evidence supporting the notion
of a fall in glycolysis and oxidative phosphorylation was observed in
the rat gastrocnemius during prolonged hypocaloric feeding. They
observed an increased fatigue concomitantly with significant falls in
PFK, SDH, and β-hydroxyacyl-CoA-dehydrogenase levels, key enzymes
indicating a decrease in glycolysis and TCA cycle activity and, in all
likelihood, decrease oxidative phosphorylation. This was subsequently
validated with the use of NMR (338, 295) for measuring muscle ATP, total
creatine, pH and Mg²⁺. However, fast and slow twitch muscles have not
been differentiated in these studies. Furthermore, while the enzyme
activity describes a diminished state arising from underfeeding, it
falls short of explaining the functional changes occurring in the
fatigued muscle (358).

Although there is sufficient evidence to suggest that muscle fatigue
during malnutrition is related to a depressed state of energetics, and
that this depressed energetics is reversed with refeeding (374, 355),
there is some question whether glycolysis is compromised in the
undernourished state (358, 149). There are, with respect to this issue,
several questions that have not been investigated: (a) glycogenolysis and glycolysis have not been studied in hypocalorically fed animals using net glycogenolysis and the metabolite concentrations of the glycolytic and the TCA pathways; (b) furthermore, no investigators have attempted to distinguish between the metabolic activity of the fast- and slow-twitch muscles nor have they correlated glycogenolysis with fatigue in malnutrition. Although attempts were made (315, 316) to study the relaxation time and contractile performance of the soleus and EDL muscles in HYPO, fasted, and control rats, enzyme activity and function were not measured in the same muscles; (c) moreover, glycogen deposits, net glycogenolysis, and glycolysis in these muscles have not been investigated within the context of refeeding. Furthermore, while changes in body composition have been studied to some extent within the context of muscle fatigue in both the underfed and refed states (73), composition and function have not been investigated concurrently within a short refeeding period, nor have mass and protein content of specific organs been measured.

3.1.1 Central Hypotheses

These questions led to the formulation of four specific hypotheses regarding the performance of muscle in undernutrition and early refeeding. These hypotheses are framed from the perspectives of muscle fatigue and body composition, muscle fatigue and muscle-type, muscle fatigue and metabolism, and substrate availability and fatigue.

3.1.1.1 Muscle Fatigue and Body Composition. Although it is known that muscle function responds more promptly to refeeding over the long term than do body composition parameters, it is not known how muscle recovers over the short term with respect to changes in body composition, muscle weight and muscle size.
HYPOTHESIS 1: Muscle function will respond earlier to refeeding than muscle mass, muscle cross-sectional area, organ mass and serum biochemistry.

3.1.1.2 Muscle Fatigue & Muscle Type.

Slow-twitch and fast-twitch muscle are physiologically distinct. Slow-twitch muscle is primarily made of oxidative fibers, is heavily vascularized and possesses an elevated number of mitochondria. Hence, a slow-twitch muscle has an elevated capacity for oxidative phosphorylation and will therefore likely rely on free fatty acids as an important fuel source. Fast-twitch muscle is primarily composed of glycolytic fibers, and relies on muscle glycogen and circulating glucose for substrate. It possesses and increased capability of utilizing the glycolytic cycle for energy production. These differences suggest the potential of a dissimilar response of muscle to malnutrition and refeeding between the two muscle types.

HYPOTHESIS 2: The specific response of muscle to malnutrition and refeeding is related, in turn, to a depression and revival in glycolysis and oxidative phosphorylation. These changes are dissimilar between fast-twitch and slow-twitch muscles.

3.1.1.3 Muscle Fatigue: Substrate and Metabolism. It is not certain whether the decrease in muscle performance that has been documented during malnutrition is the result of: (a) a deficiency in substrate availability (158); (b) a depressed substrate utilization (278,); or (c) a defective oxidative phosphorylation and glycolysis (357, 358). Saltin (365b) points out that glucose and FFA access to the cell, during exercise intensities greater than 50% \( V_{O_{2\max}} \), may be greatly diminished.
HYPOTHESIS 3: Net glycogenolysis and glycolysis, in rat muscle, will be affected differently by malnutrition and refeeding resulting in dissimilar muscle fatigue responses.

HYPOTHESIS 4: Muscle performance during malnutrition and early refeeding will remain independent of substrate availability.

3.1.2 Research Goals.

To validate the aforementioned hypotheses, the research protocols were validated from four perspectives: first, the eating pattern of the rats needed to be studied, as it is unknown how a hypocaloric liquid diet will affect this pattern. Second, body composition changes needed to be measured in the underfed state and during sequential early refeeding. Third, the function of muscle needed to be studied concurrently with the changes in body composition. Fourth, substrate and metabolism needed to be measured concomitantly with muscle function. Thus, for this research, a total of nine goals were established to provide information from the four perspectives.

3.1.2.1 Eating pattern of HYPO-fed rats. This is an important area to investigate, as it is unknown how a hypocaloric liquid diet will influence the time at which the rat eats, the duration of the eating time, and serum insulin. These are relevant questions that pertain to the issue of substrate availability.
Goal 1: To establish an animal model of undernutrition in which the feeding pattern of the rat is described both in terms of feeding time and feeding duration, and in which serum insulin levels are documented postprandially.

3.1.2.2 Body composition and diet. Changes in body composition with refeeding have been previously studied by others; however, it is not known how changes in composition are affected by hypocaloric liquid diets that are micronutrient complete, and how refeeding a calorically and micronutrient diet similar to controls affects body composition in the early introductory phase.

Goal 2: To characterize the weight loss, during underfeeding and refeeding, in terms of gross changes in body weight, organ weights and proteins, tissue hydration, and serum profile.

Goal 3: To validate the micronutrient supplemented liquid diet as a tool for weight loss and normalization of serum electrolytes.

Goal 4: To duplicate the type of diet (enteral) used in the nutritional support of malnourished patients, as well as reproduce the changes in body composition observed in non-traumatic, and non-septic malnourished patients.

3.1.2.3 Distinguishing muscle types. Distinguishing muscle types is essential in qualifying and quantifying the response of different muscles to malnutrition and refeeding. This practice cannot be done easily in the clinical setting. Muscle groups composed of many fiber types are generally used. For example the adductor pollicis muscle is composed of about 50% slow-twitch fibers. It therefore cannot serve as an adequate muscle exemplifying the action of fast-twitch muscle.
Goal 5: To isolate both the soleus (primarily slow-twitch fibers) and the EDL (primarily fast-twitch fibers). Surgically attach the muscle to a force transducer, electrically stimulate the muscle and freeze clamp them while still stimulated. The performance of one muscle will consistently be dissimilar from the other during underfeeding and refeeding.

3.1.2.4 Muscle fatigue and body composition. It is not known if there is a change in muscle function in the early refeeding phase (1 to 4 days), nor is it known how specific organs and muscles change over that refeeding period.

Goal 6: To demonstrate that the performance of muscle, during HYPO feeding and sequential refeeding, acts independently from dry muscle mass, cross-sectional area of muscle, protein and muscle glycogen.

3.1.2.5 Muscle fatigue: substrate and metabolism. The main focus is to study the performance of muscle from the perspective of fatigue in relation to substrate availability and utilization, and from the perspective of glycolytic and glycogenolytic integrity during undernutrition and early refeeding. In addition, two muscle types are differentiated in terms of function and metabolism: the soleus (slow-twitch) and the EDL (fast-twitch). Their function is analyzed within the context of malnutrition and refeeding using objective measurements of force that functionally assessed the state of mechanics and energetics.

To examine aberrations in muscle metabolism, whether it be a compensatory increase or a significant decline in metabolic activity, the following key metabolites were measured: G-6-P, F-6-P, F 1,6-diP, lactate, α-ketoglutarate, and glycogen in both the stimulated and unstimulated soleus and EDL muscles.
Goal 7: To determine if muscle performance is associated with muscle glycogen concentrations prior to stimulation.

Goal 8: To establish the extent to which the utilization of glycogen in both the soleus and EDL muscles is associated with fatigue during malnutrition and refeeding and, in so doing, to assess the activity of net glycogenolysis.

Goal 9: To determine whether each muscle type is equally sensitive to malnutrition and refeeding and whether fatigue is related to disparate metabolic changes.

3.1.3 Rationale of The Research and Methodologies.

The main rationale of this investigation is to further our understanding of the mechanisms involved in the depression and recovery of muscle function during refeeding and to validate its use as an assessment tool for malnutrition and early recovery. The functional and metabolic changes that take place in muscle during the initiation of feeding is an area that has not been studied extensively. Although it has been shown that muscle function eventually normalizes with recovery time (73, 355), it is unclear how much of muscle recovery is related to the regeneration of muscle mass and substrate reserves. Hence, it is useful to validate the importance of serum glucose and muscle glycogen in the early recovery of muscle function.

The rationale behind using the soleus and EDL muscles lies in the fact that they are composed of disparate fiber types. Fiber differentiation in vivo has not been undertaken in previous NMR work. Therefore, the effort to consider in situ fiber-specific function and biochemistry is in effect an important step.

The degree of caloric restriction, the refeeding method, and the composition of the diet are important considerations in the analysis of muscle performance, because they are perceived as key dietary
confounders that may alter metabolic processes. Complete food restriction, although frequently adopted in the literature, poses some difficulties related to possible physiological and endocrinological lag time arising with the sudden introduction of food. Hypocaloric feeding appears to be the preferred choice, as there is evidence (189) that total caloric restriction decreases energy maintenance requirements proportionally. In addition, hypocaloric diets produce a more representative model of chronic malnutrition, which is the situation typically found in the clinical setting.

Refeeding practices are of particular concern; it is clear that infusion rates, especially with respect to TPN, can provoke specific electrolyte alterations during the starve-refeed period; refeeding may ultimately affect survival if too rapid. Enteral feedings are also limiting, in that they can promote hyperglycemia, and therefore, adversely compromise normal metabolic responses. This could be an important confounder of muscle function, and as such, would justify the use of oral ad libitum food intake.

A high carbohydrate liquid diet is used in this investigation of muscle function because there is evidence that glucose uptake by the muscle can be optimized with such a diet (71).

3.2 MATERIALS AND METHODS USED

One of the original features of this research is the development and use of liquid diets suitable for feeding hypocaloric (HYPO) and control (CN) rats. Such diets contrast with the traditional solid chow diets, which leave the HYPO rats more agitated. The exact formulation of the diets are described in Annex-1.

For this research, two methodological goals distinct from the major study goals were established: one goal of the experimental methodology was to generate a model of undernutrition that originates from a
macronutrient-deficient diet. A micronutrient-complete diet is provided to reduce the risk of vitamin, electrolyte, and/or microelement deficiencies which may precipitate metabolic sequela.

The other methodological goal was to ensure that muscle fatigue was not derived from a centralized neurological impairment. To produce such a model of fatigue, the nerve must be electrically stimulated.

The predictor variable is the physiological and metabolic impact of hypocaloric dieting. The outcome variables are parameters of body composition, which include changes in body weight, organ composition and substrate availability, and parameters of muscle function, which include the maximal relaxation rate, the twitch tension, the maximal tension, fatiguability, and the ratio low frequency tension to maximal frequency tension.

3.2.1 Rats and diet

Male, adult, Wistar, rats (Charles River Laboratories, Montreal, Canada), 18 to 20 weeks of age and weighing between 350 and 380 g, were obtained for the study. The rats were free of any symptomatology and/or pathologies and exhibited normal ambulation. On arrival from the supplier, the rats were individually housed in an environmentally controlled atmosphere at an ambient temperature of 22°C with a 12-h light-dark cycle. The rats were fed a chow (Purina Laboratory rodent Diet #5001 TM) and adapted to the animal facility for an average of about 2 weeks before the start of the study.

The animals were then fed a liquid diet that was made up of a 10% amino acid mixture (Travasol-10%, electrolyte free, Baxter, Toronto, Canada), 20% lipid emulsion (Intralipid-20%, Baxter, Toronto, Canada), and a glucose solution (dextrose 50%, Baxter, Toronto, Canada), vitamins (Vitamin-1000, Sadex, Boucherville, P.Q), Ascorbic acid (Barocca-C, Roche, Montreal Canada), electrolytes and trace elements (Baxter, Toronto, Canada) (Tables 3.1. and 3.2.). The vitamin, mineral and
electrolyte constitution of the diets were similar to that reported by Hoshino et al. (197). The diets were sufficiently complete in electrolytes to prevent any significant losses in serum over the course of the 7 day depletion period (Table 5.5. chapter 5), and were internally consistent throughout the study. In addition, the macronutrient composition of the control (CN) diet was similar to that of the rat chow, and therefore duplicated diets used by many studies. The glycemic index of the food appears to be of little importance in performing muscle; Horowitz and Coyle (196) have shown that despite differing glycemic indices of meals, fatigue was not produced in physically fit men performing moderate intensity exercise at 50 to 70% VO₂max, despite significant and similar falls in plasma glucose and insulin from rest to exercise. Similarities with the rat can only be assumed.

An ideal macronutrient distribution, with more than an adequate amount of protein, was supplied to protect the integrity of the CN group and to not compromise its role as a valid comparator. Consequently, the CN diet was higher in protein and lower in carbohydrate compared to the refeeding diet (Table 3.2.). The latter diet was similar in macronutrient composition to Hoshino's (197) formulation, which was shown to be liberally taken by rats and to maintain normal rates of growth (65). The choice of a high carbohydrate liquid diet in treating the refed group meets two objectives: First, it is consistent with the practice of prescribing predominantly carbohydrate based liquid enteral feedings to hospitalized patients in surgical and chronic care wards with the expectation of maximizing recovery; and second, it has been previously shown that diets with carbohydrates as high as 70% of calories are generally capable of minimizing insulin insensitivity (369).
3.2.2 Feeding Protocol.

Prior to introducing the liquid diet, the rats were randomly assigned to either a control (CN) well fed group, or to a hypocaloric group (HYPO). The control rats were fed, ad libitum, a diet with a macronutrient content, expressed as a percentage of calories, equal to 25% protein, 64% carbohydrate, and 11% fat. The HYPO group was fed a calorically restricted diet containing 13% protein, 73% carbohydrate, 14% fat (Table 3.2.). The HYPO rats were subsequently refed (RE), after 7 days of underfeeding (Fig. 3.1.), with a diet equal in caloric density to the CN diet and similar in macronutrient composition to the HYPO diet.

The CN rats were fed ad libitum a liquid diet that is nutritionally adequate for a study of short duration such as 10 days (Tables 3.1. & 3.2.). The HYPO rats were malnourished over 7 days with a liquid diet that was identical in micronutrient content to the CN diet. The caloric density of the HYPO diet, however, was such that only 20% of the energy (calories) of the CN diet was provided to the HYPO rats (Table 3.2.). The CN diet contained 4.94 g and the HYPO diet 0.64 g of protein per 100 ml of volume. The CN diet was sufficient to promote visible gains in body weight (Figure 5.1). It has been shown that normal growth for this size rat necessitates between 2.40 - 2.85 g /d of digestible protein (5, 309). Clearly the control rats received protein in excess of that required for growth requirements. The HYPO diet by contrast received a highly restricted intake of all macronutrients. The diets were isovolemic so that the energy density was 80% lower in the HYPO diet (Table 3.2.).
TABLE 3.1. Nutrient composition of liquid diets for controls (CN), hypocaloric (HYPO) & refed (RE) rats. (Values expressed/100 ml and /100 Kcal) Nutrients are identical in all three diets.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>CN Daily intake</th>
<th>HYPO Daily intake</th>
<th>* N.R.C. N.R.C Daily intake</th>
<th>N.R.C /100 Kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>13.74 μg</td>
<td>13.74 μg</td>
<td>13.74 μg</td>
<td>12.0 μg</td>
</tr>
<tr>
<td>Nicotinic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>120.85 μg</td>
<td>120.85 μg</td>
<td>120.85 μg</td>
<td>150.0 μg</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>23.10 μg</td>
<td>23.10 μg</td>
<td>23.10 μg</td>
<td>40.0 μg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>32.92 μg</td>
<td>32.92 μg</td>
<td>32.92 μg</td>
<td>36.8 μg</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>31.57 μg</td>
<td>31.57 μg</td>
<td>31.57 μg</td>
<td>40.0 μg</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0.01 μg</td>
<td>0.01 μg</td>
<td>0.01 μg</td>
<td>0.5 μg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>5.48 i.u</td>
<td>5.48 i.u</td>
<td>5.48 i.u</td>
<td>20.0 i.u</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>630.70 μg</td>
<td>630.70 μg</td>
<td>630.70 μg</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>27.43 μg</td>
<td>27.43 μg</td>
<td>27.43 μg</td>
<td>20.0 μg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.548 i.u</td>
<td>0.548 i.u</td>
<td>0.548 i.u</td>
<td>10.0 i.u</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.024 i.u</td>
<td>0.024 i.u</td>
<td>0.024 i.u</td>
<td>0.132 i.u</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.165 μg</td>
<td>0.165 μg</td>
<td>0.165 μg</td>
<td>2.0 μg</td>
</tr>
<tr>
<td>Sodium</td>
<td>82.23 mg</td>
<td>82.23 mg</td>
<td>82.23 mg</td>
<td>22.0 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>206.47 mg</td>
<td>206.47 mg</td>
<td>206.47 mg</td>
<td>420.0 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>14.83 mg</td>
<td>14.83 mg</td>
<td>14.83 mg</td>
<td>50.0 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7.57 mg</td>
<td>7.57 mg</td>
<td>7.57 mg</td>
<td>5.00 mg</td>
</tr>
<tr>
<td>Chloride</td>
<td>313.51 mg</td>
<td>302.21 mg</td>
<td>302.21 mg</td>
<td>5.00 mg</td>
</tr>
<tr>
<td>Iodine</td>
<td>5.87 μg</td>
<td>5.87 μg</td>
<td>5.87 μg</td>
<td>2.00 μg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>12.79 mg</td>
<td>12.79 mg</td>
<td>12.79 mg</td>
<td>28.0 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>14.54 μg</td>
<td>14.54 μg</td>
<td>14.54 μg</td>
<td>50.0 μg</td>
</tr>
<tr>
<td>Zinc</td>
<td>29.20 μg</td>
<td>29.20 μg</td>
<td>29.20 μg</td>
<td>120.0 μg</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.19 μg</td>
<td>0.19 μg</td>
<td>0.19 μg</td>
<td>0.30 μg</td>
</tr>
<tr>
<td>Manganese</td>
<td>33.97 μg</td>
<td>33.97 μg</td>
<td>33.97 μg</td>
<td>20.0 μg</td>
</tr>
<tr>
<td>Selenium</td>
<td>1.16 μg</td>
<td>1.16 μg</td>
<td>1.16 μg</td>
<td>1.00 μg</td>
</tr>
<tr>
<td>Iron</td>
<td>48.77 μg</td>
<td>48.77 μg</td>
<td>48.77 μg</td>
<td>350.0 μg</td>
</tr>
<tr>
<td>Acetate</td>
<td>253.62 mg</td>
<td>215.54 mg</td>
<td>215.54 mg</td>
<td></td>
</tr>
<tr>
<td>Ca:P</td>
<td>1.16</td>
<td>1.16</td>
<td>1.16</td>
<td>1.3-1.5</td>
</tr>
</tbody>
</table>

* National Research Council's (N.R.C.) 1995 daily nutrient recommendations for rats (309b). ‡ Under normal conditions rats do not require biotin or vit C in the diet. The NRC recommendations are based on the daily consumption of 10 g of rat chow/day for a rat weighing approximately 400 g (ICN Biomedicals/Animal research diets, 1995)

‡ Riboflavin requirement is based on minimal dietary concentration =1.2 mg/kg diet/d and appropriate for short studies (309b).

† Pyridoxine requirements vary between 4-7 mg/kg diet/day (309b)

f Na requirements for maximal growth was 2.2 g/kg diet/day (309b)

g Calcium intakes of 3.5 mg/kg diet is sufficient to produce normal bone calcification (309b)
All rats were given 120 ml daily of diet, allowing CN and HYPO rats to consume *ad libitum* amounts of diet. Rats were fed in metabolic cages with drip collectors and the exact volumes consumed were measured. Controls consumed 77% of the total volume, HYPO rats consumed 85% of the total volume and re-fed rats consumed 74% of the diet's volume. By keeping the diets isovolemic, the reduction in energy intake was achieved in the HYPO group without reducing the time over which the diet was consumed. The rats in both groups appeared to be satisfied with their diet; the HYPO group did not appear hyperactive as they did when we fed hypocaloric solid chow diets (data not given). The HYPO rats lost 20% of their initial weight by day 7 (Fig. 5.1. chapter 5). The CN, while on the liquid diet, lost 5% of their starting weight by day 4 due to loss of solid fecal contents and adaptation to diet, with a subsequent 6.25% increase by day 10. The adjustment phase to the diet was included as part of the study in order to produce a normal and consistent pattern of weight loss in HYPO rats, one which initially consists of mostly fluid loss, followed by protein catabolism and fat depletion. The objective was ultimately to produce a 20 to 25% drop in weight; it was clear that the HYPO rats attained this goal while consuming 95% of the diet right from day 1 of the study. Furthermore, although the CN rats did drop 5% of their starting weight by day 4, they began afterwards to gain weight normally at a rate of about 3.74 g/d.

On the seventh day, a random selection of HYPO rats were studied and sacrificed (HYPO rats) while others were studied on days 1 (RE-1), 2 (RE-2), 3 (RE-3), and 4 (RE-4) after refeeding with a high carbohydrate diet containing an energy density identical to that of the CN diet (Table 3.2.). The repletion diet was also identical in micronutrient composition to that fed to the HYPO rats. It was however different in macronutrient composition compared to CN, in that protein was lower and carbohydrates were higher (Table 3.2.). The higher carbohydrate content (in the area of 70% of calories) seems to maximize recovery of normal
glucose access to the cell. The protein intake during this refeeding phase was 2.56 g/d, an amount that meets the animal's needs for normal growth (349).

TABLE 3.2. Caloric density, protein, carbohydrate and fat contents of liquid control (CN), hypocaloric (HYPO) & refeeding (RE) diets. Values in total intake are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Caloric Density (Kcal/ml)</th>
<th>Protein (g/100ml)</th>
<th>Carbohydrate (g/100ml)</th>
<th>Fat (g/100ml)</th>
<th>Total Intake (Kcal/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>0.84</td>
<td>4.94</td>
<td>15.81</td>
<td>1.03</td>
<td>77.7 ± 1.3</td>
</tr>
<tr>
<td>HYPO</td>
<td>0.17</td>
<td>0.64</td>
<td>3.65</td>
<td>0.26</td>
<td>17.5 ± 0.6</td>
</tr>
<tr>
<td>RE</td>
<td>0.84</td>
<td>2.57</td>
<td>18.04</td>
<td>1.31</td>
<td>75.3 ± 1.5</td>
</tr>
</tbody>
</table>

Figure 3.1. Schematic representation of feeding protocol over the 11 day study period.
3.2.3 Organ Extractions & Tissue Protein Determination.

Liver, heart, kidneys and diaphragm were excised and frozen at -20°C. Samples were weighed and lyophilized. The protein concentration of specific organs and muscles were determined by the Biuret test and read by spectrophotometer. Total organ and muscle water content was determined using the difference between wet and dry weights of samples.

3.2.4 Exposure of muscle and conditions of stimulation.

The muscle stimulation protocols were based on a selection of earlier protocols by Close (76), Edwards (112), Russell et al. (354, 357), Roland et al. (350), and more recent techniques reported by Nishio & Jeejeebhoy (315) and Hood & Parent (194).

Rats were first anesthetized by an intra-peritoneal injection of sodium pentobarbitol, 6.5 mg/100 g body weight. Supplemental doses equal to about 14% of the initial dose were administered as required. When the animals were fully sedated, all the hair of both hindlimbs and hip areas were removed using an electric clipper. The skin was then separated from the leg allowing for the soleus in one limb and the EDL in the other to be exposed with minimal dissection, while minimizing blood loss. It was necessary for the Achilles tendon to be sectioned, to minimize any interference from the gastrocnemius. The tendons of the muscles were tied with 1-0 silk connected to a force transducer. The muscles were dabbed with a mineral oil that was maintained at 33 °C. Separated skin was placed back over the muscle, then the limb was wrapped in an impermeable plastic film (Saran Wrap, Dow Chemical Co.). The sciatic nerve was exposed in the hip area and surrounded by two electrodes (Dantec 13L20 Surface Electrodes, Dantec Electronik, Denmark) which were embedded in plastic, allowing these electrodes to be solidly attached by sewing together the two ends of the plastic sheath. The electrodes were in turn connected via a stimulus isolation unit (Grass SIUS, Grass Instrument Co. Quincy Mass. USA) to a stimulator (Grass
Stimulator S84, Grass Instrument Co. Quincy Mass. USA) (Fig. 3.2.). The rats were placed on a dry base of silicone elastomer (Sylgard 184 silicone elastomer, Dow Corning, Elizabeth Town, Kentucky, USA), which overlay a double walled 0.5 in. perspex box. Between the walls circulated warm water that kept the core temperature of the rat at 37 °C. The use of a heat lamp assisted in keeping the body temperature at 37 °C and the muscle temperature at 33 °C as measured by a surface thermometer (Physitemp, Model BAT-12, Physitemp Instruments Inc. Clifton, NJ.). A nail was driven through the knee joint and pushed into the Sylgard to ensure the stability of the proximal tendon. The nail was securely tied to a steel post -- which had been incorporated into the perspex box -- using a 1-0 silk.

![Diagram of muscle stimulation setup]

**Figure 3.2.** Schematic representation of the in situ muscle stimulation.
The animals' extremities were secured to the sylgard base using pins. The rats were given (by an open mask), a gas mixture containing 60% oxygen and 40% nitrogen, which had been shown by preliminary studies to optimize muscle performance. A similar study had indicated that the blood gas and pH of these animals were normal in all nutritional states (338).

3.2.4.1 Instrumentation for measurement. The muscle tendon was connected to a transducer (Grass FT03, Grass Instrument Co, Quincy, Mass, USA) with a maximum work range of 0.05 Kg and a displacement rate of 20mm/kg using yellow springs. The transducer was connected to an A/D converter (Labmaster, Scientific Solutions Solon, Ohio) in a microcomputer with an 80386 Intel processor running at 33 MHz. The Labmaster A/D board was capable of converting the analog signal at 100 KHz. The digital output was transferred by direct memory access (DMA) to the RAM of the computer and then stored on a disk. Using a customized program, the data were displayed graphically and the digital data were analyzed to obtain force and relaxation rate.

3.2.4.2 Protocol of stimulation. Initially, it was determined that 20 V resulted in a supra-maximal stimulation using square waves 75 µs in duration. Using this voltage, the length of the muscle (Lo) needed for a maximal twitch (Pt) was determined.

While delivering electrical pulses at a train duration of 1000 ms and at a frequency of 1 Hz, maximal twitch (Pt) was determined by adjusting the muscle length. This was done by using a rack and pinion system (x-block, Narishige, Japan) which could be adjusted forward and backward to obtain the optimal length at which maximal tension was observed (Lo). After having determined Lo, the following protocols were applied to the soleus and EDL muscles. The soleus was stimulated for a duration of 2000 ms at frequencies of 10 to 100 Hz. The muscle was allowed to rest for 1 minute between stimuli. The EDL was stimulated for 500 ms at frequencies
of 10 to 150 Hz (Table 3.3.). The measured forces in both soleus and EDL were corrected for muscle weight (Po(N)/g) (357). The corrected forces at the various frequencies were used to determine the maximal isometric force and the force-frequency response of these muscles (425). In addition, the relaxation rate of the muscle was measured using the slope of relaxation after the 100 or 150 Hz stimulus was applied to the soleus and EDL, respectively, using methods published previously (425, 420). After a 5 minute rest period, the muscle was exposed to repetitive stimulations with a train duration of 500 ms (soleus) or 300 ms (EDL), at a train rate of 60 per minute, for a total of 40 contractions to induce fatigue (Table 3.3.). The loss of force over the 40 repetitive stimulations is referred to as fatigue.

In another set of rats, after fatiguing the muscle, there was a 5 minute rest period, followed by a series of stimulations again at the same frequencies. Once again, the MRR was measured at the end of these single contractions.

3.2.4.3 Measurements of function. Muscle function measurements were conducted in control (CN) rats, HYPO rats and reed rats RE1-4. Control rats were studied in a paired fashion with the HYPO and the 3rd day reed rats (RE-3). In other words, control rats were well fed for 7 days and for 10 days.

Muscle function was measured using the parameters of twitch tension [Pt(N)], maximal tetanic tension [Po(N)], the ratio of low frequency stimulation to maximal frequency stimulation [F10/Po(N) and Pt(N)/Po(N)], and fatigability.

The tension in the muscle was measured by the transducer and converted, on the computer screen, into a graphic representation of the isometric contraction. The height of the graph was converted into force from a graph standardized with set weights. A new standardized graph was
established for each day of experimental muscle function tests. A graphic illustration of how the functional measurements of both muscles were made from the computer screen, can be found in Figures 3.3.A and 3.3.B

---

Figure 3.3.A. Schematic representation of soleus twitch, tetanus and fatigue
$\text{EDL}$

**Fatigue % Height Lost from Initial Tetanus**

$\text{Fatigue} = \frac{\text{Initial HT} - \text{Final HT}}{\text{Initial HT}} \times 100$

---

**Figure 3.3.B. Schematic representation of EDL twitch, tetanus and fatigue**

$\text{EDL}$

$\text{Tetanus}$

$\text{MRR} = 0.693/t^{1/2}$

**Partial differential of two points against the best fit of the line following Marquandt's least square fit.**

---

$\text{Initial Height}$

$\text{Final Height}$

$\text{Twitch - 1 Hz}$

$\text{Height}$
3.2.5 Muscle Analysis

This section contains a description of the muscle sampling technique used in order to measure accurately muscle glycogen content and various biochemical metabolites. The sampling was done either on resting muscle, or on muscle subjected to 40 repeated tetany.

### TABLE 3.3. Muscle stimulation protocol.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Soleus</th>
<th>EDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Force-frequency measurement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of tetanus (ms)</td>
<td>2000</td>
<td>500</td>
</tr>
<tr>
<td>Rate of stimulation (Hz)</td>
<td>10, 20, 30, 50, 70, 100</td>
<td>10, 30, 50, 70, 100, 150</td>
</tr>
<tr>
<td><strong>Protocol for inducing fatigue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of stimulation (Hz)</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Duration of tetanus (ms)</td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td>Repetition rate (tetany/min)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Number of tetany</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

3.2.5.1 **Sampling technique.** The stimulated muscles were completely excised from the hindlimb while still contracting, followed by freeze clamping with narrow jaw pliers cooled in liquid nitrogen. The muscles were then lyophilized and stored at -70 °C. The unstimulated muscles were freeze clamped before the stimulation protocol began for the other
muscles. In other words, if the soleus of the right hindlimb was stimulated in one specific rat, then the soleus of the left hindlimb would serve as the unstimulated control. The same approach was used for the EDL.

3.2.5.2 Glycogen analysis. Glycogen concentrations were measured from these samples using a method reported by Harris et al. (170), a single reagent system for glucose using a glucose kit (Glucose 100 Trinder, Sigma Diagnostics. Cat.No: 315-100, St-Louis, USA, 63178). Muscles were freeze dried in a lyophilizer and 5-10 mg cross-sections were desiccated and digested using 600 µL of KOH. The digest was placed in an oven for 45 minutes at a temperature of 50°C. A 100 µl aliquot of digest was taken and added to equal volumes of HCL (0.1M) and sodium acetate (pH = 4.9). The pH so obtained is 5.6, ideal for maximal enzymatic activity and for protein precipitation. The α-amylase and amyloglucosidase enzyme preparations were added to the hydrolysate and incubated for 4 hours at 25-30°C. The homogenate was centrifuged for 25 minutes at 18-22°C and a 50 µL aliquot was taken and used in a glucose reagent system. One ml of Glucose-Trinder preparation (SIGMA, Chemical Company, St.Louis Mo) was added to the aliquot digest, to the glucose standard and to the appropriate blanks. The mixtures were read on a spectrophotometer after 18 minutes. This was done for both the stimulated and unstimulated soleus and EDL.

3.2.5.3 Metabolites analysis. The rest of the freeze dried samples were deproteinized with perchloric acid (PCA) and EDTA, and neutralized using a dye, KOH, and PCA. The stimulated and unstimulated deproteinized samples were assayed for lactate, glucose-6-phosphate, fructose-6-phosphate, fructose 1,6-di-phosphate, and α-ketoglutarate using fluorometric methodologies based on "Bergmeyer's Methods of Enzymatic Analysis" (28).
3.2.6 Blood Biochemistry

Rat weights were taken daily at 0800 h throughout the entire depletion and repletion periods. The rat was sacrificed by exsanguination. Prior to any surgical intervention and muscle function analysis, blood samples were taken from the retro-orbital vein for the determination of serum glucose and insulin. The retro-orbital method permitted blood sampling that preceded any surgical intervention, and minimized the release of epinephrine and cortisol. Samples were taken on day 7 of HYPO rats, on days 1, 2, 3, and 4 of refeeding and on day 10 for controls. Using a 5 cc syringe and a 22G needle, 3-5 ml of blood was also taken from the inferior vena cava for all other serum biochemistry values. The blood from both sources was spun and the serum extracted and stored at -20 °C. The serum was used to determine total proteins (221, 439), and transferrin concentration using the immunoturbidimetric method (Bayer, Etobicoke, Ontario, M9W 1G6), γ-glutamyl transferase (GGT) (Hitachi model 717, Boehringer-Mannheim, Montreal, Canada), bilirubin (399), and electrolytes consisting of sodium, chloride, potassium (399), phosphate (398), and calcium (239). Electrolyte measurements were conducted at the biochemistry laboratory of the Toronto General Hospital, using an Olympus A.U. 800 analyzer (Olympus Co. Dallas, USA). GGT was used in this instance as a sensitive indicator of hepatic biliary disease and liver function (168), and was measured at the Toronto Western Hospital.

Serum insulin was determined by immunoassay at the biochemistry laboratory of the TGH using a technique described by Livesey et al (262). Blood lactates as well as serum free fatty acids were determined by fluorometry.

Serum glucose was determined by the biochemistry laboratory of the Toronto General using the Olympus A.U. 800 and the method of Zilva and Pannall (439).
3.2.7 Statistical Design.

The statistical design was selected on the basis of verifying or eliminating any possible null hypothesis. Three null hypotheses can be stated within this thesis: (a) feeding a hypocaloric diet to an adult rat over a seven day period will not alter muscle function; (b) changes in visceral or somatic mass will occur concurrently with changes in muscle function; (c) refeeding will not improve muscle function or (d) muscle mass. These alternative hypotheses recognize that muscle function can increase with underfeeding, and it can also increase or decrease with refeeding. Work by Nishio et al. (313, 316) has shown that these three alternatives occur, depending on the muscle fiber type. Therefore, a two-tailed statistical design was considered appropriate.

3.2.7.1 Sample size. The sample size determination was established using muscle function data taken from the work of Nishio (313). The sample size was corrected by an adjustment factor described by Bach & Sharpe (15) and represented below:

\[
\frac{\text{df} + 3}{\text{df} + 1}
\]

where df is the degrees of freedom. Thus, the sample size needed to acquire statistical significance for the muscle function data was 12.

3.2.7.2 Analysis of data. The statistical approach to data analysis was based on the design of one control group (CN) and five experimental groups: HYPO, RE-1, RE-2, RE-3, RE-4.

An analysis of variance (ANOVA) was conducted between the six groups (CN, HYPO, RE1-4) for functional and body composition data. The ANOVA was used to determine if there was a significant difference between the six groups. It does so by calculating the mean square (between), the average difference between the group mean and the grand mean. The mean square (within) was calculated as the differences between the individual data points within a group and the group mean. Significance was
determined as an elevated F value determined as the ratio of the Sum Sq. between:Sum Sq. within. If the ANOVA was significant (p<0.05), then unpaired comparisons were made among the different experimental groups using Duncan’s New Multiple Range test for unplanned comparisons. The differences were considered significant at a p<0.05 for unstimulated glycogen and p<0.01 for all other comparisons.

An analysis of covariance (ANCOVA) is essentially a combination of regression analysis and ANOVA (383). This technique consists of establishing separate regression lines, between various independent and dependent variables, for each experimental group, including CNs. If the slopes of the regression lines are similar, an ANCOVA is performed; in such a case, a relationship between the treatment group (in this case the various diets) and the covariate can be determined. Such a relationship is established by determining a partial F-test. The covariate is also referred to as the sum of Squares due to regression. Hence, the dependent variable is adjusted using the covariate. The F-test establishes whether the covariate is significant with a p<0.01. Regression analysis was performed between refeeding days and several functional parameters, such as MRR, Po(N), fatigue, to provide an indication of recovery.

Multiple regressions were established using a variant of the Systat software package, called Mystat.
CHAPTER 4 EATING PATTERN OF RATS

The investigation into the feeding pattern of the rats was done in response to goal 1 (as described in section 3.1.2.1).

4.1 BACKGROUND

Nocturnal patterns of eating are usually followed by rats (406). It is unclear, however, whether a caloric restriction would change their eating pattern; for example, Tulp and Horton (406) showed that when 7 week old rats were fed a protein deficient, but calorically sufficient diet, they changed to a diurnal eating pattern of frequent eating. It is uncertain if such a pattern would be followed on a caloric restricted diet.

The eating pattern is of interest for two reasons: (a) muscle stimulation was conducted postprandially, and there is some evidence that muscle fatigue could be dependent on substrate availability, notably in the form of circulating serum glucose (429) arising from hepatic glycogenolysis (276). Although hepatic glycogen level falls in starvation (276), it is not well understood how hepatic glycogen reserves would be altered throughout the day in hypocalorically fed and refed rats (group RE), as there is uncertainty surrounding their eating pattern. Moreover, the concentration of hepatic glycogen is not exclusively dependent on the delivery of glucose through dietary ingestion of carbohydrate; there is evidence (328) that insulin levels are more important than serum glucose concentrations alone in heightening net hepatic glucose uptake.
The level of hepatic glycogen reserve is influential in maintaining serum glucose homeostasis. The glucose, in turn, is critical to the nervous system, and therefore, could induce central fatigue if amounts are inadequate (35), and it could play a role in sparing muscle glycogen (347). For this part of the investigation, two objectives must be met:

**Objective 1:** To determine if the rats that were hypocalorically fed a liquid diet consumed that diet in bolus feeds to satisfy their heightened appetites, or ate frequent small portions. Alterations in the rat's eating pattern may change the insulin levels that occur, which would alter glucose uptake into the muscle cells (207).

4.2 METHODS
A total of 13 male Wistar rats were randomly assigned into two hypocalorically fed groups. One group of rats was fed a hypocaloric diet in the morning (0900 h). The other group of rats were fed in the late afternoon (1600 h).

4.2.1 Feeding Protocols
The rats were given 120 ml of hypocaloric liquid diet (25 kcal) for seven days. The rats lost between 17 and 26% of their initial weight. In the AM group, the volume of consumed diet was determined between 1000-1040 h; 1100-1140 h; and 1200-1222 h. The leftover diet in the PM-fed rats was measured in the morning of the following day. Furthermore, visual inspection of the rats shortly after the diet was given revealed prompt and avid consumption of the diet, suggesting that an eating pattern similar to that of the AM-fed rats was adopted. In this group, the post-absorptive insulin levels were of considerable interest to compare with the AM fed insulin levels. The question of interest revolved around whether insulin levels in the morning were significantly different between rats, depending on the feeding time. This is critical,
as the membrane potential of the muscle is thought to be depolarized by elevated insulin levels in the hypocaloric state. Hence, the goal was to minimize changes in insulin levels with HYPO feeding.

4.2.2. Determination of Insulin levels

Serum insulin levels were determined in AM-fed rats after seven days of HYPO feeding. This was done by taking a blood sample from the retro-orbital vein of the same rat (taken from 9 AM-fed group) at 1000 h and 1600 h (Table 4.1.). Serum insulin levels were also determined at several times during the day in both the AM- and PM-fed rats (1000 h; 1200 h; 1400 h; and 1600 h). The data of the AM-fed rats were pooled and a mean established (Table 4.2.). Similarly, the data of the PM-fed rats were also pooled and a mean value measured. Statistical significance between the AM and PM rats was determined using the two-tailed Student t-test.

4.3 RESULTS

4.3.1 Eating Pattern

Hypocalorically-fed rats began consuming their diet almost immediately. On the first day, however, the rats consumed very little of the total volume in the first three hours of being presented with food. In fact, during those initial hours, only 32% of the diet was consumed. By the end of the day, however, 96% of the diet was finally ingested. After a 3 day adjustment period to the diet, 96% of the volume was consumed within the first three hours of being presented with the diet (Fig. 4.1.).

4.3.2 Serum Insulin

The levels of serum insulin did not significantly change between morning and late day in the AM fed hypocaloric rats (Table 4.1.)
TABLE 4.1. Serum insulin levels in AM fed rats (pmole/L ± SEM.)

<table>
<thead>
<tr>
<th>Group</th>
<th>10:00 am insulin n=13</th>
<th>4:00 pm insulin n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.M.</td>
<td>41.15 ± 4.09</td>
<td>63.8 ± 10.14</td>
</tr>
</tbody>
</table>

Figure 4.1. Daily cumulative liquid diet intake of HYPO rats during daylight hours over 7 days.
TABLE 4.2. Serum insulin levels in PM and AM fed rats, determined throughout day eight. Values expressed as pmole/L ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Serum insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.M fed rats</td>
<td>6</td>
<td>36.83 ± 4.98</td>
</tr>
<tr>
<td>P.M fed rats</td>
<td>4</td>
<td>125.25 ± 9.45*</td>
</tr>
</tbody>
</table>

* p<0.001

Insulin levels of those animals fed in the late afternoon were three-fold higher throughout the day than those fed in the morning (Table 4.2.)

4.4 DISCUSSION

4.4.1 Impact of Underfeeding on Feeding Pattern

The hypocaloric diet changed the feeding habit of the rats from that of a nocturnal nibbler to that of diurnal bolus feeders (Fig.4.1.). Most of the 120 ml of diet was consumed within the first 3 hours of feeding time following a 3 day adaptation period. The change over to bolus eating has been reported not to disturb patterns of glycogen synthesis in rats refed after 22 hours of starvation. Holness et al. (192) found no difference in liver glycogenesis with either ad libitum or meal fed rats.

4.4.2 Impact of Underfeeding on Insulin Levels

The significance of the rats changing to a schedule of diurnal bolus eating, and of feeding these rats in the AM is seen in terms of the insulin values; lower serum insulin in the AM fed rats persists through the day (Tables 4.1. and 4.2.). This may have an effect on muscle
energetics, as the degree of glucose uptake as well as the degree of glycogen phosphorylase activity in the unstimulated muscle would be affected by insulin concentrations (149). Insulin in concert with insulin receptors in the muscle favours glucose entry into muscle cells (207, 23, 217). The trans-endothelial transport of insulin (27), and not necessarily the insulin receptors, may be the limiting stage, although neither receptors nor trans-endothelial transport were measured in this thesis. Underfeeding may heighten insulin insensitivity (331, 174, 274) and restrict glucose access to the cell. Malnutrition creates a change in the hormonal milieu that is characterized by the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla, in addition to growth hormone and glucocorticoids, which prevent glucose uptake by the muscle (394, 390). It is unclear whether the reduced glucose uptake is the consequence of high circulating FFA ensuing from a heightened lipolysis or simply the consequence of low insulin.

In conclusion, hypocalorically-fed AM rats produced little variability in insulin levels between 1000 h and 1600 h (Table 4.1). There was, in fact, no significant difference between insulin values recorded in the morning and those values recorded in the late afternoon. Insulin levels were the lowest when the rats were fed in the morning of the previous day. This suggests that in order to maintain uniformly low insulin and hepatic glycogen concentrations, the hypocaloric diet should be given to rats at 0900 h on the day prior to the experiments. The rats do not receive any food the day of the experiment.
CHAPTER 5

THE EFFECT OF UNDERNUTRITION AND EARLY REFEEDING ON BODY COMPOSITION

The effect of undernutrition and early refeeding on body composition was addressed in goals 2, 3, 4, and 5 (see subsection 3.1.2.2). Here, some background information is provided concerning the effects of malnutrition on the erosion of body mass.

5.1 BACKGROUND

Traditionally, the effects of malnutrition and refeeding have been measured by the amount of wasting and restoration of total lean and fat mass and by changes in plasma protein levels (43, 345).

Simple malnutrition commences with a loss of liver glycogen, a physical breakdown or catabolism of protein, and by an increasing reliance on adipose tissue reserves for free fatty acids and ketones (276). The loss of lean mass takes place primarily from the visceral mass and to a lesser extent from the peripheral skeletal muscles (197). While there is agreement with respect to the pattern of tissue erosion in simple starvation and tissue deposits during recovery, the impact on body composition of hypocalorically feeding and refeeding a liquid diet with an elemental formulation is not clear. There are therefore three main objectives to be achieved in this chapter.

Objective 1: To measure during underfeeding and early refeeding, weight, protein and water changes in the heart, the soleus, EDL and diaphragm muscles, liver and kidneys, and serum protein.

Objective 2: To show that the micronutrient-supplemented liquid diet results in weight loss while maintaining normal serum electrolytes.
Objective 3: To measure the effect of the hypocaloric diet on serum lactates; insulin; FFA; glucose; and hepatic and muscle glycogen concentrations, as these are descriptive of undernutrition and they are indicators of substrate availability and metabolic activity.

5.2 MATERIAL AND METHODS

Rat weights were taken daily. The outcome variables of composition were measured using carcass analysis. The methodologies followed for diet preparation and feeding of rats are found in sections 3.2.1 and 3.2.2; body composition and serum analysis are described in sections 3.2.3, 3.2.5 and 3.2.6 of chapter 3.

Serum lactates can be indicative of liver metabolism, tissue hypoxia, and/or circulatory collapse (168).

5.3 RESULTS

Weight loss, muscle weight, cross-sectional area, serum biochemistry, weight of internal organs, muscle and liver glycogen concentrations are reported in the following sections.

5.3.1 Diet and Weight Loss.

5.3.1.1 CN rats. CN rats took 4 days before they were adjusted to the diet after which they gained on average 3.74 g/d for a period of six feeding days (Fig. 5.1). This daily mean weight gain was significant; an ANOVA performed on day-4, day-7 and day-10 rats was significant (F=6.21; df=2, 48; p<0.005). CN rats were fed, studied and killed on days 7, 8 or 10. Because compositional data was found to be similar in all groups, data was therefore pooled. CN rats ingested on average 77.1 ± 1.26% of the total volume provided. This ingested food provided a mean caloric intake of 77.7 ± 1.27 Kcal/d.
5.3.1.2 HYPO rats. The HYPO rats, on average, took 85.5 ± 2.7% of the total diet provided, corresponding to 17.50 ± 0.55 Kcal/d during the 7 days of depletion. This represented 23% of the total caloric intake of controls. By day 7 of depletion, the HYPO rats lost a mean 20% of their original weight. On the first day of refeeding (RE-1), the rats gained 7.39% body weight. This represents the most significant jump over the 4 day refeeding period (Fig. 5.1.). On that day, the mean energy intake was 73.20 ± 2.49 Kcal and was 9% below controls (P<0.01). Energy intake on refeeding did not return to CN levels until day 4 (RE-4) of repletion. The mean energy consumed by RE1-4 rats during the 4 day refeeding period, was 75.29 ± 1.48 Kcal/d.

5.3.2 Muscle Weight, Cross-Sectional Area, and Protein.

The weight of the soleus and EDL in CN did not change over the 10 days of study and therefore the data were pooled. Dry weights of both the soleus and EDL (Table 5.1.) fell (p<0.01) by 10.0% and 16.76%, respectively, in HYPO rats, concomitantly with a fall in muscle protein levels (Table 5.2.). Weights and proteins of the soleus and EDL did not return to CN levels by day 4 of refeeding.
Figure 5.1. Body weights of control (CN) (n=17), of rats (HYPO) (n=16) and of refed rats (mean n=15). Rats were refed a high carbohydrate (CHO) versus control (CN) rats fed a high protein high energy diet over 10 days. CN were paired with HYPO, RE-1 and RE-3.

The cross-sectional area (CSA) of both muscles significantly declined (p<0.01) in the HYPO rats, but unlike body weight, CSA did return to CN levels by day 4 in the soleus only (Table 5.4.).

All of the organ/muscle dry weights (Table 5.1.) significantly fell with undernutrition and did not recover with refeeding except for the liver, which normalized on the first day of refeeding. The protein values exhibit a pattern similar to the dry weights (Table 2.B) in that these values also declined with underfeeding and did not normalize by day 4 of refeeding, except as noted for the liver, which normalized by day 2.

In considering the organ and diaphragm dry weights as a percentage of the total body weight (Table 5.1.), there is no apparent differences between the treatment groups for all organs/muscle except the liver,
where the value fell with HYPO feeding, rose significantly with refeeding, and normalized by day 4. These findings confirm that no differential atrophy of these organs occurred during the experiments except in the liver of the HYPO rats.

TABLE 5.1. Internal organ/muscle dry weights in controls (CN), malnourished (HYPO) and repleted rats on days 1, 2, 3, 4 (RE). CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data were pooled. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (g)</th>
<th>Heart (g)</th>
<th>Kidneys (g)</th>
<th>Diaphragm (g)</th>
<th>Soleus (mg)</th>
<th>EDL (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=8)</td>
<td>(n=11)</td>
<td>(n=14)</td>
</tr>
<tr>
<td>CN</td>
<td>2.67 ± 0.08</td>
<td>0.26 ± 0.01</td>
<td>0.59 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>48.41 ± 2.20</td>
<td>45.12 ± 1.02</td>
</tr>
<tr>
<td>HYPO</td>
<td>2.07* ± 0.06</td>
<td>0.22* ± 0.01</td>
<td>0.50* ± 0.01</td>
<td>0.19* ± 0.01</td>
<td>43.57* ± 1.57</td>
<td>37.56* ± 0.72</td>
</tr>
<tr>
<td>RE-1</td>
<td>2.70 ± 0.18</td>
<td>0.21* ± 0.01</td>
<td>0.49* ± 0.01</td>
<td>0.20* ± 0.01</td>
<td>42.16* ± 1.50</td>
<td>39.14* ± 0.94</td>
</tr>
<tr>
<td>RE-2</td>
<td>2.77 ± 0.10</td>
<td>0.22* ± 0.01</td>
<td>0.52* ± 0.01</td>
<td>0.24* ± 0.01</td>
<td>40.88* ± 1.27</td>
<td>40.39* ± 1.01</td>
</tr>
<tr>
<td>RE-3</td>
<td>3.00* ± 0.11</td>
<td>0.21* ± 0.004</td>
<td>0.51* ± 0.01</td>
<td>0.23* ± 0.01</td>
<td>42.16* ± 1.52</td>
<td>38.88* ± 0.59</td>
</tr>
<tr>
<td>RE-4</td>
<td>2.90* ± 0.17</td>
<td>0.23* ± 0.01</td>
<td>0.51* ± 0.01</td>
<td>0.25* ± 0.01</td>
<td>41.93* ± 1.37</td>
<td>39.56* ± 0.62</td>
</tr>
</tbody>
</table>

* Different from controls (CN) p<0.01. Significance was determined by ANOVA performed on all 6 groups. If significant with p<0.05, a Duncan's New Multiple range test for unplanned comparisons was performed at p<0.01.
TABLE 5.2. Total protein values for the heart, diaphragm, liver, kidneys, soleus and EDL. CN were paired with HYPO, RE-1, and RE-3. No significant difference was found between CN groups, so the data were pooled. Measurements are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart (mg)</th>
<th>Diaphragm (mg)</th>
<th>Liver (g)</th>
<th>Kidneys (mg)</th>
<th>Soleus (mg)</th>
<th>EDL (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>(n=12)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>CN</td>
<td>196.23</td>
<td>239.17</td>
<td>2.31</td>
<td>488.32</td>
<td>45.87</td>
<td>39.49</td>
</tr>
<tr>
<td></td>
<td>±6.66</td>
<td>±11.78</td>
<td>±0.08</td>
<td>±15.26</td>
<td>±2.22</td>
<td>±0.98</td>
</tr>
<tr>
<td>HYPO</td>
<td>171.11*</td>
<td>177.28*</td>
<td>1.76*</td>
<td>427.47*</td>
<td>38.45*</td>
<td>34.36*</td>
</tr>
<tr>
<td></td>
<td>±4.91</td>
<td>±5.41</td>
<td>±0.05</td>
<td>±9.30</td>
<td>±1.07</td>
<td>±0.86</td>
</tr>
<tr>
<td>RE-1</td>
<td>162.15*</td>
<td>172.21*</td>
<td>2.04*</td>
<td>409.38*</td>
<td>38.35*</td>
<td>35.77*</td>
</tr>
<tr>
<td></td>
<td>±2.92</td>
<td>±5.89</td>
<td>±0.14</td>
<td>±11.83</td>
<td>±1.07</td>
<td>±1.05</td>
</tr>
<tr>
<td>RE-2</td>
<td>163.45*</td>
<td>201.23*</td>
<td>2.31</td>
<td>433.61*</td>
<td>40.16*</td>
<td>36.31*</td>
</tr>
<tr>
<td></td>
<td>±5.49</td>
<td>±8.18</td>
<td>±0.08</td>
<td>±16.84</td>
<td>±1.93</td>
<td>±0.87</td>
</tr>
<tr>
<td>RE-3</td>
<td>159.91*</td>
<td>199.33*</td>
<td>2.28</td>
<td>406.11*</td>
<td>39.40*</td>
<td>35.40*</td>
</tr>
<tr>
<td></td>
<td>±4.02</td>
<td>±10.31</td>
<td>±0.12</td>
<td>±17.21</td>
<td>±1.35</td>
<td>±0.80</td>
</tr>
<tr>
<td>RE-4</td>
<td>174.72*</td>
<td>210.82*</td>
<td>2.26</td>
<td>419.58*</td>
<td>35.40*</td>
<td>35.13*</td>
</tr>
<tr>
<td></td>
<td>±5.75</td>
<td>±6.59</td>
<td>±0.06</td>
<td>±7.25</td>
<td>±0.80</td>
<td>±0.94</td>
</tr>
</tbody>
</table>

* Different from controls (CN) p<0.01. Significance was determined by ANOVA performed on all 6 groups. If significant with p<0.05, a Duncan's New Multiple range test for unplanned comparisons was performed at p<0.01.
TABLE 5.3. Internal organ dry weights, expressed as a percentage of total body weight in controls (CN), malnourished (HYPO) and repleted rats on days 1, 2, 3, 4 (RE). CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data was pooled. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver % B.WT (n=10)</th>
<th>Heart % B.WT (n=9)</th>
<th>Kidneys % B.WT (n=9)</th>
<th>Diaphragm % B.WT (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>0.554 ±0.044</td>
<td>0.057 ±0.006</td>
<td>0.161 ±0.003</td>
<td>0.077 ±0.002</td>
</tr>
<tr>
<td>HYPO</td>
<td>0.466 ±0.025</td>
<td>0.068 ±0.002</td>
<td>0.173 ±0.003</td>
<td>0.070 ±0.003</td>
</tr>
<tr>
<td>RE-1</td>
<td>0.726* ±0.062</td>
<td>0.061 ±0.002</td>
<td>0.158 ±0.005</td>
<td>0.067 ±0.002</td>
</tr>
<tr>
<td>RE-2</td>
<td>0.853* ±0.058</td>
<td>0.065 ±0.002</td>
<td>0.170 ±0.005</td>
<td>0.075 ±0.003</td>
</tr>
<tr>
<td>RE-3</td>
<td>0.838* ±0.042</td>
<td>0.062 ±0.001</td>
<td>0.166 ±0.005</td>
<td>0.072 ±0.002</td>
</tr>
<tr>
<td>RE-4</td>
<td>0.585 ±0.029</td>
<td>0.065 ±0.001</td>
<td>0.163 ±0.003</td>
<td>0.080 ±0.005</td>
</tr>
</tbody>
</table>

* Different from controls (CN) p<0.01. Significance was determined by ANOVA performed on all 6 groups. If significant with p<0.05, a Duncan’s New Multiple range test for unplanned comparisons was performed at p<0.01.
TABLE 5.4. Cross-sectional area (CSA) of soleus and EDL muscles expressed as cm$^2$ for the different feeding groups: CN (controls), HYPO (depleted), and RE-1 to RE-4 (refed). CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data were pooled. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-2</th>
<th>RE-3</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=8)</td>
<td>(n=9)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>SOLEUS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cm$^2$)</td>
<td>0.136 ± 0.006</td>
<td>0.118* ± 0.003</td>
<td>0.126* ± 0.004</td>
<td>0.123* ± 0.003</td>
<td>0.125* ± 0.003</td>
<td>0.129 ± 0.004</td>
</tr>
<tr>
<td>EDL</td>
<td>(n=10)</td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>(cm$^2$)</td>
<td>0.130 ± 0.004</td>
<td>0.113* ± 0.003</td>
<td>0.122* ± 0.002</td>
<td>0.123* ± 0.003</td>
<td>0.121* ± 0.002</td>
<td>0.117* ± 0.003</td>
</tr>
</tbody>
</table>

* Different from controls (CN) p<0.01 Significance was determined by ANOVA performed on all 6 groups. If significant with p<0.05, a Duncan's New Multiple range test for unplanned comparisons was performed at p<0.01

5.3.3 Serum biochemistry.

The liquid diet was sufficiently complete in mineral content to ensure stable serum electrolyte concentrations in all groups of rats. No significant differences in serum electrolytes values were observed between CN, HYPO and refed rats (Table 5.5.). Serum phosphate, bilirubin and GGT (τ-glutamyl transferase) values were also similar in CN, HYPO and RE1-4 rats. GGT mean value was 0, indicating that rats had a normal liver function.

The serum biochemistry for transferrin, total proteins, insulin, and glucose (Table 5.6.) did not differ over the 10 days of study in CN and the data were pooled. In the HYPO rats, serum insulin and glucose levels were lower as compared with CN (P<0.01). Conversely, in RE-1, insulin and glucose levels significantly improved, but failed to return to CN levels by day 4 of refeeding P<0.01 (Table 5.6.). The glucose:insulin ratio (Table 5.7.) showed, in addition, an important rise in HYPO rats.
which normalized in the RE-1 rats. The F.F.A. levels, however, did not change in the HYPO rats, but became significantly lower in the RE-1 and RE-4 groups. Similarly, serum lactates also followed an identical pattern to that of F.F.A. Although total plasma proteins were not different between groups, serum transferrin, a plasma protein known to respond to protein depletion (267), fell significantly in HYPO, returned to CN in RE-3 and rose significantly above the CN value in RE-4.

**TABLE 5.5.** Mean serum electrolytes, P04, Blrb and GTG for control (CN), underfed (HYPO), and refed (RE) groups on days 1 to 4. Values are expressed in umol/L for Blrb and mmol/L for the others. CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data were pooled. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Serum</th>
<th>CN (n=15)</th>
<th>HYPO (n=6)</th>
<th>RE-1 (n=8)</th>
<th>RE-2 (n=13)</th>
<th>RE-3 (n=11)</th>
<th>RE-4 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>132.9 ±2.5</td>
<td>142.3 ±1.3</td>
<td>138.0 ±2.0</td>
<td>139.0 ±1.5</td>
<td>136.3 ±3.0</td>
<td>141.3 ±2.6</td>
</tr>
<tr>
<td>K</td>
<td>5.4 ±0.1</td>
<td>5.1 ±0.1</td>
<td>5.7 ±0.3</td>
<td>5.5 ±0.3</td>
<td>6.0 ±0.3</td>
<td>6.1 ±0.2</td>
</tr>
<tr>
<td>Cl</td>
<td>99.3 ±1.4</td>
<td>105.5 ±1.1</td>
<td>103.7 ±2.0</td>
<td>100.7 ±0.8</td>
<td>99.5 ±1.7</td>
<td>101.8 ±1.9</td>
</tr>
<tr>
<td>Ca</td>
<td>2.24 ±0.07</td>
<td>2.31 ±0.08</td>
<td>2.31 ±0.08</td>
<td>2.27 ±0.06</td>
<td>2.25 ±0.10</td>
<td>2.42 ±0.07</td>
</tr>
<tr>
<td>Mg</td>
<td>1.02 ±0.02</td>
<td>1.24 ±0.04</td>
<td>1.02 ±0.05</td>
<td>1.07 ±0.04</td>
<td>1.08 ±0.07</td>
<td>1.00 ±0.04</td>
</tr>
<tr>
<td>P04</td>
<td>3.14 ±0.34</td>
<td>3.28 ±0.33</td>
<td>2.89 ±0.29</td>
<td>2.95 ±0.15</td>
<td>3.00 ±0.30</td>
<td>3.35 ±0.24</td>
</tr>
<tr>
<td>Blrb</td>
<td>6.5 ±0.3</td>
<td>6.8 ±0.5</td>
<td>7.7 ±0.8</td>
<td>8.1 ±0.53</td>
<td>8.2 ±0.54</td>
<td>8.2 ±1.0</td>
</tr>
<tr>
<td>GTG</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ANOVA was performed between all 6 groups and no significant differences were observed.
TABLE 5.6. Serum transferrin, total protein, insulin and glucose levels were determined for the controls (CN), depleted (HYPO) and repleted rats (RE) on days 1, 2, 3, 4 of refeeding. CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data was pooled. Measurement values expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Transferrin (g/L)</th>
<th>Total Proteins (g/L)</th>
<th>Insulin (pmol/L)</th>
<th>Glucose (Mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN (n=10)</td>
<td>1.54±0.04</td>
<td>39.07±2.07</td>
<td>143.6±10.9</td>
<td>8.54±0.69</td>
</tr>
<tr>
<td>HYPO (n=8)</td>
<td>1.32±0.02*</td>
<td>45.86±2.20</td>
<td>43.9±5.5*</td>
<td>6.38±0.44*</td>
</tr>
<tr>
<td>RE-1 (n=8)</td>
<td>1.33±0.04*</td>
<td>46.25±3.33</td>
<td>111.7±10.8†</td>
<td>8.38±0.68</td>
</tr>
<tr>
<td>RE-2 (n=10)</td>
<td>1.39±0.02*</td>
<td>40.08±1.49</td>
<td>110.7±37.6†</td>
<td>7.03±0.40*</td>
</tr>
<tr>
<td>RE-3 (n=10)</td>
<td>1.54±0.04</td>
<td>41.67±2.03</td>
<td>93.8±12.1†</td>
<td>7.09±0.25*</td>
</tr>
<tr>
<td>RE-4 (n=9)</td>
<td>1.63±0.05*</td>
<td>39.22±3.90</td>
<td>101.6±13.9†</td>
<td>7.35±0.32†</td>
</tr>
</tbody>
</table>

* Different from controls (CN) p<0.01. † Significantly different from controls (CN) and HYPO p<0.01. Significance was determined by ANOVA performed on all 6 groups. If significant with p<0.05, a Duncan’s New Multiple range test for unplanned comparisons was performed at p<0.01.
TABLE 5.7. Serum biochemistry values for lactates, free fatty acids (FFA), insulin, and glucose. Values are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose/Insulin (Mmol/Pmol) n=9</th>
<th>F.F.A (mmol/L) n=8</th>
<th>Lactate (mmol/L) n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>0.062±0.006</td>
<td>0.91±0.06</td>
<td>2.90±0.19</td>
</tr>
<tr>
<td>HYPO</td>
<td>0.160±0.020*</td>
<td>0.84±0.06</td>
<td>3.00±0.31</td>
</tr>
<tr>
<td>RE-1</td>
<td>0.060±0.008</td>
<td>0.59±0.08*</td>
<td>1.80±0.18*</td>
</tr>
<tr>
<td>RE-2</td>
<td>0.089±0.005*</td>
<td>0.79±0.09</td>
<td>N/A</td>
</tr>
<tr>
<td>RE-3</td>
<td>0.074±0.005*</td>
<td>0.66±0.07*</td>
<td>N/A</td>
</tr>
<tr>
<td>RE-4</td>
<td>0.074±0.007*</td>
<td>0.61±0.07*</td>
<td>2.36±0.28*</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
† Significantly different from HYPO with a p<0.01 N/A: Not available

5.3.4 Weights of the liver, heart, kidneys and diaphragm.

The weights of the liver, heart, kidneys, and diaphragm did not differ over the 10 days of the study in the CN group; therefore, the data were pooled for these rats. The dry weights of the liver, heart, diaphragm and kidneys (Table 5.1.), fell significantly (p<0.01) in HYPO as compared with CN. On refeeding, the liver weights increased (p<0.01) in RE-1 to CN levels, while the weights of none of the other organs returned to normal levels by the 4th day of refeeding (p<0.01). A significant 3.6% increase in water was observed in the diaphragm in the malnourished state. For the most part, however, water content of the viscera and muscles changed very little with HYPO feeding and refeeding (Table 5.8.). The total proteins of all organs followed the same pattern as their dry weights (Table 5.2.), although liver proteins did not exceed CN values in RE3-4 as did the liver dry weight.
The dry weights of both the soleus and EDL (Table 5.1.) significantly fell \((p<0.01)\) in HYPO by 10.0% and 16.76% respectively. Concomitantly muscle protein levels fell (Table 5.2.). The weights of the soleus and EDL did not return to CN levels after 4 days of refeeding (RE-4).

**TABLE 5.8.** Percent water content of muscles and viscera in control fed rats (CN), after 7 days of hypocaloric feeding (HYPO) and 4 successive days of refeeding (RE1-4). CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data were pooled. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Organs</th>
<th>CN (n=11)</th>
<th>HYPO (n=11)</th>
<th>RE-1 (n=9)</th>
<th>RE-2 (n=11)</th>
<th>RE-3 (n=11)</th>
<th>RE-4 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>70.61 ±0.42</td>
<td>69.82 ±0.17</td>
<td>69.87 ±0.51</td>
<td>68.50 ±0.48</td>
<td>66.63 ±0.73</td>
<td>68.09 ±0.75</td>
</tr>
<tr>
<td>Heart</td>
<td>75.93 ±0.22</td>
<td>74.97 ±0.61</td>
<td>75.21 ±0.42</td>
<td>75.30 ±0.50</td>
<td>75.75 ±0.20</td>
<td>74.82 ±0.10</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>71.57 ±0.50</td>
<td>75.15* ±0.46</td>
<td>75.65* ±0.35</td>
<td>74.29* ±0.64</td>
<td>74.85* ±0.33</td>
<td>73.72 ±0.09</td>
</tr>
<tr>
<td>Kidneys</td>
<td>74.90 ±0.28</td>
<td>74.58 ±0.27</td>
<td>75.16 ±0.43</td>
<td>75.19 ±0.37</td>
<td>75.39 ±0.35</td>
<td>75.07 ±0.19</td>
</tr>
<tr>
<td>Soleus</td>
<td>77.61 ±0.36</td>
<td>76.77 ±0.41</td>
<td>77.61 ±0.57</td>
<td>78.16 ±0.45</td>
<td>78.32 ±0.36</td>
<td>77.57 ±0.37</td>
</tr>
<tr>
<td>EDL</td>
<td>77.05 ±0.24</td>
<td>76.65 ±0.30</td>
<td>76.75 ±0.53</td>
<td>77.56 ±0.48</td>
<td>77.15 ±0.30</td>
<td>77.61 ±0.49</td>
</tr>
</tbody>
</table>

* Significantly different from controls (CN) \(p<0.01\). Significance was determined by ANOVA performed on all 6 groups. If significant with \(p<0.05\), a Duncan's New Multiple range test for unplanned comparisons was performed at \(p<0.01\).

5.3.5 **Muscle glycogen concentrations**

The glycogen in the muscles did not differ over the 10 days of study in the CN groups and the data were pooled. Seven days of hypocaloric feeding produced a significant drop in glycogen concentrations in both the EDL and soleus muscles \((p<0.05)\). This drop is also indicative of a decreased availability in free glycogen; as the dry mass of both the
soleus and EDL decreased with hypocaloric feeding. Therefore, the reduced mmol of glucose/dry weight of muscle, in addition to the diminished muscle weight, suggest an overall loss of free available muscle glycogen. Such an interpretation has also been made by others (58, 81b, 440), who have worked with starved and undernourished rats. Their glycogen values were either expressed per dry weight or per wet weight, and were significantly reduced compared to unstarved rats. Their conclusions related to diminished glycogen concentrations were unchallenged, despite in some cases, not reporting the total dry weight of the muscles. The main assumption, presumably accepted by the scientific community, was that glycogen remains uniformly distributed throughout the muscle in times of starvation. A similar assumption concerning uniformity is also made in this thesis, on the basis that glycogen accumulates in close proximity to the S.R. (340b) and that the latter is abundantly present in the cell, in concentrations that vary depending on the muscle type (340b).

In RE-1 rats, glycogen in both muscle-types increased markedly. However, concentrations fell in the soleus of RE2-3 rats, and then returned to control levels in RE-4 rats. The EDL, by contrast, maintained the levels found in RE-1 rats until day 3 of refeeding, after which concentrations significantly rose (p<0.05) as shown in Figures 5.2. and 5.3.
Figure 5.2. Glycogen concentrations in the unstimulated soleus of controls (CN), 7 day depleted (HYPO) and days 1, 2, 3, 4 refed (RE-1 to RE-4). *Significantly different from CN p<0.05; + Significantly different from HYPO p<0.05.
Figure 5.3. Glycogen concentrations in unstimulated EDL in CN, HYPO and RE-1 to RE-4. CN were paired with HYPO, RE-1 and RE-3. No significant difference was found between these CN groups, so the data were pooled.

* Significantly different from CN p<0.05; † Significantly different from HYPO p<0.05; and ‡ Significantly different from all groups p<0.05

5.3.6 Liver glycogen

Liver glycogen concentrations fell dramatically in the HYPO rats. The values in Table 5.9. describe a sharp fall on the 7th day, with an immediate rebound on the first day of refeeding (RE-1). Interestingly, the values subsequently declined significantly again from CN values on the 4th day of refeeding (RE-4), although they still remained elevated compared to the HYPO glycogen values. These values are consistent with
the range of values published by Calder et al. (58). The loss of liver glycogen is also more impressive than the glycogen losses observed in both the soleus and EDL muscles. This is expected as liver glycogenolysis is stimulated by epinephrine, glucagon, and cortisol (310, 296b), all of which help maintain serum glucose homeostasis and providing extrahepatic tissue with glucose, a necessary substrate for metabolism (58). In contrast, muscle glycogen is normally a valuable source of internal substrate, that feeds G-6-P through the glycolytic cycle (310). However, in unstimulated muscle, Conlee et al. (81b) and Zorzano et al. (400) observed a 36% decline in unstimulated muscle glycogen, while Calder et al. (58), in discussing earlier work, indicate an 80% fall in liver glycogen during starvation. Hence, a single muscle’s need for glycogen is small in comparison to the extensive demand for liver glycogen during starvation. Furthermore, during periods of food deprivation, the greater stability of muscle glycogen in comparison to liver glycogen, has been previously shown by others (1b), and is now generally accepted as a standard biochemical observation (81b), although diurnal variations do exist and can vary considerably within different muscle types.

TABLE 5.9. Liver glycogen levels in control well fed (CN), 7 day hypocalorically fed (HYPO), 1st and 4th day refed (RE-1 & RE-4). Values are expressed as a mean mmol glucose/Kg dry weight ± SEM

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=4</td>
<td>n=4</td>
<td>n=3</td>
<td>n=3</td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>675.6 ± 154.4</td>
<td>27.5 ± 5.3</td>
<td>716.9 ± 222.3</td>
<td>285.8 ± 43.8</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
+ Significantly different form HYPO with a p<0.01
5.4 DISCUSSION

In this section, the implications of the changes in body composition, the diets used in the study, and the changes in body, muscle and organ weights along with the liver glycogen, muscle glycogen and serum biochemistry are presented separately.

5.4.1 Changes in body composition

5.4.1.1 Body composition in CN. In control animals, the body weights significantly increased following a 4 day adjustment period; an ANOVA performed on day-4, day-7 and day-10 rats was significant (F=6.21; df=2, 48; p<0.005). In addition, the rats did not show a change in muscle weight, muscle and liver protein, and muscle glycogen. Therefore, the CN rats were stable over the period of study, and all other groups could be referred to the pooled data for the CN rats. Adult male rats had been specifically chosen to minimize differential growth changes in lean mass over time.

5.4.1.2 Changes in body & muscle weights. In contrast, hypocaloric feeding produced a 20-25% fall in the weight of the body, and a mean 13.4% drop in muscle dry weights. These results show that the soleus and EDL, respectively, lost 16.2% and 13.0% protein with malnutrition. This demonstrates that both muscles, and therefore both fiber types (slow & fast-twitch), are equally susceptible to protein catabolism during a caloric deficit.

Refeeding the high carbohydrate diet resulted in a slight increase in body weight within the first 24 h (Fig. 5.1.), but it still remained below CN by day 4. Dry weights for the soleus and EDL also remained below CN values.

5.4.2 Diets used in study

The CN diet gave 4.94 g of protein per 100 g of liquid diet and 276 kcal/kg body weight of energy, an amount considered adequate for maximal
growth (309). The diet provided sufficient micronutrients to ensure acceptable rat growth rates in a previous study (197). The HYPO diet was significantly deficient in protein-energy, but contained all other nutrients in the same amounts as fed to CN, thereby maintaining serum electrolyte concentrations similar to CN (Table 5.5.). Thus, we had a model which was purely deficient in protein energy, and the data were not confounded by a micronutrient or electrolyte deficiency. The refeeding diet (made of high carbohydrate), designed to the keep volume and micronutrients constant, was higher in carbohydrates (CHO) than the CN and lower in protein and was fed to rats on the four consecutive days following underfeeding. However, the refeeding diet could not be considered as being absolutely protein deficient, because it contained 2.56 g of protein, an amount which is in the vicinity of the N.R.C’s (309) recommended 2.85 g, required for normal growth in adult rats. It was also sufficient to normalize serum transferrin levels, a widely used marker of protein nutrition (401), within three days. Thus, the feeding protocol allowed a measurement of the effects of a protein-energy deficit, as well as the effects of refeeding a high carbohydrate diet.

5.4.3 Changes in body, muscle and organ weight

The effect of hypocaloric feeding was a fall in the weight of the HYPO group’s body, muscles, heart, kidneys, diaphragm and liver. There is no quantitative evidence that weight loss or protein erosion were more substantive in the viscera than in the muscle tissue, as the results reveal a mean 20.3% loss in muscle weight and a 17.5% in mean organ weight; similarly, mean protein erosion were 18.4% and 16.2% respectively. These results, along with those reported by Hoshino et al. (197), do not support the notion of a peripheral to central translocation of amino acids during trauma, as suggested by Levenson et al. (256).
Refeeding the HCD resulted in a slight increase in body weight over the first 24 h (Fig. 5.1.), which remained below CN by day 4. Dry weights for the soleus, EDL, kidneys, diaphragm and heart also remained below CN (Table 5.1.). However, the liver weight did return to CN values in RE-1 and surpassed CN in RE-3 and RE-4. The fall in liver weight in the malnourished state has also been observed elsewhere (183), and is in keeping with an extensive glycogen breakdown taking place to ensure blood glucose homeostasis (206) as well as the protein catabolism observed in this study. The latter is normally suspected because of the decreased synthetic rate of some of the liver proteins during malnutrition, and clinically observed as lower serum protein values (368).

The rapid upswing on day 1 of refeeding is in part explained, as our results have shown, by protein synthesis (Table 5.2.). The rest of the weight increment, (55.5% on day 1) is likely a combination of fat synthesis (184) and glycogenesis (206), with edema not playing an important role (0.07%) (Table 5.8.). However, no attempt was made to distinguish between intracellular and extracellular water. Protein synthesis in the liver was clearly more responsive to refeeding than the other observed organs, with total protein returning to CN levels in RE-2 (Table 5.2.). These results, furthermore, show that the increased dry weight observed in RE-3 and RE-4 rats was not related to protein, but was likely due to heightened glycogen reserves. The transferrin levels also responded to the HCD. The data show that restoration of transferrin levels and liver weight does not imply a parallel restoration of muscle and other internal organs. Peripheral and visceral mass (with the exception of the liver) are, in fact, not restored during this early refeeding period.

In addition, the protein content of the soleus and EDL, as well as that of the diaphragm, heart and kidneys, were also not repleted at a time when transferrin levels had exceeded the CN levels (Table 5.2.).
The results (16.2% and 13% protein loss in the soleus and EDL, respectively) demonstrate that both muscles, and therefore, both slow-twitch and fast twitch fibers (type I and II) are equally susceptible to caloric deficits. There were no histological data that looked at myofibrillar integrity, and therefore, fiber size could not be observed. This finding in adult rats is further completed by observations (266, 197) that describe a selective atrophy of fast-twitch fibers in malnutrition. It does contrast, however, with work done by Li and Goldberg (259) on incubated soleus and EDL of malnourished rats. They showed that although the soleus was more active in protein synthesis and degradation, the rate of protein breakdown was undisturbed by fasting. In the EDL, by contrast, there was a greater protein loss due to a reduction in protein synthesis; a reduction tied to less muscle RNA (259). This kind of synthesis and degradation was however, not observed in the present study.

5.4.4 Liver glycogen

The storage of liver glycogen is dependent on whether food is restricted and then reintroduced (82), on postprandial timing (64), on the composition of the rat diet (71, 82), on the route of food delivery (intraduodenal or peripheral) (376), on changes in insulin levels (328), and on diurnal fluctuations in liver glycogen concentration (81b, 367b).

The data show a significant decline in liver glycogen with hypocaloric feeding, which is consistent with simple starvation (276, 64). The rat model produced here depicts a malnourished state with significantly lower serum insulin and glucose levels (Table 5.6.), which are derived from the fall in liver glycogen (Table 5.9) and the restricted glucose in the diet.

Recent evidence suggests that elevated insulin levels are in fact critical to glucose uptake by the liver (328). The glucose:insulin ratio reported herein, suggests a disproportionate level of glucose for the
amount of secreted insulin in underfed rats. This disproportionately high ratio was normalized on the first day of refeeding. On subsequent refeeding days (RE-2, RE-3, RE-4), however, this ratio became (once again) higher than controls. By contrast, liver glycogen concentrations still remain abnormally low by day 4 of refeeding. These results suggest that insufficient glucose was entering the cell, thereby affecting liver glycogenesis, despite choosing an oral route of glucose delivery to maximize liver glycogenesis (209).

The glycogen concentration on the first day of refeeding (Table 5.9) had very large standard errors. In some cases, the glycogen concentration was clearly higher than controls. Supernormal concentrations have been reported (299) in refeeding 42-hour fasted dogs. The contribution of dietary glucose to net hepatic glucose utilization has been shown to be significantly greater than in well nourished controls (299). They found a 53% contribution versus a normal 25-30% contribution. Furthermore, Huang et al (198) showed that the serum glucose contribution to the direct glucose to glucose 6-phosphate; to glycogenesis pathway, varied between 30% and 80%. The more elevated standard error of the mean in the RE-1 rats suggests considerable variability in glycogen synthase activity. In addition, the diurnal glycogen rhythms in the liver have been reported to cause variations between 30-50% (83, 367b) in glycogen levels between 0700 h and 1900 h. Conlee et al. (81b) report, however, that glycogen concentrations peak at 1200 h with very large error bars, suggesting that between 0700 h and 1400 h the variance is of little significance. Preliminary work with the HYPO rats fed at 0900 h, demonstrated very low liver glycogen concentration that fluctuated very little throughout the day (data not included).

The liver's reaction to hypocaloric feeding and refeeding is descriptive of normal metabolic activity. These results suggest that glucose concentrations, while significantly lower in the hypocaloric
state, are not the limiting factor in replenishing hepatic glycogen reserves; rather, a marked insulin insufficiency relative to glucose appears to be responsible. This finding suggests that simply refeeding, and thus heightening substrate availability, is not singularly associated with the normalization of metabolic activity.

5.4.5 Muscle glycogen

Glycogen concentrations in unstimulated muscle followed similar patterns in both muscles (Fig. 5.2. and 5.3.), and hence, both fast-twitch and slow-twitch muscles appear to be affected in a similar manner by malnutrition and the refeeding process. The decline of glycogen content in the muscles of the hypocalorically fed rats is supported by others (440, 343). The literature describes diurnal rhythms in muscle glycogen concentrations, causing 20-40% variability (81b) between 0700 h and 1900 h. These rhythms are not likely to distort the current findings for several reasons. First, Saubert et al. (367b) reports that the most significant rates of glycogen loss were measured between 0630 h and 0830 h in all 3 muscles (gastrocnemius white and red; soleus). Second, only a 17% fall in glycogen levels occurred in the soleus within the same 12 hour range (previously described), and no visible fall in glycogen was observed in the white gastrocnemius. The stable glycogen concentrations in white muscle was also reported by Conlee et al (81b). Third, all experiments were conducted between 0900 h and 1700 h, a time range subject to the least amount of diurnal change. Fourth, Conlee et al. (81b) also studied fasted rats, and concluded that glycogen concentrations did not vary between morning and afternoon measurements. Fifth, the standard errors reported herein are quite low (2-7% of mean), thereby suggesting that variability was minimized. This contrasts with the large standard errors reported by Saubert et al (367b), i.e 5-28% of mean. In considering why glycogen levels in muscle would have fallen in malnutrition, several mechanisms proposed in the literature must be
considered: (i) the notion of insulin resistance or reduced ability of glucose to enter the cell and glyconeogenesis, and (ii) a heightened glycogenolysis for blood glucose homeostasis.

5.4.5.1 Low insulin levels and glyconeogenesis. Leighton et al. (252) suggest that the minimum insulin levels necessary for the stimulation of lactate and glycogen synthesis are heightened by the influence of glucocorticoids, thereby indicating an insulin resistance is taking place with adrenal gland secretions. Similarly, Kozowski et al. (238) showed a 40% decrease in exercise endurance time, using rats with hyper-adrenalinemia. In the pre-exercise state, they found that glycogen and PCr, in both fast-twitch and slow-twitch muscles, had fallen in comparison to controls. Hence, they realized that not only is substrate availability a pivotal issue of glycogen content, but equally important is the hormonal milieu (85).

The data in the present study suggest a strong link between a high glucose: insulin ratio and muscle glycogen reserves in the soleus. Hypocaloric feeding caused a dramatic rise in the glucose:insulin ratio concurrently with a significant fall in both the EDL and soleus glycogen. On the first day of refeeding, normal and above normal glycogen concentrations were found in the soleus and EDL, respectively. This significant replenishment of glycogen reserves takes place despite the fact that with refeeding less glucose is available peripherally; most is taken up by the liver (299). Nevertheless, this coincided with a normalization of the glucose:insulin ratio. However, when this ratio subsequently fell on the second, third, and fourth days, only the soleus glycogen became sub-optimal. The EDL muscle was somehow able to maintain elevated glycogen levels. This introduces the possibility that 3-carbon intermediates such as lactate and pyruvate may form glycogen in the EDL muscle (glycogenesis). Similarly, Bonen et al. (47) found that in the soleus incubated in vitro with glucose, muscle glycogenesis was elevated, and was further stimulated by elevated insulin levels; the
latter failed, however, to influence glyconeogenesis. Lactate, by contrast, acted as a notable glyconeogenic precursor in fast-twitch muscle. Conversely, they point out that while corticosterone does slow down glycogenesis, it has no affect on glyconeogenesis. Furthermore, they showed that, in the perfused rat hindquarter, glyconeogenesis correlated strongly with the number of fast-twitch fibers in the muscle; they were able to show that only 5% of lactate in the soleus and 32% in the EDL was directed towards glycogen synthesis. James et al. (211) have proposed, in addition, that insulin sensitivity is more pronounced in muscles with an elevated proportion of oxidative fibers. Sensitivity is defined in this instance, as an increased ability for peripheral glucose disposal in muscle, which may be associated with an elevated cell-surface receptor number (212). In addition, the activity of the insulin receptor, tyrosine kinase, appears to be greater in red slow-twitch muscle and associated with a greater incorporation of glycogen into muscle (212). Hence, insulin insensitivity, in malnutrition, likely influences slow-twitch fibers, while the EDL by-passes this insensitivity by relying on glyconeogenesis to maintain normal glycogen reserves (310). It is also possible that the fall in EDL glycogen during underfeeding, rather than being related to an insulin insensitivity, may be the result of a preferential dependence of fast-twitch fibers for serum glucose homeostasis (364). This possibility will be discussed in the next subsection.

The likelihood of an insulin resistance in muscle is further strengthened on the basis that the conditions that encourage glycogenolysis -- low glucose, low insulin, elevated glucagon and epinephrine -- usually discourage glycogenesis (379), and therefore, interfere with the cellular uptake of glucose and the activation of \textit{glycogen synthase D} (85).

A key factor in this discussion on insulin resistance is an understanding of the many steps involved in the process. A recent review by
Bergman et al. (27) has suggested the rate-limiting step is the trans-endothelial transport of insulin and not the binding to the receptors. This finding suggests that undetected differences in receptor binding does not preclude the possibility of insulin resistance, and that glucose uptake must be considered in the equation. Chisholm and O’Dea (71), while working with high and low fat diets, could not find any difference in insulin binding to the soleus between the treatment groups, nor could they find a difference in the extent of carbon-14 glucose incorporation into glycogen or lipid. This group related the observed glucose intolerance to a diminished hepatic glucokinase activity. Grimditch et al. (161) did uncover, however, an association between a high fat diet and insulin resistance in muscle.

The findings of the present study (Table 5.7.) support the notion of insulin resistance in the sense that there is proportionally less insulin available for glucose uptake into the cell. It is more difficult to support the theory of an ineffectual glucokinase, since the latter has an elevated Km, and hence, responds only in instances of a glucose load. The hypocaloric diet in the current study does not offer such a load. The attention, rather, could be placed on hexokinase activity, which is present in both muscle and liver, and is inhibited by an elevated level of glucose-6-phosphate (149). However, hexokinase measurements were never done in this study, limiting any conclusions from this perspective. Nevertheless, the persistence of an elevated glucose:insulin ratio with refeeding could be related to both an insulin insensitivity (descriptive of poor glucose access to the cell) and an inhibited hexokinase enzyme, observed with a build-up of G,6-P (310). This would have to be verified in a future study.

5.4.5.2 Increased muscle glycogenolysis. Although the hormonal milieu created by malnutrition (42) is conducive to glycogenolysis (251), one would expect this metabolic pathway to be most active in the liver, as the latter readily maintains blood glucose homeostasis via the
action of G,6-Phosphatase. However, there is evidence that the muscle can participate in this process. Working with starved mice, Sakaida et al. (364) showed that muscle glycogen did not fall until after 16 hours postprandially. Moreover, they concluded that the glycogen primarily found in fast-twitch glycolytic fibers (type IIB) was used for blood glucose homeostasis only after liver glycogen had been fully depleted.

The results of the present study confirm that the fast-twitch EDL was more affected, with a 30% drop in glycogen. The slow-twitch soleus did, however, suffer a glycogen loss of 19%, which suggests that glycogen phosphorylase was active in the fast twitch (type II) fiber, and that glycogen synthase was slow in the slow-twitch (type I) fiber. Why one muscle would be involved in this process more than another is not clear. A likely explanation could be that the EDL (more than the soleus, because of its glycolytic nature) produced more lactate, which through the Cori cycle would sustain blood glucose levels as a gluconeogenic substrate. However, as shown in Chapter 6, the lactate levels in both unstimulated muscles were not different from each other.

### 5.4.6 Serum Biochemistry

The biochemical parameters traditionally used in the assessment of malnutrition (Table 5.6.) have reacted in accordance with what is normally observed in unstressed starvation (42, 356). The hypocaloric diet, however, likely diminished the expected rise in FFA concentration normally seen with starvation, and perhaps prevented a more acidic milieu as depicted by normalized blood lactate levels (Table 5.7.). In addition, the hypocaloric diet was sufficiently complete in micronutrients to curtail any drops in electrolytes levels (Table 5.5.), thereby making this model truly representative of a macronutrient, energy-deficient state.

In conclusion, undernutrition (produced by a micronutrient complete diet) generated in the rat changes in body composition and in substrate
availability that typified unstressed malnutrition. This model did contrast with the idea that skeletal muscle erosion would be less than the erosion of visceral mass (368). This model did however, prevent significant losses in serum electrolyte concentration; thereby, providing a setting that minimizes the likelihood of a dietary-based electrolyte deficiency affecting the trans-membrane potential of the muscle.

In addition, refeeding primarily fostered protein synthesis in the liver over other organs, with no visible accretion of protein in skeletal muscle, heart or diaphragm in the early phase of refeeding.

The data presented herein suggest that insulin insensitivity is likely taking place during malnutrition, and is causing glycogen levels in the soleus to remain sub-optimal after the first day of refeeding. The impact of this insulin insensitivity on muscle function is discussed in the next chapter.
CHAPTER 6

EFFECTS OF HYPOCALORIC FEEDING AND HIGH CARBOHYDRATE REFEEDING ON IN SITU TETANIC MUSCLE TENSION, MAXIMAL RELAXATION RATE, FATIGUE, AND GLYCOLYSIS IN SLOW-TWITCH AND FAST-TWITCH MUSCLE IN RATS.

The effects of hypocaloric feeding and refeeding on muscle performance and glycolysis were investigated in accordance to my research goals 6, 7, and 8 (see section 3.1.2.4). In this chapter, information is provided on muscle function and malnutrition.

6.1 BACKGROUND

Although refeeding in malnutrition has been investigated in animals and humans from the perspective of muscle function and body composition (73, 184, 214, 338, 356, 409), no studies to date have looked at the alterations of the glycolytic metabolites in slow-twitch and fast-twitch muscles concurrently with performance and with glycogen concentrations and utilization. In addition, while fasting and hypocaloric feedings have been used in many studies, none have utilized liquid control, hypocaloric and refeed diets that contain identical micronutrients concentrations to investigate sequentially both the underfed and refed states. Sufficient evidence exists to suspect that a diminished muscle size, due to protein erosion, may be significantly influential in precipitating fatigue during malnutrition. There is also significant work suggesting that slower metabolic pathways, due to slower enzymes, may be a contributing factor in the development of fatigue. In addition, there is also some indication that insulin may play an influential role.
in the skeletal muscle's metabolic capacity (214, 440), although it is unclear to what extent insulin concentrations affect the performance of both muscle fiber types.

For this chapter on the effects of undernutrition and early sequential refeeding on muscle fatigue and glycolysis, seven objectives are presented which collectively support the notion that fatiguability is not equally observed in both fiber types, and that it stems from an irregularity in glycolysis and glycogenolysis.

**Objective 1.** To determine the fatiguability of the muscles when rats are hypocalorically fed and then refed a calorically and nutritionally complete diet.

**Objective 2.** To demonstrate that muscle function parameters, such as muscle twitch, tetanic tension, maximal relaxation and fatigue, act independently from body composition parameters that include body weight, muscle and organ wet, and dry weights, as well as serum proteins.

**Objective 3.** To establish whether muscle fatigue is the result of myofibrillar damage that compromises the recovery of tetanic contractions. Post-fatigue evaluations of Po(N), Po(N)/g, Pt:Po(N) and MRR were conducted for two reasons: first, to determine if post-fatigue values return to pre-fatigue values; and second, to establish if the delta between pre- and post-fatigue measurements were different between CN, HYPO, RE-1 and RE-4 rats.

**Objective 4:** To measure glycogen in stimulated and unstimulated muscle and to use net glycogenolysis values as evidence of glycogenolytic activity. Comparing the soleus with the EDL will provide evidence for fiber type specificity and preferential fuel utilization. Any significant change in net glycogenolysis taking place with malnutrition and refeeding can be associated with the development of fatigue.
Objective 5. To utilize the ratio of fructose 1,6-diphosphate:fructose-6-phosphate as an indicator of glycolytic activity as well as an indirect measure of PFK activity. To support the use of this ratio with the G,6-P:lactate ratio. To assess the relative oxidative phosphorylation activity with the use of the △lactate:△glycogen ratio. Any increase in lactate produced relative to glycogen utilized would be interpreted as an indicator of the diminished oxidation of pyruvate. To use an increase in glucose-6-phosphate as a possible indicator of hexokinase activity (149, 310). To measure α-ketoglutarate concentrations in muscle as an indicator of TCA cycle activity (310, 346) and to reinforce any findings by using △α-ketoglutarate: △glycogen ratio. The reliance on α-ketoglutarate has three specific advantages as a measure of oxidative phosphorylation: First, the conversion of α-ketoglutarate to succinyl CoA is an analogous reaction to the conversion of pyruvate to acetyl CoA. Second, it uses the same co-enzymes. And third, it is a non-equilibrium reaction, and an important flux generating step (310). A slow down of one reaction would likely slow down the other (251).

Objective 6. To use the maximal relaxation rate (MRR) as an indication of the muscle’s energetics, and to relate any changes to substrate availability and utilization.

Objective 7: To establish to what extent the lower insulin and glucose concentrations affect the performance of the muscle.

Objective 8: To determine liver metabolite concentrations, thereby supplying evidence that the functional and metabolic changes occurring in the muscle of hypocalorically-fed rats were not the consequence of an anoxic animal.
6.2 MATERIAL and METHODS

For diet, surgical isolation of muscles, muscle stimulation and biochemical assays of tissues see sections 3.2.1, 3.2.2, 3.2.3 and 3.2.4 of Chapter 3.

Post-fatigue evaluation of muscle function, in a subset of rats, consisted of measuring Po(N), Po(N)/g, MRR and Pt:Po(N) after a 5 minute rest period following 40 tetanic contractions. See the stimulation protocol section 3.2.4.

Another subset of rats was used in order to measure both the function and the total weight of the muscle. A regression of tetanic tension vs. muscle weight was done in this group. This was necessary because in subsequent groups, muscle extraction was done by freeze-clamping the muscle while it was still contracting, which made it difficult to extract the entire muscle. The goal was to measure metabolites and glycogen levels accurately.

6.3 RESULTS

6.3.1 Muscle Tension Development

The muscle's individual 1 Hz twitch contraction (Pt), 10 Hz single contraction (F10(N)), and single tetanic contraction (Po(N)) were measured in both the soleus and EDL muscles. These contractions are direct measures of muscle mechanics, and as such, authenticate the integrity of the cross-bridging of the thick and thin filaments, of excitation-contraction coupling, and of the neuromuscular transmission. Tables 6.1.A and 6.1.B., display a wide spectrum of function tests, which depict the influence of various nutritional states on functional integrity.

The muscle function parameters given in these tables did not change significantly over the 10 days of the study in the CN animals, and therefore the data were pooled. Here, the results of the soleus muscle and the EDL muscle are discussed separately.
6.3.1.1 *Soleus muscle*. The maximal force of contraction (Po(N)) of the soleus (Table 6.1.A) was affected by malnutrition as Po(N) dropped significantly (p<0.01) in the HYPO rats. Correcting Po(N) for wet weight (Po(N)/g) normalized the tension between the controls and the hypocalorically-fed rats. Yet, the regression of Po(N) vs. muscle wet weight in the CN and HYPO rats (subset of rats) was not significant (F=1.85; df=1, 19; p=N.S.), even though Po(N) corrected for weight (Po(N)/g) did in fact normalize in HYPO rats. This suggests that the physical wasting of this muscle may not be a limiting factor. Furthermore, the Po(N) did not correlate with the muscle dry weight (F=1.44; df=1,13; p=N.S.) in CN and HYPO rats.
TABLE 6.1.A The effect of nutritional state on function of *soleus* expressed as a single twitch (Pt), a twitch to tetanus ratio (Pt/Po(N)), a single tetanic contraction measured in Newtons (Po(N)), a tetanus corrected for wet weight (Po(N)/g), a maximal relaxation rate (MRR) (mm/ms), and fatigue (% force lost from initial contraction) Values are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Feeding group</th>
<th>CN</th>
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<th>RE-2</th>
<th>RE-3</th>
<th>RE-4</th>
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<td><strong>Function</strong></td>
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<td></td>
</tr>
<tr>
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<td>0.26*</td>
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<td>0.36</td>
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<tr>
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<td>0.88</td>
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<td>±0.06</td>
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<tr>
<td>Po(N)</td>
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<td>1.32*+</td>
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<td>Po(N)/g</td>
<td>7.69</td>
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<td>9.16*</td>
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<td>1.63*</td>
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<td>32.80</td>
<td>35.85*</td>
<td>34.06*</td>
</tr>
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<td>±2.82</td>
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</tr>
<tr>
<td>F10/Po(N)</td>
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<td>0.51</td>
<td>0.54</td>
<td>0.45</td>
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<td>0.53</td>
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</tr>
<tr>
<td>Pt/Po(N)</td>
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<td>0.19</td>
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<td>0.21</td>
<td>0.18</td>
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<td>±0.01</td>
<td>±0.005</td>
<td>±0.03</td>
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* Different from CN with a p<0.01
+ Different from HYPO with a p<0.01
Significance was determined by ANOVA performed on all 6 groups.
If significant with a p<0.05, Duncan's New Multiple range test for unplanned comparisons was performed with a p<0.01
Mean n: refers to the mean number of muscles
TABLE 6.1.8. Function of EDL expressed as a single twitch (Pt), a twitch to tetanus ratio (Pt/Po(N)), a single tetanic contraction measured in Newtons (Po(N)), a tetanus corrected for wet weight (Po(N)/g), a maximal relaxation rate (MRR) (mm/ms), and fatigue (% force lost from initial contraction). Values are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Feeding group</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-2</th>
<th>RE-3</th>
<th>RE-4</th>
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<td>n=18</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pt(N)</td>
<td>0.44 ±0.02</td>
<td>0.40 ±0.02</td>
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<td>0.42 ±0.03</td>
<td>0.38 ±0.03</td>
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<td>F10(N)</td>
<td>0.49 ±0.02</td>
<td>0.45 ±0.02</td>
<td>0.48 ±0.02</td>
<td>0.47 ±0.03</td>
<td>0.47 ±0.03</td>
<td>0.50 ±0.02</td>
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<tr>
<td>Po(N)</td>
<td>2.62 ±0.11</td>
<td>2.06* ±0.05</td>
<td>2.19* ±0.07</td>
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<td>2.38* ±0.07</td>
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<tr>
<td>Po(N)/g</td>
<td>14.91 ±0.46</td>
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<td>13.69* ±0.41</td>
<td>14.80 ±0.41</td>
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<td>MRR(mm/ms)</td>
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<td>5.25* ±0.24</td>
<td>5.47* ±0.36</td>
<td>6.55 ±0.44</td>
<td>6.17* ±0.40</td>
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<td>Fatigue</td>
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<td>55.12 ±3.57</td>
<td>48.57* ±2.92</td>
<td>75.24*+ ±4.81</td>
<td>75.84*+ ±2.72</td>
<td>65.00*+ ±4.99</td>
</tr>
<tr>
<td>F10/Po(N)</td>
<td>0.19 ±0.01</td>
<td>0.23 ±0.02</td>
<td>0.23 ±0.02</td>
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<tr>
<td>Pt/Po(N)</td>
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<td>0.20* ±0.02</td>
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<td>0.16 ±0.01</td>
<td>0.16 ±0.01</td>
<td>0.21* ±0.01</td>
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* Significantly different from CN with a p<0.01. Significance was determined by ANOVA performed on all 6 groups. If significant with a p<0.05, Duncan's New Multiple range test for unplanned comparisons was performed with a p<0.01
+ Significantly different from HYPO rats with a p<0.01
TABLE 6.2.A. Post-fatigue function of soleus expressed as a single twitch (Pt2), a single tetanic contraction measured in Newtons (Po(N2)), a tetanic contraction corrected for weight Po(N)2/g, and a maximal relaxation rate (MRR2) (mm/ms). Values are expressed as means ± SEM. Only a subset of the population underwent post-fatigue measurements.

<table>
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<tr>
<th>Feeding days</th>
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<tr>
<td>Pt(N)2</td>
<td>0.25</td>
<td>0.28</td>
<td>0.24</td>
<td>0.25</td>
<td>0.29</td>
<td>0.26</td>
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<tr>
<td>Po(N)2</td>
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<td>1.87</td>
<td>1.40</td>
<td>1.71</td>
<td>1.85</td>
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<tr>
<td>Pt/Po2</td>
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<tr>
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<td>±0.72</td>
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<tr>
<td>MRR2 (mm/ms)</td>
<td>1.51*</td>
<td>1.66*</td>
<td>1.95+</td>
<td>1.69*</td>
<td>1.17*</td>
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* Significantly different from pre-fatigue values using t-test with a p<0.05
† Significant change from pre-fatigue values in this group compared to controls by ANOVA with a p<0.05 and by Duncan's New Multiple Range with a p<0.01
Post-fatigue function of the EDL expressed as a single twitch (Pt2), a single tetanic contraction measured in Newtons (Po(N2)), a tetanic contraction corrected for weight Po(N)/g, and a maximal relaxation rate (MRR2) (mm/ms). Values are expressed as means ± SEM. Only a subset of the population underwent post-fatigue measurements.

<table>
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<th>Feeding days</th>
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<tr>
<td>Pt(N)2</td>
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<tr>
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<td>MRR2 (mm/ms)</td>
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<td>±0.44</td>
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<td>±0.58</td>
<td>±0.28</td>
<td>±0.23</td>
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</table>

* Significantly different from pre-fatigue values using t-test with a p<0.05
† Significant change from pre-fatigue values in this group compared to controls by ANOVA with a p<0.05 and by Duncan's New Multiple Range with a p<0.01

A post-fatigue recovery of pre-fatigue tetanic tension measured both as Po(N) and Po(N)/g values, was also observed in all of the groups (Table 6.2.A). Upon refeeding, the tension, further suffered a significant fall from values observed in the HYPO rats, even though muscle dry weight (Chapter 5, Table 5.1.), protein (Chapter 5, Table 5.2.) and cross-sectional area (Chapter 5, Table 5.4.) did not demonstrate any changes. The tension, however, did normalize by day 4 of refeeding, despite persistent subnormal muscle proteins and dry weights. A regression of muscle dry weight vs. refeeding days (F=0.025; df=1, 39; p=N.S.) was not significant.

The inter-rat variability of the soleus with regard to the Po(N) in the CN, HYPO, and refed rats, was also very weakly correlated with the
wet muscle weight ($r=0.39; \ F=10.07; \ df=1, 57; \ p<0.005$) (Figure 6.1.) in a subset of rats. In the HYPO and refed groups, the correlation of $Po(N)$ with muscle wet weight was about the same ($r=0.41; \ F=8.81; \ df=1,45; \ p<0.01$) again in a subset of rats. Within the HYPO group in particular, however, no variation in $Po(N)$ was attributed to differences in muscle wet weight ($F=2.04; \ df=1,7; \ p=N.S.$). It was not until the 4th day that inter-rat variability, within this group, was associated with muscle weight ($r=0.74; \ F10.18; \ df=1,9; \ p<0.05$).

When $Po(N)$ was correlated with insulin concentrations in the HYPO rats, the relationship was strongly negative ($r=-0.91; \ F=43.53; \ df=1,10; \ p<0.005$). A strong negative correlation was also present between $Po(N)/g$ and insulin ($r=-0.84$). This highly significant association ($f=21.62; \ df=1,10; \ p<0.001$) described a potentiation of tension when insulin levels were at their lowest values. Furthermore, the correlation values progressively declined with refeeding (HYPO, $r=-0.84, \ p<0.005$; RE-1, $r=-0.65, \ p<0.025$; RE-4, $r=-0.54, \ p<0.05$). A regression of $Po(N)$ of all the groups of rats vs. insulin concentrations reveals no significant correlation ($r=-0.089; \ F=0.366; \ df=1,46; \ p<0.548$).

A multiple regression of $Po(N)$ vs. insulin and muscle wet weight (MUSCWWT) was also conducted for all groups of rats using the following equation:

$$Po(N) = 0.818 - (0.001 \text{ insulin}) + (0.005 \text{ MUSCWWT}).$$

The results indicate that only the muscle wet weight significantly contributed to the prediction of $Po(N)$ ($r=0.41; \ F=4.70; \ df=2,45; \ p<0.014$). This result closely approximated the correlation calculated in linear regression, which indicates little influence of insulin concentration in the outcome of the single tension overall.
The lower-frequency stimulation of 10 Hz (F10(N)) in the soleus, described in Table 6.1.A, produced a tension that remained unaffected by underfeeding and refeeding. In fact, no statistical difference, as calculated by an ANOVA, was found between the forces produced at 10 Hz.
in CN, HYPO and refeed (RE) rats in both muscle types. This contrasts with a significant fall in twitch tension, generated by 1 Hz (Pt), in the HYPO and RE-1 rats. However, when corrected for Po(N), there was no longer any difference.

The ratio F10/Po, an indicator of low and high-frequency fatigue, was unaffected by underfeeding and refeeding in either the soleus (Table 6.1.A) or the EDL (Table 6.1.B) muscles, thereby giving some indication that no abnormality in neuromuscular transmission and excitation-contraction coupling was occurring (121). Furthermore, a return to pre-fatigue values was also observed for the post-fatigue ratio Pt:Po(N) (Table 6.2.A).

6.3.1.2 EDL muscle. Similar to the maximal tetanic contraction (Po(N)) in the soleus, the EDL’s tetanus was significantly compromised (Table 6.1.B) in the main nourished state and on the first day of refeeding (RE-1). When corrected for the wet weight of muscle, the tension in both groups (Po(N)/g) remained abnormally low, and contrary to the soleus, the EDL’s Po(N) did not recover by day 4 of refeeding, although its Po(N)/g did recover. This improvement took place despite the fact that, like the soleus, the EDL’s dry weight, cross-sectional area, and protein level did not recover with refeeding. Furthermore, the Po(N) was not correlated with the dry weight of the muscle in the CN and HYPO rats (F=1.70; df=1, 12; p=N.S.). Post-fatigue tension also returned to pre-fatigue values in all groups (Table 6.2.B).

The variability in the EDL Po(N) among all groups (CN, HYPO and refeed) of rats (Fig. 6.2.) was positively correlated with muscle wet weight (r=0.73; F=58.68; df=1, 53; p<0.0001). A regression done only in HYPO, RE-1, RE-2, RE-3, RE-4 rats was also positively correlated (r=0.56; F=19.05; df=1, 43; p<0.005) with wet muscle weight, however, the association was not as strong.
A regression of Po(N) vs. muscle wet weight in the CN and HYPO rats, showed a significant correlation ($r=0.77; F=54.65; df=1, 17; p<0.001$). The correlation was most notably pronounced between the Po(N) and the muscle wet weight in the HYPO group ($r=0.80; F=10.25; df=1, 7; p<0.025$).

![Figure 6.2. Linear regression of the EDL Po(N) with muscle wet weight in CN, HYPO, RE-1, RE-2, RE-3 and RE-4. (Subset of rats)](image)

$\text{EDL}$

$\text{Po(N)}$ (Single tetanus)

Muscle wet weight (mg)

$r = 0.73 (F=58.68; df=1,53; p<0.005)$

The Po(N)/g in the EDL of the HYPO rats also correlated with insulin levels ($r=0.63; F=6.00; df=1,10; p<0.05$), but in contrast to what was observed in the soleus, the relationship was positive and the probability of a false positive was greater ($p<0.05$). This in effect means that there was a tendency towards a heightened muscle tetanus with
higher insulin values. It is noteworthy that the Po(N) in the HYPO rats did not correlate with insulin ($F=0.83; df=1,12; p=N.S.$).

Like the soleus, a multiple regression of Po(N) vs. insulin and muscle wet weight was conducted over all rat groups using the following equation:

$$Po(N) = -0.399 + (0.001 \text{ insulin}) + 0.016 (\text{MUSCWT})$$

The results revealed that only the muscle wet weight significantly contributed to the prediction of Po(N) ($r=0.685; df=2,39; p<0.0005$).

### 6.3.2 Maximal Relaxation Rate (MRR)

The EDL and soleus (Table 6.1.A & 6.1.B) exhibited a significant fall ($p<0.01$) in the MRR in the HYPO animals. In the soleus, no correlation existed between the Po(N)/g and the MRR ($F=0.306; df=1,57; p=N.S.$), whereas in the EDL such an association was found ($r=0.41; F=10.06; df=1,50; p<0.005$). In addition, a significant fall in post-fatigue MRR of both muscles was also observed in the CN, HYPO, RE-2, RE-3 and RE-4 rats. However, only in the soleus of the RE-1 rats was the post-fatigue MRR similar to the pre-fatigue MRR, as determined by a Student t-test. This small difference was also significant between all 5 groups, as was determined by an ANOVA ($p<0.01$). This indicates that no further decline in MRR occurred with fatigue (Tables 6.2.A and 6.2.B).

In the EDL, although a post-fatigue decline in MRR was observed in the HYPO and RE-1 rats, this fall was not as significant as that of the other groups, as determined by an ANOVA and by Duncan’s New Multiple Range test ($p<0.01$) (Table 6.2.B). The EDL’s MRR never fully reached the CN values by day 4 of refeeding, whereas recovery was observed in the soleus. Furthermore, a regression of MRR vs. refeeding days, in both the soleus and the EDL, was not significant. Yet, it was previously shown
(chapter 5), that both serum insulin (F=5.27; df=1, 63; p<0.05) and the glucose:insulin ratio (F=14; df=1, 33; p<0.005) significantly improved with refeeding. This indicates that some factor(s) other than refeeding time were influential in the recovery of the MRR observed in the soleus.

In keeping with this notion of secondary factors aiding the recovery process, the involvement of insulin was explored. A linear regression of the MRR of the soleus of the CN, HYPO, RE-1, RE-2, RE-3, RE-4 rats vs. serum insulin concentrations (Fig. 6.3.) was done. The results show a weak negative correlation (r=-0.34) that is highly significant (F=10.29; df=1,81; p<0.005). The coefficient of determination, r², links 11% of the variability of the MRR to serum insulin concentrations. The best fit of the regression slope was obtained with a linear equation expressed as: $y = 2.41 - 0.0026x$ and is graphically represented in Figure 6.3.

An ANCOVA conducted over all 5 experimental groups, revealed regression slopes that were homogeneous ( F= 1.90 df= 5, 70; p= N.S.). Moreover, the covariate, insulin, was highly significant (F=5.41; df=5, 75, p<0.005), indicating that insulin alone was not responsible for the decline in MRR. In addition, the ratio of F1,6-diP:F,6-P in the unstimulated soleus correlated weakly with MRR (r=0.42; df=1,23;p<0.05). In the EDL, such a correlation was not significant (F=1.08; df=1,23; p=N.S).

A linear regression was calculated between the MRR of the soleus in the HYPO rats and serum insulin concentrations (Fig. 6.4.). A negative correlation was again calculated (r=-0.75) and its significance was remarkable (F=12.54; df=1,11; p<0.005). This reveals a very strong association between insulin and MRR in the underfed state. The best fit was attained using the equation expressed as: $y = 3.08 - 0.012x$. The graph shows the trend.
Figure 6.3. Linear regression of MRR of the soleus vs. insulin for control (CN), underfed (HYPO) and refed (RE) rats.
Figure 6.4. Linear regression of MRR of the soleus vs. insulin for underfed (HYPO) rats.
A regression line was drawn for the MRR of the EDL and serum insulin concentrations (Fig. 6.5). Contrary to the soleus, no significant correlation \( (F=2.10; df=1.54; p=\text{N.S}) \) could be found.

Figure 6.5. Linear regression of MRR of the EDL vs. insulin for control (CN), underfed (HYPO) and refed (RE) rats.
In addition, a regression of the MRR vs. the glucose:insulin ratio was found to be insignificant (F=2.46; df=1,42; p=N.S) showing that insulin insensitivity was not influential in the observed changes in the MRR of the EDL.

### 6.3.3 Muscle Fatigue

The force of the soleus in the HYPO rats, after 40 repetitive stimulations, fell 25% more than seen in the CN values (p<0.01) (Table 6.1.A). Refeeding did not cause the muscle fatiguability to normalize; a regression of fatigue vs. refeeding days was not significant (F=1.63; df=1, 62; p=N.S.). Furthermore, a relationship could not be found between insulin and fatigue (F=3.12; df=1,67; p=N.S) (Fig.6.6.), nor between the glucose:insulin ratio and fatigue (F=1.04; df= 1, 39; p=N.S.). A regression of fatigue in the CN and HYPO rats vs.insulin concentrations showed similar slopes. An ANCOVA revealed that the covariate, insulin, was significant, thereby indicating that once corrected for insulin, changes in fatigue were still occurring (adjusted mean: F=27.92; p<0.0001).

Underfeeding did not appear to affect the fatiguability of the EDL, when compared with well fed control rats (Table 6.1.B). Following one day of refeeding, the muscle's endurance further deteriorated (greater fatigue) until day four (RE-4). Hence, it became apparent that refeeding did not normalize the fatiguability of this muscle within this early refeeding period. Moreover, a significantly negative correlation (r=-0.31) was found with the 4 refeeding days (F=6.60; df=1, 61; p<0.025). This relationship demonstrated that fatigue worsened with refeeding, despite no further significant depression in muscle dry mass (F=2.5; df=1, 44; p=N.S.), protein (F=0.022; df=1, 73; p=N.S.) and cross-sectional area (F=0.26; df=1, 42; p=N.S.). This increase in fatiguability appears to be associated with a fall in insulin (r=-0.58; F=29.78; df=1, 57; p<0.005) (Fig.6.7.). When all groups were considered...
together, the regression was especially strong in the RE-4 rats ($r=-0.67$; $f=9.9$; df=1, 13; $p<0.01$). The fatiguability was not associated with any changes in the glucose:insulin ratio ($F=1.04$; df=1, 39; $p=N.S.$).

**SOLEUS**

![Graph showing relationship between insulin and fatiguability](image)

$r=-0.21 (F=3.11; p=N.S)$

*Figure 6.6. Regression of soleus fatiguability vs. serum insulin concentrations from CN, HYPO, RE1, RE2, RE3, and RE4 rat feeding groups.*
Figure 6.7. Regression of EDL fatiguability vs. serum insulin concentrations from CN, HYPO, RE1, RE2, RE3, and RE4 rat feeding groups.

6.3.3.1 Muscle glycogen and fatigue.

In unstimulated muscles, the glycogen of the well fed CN rats did not differ over the 10 days of study, and therefore, the data were pooled. For the HYPO rats, seven days of hypocaloric feeding produced a
significant drop in glycogen concentrations in only the soleus muscle (p<0.05) (Tables 6.3.A & 6.3.B). The EDL does appear, however, to be susceptible to glycogen loss in underfeeding, as the unstimulated concentrations, previously measured in the EDL (Fig. 5.3. Chapter 5), did show a fall in glycogen in the HYPO rats.

**TABLE 6.3.A.** Glycogen concentrations in unstimulated, stimulated and utilized glycogen in soleus muscles in controls (CN), 7 day hypocaloric (HYPO) and 1st (RE-1) and 4th (RE-4) day refed. Values are expressed as means mmol glucose/Kg dry weight ± SEM.

<table>
<thead>
<tr>
<th>Feeding group</th>
<th>CN (Mean n)</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimul.</td>
<td>n=8</td>
<td>n=7</td>
<td>n=6</td>
<td>n=8</td>
</tr>
<tr>
<td>105.72 ±7.67</td>
<td>70.11* ±2.39</td>
<td>100.00 ±8.33</td>
<td>83.78* ±4.89</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>86.83 ±6.05</td>
<td>65.67* ±2.78</td>
<td>87.50± ±9.61</td>
<td>61.57* ±6.30</td>
</tr>
<tr>
<td>Utilized</td>
<td>25.06 ±0.94</td>
<td>4.44* ±2.89</td>
<td>12.44* ±6.89</td>
<td>19.83 ±2.94</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
† Significantly different from HYPO with a p<0.01
TABLE 6.3.B. Glycogen concentrations in unstimulated, stimulated and utilized glycogen in the EDL muscle in controls (CN), 7 day hypocaloric (HYPO) and 1st (RE-1) and 4th (RE-4) day reed. Values are expressed as means mmol glucose/Kg dry weight ± SEM.

<table>
<thead>
<tr>
<th>Feeding group</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Mean n)</td>
<td>n=7</td>
<td>n=7</td>
<td>n=5</td>
<td>n=8</td>
</tr>
<tr>
<td>Condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstim.</td>
<td>161.67 ± 13.39</td>
<td>142.11 ± 12.67</td>
<td>217.78* ± 13.39</td>
<td>166.00+ ± 11.22</td>
</tr>
<tr>
<td>Stimulated</td>
<td>89.22 ± 5.50</td>
<td>54.61* ± 8.00</td>
<td>125.94*+ ± 20.05</td>
<td>87.94+ ± 10.28</td>
</tr>
<tr>
<td>Utilized</td>
<td>67.50 ± 4.61</td>
<td>95.28* ± 9.67</td>
<td>103.50* ± 6.17</td>
<td>76.89 ± 8.17</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
+ Significantly different from HYPO with a p<0.01

The glycogen levels found in these muscles were similar to those values published by Terjung et al. (395).

In the first day refeed group (RE-1), the glycogen concentration in both muscles was increased markedly; the concentration normalized in the soleus, whereas the level rose above the CN values in the EDL with refeeding. However, the concentrations in both the soleus and EDL fell again in RE-4. This drop represented a return to normal values for the EDL, and a subnormal readjustment for the soleus.

In the soleus muscle, no correlation was established between the glycogen concentration and refeeding days (F=0.81; df=1,26; p=N.S), nor was there a link between fatigue and glycogen levels (F=0.37; df=1,12; p=N.S). Similarly, glycogen concentrations in the EDL did not correlate with refeeding days (F=0.036; df=1,20; p=N.S) or with fatigue (F=0.64; df=1,9; p=N.S). Hence, there appears to be no association between the fatiguability of either muscle and the concentration of glycogen in the muscle prior to stimulation.

Net glycogenolysis was considered in each muscle (Tables 6.3.A & 6.3.B); this would give a clear view of the importance of glycogeno-
lysis, throughout hypocaloric feeding and refeeding, in sustaining muscular tension. In malnutrition, net glycogenolysis in the soleus was greatly decreased (p<0.01), and then normalized by day 4 with refeeding. Although there was a significant correlation between refeeding and glycogenolysis, in only the soleus (r=0.48; F=6.99; df=1, 24; p<0.025), no relationship could be established between glycogenolysis and fatigue, or between glycogenolysis and insulin concentrations (F=0.14; 1, 13; p=N.S.).

In the EDL, rather than decline, glycogenolysis was significantly increased in the HYPO rats, remained elevated until day 1, and normalized on day 4 of refeeding. There was, however (much like the soleus) no visible relationship between glycogenolysis and fatigue or insulin concentrations and glycogenolysis.

6.3.3.2 Muscle Metabolites. The metabolite concentrations in the soleus (Tables 6.4.A & 6.4.B) and EDL (Tables 6.5.A & 6.5.B) give a more complete picture of the efficiency of the various pathways during the unstimulated and stimulated states. The changes in metabolites (delta values) and the delta metabolic ratios are important components of this picture.

In the unstimulated slow twitch muscle (soleus), malnutrition does appear to reduce glycolysis and glycogenolysis significantly, as the ratio F1,6-diP:F6-P significantly declined in the HYPO rats (Table 6.4.A). In addition net glycogenolysis significantly fell in these same rats (Table 6.3.A). The fall in F1,6-diP:F6-P ratio further worsened with the initiation of refeeding in the RE-1 rats (p<0.01) and persisted below the CN values until the fourth day of the refeeding period. Similarly, the lactate levels also fell (p<0.01) and remained at subnormal concentrations during the entire refeeding protocol. Such a fall in lactate generally suggests a suboptimal glycolysis (268), since
lactate, is in fact, the end product of anaerobic glycolysis (310). It must, however, be considered along with other metabolites for a more accurate picture.

No changes in G-6-P and F-6-P were observed throughout this experiment. G-6-P is known to play a number of possible roles: (a) it can allosterically inhibit hexokinase (310), and (b) allosterically activate the phosphorylated form of glycogen synthase (411b). There is however, no evidence presented here that argues in favor of any of these possibilities, as the G-6-P concentrations did not vary between experimental groups (Table 6.4.A). Similarly, changes in F-6-P concentrations can suggest an internal regulation of PFK activity (64).

In this thesis, no unusual or abnormal control of PFK can, however, be justified strictly on the basis of the uniform F-6-P values reported herein (Table 6.4.A). The levels of α-ketoglutarate were unremarkable until the 4th day of refeeding, at which point concentrations rose significantly (p<0.01). Although α-ketoglutarate, in itself, is not considered a determining indicator of citric acid cycle activity, it is the primary substrate in a non-equilibrium flux generating step within the TCA cycle namely, α-ketoglutarate to succinyl CoA. Furthermore, it is a biologically irreversible reaction (251). α-ketoglutarate is catalyzed by an α-keto-glutarate dehydrogenase-complex following three separate reactions (310). This enzyme-complex has been described as a key indicator of the maximum capacity of the TCA cycle (310). However, this enzyme complex was not measured in this thesis. This measurement is not the only acceptable approach in unstimulated muscle, since the TCA cycle appears to have two clearly defined flux-generating steps that support the notion of two physiological pathways within the TCA cycle: Acetyl-CoA to α-ketoglutarate; and α-ketoglutarate to oxaloacetate (310). Within such a paradigm, and given that substrate kinetics were
not done, the unchanged concentration of $\alpha$-ketoglutarate, in HYPO-fed rats, could suggest that the TCA cycle is similar to control fed rats, and thus unaffected in this muscle.

In the unstimulated slow-twitch muscle (EDL), malnutrition and refeeding do not appear to produce any important changes in the activity of the rate-limiting glycolytic enzyme P.F.K., as the ratio F1,6-diP:F6-P remained unchanged (Table 6.4.B) by nutritional status. Although the enzyme activity was not directly measured, this ratio has been used by others (360, 414, 440) to imply that changes in PFK activity had occurred. Lower lactate and $\alpha$-ketoglutarate levels were also observed ($p<0.01$) with underfeeding and with subsequent refeeding, whereas in a manner similar to the soleus, G,6-P and F,6-P remained at CN levels. Caution must be used when interpreting a fall in $\alpha$-ketoglutarate, since no substrate kinetics were conducted; such a fall could result from a less active TCA cycle, but could also be the result of an accelerated TCA cycle. Further clarification could be made using other metabolites. Lower lactate levels in the fast-twitch muscle, much like what was observed in the soleus, can suggest either a slower glycolysis or a more efficient TCA cycle. The use of other metabolites can help bring some clarification to this question.
TABLE 6.4.A  Metabolite levels in the unstimulated soleus, in controls (CN), 7 day HYPO fed (HYPO) and first day (RE-1) and fourth day (RE-4) re-fed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>n=6</td>
<td>n=6</td>
<td>n=5</td>
<td>n=7</td>
</tr>
<tr>
<td>Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>9.01</td>
<td>5.36*</td>
<td>5.92*</td>
<td>4.39*</td>
</tr>
<tr>
<td></td>
<td>±1.07</td>
<td>±1.15</td>
<td>±0.53</td>
<td>±0.60</td>
</tr>
<tr>
<td>G,6-P</td>
<td>0.93</td>
<td>0.76</td>
<td>0.86</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>±0.08</td>
<td>±0.12</td>
<td>±0.06</td>
<td>±0.11</td>
</tr>
<tr>
<td>F,6-P</td>
<td>0.19</td>
<td>0.16</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
</tr>
</tbody>
</table>
| F,1,6-diP      | 0.17| 0.06*| 0.06*| 0.09*∥
|                | ±0.02| ±0.01| ±0.004| ±0.02|
| F1,6-diP/F6-P  | 1.45| 0.78*| 0.26*§| 0.60*|
|                | ±0.29| ±0.24| ±0.02| ±0.09|
| α-Ketog        | 0.29| 0.31 | 0.36 | 0.52*|
|                | ±0.06| ±0.05| ±0.07| ±0.05|

* Significantly different from CN rats with a p<0.01 using ANOVA & Duncan’s New Multiple range test.
∥ Significantly different from RE-1 rats with a p<0.01
§ Significantly different from HYPO rats with a p<0.01
TABLE 6.4. B Metabolite levels in the unstimulated EDL, in controls (CN), 7 day HYPO fed (HYPO) and first day (RE-1) and fourth day (RE-4) refeed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td></td>
<td>7.90 ± 1.13</td>
<td>4.07 ± 0.64</td>
<td>5.10 ± 0.74</td>
<td>4.27 ± 0.65</td>
</tr>
<tr>
<td>G,6-P</td>
<td></td>
<td>0.65 ± 0.07</td>
<td>0.69 ± 0.12</td>
<td>0.62 ± 0.05</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>F,6-P</td>
<td></td>
<td>0.21 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>F,1,6-diP</td>
<td></td>
<td>0.14 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>F1,6-diP/F6-P</td>
<td></td>
<td>0.84 ± 0.10</td>
<td>0.74 ± 0.10</td>
<td>0.84 ± 0.10</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>α-Ketog</td>
<td></td>
<td>0.36 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>0.24 ± 0.03</td>
<td>0.26 ± 0.03</td>
</tr>
</tbody>
</table>

* Significantly different from CN rats with a p<0.01 using ANOVA & Duncan’s New Multiple range test.

The stimulated soleus muscle (Table 6.5.A) suffered a very noticeable decline (p<0.01) in lactate levels as well as in the ratio F1,6-diP:F,6-P in HYPO animals, following repeated tetanic contractions. Lactate values continued to be subnormal until day 4 of the refeeding period, while the F1,6-diP:F,6-P ratio did seemingly normalize with refeeding. Meanwhile, the G,6-P, F,6-P and α-ketoglutarate concentrations remained unchanged.

The lactates in the stimulated EDL, by contrast (Table 6.5.B), were significantly greater in the HYPO and RE-1 rats (p<0.01). This rise took place despite the significantly lower lactate levels in the unstimulated EDL of HYPO rats, which never normalized with refeeding.
In the EDL, G,6-P rose significantly in the RE-1 rats and did not return to normal in RE-4 rats. This rise was independent of initial baseline concentrations, which (as previously shown Table 6.4.B) were unaffected by nutritional status. The rise in G,6-P occurred while F,6-P, α-ketoglutarate and the ratio F,1,6 di-P:F,6-P were not altered.

TABLE 6.5.A Metabolite concentrations in stimulated soleus muscles of controls (CN), 7 day hypocalorically fed (HYPO), first day (RE-1) and fourth day (RE-4) refed rats. Values are in mmole/kg dry weight and expressed as a mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>n=7</td>
<td>n=8</td>
<td>n=6</td>
<td>n=8</td>
</tr>
<tr>
<td>Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>10.80 ±1.05</td>
<td>8.00* ±0.82</td>
<td>6.72* ±1.67</td>
<td>6.07* ±1.06</td>
</tr>
<tr>
<td>G,6-P</td>
<td>1.29 ±0.24</td>
<td>1.10 ±0.11</td>
<td>1.17 ±0.08</td>
<td>0.81 ±0.11</td>
</tr>
<tr>
<td>F,6-P</td>
<td>0.32 ±0.06</td>
<td>0.32 ±0.05</td>
<td>0.25 ±0.03</td>
<td>0.26 ±0.06</td>
</tr>
<tr>
<td>F,1,6-diP</td>
<td>0.53 ±0.09</td>
<td>0.26* ±0.03</td>
<td>0.29* ±0.06</td>
<td>0.41 ±0.05</td>
</tr>
<tr>
<td>F16diP/F6P</td>
<td>2.62 ±0.47</td>
<td>0.81* ±0.20</td>
<td>1.70 ±0.33</td>
<td>2.75 ±0.74</td>
</tr>
<tr>
<td>α-Ketog</td>
<td>0.93 ±0.08</td>
<td>0.95 ±0.11</td>
<td>1.10 ±0.11</td>
<td>0.93 ±0.10</td>
</tr>
</tbody>
</table>

* Significantly different from CN rats with a p<0.01 using ANOVA & Duncan's New Multiple range test.
TABLE 6.5.B  Metabolite concentrations in stimulated EDL muscles of controls (CN), 7 day hypocalorically fed (HYPO), first day (RE-1) and fourth day (RE-4) refeed rats. Values are in mmole/kg dry weight and expressed as a mean ± SEM.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Feeding groups</th>
<th>Mean n</th>
<th>CN n=7</th>
<th>HYPO n=6</th>
<th>RE-1 n=6</th>
<th>RE-4 n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td>63.57</td>
<td>75.58*</td>
<td>82.30*</td>
<td>67.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±4.27</td>
<td>±5.09</td>
<td>±2.95</td>
<td>±4.54</td>
<td></td>
</tr>
<tr>
<td>G,6-P</td>
<td></td>
<td></td>
<td>7.96</td>
<td>8.01</td>
<td>17.69*+</td>
<td>12.14*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.89</td>
<td>±1.98</td>
<td>±1.87</td>
<td>±2.49</td>
<td></td>
</tr>
<tr>
<td>F,6-P</td>
<td></td>
<td></td>
<td>4.58</td>
<td>3.40</td>
<td>4.80</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.88</td>
<td>±0.65</td>
<td>±0.62</td>
<td>±0.61</td>
<td></td>
</tr>
<tr>
<td>F,1,6-diP</td>
<td></td>
<td></td>
<td>0.34</td>
<td>0.18*</td>
<td>0.25</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.05</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.01</td>
<td></td>
</tr>
<tr>
<td>F16diP/F6P</td>
<td></td>
<td></td>
<td>0.062</td>
<td>0.059</td>
<td>0.053</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.009</td>
<td>±0.011</td>
<td>±0.003</td>
<td>±0.030</td>
<td></td>
</tr>
<tr>
<td>α-Ketog</td>
<td></td>
<td></td>
<td>1.25</td>
<td>1.41</td>
<td>1.40</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.10</td>
<td>±0.13</td>
<td>±0.06</td>
<td>±0.17</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
† Significantly different from all groups with a p<0.01

Key metabolite ratios (Tables 6.6.A & 6.6.B) in the stimulated muscles provide further insight into the dynamic metabolic flux that may be taking place within the muscles. In the soleus (Table 6.6.A), the lactate:glycogen ratio dropped significantly in the HYPO rats, indicating that for every glycogen unit present, less residual lactate is found. The dramatic fall of this ratio reveals a disproportionate relationship between glycogen and lactate with underfeeding. However, upon refeeding, this ratio fell even more, and remained below the CN and HYPO values even in the RE-4 rats (p<0.01). Lactate and glycogen re-harmonize at a much weaker intensity with refeeding; even though glycogen normalized in the RE-1 rats, very little lactate is found. In the RE-4 rats, despite subnormal glycogen, lactate is also proportionally lower.
The ratio G,6-P:lactate, in contrast, rose significantly (p<0.01) in the soleus of the underfed rats, it further increased significantly with refeeding, and remained elevated by day 4. The rise of this ratio, in the HYPO rats, resulted from a disproportionate presence of G,6-P for the amount of lactate present. With refeeding, there was a greater build up of G,6-P with respect to lactate.

The ratio α-ketoglutarate:lactate rose significantly in the HYPO, RE-1 and RE-4 rats (p<0.01). There was a disproportionate build-up of α-keto glutarate for every unit of lactate present in the muscle.

In the EDL (Table 6.6.B), the ratio lactate:glycogen rose (p<0.01) in the HYPO rats, and significantly (p<0.01) fell back to normal with refeeding. The ratio α-ketoglutarate:lactate, on the other hand, was not affected by the underfeeding and refeeding processes. The G,6-P:Lactate ratio, in contrast to the soleus observations, fell significantly in the HYPO rats, indicating a disproportionate build up of lactate relative to G,6-P, possibly describing a greater glycolytic flux than in CN.

TABLE 6.6.A Metabolite ratios in stimulated soleus muscles of controls (CN), 7 day hypocalorically fed (HYPO), first day (RE-1) and fourth day (RE-4) refed rats. Values are in mmole/kg dry weight and expressed as a mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>n=6</td>
<td>n=7</td>
<td>n=5</td>
<td>n=7</td>
</tr>
<tr>
<td>Lact/glycog</td>
<td>0.82±0.11</td>
<td>0.64±0.06</td>
<td>0.020±0.005</td>
<td>0.043±0.009</td>
</tr>
<tr>
<td>G,6-P/Lact</td>
<td>0.08±0.01</td>
<td>0.14±0.01</td>
<td>0.17±0.04</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>α-ketog/Lact</td>
<td>0.08±0.01</td>
<td>0.11±0.01</td>
<td>0.19±0.04</td>
<td>0.14±0.05</td>
</tr>
</tbody>
</table>

* Significantly different from CN with p<0.01
† Significantly different from HYPO with p<0.01
‡ Significantly different from RE-1 with p<0.05
TABLE 6.6.B Metabolite ratios in stimulated EDL muscles of controls (CN), 7 day hypocalorically fed (HYPO), first day (RE-1) and fourth day (RE-4) refed rats. Values are in mmole/kg dry weight and expressed as a mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n Ratios</td>
<td>n=7</td>
<td>n=8</td>
<td>n=6</td>
<td>n=8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>4.47 ±0.64</th>
<th>7.86* ±0.51</th>
<th>3.77 ±0.78</th>
<th>4.21 ±0.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lact/glycog</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G,6-P/Lact</td>
<td>0.19 ±0.04</td>
<td>0.12* ±0.03</td>
<td>0.23 ±0.02</td>
<td>0.22 ±0.02</td>
</tr>
<tr>
<td>α-ketog/lact</td>
<td>0.018 ±0.002</td>
<td>0.019 ±0.002</td>
<td>0.017 ±0.0004</td>
<td>0.017 ±0.002</td>
</tr>
</tbody>
</table>

* Significantly different from CN with p<0.01

The delta (Δ) metabolites in the soleus (stimulated - unstimulated metabolite concentrations), resulting from repeated stimulation further completes the metabolic picture within the muscle; the delta provides quantifiable changes in metabolite concentrations, that are uniquely attributed to net flux activity through a metabolic pathway. This is an important set of data because it is uncertain whether a small metabolite concentration, measured in the stimulated state, is the consequence of a small increment or net flux, or the consequence of a large net flux from a smaller baseline unstimulated value. The significance of these delta values can be further heightened using the net glycogenolysis values, shown previously in Tables 6.3.A & 6.3.B; these describe the net flux through glycogenolysis.

The changes taking place in the soleus are displayed in Table 6.7.A. In this highly oxidative muscle, the metabolite concentrations increased with stimulation, as the stimulated minus the unstimulated concentrations yielded positive (+) values. Stimulation consisted of maximal frequency electrical pulses, given at a rate of 1 pulse/sec for 40 seconds.
The delta values of all metabolites were of similar magnitude between treatment groups in this muscle, except for a significant reduction of \( \Delta G,6\text{-P} \) in the HYPO through to the RE-4 rats, a fall of \( \Delta F1,6\text{-diP} \) in the HYPO and RE-1 rats, and a fall in \( \Delta F,6\text{-P} \) in the RE-1 and RE-4 rats.

**TABLE 6.7.A.** Delta metabolite levels in the soleus, obtained by subtracting individual concentrations in unstimulated from concentrations in stimulated muscles in controls (CN), 7 day HYPO and 1st day (RE-1) and 4th day (RE-4) refeed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>n=6</td>
<td>n=6</td>
<td>n=5</td>
<td>n=7</td>
</tr>
<tr>
<td>( \Delta ) metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>+1.33 ±1.97</td>
<td>+1.93 ±0.80</td>
<td>+1.13 ±1.43</td>
<td>+1.72 ±1.10</td>
</tr>
<tr>
<td>G,6-P</td>
<td>+1.10 ±0.31</td>
<td>+0.60* ±0.14</td>
<td>+0.37* ±0.11</td>
<td>+0.28* ±0.07</td>
</tr>
<tr>
<td>F,6-P</td>
<td>+0.20 ±0.02</td>
<td>+0.20 ±0.04</td>
<td>+0.02* ±0.03</td>
<td>+0.07* ±0.05</td>
</tr>
<tr>
<td>F1,6-dip</td>
<td>+0.46 ±0.13</td>
<td>+0.22* ±0.02</td>
<td>+0.23* ±0.06</td>
<td>+0.39 ±0.04</td>
</tr>
<tr>
<td>( \alpha )-KETOG</td>
<td>+0.65 ±0.08</td>
<td>+0.83 ±0.13</td>
<td>+0.80 ±0.16</td>
<td>+0.56 ±0.10</td>
</tr>
</tbody>
</table>

* Significantly different from CN rats with a \( p<0.01 \)

An examination of the delta (\( \Delta \)) metabolites in the EDL revealed significant increments in muscle lactate (Table 6.7.B) in the HYPO and RE-1 rats, while a significant production of \( \alpha \)-ketoglutarate was also reported in the HYPO and RE-1 rats. On the first day of refeeding, there was a notable climb (\( p<0.01 \)) in the delta G,6-P levels which were again observed on day 4.
TABLE 6.7.B. Delta metabolite levels in the EDL, obtained by subtracting individual concentrations in unstimulated from concentrations in stimulated muscles in controls (CN), 7 day HYPO and 1st day (RE-1) and 4th day (RE-4) refed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n metabolites</td>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>Δ Lactate</td>
<td>+55.41</td>
<td>+72.12*</td>
<td>+77.38*</td>
<td>+63.42</td>
</tr>
<tr>
<td></td>
<td>±3.54</td>
<td>±5.74</td>
<td>±2.82</td>
<td>±4.58</td>
</tr>
<tr>
<td>Δ G,6-P</td>
<td>+6.75</td>
<td>+6.37</td>
<td>+17.07*</td>
<td>+14.75*</td>
</tr>
<tr>
<td></td>
<td>±1.25</td>
<td>±1.73</td>
<td>±1.85</td>
<td>±1.80</td>
</tr>
<tr>
<td>Δ F,6-P</td>
<td>+4.94</td>
<td>+3.23</td>
<td>+4.64</td>
<td>+3.10</td>
</tr>
<tr>
<td></td>
<td>±0.79</td>
<td>±0.64</td>
<td>±0.61</td>
<td>±0.61</td>
</tr>
<tr>
<td>Δ F1,6-diP</td>
<td>+0.15</td>
<td>+0.09</td>
<td>+0.12</td>
<td>+0.11</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.01</td>
</tr>
<tr>
<td>Δ α-KETOG</td>
<td>+0.78</td>
<td>+1.22*</td>
<td>+1.19*</td>
<td>+0.84</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.12</td>
<td>±0.10</td>
<td>±0.07</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
+ Significantly different from HYPO with a p<0.01

Whereas the delta metabolite values can indicate product flux, they also reveal congruence or discordance in the pathway flux activity. This information cannot be obtained by establishing direct metabolite ratios in either the stimulated or unstimulated muscle. With this in mind, delta metabolite values were measured and a ratio established with one another as means of quantifying the activity of the glycolytic, glycogenolytic, and TCA pathways in relation to one another. However, caution is necessary when interpreting these ratios; a rise or fall in delta values need to be supported by static ratios in stimulated muscle in order to verify if such rises or falls in product can be observed as product build up at a rate-limiting step. Given this cautionary measure, it is generally understood that in stimulated muscle, most pathway metabolite intermediates remain more or less unchanged under normal conditions. There is, nevertheless, a rise in substrate flux (310).
The soleus muscle (Table 6.8.A) showed no significant increases or declines in these ratios with the different nutritional states, except for an increase in the $\Delta \alpha$-ketoglutarate:glycogen ratio ($p<0.01$) in the HYPO and RE-1. This translates into a more elevated $\alpha$-ketoglutarate flux activity relative to glycogenolysis in the HYPO and first day refeed rats. An aberration in $\alpha$-ketoglutarate dehydrogenase activity is not entertained in this case, as there were no increase observed in the stimulated soleus, or any differences in the delta $\alpha$-ketoglutarate values seen between the various groups (Tables 6.5.A & 6.7.A).

**TABLE 6.8.A.** Delta metabolite ratios in the soleus, obtained by taking delta values in one rat and forming a ratio with the delta of another metabolite in the same muscle in controls (CN), 7 day HYPO and 1st day (RE-1) and 4th day (RE-4) refeed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n</td>
<td>n=5</td>
<td>n=5</td>
<td>n=4</td>
<td>n=5</td>
</tr>
<tr>
<td>$\Delta$ ratios</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$Lactate/$\Delta$Glycogen</td>
<td>0.19 ±0.45</td>
<td>-1.54 ±1.44</td>
<td>0.41 ±0.29</td>
<td>0.91 ±0.37</td>
</tr>
<tr>
<td>$\Delta$α-Keto/$\Delta$Glycogen</td>
<td>0.15 ±0.02</td>
<td>0.64* ±0.16</td>
<td>0.33*± ±0.14</td>
<td>0.17 ±0.04</td>
</tr>
<tr>
<td>$\Delta$F1,6-diP/$\Delta$F,6-P</td>
<td>2.20 ±0.94</td>
<td>1.49 ±0.51</td>
<td>1.40 ±0.42</td>
<td>1.77 ±0.51</td>
</tr>
<tr>
<td>$\Delta$Lact/$\Delta$α-ketog</td>
<td>1.82 ±4.45</td>
<td>2.29 ±1.26</td>
<td>-0.14 ±2.77</td>
<td>5.54 ±4.49</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a $p<0.01$
† Significantly different from HYPO with a $p<0.01$

In the EDL, (Table 6.8.B) none of the ratios were disturbed with either underfeeding or refeeding. Disproportionate build ups in lactate compared to utilized glycogen could not be found, and yet, as shown in 6.3.B, there were in fact significant increases in the amount of glycogen used by the EDL in the HYPO and RE-1 rats.
TABLE 6.8.B Delta metabolite ratios in the EDL, obtained by taking delta values in one rat and forming a ratio with the delta of another metabolite in the same muscle in controls (CN), 7 day HYPO and 1st day (RE-1) and 4th day (RE-4) refeed rats. Values for metabolites are in mmole/Kg dry weight and are expressed as a Mean ± SEM.

<table>
<thead>
<tr>
<th>Feeding groups</th>
<th>CN</th>
<th>HYPO</th>
<th>RE-1</th>
<th>RE-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n Δ metabolites</td>
<td>n=5</td>
<td>n=5</td>
<td>n=4</td>
<td>n=6</td>
</tr>
<tr>
<td>ΔLactate/ΔGlycogen</td>
<td>4.66 ±0.50</td>
<td>4.87 ±0.84</td>
<td>4.32 ±0.35</td>
<td>4.95 ±0.42</td>
</tr>
<tr>
<td>Δα-keto/ΔGlycogen</td>
<td>0.07 ±0.01</td>
<td>0.07 ±0.01</td>
<td>0.07 ±0.01</td>
<td>0.08 ±0.02</td>
</tr>
<tr>
<td>ΔF1,6-diP/ΔF,6-P</td>
<td>0.03 ±0.01</td>
<td>0.03 ±0.01</td>
<td>0.03 ±0.00</td>
<td>0.04 ±0.01</td>
</tr>
<tr>
<td>ΔLact/Δα-ketog</td>
<td>67.39 ±9.64</td>
<td>59.21 ±9.60</td>
<td>65.63 ±4.04</td>
<td>71.32 ±14.15</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
† Significantly different from HYPO with a p<0.01

6.3.3.3 Liver Metabolites. Contrary to the soleus, the liver (Table 6.9.) did not produce less lactate in the HYPO rats. However, less G,6-P was found in the liver of the underfed rat (p<0.01). Upon introducing a calorically dense diet in the RE-1 rats, both lactate and G,6-P noticeably climbed above the CN and HYPO values (p<0.01). No variations in other metabolites could be observed with underfeeding and refeeding. The fall in G,6-P in the HYPO rats suggest a reduced influx of glucose into the liver. This makes sense in light of the lower blood glucose concentrations (Table 5.6), the abundance of glucokinase and its elevated Km (310). In RE-1 rats, by contrast, the rise in G,6-P can suggest three possibilities: i) rapid glycogenolysis, ii) rapid and efficient glucose phosphorylation, and iii) an increased glyconeogenesis (310).
TABLE 6.9. Liver lactate, G-6-Phosphate, F-6-Phosphate, F-1,6 di-Phosphate, and α-ketoglutarate levels, expressed as mmole/Kg dry weight on the various feeding days. Values are represented as means ± SEM.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>CN n=8</th>
<th>HYPO n=8</th>
<th>RE-1 n=5</th>
<th>RE-4 n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>1.00 ±0.11</td>
<td>0.61 ±0.20</td>
<td>1.85*±0.44</td>
<td>0.44*±0.24</td>
</tr>
<tr>
<td>G,6-P</td>
<td>0.98 ±0.14</td>
<td>0.44*±0.08</td>
<td>1.59*±0.39</td>
<td>0.42*±0.15</td>
</tr>
<tr>
<td>F,6-P</td>
<td>0.25 ±0.05</td>
<td>0.11 ±0.03</td>
<td>0.31 ±0.09</td>
<td>0.15 ±0.06</td>
</tr>
<tr>
<td>α-Ketog</td>
<td>0.50 ±0.06</td>
<td>0.35 ±0.01</td>
<td>0.59 ±0.06</td>
<td>0.52 ±0.09</td>
</tr>
<tr>
<td>G,6-P/Lact</td>
<td>0.96 ±0.15</td>
<td>1.72 ±0.65</td>
<td>0.96 ±0.35</td>
<td>2.87 ±1.12</td>
</tr>
</tbody>
</table>

* Significantly different from CN with a p<0.01
† Significantly different from HYPO with a p<0.01

6.3.4 Serum Biochemistry

Lactate levels in the serum were unperturbed by the effect of the anesthetic and the various feeding protocols (Table 5.7. Chapter 5).

Free fatty acids (FFA) levels remained similar to the CN values in the HYPO rats, markedly declined with the introduction of a full diet, normalized again on the second day (RE-2), and fell again on days 3 and 4 of the refeeding schedule (Table 5.7. Chapter 5).

Glucose levels declined with malnutrition, as did the insulin level (Table 5.6. Chapter 5). The glucose:insulin ratio (Table 5.7. chapter 5) suggests, however, an insulin insensitivity in malnutrition, which corrected itself on day 1 of refeeding, but plummeted for the rest of the refeeding period.

6.4 DISCUSSION

The direction of this discussion revolves around establishing whether the main hypotheses are proven to be valid. First, it was successfully
demonstrated that the performance of the muscle was independent of body composition, muscle weight and muscle size. Despite a sub-optimal muscle dry weight, muscle cross-sectional area, organ weight and body weight the tetanic tension in the soleus normalized, and fatiguability remained unchanged in the case of the EDL.

Second, the influence undernutrition and refeeding on the response of the two distinct muscle types to electrical stimulation were shown to be dissimilar. This was seen in the instance of the soleus tetanus corrected for wet weight normalizing in the hypocaloric state, but remaining sub-optimal in the case of the EDL. Underfeeding caused the fatiguability of the soleus to increase, whereas it was unchanged in the EDL metabolic repercussions of malnutrition and early.

Third, it has been demonstrated that net glycogenolysis and glycolysis, in rat muscle, is affected differently by malnutrition and refeeding resulting in dissimilar muscle fatigue responses: (a) net glycogenolysis increased in the EDL of the underfed rat, whereas it fell in the soleus. (b) the integrity of the glycolytic cycle appeared to be compromised in the soleus of the underfed rat (using the low F,6-P:F1,6-diP ratio), whereas the EDL exhibited no disturbance of glycolysis.

Fourth, it has been shown that muscle performance, remained independent of substrate availability. In fact, despite the normalization of serum glucose and soleus glycogen concentrations on the first day of refeeding, fatiguability of the slow-twitch muscle did not normalize. Furthermore, despite sub-optimal blood sugar levels in the HYPO rats, the endurance of the fast twitch muscle remained normal.

The validation of these four hypotheses has given further clinical credibility for use of muscle function as an objective measure of either deterioration in nutritional status during malnutrition, or of the improvement occurring with refeeding. The size and weight of the muscle in the malnourished and refed state was studied in chapter 5. In this section, the focus is specifically placed on substrate and metabolism.
First, the multifaceted influences that affect muscle performance during underfeeding and refeeding are considered. This frame is supported, first, by a discussion of the key factors that affect muscle fatigue in the malnourished rat; second, by an overview of the sensitivity of muscle function parameters to the rat's nutritional status in comparison to the published literature. In the following five subsections, each functional parameter of muscle will be discussed within the framework of this model: (a) maximal tetanic contraction during under-nutrition and refeeding, (b) maximal relaxation rates during undernutrition and refeeding, (c) glycogen and metabolites in relation to the fatigue of the soleus, (d) glycogen and metabolites in relation to EDL fatigue, and (e) serum insulin and muscle fatigue. Finally, the relevancy of these data to blood biochemistry and liver metabolism will be examined in the last section of the discussion.

6.4.1 Key Influences on Fatigue in Malnourished Rat Model

The key influences on fatigue in the malnourished rats have been compared to studies by various researchers. The comparisons indicate that the key components related to the performance of muscle are: (a) the size and weight of the muscle, (b) the availability of substrate, and (c) muscle metabolism. These components have been extracted from four lines of study as explained below.

First, the defects in neuro-muscular transmission (high-frequency fatigue) (219) and in excitation-contraction coupling (low-frequency fatigue) (35) did not likely further compromising the force of either muscles. In the hypocaloric or refeed states, the ratio F10:Po(N) did not change in either muscle (Tables 6.1.A. & 6.1.B). This ratio has been used by others (354) in establishing relative failures or potentiation of high frequency and low frequency stimulations. Ancillary to this argument is the fact that post-fatigue recovery of the Pt:Po(N) in both muscles was also complete (Tables 6.2.A & 6.2.B) in all groups, in that
they returned to pre-fatigue values. Furthermore, the post-fatigue recovery of the twitch contractions (Pt) in both muscles (Tables 6.2.A & 6.2.B) suggest that no damage to the T-system or to the sarcolemma actually took place, and that the affinity of Ca\textsuperscript{2+} for troponin (219) was unchanged. Importantly, in this study, fatigue related to a failure in central drive has been prevented by electrically stimulating the sciatic nerve.

Second, the changes in membrane potential were not investigated here. Therefore, the soleus' performance must be considered within the context of a possible membrane depolarization. However, the fact that the Po(N)/g did not fall in the soleus during underfeeding, or with refeeding, suggests that no significant depolarization of membrane potential took place in this study. Moreover, based on findings by Fong et al. (130), only the unstimulated soleus appears to be susceptible to a change in membrane potential in the malnourished rats.

Third, in keeping with these findings, the areas of substrate and metabolism become likely avenues of investigation, as these have been prominently featured as possible causes of fatigue in other studies (17, 74, 357, 358, 440, 177, 237).

Fourth, muscle wasting has long been associated with malnutrition (323); the catabolism of somatic proteins takes place with varied intensity, resulting in structural and ultrastructural deterioration (358, 95, 151, 308). Research by Lopes et al. (266) and Pichard (338) reaffirm the view that there is, in addition, deterioration of muscle function with malnutrition that seemingly has a direct relationship with physical wasting. The implications of using function for diagnosis is sequential logic. This is particularly true if one considers the importance of skeletal muscle in the body; it comprises more than 40% of the body mass in adults (278).
The novelty of the findings presented herein can be seen at several levels: (a) in terms of the early changes in MRR, Po, and Po/g that were observed in the soleus and only partly in the EDL during refeeding; (b) in terms of persistence of fatigue after 4 days of refeeding in both the slow-twitch and fast-twitch muscles, despite a full recovery of glycogenolysis in these muscles; (c) in terms of the association of fatigue with malnutrition in only the slow-twitch muscle and of the onset of fatigue with refeeding in only the fast-twitch muscles; and (d) fatigue in the fast-twitch muscle is significantly and negatively correlated with insulin, whereas it is not in the slow-twitch muscle.

The disparate response of muscle function to underfeeding and refeeding, depending on whether slow-twitch or fast-twitch muscles are being stimulated, and what kind of stimulation parameter is being used, leads one to ask two critical questions: Is there a parameter of function that can be used more than another as a determinant of nutritional status? Is one muscle type more sensitive to the changes in nutritional status than another muscle type? These questions are answered in the following sections.

6.4.2 Sensitivity of Muscle Function Parameters to Nutritional Status

Sensitivity is defined in this context as the ability of the functional parameters to respond to changes in nutritional status (200). The intent of this section is to represent conceptually the influences of various factors in the performance of muscle and to determine, first, which functional parameters of function are most sensitive to nutritional status and, second, which muscle type, if any, is most reliable. This is done (a) by considering the correlations existing between specific muscle function parameters and the availability of glucose, glycogen, glycogenolysis, insulin, and free fatty acids; and (b) by studying the activity and fluxes through glycogenolysis, through
glycolysis, and to a lesser extent through the T.C.A. cycle; and (c), by studying the slow-twitch soleus and the fast-twitch EDL muscles concurrently during depletion and repletion.

The literature has provided some data that suggest that some of the parameters of function may not be useful in the clinical setting. Shizgal et al. (374) found that absolute force declined with underfeeding and responded very rapidly to refeeding, suggesting a dependency on critical substrate reserves. In their discussion, they considered the dependency of function on substrate availability to diminish its sensitivity to nutritional status. Zeiderman and McMahon (435) found the sensitivity of the force frequency curve to post-surgical outcome to be 47%, the sensitivity of MRR to be 53%, and the sensitivity of both the MRR and force frequency curve to be 37%. In contrast, Brough (52) reported a sensitivity of 82% for the MRR. These results indicate that absolute tetanus is a poor predictor of post-surgical complications, and therefore, is insensitive to nutritional status. The results, however, characterize the MRR as more or less reflective of nutrition. The MRR and the bioenergetics have been previously linked (96, 97). This association suggests that if changes in metabolism and in calcium flux can be reliable as a nutritional index, then MRR should (in theory) be quite sensitive. Yet, this has been difficult to show consistently. It is the intention here to define more clearly the reasons for the inconsistency in results.

In addition, the endurance of the muscle is known to be directly dependent on substrate availability and on the muscle's ability to use this substrate. A loss of endurance due to fatigue would be reflective of either a compromised metabolism, a lack of substrate, or both. The data presented herein describe a scenario, around which the availability of substrate is not always the critical issue.
To facilitate the process of integrating the present findings within a comprehensive scheme, the key relationships between the three main functional parameters must be described.

6.4.2.1 *Tetanus (PoN).* In the malnourished rat, the soleus' fall in Po(N) is not significantly related to the erosion of muscle mass that ensues from undernutrition; rather, the aberration appears related more to the serum insulin concentration. This suggests that the tetanus may be unreliable as a parameter of nutritional status because of the strong link with insulin, a hormone that fluctuates greatly with blood sugar, and therefore carbohydrate absorption.

In contrast, the EDL's fall in tetanus during underfeeding was strongly associated with muscle mass ($r=0.77; F=54.65; df=1, 17; p<0.005$). In addition, the force corrected for weight was potentiated by insulin concentrations in the HYPO rats ($r=0.63, F=6.00; df=1,10$ $p<0.05$).

6.4.2.2 *Maximal relaxation rate (MRR).* The soleus MRR in the HYPO rats was also negatively affected by the insulin concentrations. Furthermore, the disassociation from Po(N)/g further validates that MRR is, in fact, reflective of this muscle's energetics. The EDL MRR, however, did not correlate with insulin.

6.4.2.3 *Muscle fatigue.* The soleus' fatigue was not associated with the insulin concentrations, whereas a fairly strong negative correlation was found in the case of the EDL ($r=-0.58; F=29.78; df=1,57; p<0.005$). Glycogen concentrations in the muscle appear to be related to the soleus' fatigue, but this is no longer apparent with refeeding. The EDL fatigue does not at any time relate to glycogen concentration.

The following sections describe and discuss, in more detail, how these specific parameters fluctuated with underfeeding and refeeding.
6.4.3 Maximal Tetanic Contraction

The muscle function of both the soleus and EDL bear striking similarities; both are negatively affected by hypocaloric feeding. However, the functional status during underfeeding is important for understanding the subsequent effect of refeeding.

6.4.3.1 Tetanus and underfeeding in the soleus. In the present study, the tetanic force (Po(N)) of both the soleus and the EDL are diminished in the hypocaloric state, indicating several possible scenarios: (a) a depletion of PCr reserve (266, 214, 337); (b) a fall in pH (363, 70, 167); (c) a decrease in Ca²⁺ release from the S.R., from the T-system or from a decreased affinity of Ca²⁺ for troponin (278); (d) a loss of muscle mass and/or myofibrillar damage (434, 109, 159, 285); and (e) membrane depolarization (130, 336).

A loss of PCr has been described by Pichard et al. (338) in studying the unstimulated muscle in malnourished rats, and by Mijan de la Torre et al. (295) in stimulated muscle of malnourished rats. In addition to a lower PCr, both groups of researchers found abundant free ADP (FADP) and a reduced ∆G_{ATP} during malnutrition. They showed (338, 295) that the ATP in the muscle remains constant and unperturbed by hypocaloric or fasted states. This ATP is essentially maintained at the expense of the PCr through the creatine kinase reaction. The fall in both the soleus' and EDL's Po is therefore likely related to a fall in PCr, but not from a decline in cellular ATP concentrations. Furthermore, it is a well established physiological fact (214, 337) that the energy of a single tetanus, lasting less than 2 seconds, is derived from the hydrolysis of ATP buffered by phosphocreatine (PCr) reserves in the muscle.

A fall in pH can cause a fall in PCr concentrations. However, such a fall does not necessarily imply a reduced tension in all situations; Sahlin et al. (361) have suggested that an abundant FADP, in concert
with a lower pH, could push the CK reaction forward and potentiate the force. The magnitude of the drop in pH is of importance, however, as others (103, 125) have reported a decrease in tetanic contraction with a fall in pH from 7.0 to 6.6. Such a fall in pH is not likely to have occurred in the present study, as lactate levels did significantly fall with underfeeding in both types of unstimulated muscles (Tables 6.4.A & 6.4.B.). Similarly, a compromised muscle enzyme activity (70) or altered Ca\(^{2+}\) affinity for troponin (44) did not likely occur because of a fall in pH. The impact of undernutrition on changes in calcium flux and calcium affinity can also be considered. However, because muscle pH is not likely to have changed to any significant degree in either the soleus or the EDL during the single tetanic contraction, there is no basis for assuming that the affinity of Ca\(^{2+}\) for troponin has changed in these muscles. In addition, a normalized tetanic tension was found in only the soleus, once corrected for wet weight loss. This suggests as well that Ca\(^{2+}\) release from either the S.R. or the T-system is normal in the soleus, muscle but abnormal in the EDL.

The impact of the loss of muscle mass and myofibrillar damage on the fall in tension observed in the soleus muscle was likely tied, to some degree, to the erosion in muscle mass; the tension did normalize once it was corrected for weight (Table 6.1.A). This finding also supports the notion that myofibrillar damage, in the soleus, did not occur. Hence, variance within the HYPO group was not linked to muscle mass; no significant correlation could be made between muscle weight and the Po(N) in the HYPO group. Such a lack of relationship between structure and tension is supported by other investigators, who have found myofibrillar integrity not to be compromised in the soleus with malnutrition (197) despite a significant loss of protein. This raises the possibility that some other factor, such as insulin, may be influential in the soleus' single tetanus (Po(N)). This is discussed in the following subsection.
In contrast, the loss of force observed in the EDL, with underfeeding, appears to be not only linked to the loss of wet mass \( (r=0.77; F=10.25; \text{df}=1, 17; p<0.005) \), but also to myofibrillar damage; the Po(N) did not normalize once it was corrected for wet weight (Table 6.1B). Moreover, 63% \( (r=0.80; p<0.025) \) of the Po(N) variability in the HYPO rats appears to be linked to the weight of the muscle. For all the experimental groups, the weight of the EDL appears to explain 53% of the between-rat variability in Po(N) \( (r=0.73; p<0.005) \). The relationship between malnutrition and myofibrillar damage to fast-twitch muscles has been documented by others in fasting obese subjects (354) and in hypocaloric rats (197).

This suggests, in addition, that the EDL muscle is more independent from the influences of external substrates and hormones compared to the soleus. This is in agreement with the findings of Rennie et al. (344), which suggest that the glucose-fatty acid cycle is influential only in the slow twitch muscles. However, the loss of muscle mass does not fully explain why there is a fall in the EDL's Po(N) in the HYPO rats.

**Membrane depolarization**, to some extent, may be influential in causing a depression in tension. In the soleus, the relationship of Po(N) with serum insulin levels within the HYPO group was very strong \( (r=-0.91; p<0.005) \). Once Po(N) was corrected for weight, the correlation with insulin remained strong \( (r=-0.84; \text{df}=1,10; p<0.005) \), and gave a sense of the minimal effect of muscle weight variability on Po(N) in the soleus muscle of this HYPO group. The difference in the coefficient of variability \( (r^2) \) between Po(N) and Po(N)/g was 12%, therefore indicating that, in the underfed rats, only 12% of the variability in Po(N) between the HYPO rats was explained by muscle weight differences.

The unexpected relationship between insulin and Po(N)/g cannot be explained with certainty, although it is known that potassium-depleted muscles are more susceptible to depolarization by insulin (321); Pichard et al. (336) reported a lower intracellular potassium activity \( (a[K^-]) \).
and membrane potential in only the soleus muscle of the malnourished rat. Both of these indices normalized by the 7th day of refeeding. Similarly, the data presented herein suggest that a depolarization of the soleus muscle has taken place in the HYPO rats, and that this depolarization recovered by day 4.

In the present study, although the single correlation between tetanic tension and insulin suggests a significant role played by insulin in the HYPO rats, the multiple regression analysis of wet weight and insulin vs. all rats, still finds muscle weight as the best predictor of outcome.

In the EDL, in contrast to the soleus, there was a strong positive correlation between insulin and $\text{Po(N)/g}$ in HYPO rats ($r=0.65; \text{df}=1,10; p<0.05$). This relationship describes a rise in force with increasing levels of insulin in the underfed state. Why this occurs is unclear; however, based on the previous work by Pichard et al. (336), this muscle did not have a diminished $a[K^-]$; therefore, the normal action of insulin would be to potentiate Na$^+$- K$^-$ ATPase activity (75), which is likely to be abated by underfeeding. Hence, there is likely not to be correlational evidence linking the abated $\text{Po(N)}$ in the EDL with membrane depolarization. Furthermore, multiple regression analysis of wet weight and insulin showed (as in the case of the soleus) that wet weight was still the best predictor of outcome.

6.4.3.2 Tetanus and refeeding. The $\text{Po(N)}$ of the soleus fully recovered by the 4th day of refeeding (Table 6.1.A). Yet the lost muscle mass that ensued from underfeeding did not recover or improve in any way. A regression of dry muscle mass vs. the 4 refeeding days has shown no significance ($F=0.025; \text{df}=1,39; p=N.S$). Over the complete 4 day refeeding period, only 17% of inter-rat variability of $\text{Po(N)}$ could be linked to the muscle wet weight.

While the relationship between $\text{Po(N)}$ and muscle weight is quite weak
in both muscles, it is also evident that the association between the soleus Po(N)/g and insulin weakens with refeeding (HYPO: \( r = -0.84; \text{df}=1, 10; p<0.005 \)), (RE-1: \( r = -0.65; \text{df}=1, 12; p<0.025 \)), (RE-4: \( r = -0.30; \text{df}=1, 15; p<0.05 \)), whereas the impact of variable muscle mass on Po(N) heightens with refeeding; muscle wet weight explains 56% of the inter-rat variability in the 4th day refed rats. Hence, the reasons for the recovery of tension in the soleus appears to be tied to the decreasing negative effect of insulin on Po(N) with refeeding, while the persistent low tension observed in the EDL seems to be linked, to some extent, to the loss in muscle wet weight. The relationship is not clear, however, as myofibrillar damage and muscle mass erosion have both been proposed (185, 358) as affecting the contractile machinery, such as cross-bridge attachment/detachment. Insulin was not likely influential in the Po(N) of the EDL muscle during refeeding; no correlation could be found (\( F=1.41; \text{df}=1, 56; p=N.S. \)).

Measuring the Po(N) in malnourished patients would pose some difficulties if one assumes that the rat model bears similarities with the human biological system. First, insulin concentrations resulting from the ingestion of food could cause a depolarization of the slow-twitch muscle membrane and a decrease in the tension. Minimal variability between patients could be ensured by conducting these measurements in a fasted state, as opposed to a hypocaloric state. Second, the fast-twitch muscles may be a more objective measure of nutritional status, since its Po(N) correlates with muscle wet weight. However, while up to 40% variability in Po(N)/g can still be attributed to variable insulin levels in the hypocaloric group, there is, however, no apparent association between Po(N) and insulin in the HYPO rats.
6.4.4 *Maximal Relaxation Rate in Undernutrition and Refeeding*

The MRR fell significantly in both muscles of the HYPO rats (p<0.01). There are several uncertainties, however, associated with such a depression. It is unclear if the MRR, in the instance of underfeeding and possible muscle wasting, is a reliable index of energetics, as was suggested earlier by Dawson et al (96, 97) in well fed rats. It is unknown to what extent muscle glycogen offers protection to the muscle from the perspective of preserving myofibrillar integrity during damaging, high-intensity stimulations, and therefore, to what extent it offers protection to the S.R.

The influence of insulin on MRR is still unresolved from the perspective of the muscle's energetics. This is a question of particular significance, because the action of insulin facilitates the access of important substrate to the muscles, which may affect metabolic flux through glycolysis.

6.4.4.1 MRR and muscle wasting. The MRR, normally seen as an indicator of fatigue that is independent of muscle contraction, significantly fell in both the soleus and EDL muscles. This occurred even though the response of Po(N)/g to malnutrition varied, depending on whether it was a fast-twitch or slow-twitch muscle. In the soleus, no correlation exists between MRR and contraction (Po(N)/g) (F=0.306; df=1, 57; p=N.S), whereas an association is found between MRR and the EDL's contraction (Po(N)/g) (r=0.41; F=10.06; df=1, 50; p<0.005). This finding suggests that MRR in the EDL may not be strictly an index of free energy in malnutrition. The cases of the soleus and EDL muscles are looked at separately in the following subsection on MRR and muscle energetics.
6.4.4.2 MRR and muscle energetics.

In the soleus, the fall in MRR implies an aberration in Ca\(^{2+}\) flux into the sarcoplasmic reticulum (S.R) after a single tetanic contraction (Tables 6.1.A & 6.1.B). The reason for this disturbance in flux is not likely related to intracellular ATP concentrations, as these have been shown to be unchanged throughout malnutrition (338), but more likely to a fall in \(\Delta G_{ATP}\), as reported by others (337). A similar finding, in normally fatigued muscle, was also made by Dawson et al. (96), who in addition linked \(\Delta G_{ATP}\) with the relaxation rate of the muscle, therefore associating a slower MRR with a slower aerobic and/or anaerobic metabolism. A fall in \(\Delta G_{ATP}\) will have repercussions directly on the cross-bridge cycling, because energy-dependent enzymes like Ca\(^{2+}\)-ATPase, Ca\(^{2+}\)Mg\(^{2+}\)-ATPase, and myofibrillar-ATPase (278) will also likely be affected by a fall in the free energy change of ATP. Although there is some in vitro evidence suggesting that Ca\(^{2+}\)-ATPase in slow-twitch muscle is modulated by the phosphorylation activity of Ca\(^{2+}\)/calmodulin protein kinase (174b), there is insufficient in situ evidence to establish convincingly whether phosphorylation/dephosphorylation events are actually occurring in excitation-contraction coupling in skeletal muscle or heart (166b). The slowing of the MRR during underfeeding is therefore, not likely related to the phosphorylation of key energy-dependent enzymes. Consequently, neither the in situ phosphorylation of these isozymes, nor the phosphorylation of Ca\(^{2+}\) pumps, Ca\(^{2+}\) channels and of the regulatory protein, phospholamban (174b), all considered key proteins in the modulation of Ca\(^{2+}\) efflux from and influx to the S.R. (174b), were conducted in this thesis. However, from work arising from this thesis, there is strong evidence supporting a fall in energy-dependent enzymes during malnutrition. The hearts of these HYPO rats were analyzed for Ca\(^{2+}\)-ATPase and myofibrillar-ATPase: the results show that both fell significantly (319). Furthermore, sarcoplasmic reticulum calcium uptake, in the heart of these rats, was also diminished in the
HYPO rats. This decrease persisted until the second day of refeeding (319). This analysis, however, was not done on the skeletal muscles. Nevertheless, these findings suggest that the access of calcium to the S.R. would likely be compromised in underfeeding, thereby resulting in a rise in cytosolic calcium. A marked rise in intracellular Ca\(^{2+}\) (p<0.01) of the fast-twitch gastrocnemius was, in fact, observed by Russell et al. (357). This rise was related to the possible slowdown of the Ca\(^{2+}\)Mg\(^{2+}\)ATPase. The latter partly ensures Ca\(^{2+}\) efflux from the cell (214) and influx to the S.R. (222b).

In keeping with the notion that MRR is reflective of the energy status of the muscle, evidence is presented regarding the correlation between the MRR and the ratio of the concentrations of F,1,6-diP:F,6-P in the unstimulated soleus (r=0.42; F=4.73; df=1, 23; p<0.05). While such a correlation is hardly sufficient to establish causation, it does nevertheless suggest some mild influence of energetics on MRR. Further study of this correlation is required, however. What is known is that the ratio F1,6diP:F,6-P is significantly lower in unstimulated and stimulated HYPO rats (Tables 6.4.A & 6.5.A). Moreover, a fall in this ratio can be suggestive of a suppressed PFK activity (121, 388b), which is an aberration that would compromise the glycolytic capability of the muscle and therefore its energetics. This reinforces the argument that an energy deficit partly explains the fall in MRR in the slow-twitch muscle during undernutrition. By contrast, no correlation could be established between MRR and F1,6diP:F,6-P ratio in the unstimulated EDL. This raises the likelihood that some other factors may be quite influential in modulating the MRR of fast-twitch muscles. Such factors were not studied in this thesis.

The delta MRR that was measured in the soleus, during HYPO feeding, was the same as controls; this indicates that the slower MRR observed during underfeeding can further decline after fatigue. The cause was likely related to a decline in \(\Delta G_{ATP}\) (96) and/or a diminished excitation-
contraction coupling (121). These same causes could also be attributed to the slower MRR in post-fatigue CN rats.

The MRR appears to be a functional parameter that objectively measures the energetics of a muscle. For MRR to be considered a useful parameter in the assessment of nutritional status, there must be the understanding that MRR cannot directly fluctuate with substrate availability, such as glucose and glycogen. This is discussed in another subsection.

In the EDL muscle, a fall in the MRR, unlike in the soleus, does not appear to be linked to muscle energetics; no correlation was found between the EDL's MRR and F,1,6-diP:F,6-P (F=1.08; df=1,23; p= N.S). A relationship was found between α-ketoglutarate and MRR in the stimulated EDL (r=0.51; F=7.02; df=1, 21; p<0.025), and between the ratio α-α-ketoglutarate: glycogen and MRR (r=0.50; F=6.19; df=1, 20; p<0.025). Although not considered as strong correlations that are able to explain the slower MRR, they do suggest some degree of association between MRR and oxidative metabolism. However, this only explains intra-group variations, because α-ketoglutarate did not change between feeding groups (Table 6.5.B), nor did the ratio α-α-ketoglutarate:glycogen (Table 6.8.B), despite a significant fall in MRR. Hence, there is some evidence of a disassociation between MRR and muscle energetics in the fast-twitch muscle; a possibility also discussed by Russell et al. (258). Rather, the literature (266, 358) suggests that prolonged hypocaloric feeding produces selective type-II (fast-twitch) fiber atrophy. A slower MRR could then result from myofibrillar damage that negatively affects the cross-bridge cycling and/or the S.R. membrane, thereby preventing calcium ion uptake and release. Evidence for an attenuation of cross-bridge kinetics is supported by two observations: first, the EDL's MRR and Po(N) do not recover after 4 days of refeeding, although the recovery of both of these parameters is observed by day 4 in the soleus. Second, post-fatigue MMR values (Table 6.2.B) in the EDL
of the HYPO and RE-1 rats changed significantly less than in the CN. This can be explained by the abated cross-bridge kinetics resulting from hypocaloric feeding. In such an underfed state, fast-twitch muscle is known to be susceptible to myofibrillar damage \(^{(137, 143)}\). Hence, the significantly smaller drop in post-fatigue MRR could be due to pre-fatigue starvation-induced myofibrillar damage that is extensive enough to cause a fall in S.R. calcium-ATPase pumping. This is an expected occurrence in light of the fall in \(\Delta G_{\text{ATP}}\) \(^{(96, 357, 65, 236)}\) that is reported to occur with a slower MRR. However, the contribution of this diminished pumping action to the reduced post-fatigue MRR has been argued to be small \(^{(417)}\). In fact, work by Westerblad and Allen \(^{(417)}\) revealed that the major cause of the slowing of relaxation rate in the fatigued mouse muscles is a reduced myofibrillar Ca\(^{2+}\) sensitivity, as opposed to strictly a reduction in S.R. Ca\(^{2+}\) pumping. The findings presented here cannot discriminate between these two causes, although the full recovery of post-fatigue MRR values to pre-fatigue values in the RE-2 and RE-3 rats suggests a likely faster normalization of post-fatigue cellular ATP concentrations with refeeding.

6.4.4.3 MRR and muscle glycogen. Glycogen has also been proposed as a critical factor in the disruption of the MRR in muscle \(^{(158)}\). Surfacing from the literature are two models: (a) Enteman's model \(^{(124)}\) of sarcoplasmic reticulum-glycogenolytic complex disruption, in which a loss of glycogen is thought to disturb S.R. activity; and (b) an inhibition, by glycogenolytic enzymes \(^{(50)}\), of calcium reentry into the S.R. The glycogen data suggests that Enteman's model may be applicable in both muscles during underfeeding; glycogen concentrations fell significantly in both muscles after repetitive stimulations, suggesting that the loss of glycogen may have exposed an unprotected S.R. to the damaging effects of the contractions, and therefore compromised normal S.R. activity (Tables 6.3.A and 6.3.B). This protective role of muscle
glycogen has also been described by Green (158). This is in part supported by the work of Russell et al. (358), in which they reported Z band degeneration in the gastrocnemius of hypocalorically fed humans; it is similarly supported by the findings of Brautigan et al. (50).

The post-fatigue data (Table 6.2.A) in the soleus describes a significant fall in post-fatigue MRR in the CN, HYPO and RE-1 to RE-4 rats. There is, however, no indication that the HYPO rats, more than the other groups of rats, experienced a significant worsening of post-fatigue MRR because of a greater glycogen loss and myofibrillar damage. Had such damage occurred, a greater delta MRR (pre-fatigue MRR minus post-fatigue MRR) would have been observed in the soleus of the HYPO rats. The data did not reveal such a situation. In the EDL, post-fatigue data (Table 6.2.B) reveals a significant decline in the MRR from pre-fatigue values in the CN, HYPO and RE-1 rats. However, contrary to the soleus, the EDL’s change in MRR (pre-fatigue MRR minus post-fatigue MRR) was significantly smaller in the HYPO and RE-1 rats compared to all other groups (CN, RE-2, RE-3, RE-4). Therefore, the notion that a glycogen loss from the muscle, because of malnutrition, would have caused S.R damaged is not entirely supported by the data. The smaller A MRR is in fact evidence of no S.R damage in the fast-twitch muscle.

Furthermore, the recovery of the soleus’ MRR occurs despite sub-optimal glycogen concentration (Table 6.3.A), thereby indicating that Enteman’s model of glycogen protection is not applicable for this group. Similarly, in the EDL muscle, the Enteman model does not appear to be applicable to the RE-4 rats, as sub-optimal MRRs persisted despite normalized glycogen.

Likewise, the model suggesting an interference of calcium reentry into the S.R. by glycogenolytic enzymes (50) cannot be upheld in either muscle. Glycogenolysis was not disrupted in the EDL muscle (Table 6.3.B) of the HYPO rats, and therefore Ca²⁺-dependent glycogen phosphorylase would be present in sufficient amounts in the control animals as well as
in the HYPO and refed rats. Yet, the HYPO and refed rats showed a slower MRR than the CN rats. Furthermore, the slower MRR in the soleus took place despite a significantly diminished presence of glycogen phosphorylase (assumed to occur because of the slower glycogenolysis).

The significant fall in MRR, observed in the HYPO and refed rats, raises the likelihood of an abnormality in calcium flux arising from a diminished free energy ($\Delta G_{ATP}$) in the case of both the soleus and EDL muscles. The findings presented here do not support the notion that the depression in the EDL’s MRR of the HYPO and refed rats was the result of S.R damage.

These findings suggest that the MRR of both the fast-twitch and slow-twitch muscles could serve as an accurate measure of nutritional status during underfeeding and refeeding. This argument is justified on the basis of two findings: first, the MRR of both muscles fell with underfeeding, but did not normalize with serum glucose (Table 5.6, chapter 5) or the glucose:insulin ratio (Table 5.7, chapter 5) on the first day of refeeding; second, there appears to be a dissociation between glycogen reserves in the muscle and the MRR. The second of these findings is a good thing, because MRR otherwise would be influenced by substrate that is not necessarily representative of nutritional status.

6.4.4.4 Serum insulin and muscle MRR. An intriguing possibility is whether the accessibility of glucose to the muscle, a critical substrate for glycolysis, would correlate with the MRR and with the fatiguability of the muscle. In this context, insulin was considered as an independent variable and a possible confounder. This possibility is discussed separately for the cases of soleus and EDL muscles.

In the soleus muscle, it is generally accepted that the MRR of the muscle is strongly linked with the $\Delta G_{ATP}$. This was first hypothesized by Dawson et al. (96). They theorized that a fall in $\Delta G_{ATP}$ would slow the pumping of calcium back into the endoplasmic reticulum. This theory has
since received support from other investigators (65, 236, 357). The regression shown in Fig 6.3. indicates that as insulin increases, the MRR of the muscle falls. This implies that the higher the insulin levels in the malnourished state, the more compromised the muscle becomes with respect to its ability to relax (p<0.01). Recognizing that glycolysis is compromised in this muscle during malnutrition, it makes sense that any increase in the access of glucose to the cell, such as through insulin, would further compromise the state of energetics. The soleus, in this instance, is unable to process the acquired substrate because of a possibly compromised PFK (Tables 6.4.A and 6.5.A).

In the EDL muscle, in contrast to the soleus, no correlation could be established between the EDL’s MRR and insulin concentrations. The regression, as shown in Figure 6.5., graphically represents the weakness of this association. There is also a weakness in the link between MRR and muscle metabolism, as supported by the undisturbed glycogenolysis in this muscle. This point is further made by the undisturbed ratio of \( \text{keto:}\text{glycogen} \) reported in Table 6.8.B. The stability of this ratio throughout the underfeeding and refeeding illustrates the dissociation of MRR from oxidative phosphorylation. The suppressed MRR that was observed in the HYPO and refeed rats, therefore, cannot be explained by a diminished oxidative phosphorylation, glycolysis or glycogenolysis. This contrasts with the findings of Pichard et al. (338) and Mijan de la Torre (295), who reported a fall in oxidative phosphorylation in underfed rats. The possibility does exist that the depressed MRR that was found in the EDL was not a reflection of metabolic changes, but rather the result of damaged myofibrillar tissue which may have affected the affinity of calcium for troponin protein (known as TpC). This would in effect compromise the contraction of the muscle. Likewise, poor myosin-ATP content, the result of myosin breakdown, would prevent relaxation from occurring (278).
The loss of these key proteins could explain why the Po(N)/g had overtly fallen in the HYPO and refed rats in the EDL, but not in the soleus (Tables 6.1.A and 6.1.B). Seemingly, some myofibrillar recovery would have taken place by the second day of refeeding as evidenced by the recovery of the Po(N)/g of the EDL on day 2 (Table 6.1.B). The subsequent fall on day 3 may be due to a readjustment of electrolyte flux; this suggests a change in membrane potential.

Overall, the MRR of both the fast-twitch and slow-twitch muscles appears to be an objective functional parameter of nutritional status; its sensitivity can be heightened by ensuring patients undergo a 12 hour fast prior to testing.

6.4.5 Glycogen and Metabolites in Relation to Muscle Fatigue in the Soleus

The performance of both muscles over 40 repeated tetani was altered in the malnourished state. This specific functional test assesses the metabolic capacity of the muscle. Therefore, the increased fatiguability observed in the soleus suggests that a deficient glycogen reserve may be a cause, accepting as a premise that PCR does not play a role of any significance beyond 2 seconds of stimulation (337). The EDL, on the other hand, did not, at first glance, suffer from an increased fatigue in the underfed state. However, the percent force lost from the original tetanus, although similar to the CN, does not refute the likelihood of myofibrillar damage due to hypocaloric feeding. The initial tetanus in the HYPO rats was, in fact, significantly smaller than CN. It does suggest that the metabolic activity was not compromised in the fast-twitch muscle of the HYPO rats. With refeeding, however, the EDL's performance actually declined significantly, as determined by regression analysis ($r=-0.31; F=6.60; df=1, 61; p<0.025$).
The dissimilar reaction of these two distinct muscle types to the fatiguing process argues in favour of the need to distinguish between muscle types when conducting such a functional assessment of nutritional status. The basis for advising such a cautionary note stems from the dissimilar functional data that is presented herein, and from the dissimilar muscle biochemical findings. If one is to accept the view that muscle function can be used as an accurate measure of nutritional status, four factors need to be considered: first, changes in function must occur in the depleted state; second, the performance should not strictly be related to muscle glycogen and to serum glucose concentrations; and third, the reason for the fall in tension must relate back to some aberration to one or several biochemical pathways. The discussion, therefore, will next focus on the fatiguability of the soleus muscle in relation to glycogen reserve.

6.4.5.1 Unstimulated soleus glycogen and performance.
The importance of a generous glycogen supply in prolonged exercise is still considered a critical factor, whether one is arguing in favour of a non-metabolic, or a metabolic, basis to fatigue (158). Furthermore, the reliance of the soleus on glycogen is not questioned with respect to stimulation protocol, since it is known that stimulation rates exceeding 30 tetany/minute (270) is regarded as anaerobic activity. This experiment stimulated muscle at a rate of 60 tetany/minute. In keeping with this premise, the low glycogen concentrations measured in the unstimulated soleus muscles of the HYPO rats (Fig. 5.3. chapter 5) appear to explain the observed fall in muscle resistance during malnutrition; a 25% increase in fatigue occurred in muscles that suffered a mean 20% drop in glycogen reserves prior to stimulation. A fall was also reported by Zorzano et al. (440), although the extent was greater because of the severity of the diet restriction. However, a dissimilarity between glycogen and performance is reported in this study.
based on two observations: first, the persistent fatigue observed on the first day of refeeding contrasts with the abundance of glycogen found in this muscle (Fig. 5.3, chapter 5). Second, fatigue normalized on day 2 despite subnormal glycogen concentration. Even though fatigue on day 4 of refeeding was occurring concurrently with subnormal glycogen concentrations (Table 6.3A) no correlation could be made overall between unstimulated glycogen concentrations and muscle fatigue (F=0.37; df=1, 12; p=N.S.)

The dissociation between muscle fatigue and glycogen content is an important finding because it implies that the presence of glycogen, as a stored substrate reserve, is not critical for the optimal performance of the muscle, at least during refeeding. Furthermore, the muscle became significantly more fatiguable even though muscle glycogen did not get fully depleted during exercise. Again, no correlation was possible between stimulated muscle glycogen and fatigue (F=0.37; df=1,12; p=N.S.). The dissociation between fatigue and glycogen is further observed with the introduction of full calories on the first day; fatigue did not normalize in this muscle despite a supersaturation of muscle with glycogen. Moreover, glycogen reserves were sub-optimal after 4 days of refeeding. Hence, in malnutrition, refeeding does not necessarily replenish glycogen in linear fashion.

Unresolved at this point, however, is the degree of glycogenolysis taking place in the HYPO, RE-1, and RE-4 rats. Rennie et al. (344) found that minimal glycogenolysis was taking place at the same time that lower glycogen content was observed in muscle. Following Rennie et al.’s logical observation, perhaps the lower muscle glycogen observed in the soleus implies a slower glycogenolysis in the HYPO rats. This question will be explored in the following subsection.
6.4.5.2 Glycogenolysis and metabolites in the soleus.

The findings presented here support the notion advanced by Rennie et al. (344) that during malnutrition glycogenolysis was minimally active in the soleus (Table 6.3.A). This decrease coincided with a visible decline in the performance of the HYPO rats. The heightened fatigue was associated with a rise in the ratio \(\Delta\alpha\text{-ketoglutarate}/\Delta\text{glycogen}\) (Table 6.8.A) in the HYPO rats. This altered ratio implies that although glycogenolysis may have fallen substantially, the flux through the TCA cycle was maintained, thereby suggesting that the soleus is relying more heavily on FFA for energy. It is not surprising, then, that fatigue occurred; the rate of cellular uptake of FFAs is likely insufficient to sustain an oxidation capable of meeting the energy needs of high intensity exercise (341). Furthermore, FFAs do not provide enough citric acid cycle intermediates to meet the heavy work requirements (310).

Although refeeding caused a rise in net glycogenolysis, it still was below CN values. This suggests a continued reliance on FFA. This is also supported by the elevated \(\Delta\alpha\text{-ketoglutarate}/\Delta\text{glycogen}\) ratio in the RE-1 rats.

By the fourth day of refeeding, a greater fatigue still persisted even though the \(\Delta\alpha\text{-ketoglutarate}/\Delta\text{glycogen}\) ratio normalized, and the FFA levels had diminished. There was, however, still some evidence that there may be a proportionally greater reliance on oxidative phosphorylation still occurring in the muscle after 4 days of refeeding as well as a persistently sub-optimal glycolysis. This is supported by five factors: (a) the disproportionate build up of glucose compared to insulin on this day (Table 5.7. chapter 5), which suggests that glucose uptake into the cell may be insufficient to supplement glycogenolysis -- glucose, rather than spare glycogen, is thought to supplement the process of metabolizing carbohydrate and, therefore, to prolong endurance (91); (b) the sub-optimal insulin concentrations (Table 5.6, Chapter 5); (c) the elevated G,6-P:Lactate ratio in stimulated muscle
(Table 6.6.A.); (d) the elevated α-keto-lactate ratio (Table 6.6.A.); and (e) the sub-optimal lactate:glycogen ratio, which indicates less lactate was produced relative to the amount of glycogen available after contractions (Table 6.6.A.).

6.4.5.3 Metabolites in unstimulated soleus. The data demonstrate that malnutrition has two important effects on muscle metabolism. First, it slows down the overall glycolytic pathway as substrate becomes more scarce, and as glucose access to the muscle is diminished because of reduced insulin levels as well because of a possible insulin resistance or reduced glucose entry into the cell (Tables 5.6. and 5.7. Chapter 5). This is evidenced by the lower lactates (P>0.01) in the HYPO rats, which persisted throughout the 4 day refeeding period (Table 6.4.A.). Second, there is some indication that PFK activity significantly fell in the malnourished state, as demonstrated by the smaller F1,6-diP:F6-P ratio (Table 6.4.A.).

This ratio has previously been used by other investigators (148, 440) as a means of indirectly reflecting PFK activity; it is considered critical in the control of glycolysis (324). There is no evidence of an abnormal allosteric regulation of the reaction: F,6-P --> F,1,6-diP, as the concentration of F,6-P in the HYPO rats was not different from the CN rats. Furthermore, work by Kurland et al. (240) in rat muscle established the Km of the enzyme 6-phosphofructo-2-kinase at 0.33 mmol; also referred to as the PFK-2 enzyme, it catalyses the synthesis of F2,6-biP from F,6-P, thereby positively stimulating PFK (64). Based on the graph of this enzyme's activity relevant to the concentrations of F,6-P, it was possible to transpose the concentration of this metabolite found here (Table 6.4.A.), and estimate an activity level in the muscles of these rats.

The result indicated that a downward flux through glycolysis was in fact occurring at a rate that was about 25% of Vmax. It is not likely that any significant upward flux was taking place at the same time;
previous work by Opie and Newsholme (324) found that the PFK activity in both red and white muscle was significantly dominant, and concluded that F1,6-diPhosphatase was not controlling the phosphorylation of F,6-P. In fact, the lower energy stores in the malnourished rats suggest that the F-1,6 diPhosphatase would likely be inhibited (64). The PFK enzyme likely became significantly inactive on the first day of refeeding, as this same ratio fell dramatically (p<0.01) from the HYPO values and never normalized, even with 4 days of refeeding. The concentrations of F,6-P did not differ from the CN values during the refeeding process; this suggests that allosteric regulation had not changed with HYPO feeding or refeeding.

These findings imply that the state of energetics of the unstimulated muscle is likely compromised with malnutrition, and that the initiation of feeding further compromises the metabolic capacity of the muscle. The increased depression of the F1,6-diP:F-6-P ratio in the RE-1 rats, coincides with a persistent fatigue in the stimulated muscle.

6.4.5.4 Metabolites and function in the stimulated soleus.

In the hypocaloric rats, muscle stimulation produced similar results to those found in the unstimulated muscle. Again, glycolytic activity, as described by the F1,6-diP:F6-P ratio (Table 6.5.A) and the G,6-P:lactate ratio (Table 6.6.A) was markedly inhibited with hypocaloric feeding, whereas with refeeding there was a normalization of the F1,6-diP:F6-P ratio, but not of the G,6-P:lactate ratio on day 1. This latter ratio, is recommended by Hultman et al. (204) as an effective monitor of the glycolytic pathway activity and as a means of assessing the impairment of PFK activity in humans. This enzyme has been shown to be rate limiting in the process of glycolysis (226).

The data shown here (Tables 6.5.A and 6.6.A) do support the notion of an impaired glycolysis in the HYPO rats, an impairment that may be
localized at the rate-limiting enzyme PFK. There seems to be, by contrast, less evidence of simply a change in allosteric regulation. The concentration of P-6-P that was found in the soleus (Table 6.5.A) illustrates this latter point; it was very close to the Km established by Kurland et al. (240) in rat muscle. This level of metabolite would be sufficient to affect a normal allosteric regulation descriptive of a downward flux through glycolysis.

Other factors also lead to a similar conclusion. First, ATP concentrations did not fall and free ADP levels significantly rose in previously published data on rats that were hypocalorically fed for 7 days (338), and 21 days (357). It is generally accepted that a rise in ATP levels would inhibit PFK (64), that elevated free ADP is an expression of diminished energy stores, and therefore stimulates the enzyme PFK (251). Second, Nishio et al. (316) and Russell et al. (357) found a decreased PFK activity in the soleus and gastrocnemius of hypocalorically fed rats. Third, there was a rise in lactic acid concentrations in the stimulated soleus and EDL muscles compared to the levels found in the unstimulated muscles, which suggests a downward flux through glycolysis. Hence, the data describes a slowdown in glycolytic flux, which suggests the likelihood of an abnormal PFK activity.

The reasons for the decreased enzymatic activity was not specifically established in this thesis; however there is some room for speculation: first, Mahrenholz et al. (272) demonstrated, in sheep heart, that Ca²⁺/calmodulin protein kinase and cyclic AMP-dependent protein-kinase phosphorylated PFK and altered its activity. Consequently, there was a heightened sensitivity of PFK to ATP concentrations, thereby making PFK more likely to shut down. They also observed an increase in the Km of PFK for F,6-P; this indicates a diminished affinity of the PFK enzyme for the substrate. The protein kinase C, however, failed to phosphorylate the PFK. Mahrenholz et al. (272) conclude that by combining all kinase proteins, enzyme activity did not change overall, nor did the
stoichiometry of phosphorylation. Second, Lan and Steiner (242) found that both calmodulin and troponin C bound and deactivated PFK. There is no sense, however, why there would be a greater deactivation and phosphorylation occurring during undernutrition. Third, Chasiotis et al. (70) point out the susceptibility of glycogenolytic and glycolytic enzymes to lower pH. This was confirmation of earlier findings by Bergstrom et al. (31).

While the lactate concentrations did increase in both stimulated muscles in the present study, the soleus of the HYPO rats did not have greater lactate levels, but rather, lactate concentrations were significantly lower (Table 6.5.A). Further work would be necessary in order to establish if hypocaloric feeding does, in fact, raise the Km of PFK for F,6-P, and if there is a significant phosphorylation of PFK.

There is also evidence of a diminished substrate flux through glycolysis resulting from a decreased net glycogenolysis, a finding that carries an obvious repercussion on fatiguability. This is illustrated in three ways: (a) by the sub-optimal glycogenolysis seen in the HYPO and RE-1 rats and the persistent fatigue observed on these days; (b) by a significant fall in the glycolytic flux, as illustrated by the reduction in $\Delta F,1,6\text{ di},\Delta F,6\text{-P}$ and in $\Delta G,6\text{-P}$ (Table 6.7.A), measurements which parallel the fall in glycogenolysis; and (c) by a significant fall in the lactate:glycogen ratio (Table 6.6.A), indicating a disproportionate build up of glycogen relative to the lactate produced.

Although there appears to be a greater reliance on oxidative phosphorylation in the HYPO and refed rats, as indicated by the rise in the $\alpha$-ketogl: lactate ratio (Table 6.6.A), the disproportional build up of $\alpha$-ketoglutarate can also be the consequence of a slowdown in $\alpha$-ketoglutarate dehydrogenase activity. This is a theory that has previously received some support (7), and that ultimately describes a slowdown in oxidative phosphorylation (310). By contrast, the $\Delta$Lactate:$\alpha$-ketoglutarate ratio (Table 6.8.A) did not change significantly with HYPO
feeding and refeeding. A possible explanation for this unchanged ratio may reside in the fact that the enzyme α-ketoglutarate dehydrogenase uses the same co-factors as pyruvate dehydrogenase, and the reaction mechanism behind the conversion of α-ketoglutarate to succinyl CoA is similar to the reaction converting pyruvate to Acetyl CoA (64). Hence, a specific slowdown of α-ketoglutarate dehydrogenase is likely occurring at the same time as a slowdown of pyruvate dehydrogenase, thereby causing a proportional build-ups of both lactate and α-ketoglutarate.

The data suggest that there may be a defective PFK during malnutrition, a diminished glycogenolysis, and a slowdown in oxidative phosphorylation, all of which may be significant contributors to the muscle fatigue. In addition, there is an elevated glucose:insulin ratio indicating a disproportionate build up of glucose relative to insulin.

In refed rats, several things happened on the first day of refeeding: (a) Glucose and the glucose:insulin ratio normalized. (b) Glycogenolysis remained sub-optimal. (c) Serum FFA levels significantly fell, and therefore, hormonal milieu did not favour β-oxidation of fat. (d) The α-ketoglutarate: glycogen ratio significantly fell from the HYPO values (p<0.01), but still remained greater than CNs, which implies that TCA flux was still proportionally greater than glycogenolysis; in addition, TCA activity remained greater than glycolysis on that first day, as demonstrated by the rise in the α-keto-glutarate:lactate ratio. (e) PFK activity was normal in the RE-1 rats as evidenced by the normalized F,1,6-diP:F,6-P ratio (Table 6.5.A). However, although PFK appears to have normalized, the glycolytic flux had not, as shown by the fall in the ΔG,6-P, ΔF1,6-diP and ΔF,6-P. Finally (f), the G,6-P: lactate ratio still remained greater than the CN, thus supporting either a greater reliance on aerobic oxidation, a decreased/impaired glycolysis or both.

The source and availability of the fuels feeding these pathways are critical in understanding the compensatory mechanism that may be taking
place in the HYPO and refeed rats. The source of substrate used in
glycolysis is mostly glycogen, with glucose playing a very little role.
There are two reasons that justify this statement: first, although the
glucose:insulin ratio is normal on the first day, there is no apparent
signs of increased glucose flux into the muscle, as evidenced by the
normal G,6-P and AG-6-P; second, glycogenolysis is still occurring in
the HYPO rats, albeit at a reduced rate, despite the reduced
availability of glucose. Similarly the delta metabolite values are
equally quite low (Table 6.7.A).

Despite what seems to be a normal PFK activity (Table 6.5.A), there
is a persistent fatigue after one day of refeeding. There are seemingly
three reasons why this fatigue would persist: (a) Glycogenolysis is
still not fully recovered, thereby alluding to a greater reliance on TCA
flux. Based on the observations of the HYPO rats, the TCA cycle appears
incapable of metabolizing at a fast enough rate, the end products of
glycolysis and FFA oxidation, needed to sustain performance. (b) The
glycolytic flux is persistently low. Although glucose could
theoretically enter the muscle cell without any difficulty, based on the
normalization of the glucose:insulin ratio, and supplement the reduced
glycogenolysis, there is no evidence of this occurring. There is, in
fact, still reduced substrate flux through glycolysis (Table 6.7.A). (c)
There is a reduced, but continued, dependence on FFA oxidation. The fall
of the ratio α-ketoglutarate:glycogen from HYPO values (Table 6.8.A),
can indicate that glycogenolysis is significantly improved, while the
the TCA flux remained unchanged with refeeding. α-ketoglutarate has been
used previously as an index of oxidative metabolism (348). Compared to
the CNS, the ratio is still significantly greater, indicating that FFA
oxidation is still contributing proportionally more than in the CN rats,
despite the abundant supply of glycogen, insulin, and glucose.

While glycolysis and glycogenolysis continue to be sub-optimal on the
first day of refeeding, there is little evidence of an abated oxidative
metabolism on the first day (Table 6.6A). Although an elevated α-keto-glutarate:lactate ratio in the RE-1 rats, from the outset, suggesting that the abnormal build-up of α-ketoglutarate may be due to a slowdown in α-ketoglutarate dehydrogenase, as previously suggested in the case of the HYPO rats, it is likely related to low lactate levels (Table 6.5A). Moreover, such a slowdown would also imply a faltering pyruvate dehydrogenase enzyme because of the similarity in reaction process and co-factors (251). Hence, a slowdown of α-ketoglutarate dehydrogenase would also imply a slower pyruvate dehydrogenase. There is however, no evidence of a build-up of lactate (Tables 6.5A & 6.7A), or even a disproportionate accumulation in comparison to some other metabolites (Tables 6.6A & 6.8A). Hence, the persistence of a subnormal performance appears to be taking place because of a glycogenolysis that has not totally recovered.

In the RE-4 rats, fatigue did not normalize. Although the α-ketoglutarate:glycogen ratio did recover, there are still three parameters that remain abnormal: (a) the G,6-P:lactate ratio; (b) the α-ketoglutarate:lactate ratio; and (c) the glucose:insulin ratio.

Admittedly, assessing α-ketoglutarate by fluorometric determination of NADH holds limitations in that this method is unable to discriminate between the NADH that is specifically located in the mitochondria and that found in the cytosol (154). Hence, the interpretation regarding the state of oxidative phosphorylation can only be conjectural. The value of such interpretations can be heightened, however, in light of the findings in the EDL muscles.

6.4.6 Glycogen and Metabolites in Relation to Muscle Fatigue in the EDL

Chronic underfeeding in rats does not appear to further worsen the performance of the EDL muscle during repeated stimulation. There appears to be a metabolic adaptation to caloric deprivation taking place in the
fast-twitch muscles. This observation is based on the percentage of force lost that incurred in the HYPO rats; it was the same as controls. The reasons for the unabated fatigue are discussed in terms of the unstimulated EDL glycogen, glycogenolysis in the EDL, metabolite levels, and their effects on function. Determining whether important confounders were acting in the HYPO state is essential, if the usefulness of the EDL as a valid determinant of nutritional status is to be decided.

6.4.6.1 Unstimulated EDL glycogen. Based on unstimulated glycogen concentrations reported in Table 6.3.B, glycogen levels did not fall in the unstimulated EDL of the HYPO rats. This contrasts with glycogen concentrations reported in Figure 5.4. in chapter 5, which were significantly lower than controls. The reasons for this inconsistency are not clear; however, the relationship between insulin and fatigue (Figure 6.7.) must be considered in this discussion. The negative correlation (r=-0.58) describes a diminished fatigue with higher insulin levels. The higher insulin would seemingly favour easier access of glucose to the muscle, and consequently, a greater glycogen deposit. The glycogen would, in turn, ensure a normal fatiguability.

Similar to the soleus, there appears also to be a dissociation between muscle glycogen content and fatigue in the EDL during the refeeding process; although glycogen concentrations significantly climbed in RE-1 rats, and with it muscle endurance, fatigue set in on the second day of refeeding (RE-2), even though glycogen reserves were still super-normal (Fig. 5.3. chapter 5). Moreover, despite the full recovery of muscle glycogen reserves by day 4, fatigue still persisted in this muscle (Table 6.3.B). Glycogen concentrations in the unstimulated EDL were not correlated with fatigue (F=0.64; df=1,9; p=N.S). These findings challenge previous work (29, 177) that link glycogen concentrations with endurance. While it appears this relationship may hold true for the HYPO and RE-1 rats, refeeding malnourished rats appears to disharmonize
fatigue and glycogen. This argues in favour of the idea that fatigue is not reversed by simply replenishing glycogen reserves, and suggests that glycogen concentrations do not reflect the potential for glycogenolysis.

It could be argued, at this point, that the ability of the muscle to utilize glycogen in the instances of malnutrition and refeeding may be the limiting factor, rather than the actual glycogen content in the muscle. This is discussed in the next subsection.

6.4.6.2 Glycogenolysis and Metabolites in the EDL. Net glycogenolysis in the EDL muscle was heightened with underfeeding; it remained elevated on the first day of refeeding and normalized by the 4th day of refeeding (Table 6.3.B). The compensatory reliance of the fast-twitch muscle on glycogen during underfeeding is an expected outcome, in view of the diminished glucose and insulin levels which ensued with underfeeding, and because of the recognized minor importance of serum FFA to this muscle. This is also supported by the significant fall in the G,6-P:lactate ratio (Table 6.6.B).

This kind of metabolic adaptation makes the nutritional consequences of underfeeding undetectable; the nutritional status does not negatively affect the glycogenolytic capacity of this muscle. It is noteworthy that most of the anaerobic ATP elicited during repetitive tetanic contractions would primarily be derived from glycolysis (60). This supports the necessity of an unaltered pathway in order to maintain tension, as was reported herein during malnutrition and in RE-1 rats.

The persistent fatigue on day 4 is not apparently tied to glycogenolysis, but with a persistent excess of circulating glucose relative to the decreased insulin (Table 5.7. chapter 5). This is supported by the strong negative correlation that was found between the EDL's fatigue and insulin in the RE-4 rats \( r=-0.67;F=9.9; df=1, 13; p<0.01 \). It is noteworthy that the insulin concentrations had not recovered by day 4 (Table 5.7. chapter 5).
6.4.6.3 Metabolite levels in unstimulated EDL. The metabolic adaptability of this muscle is discussed, in this subsection, using metabolite levels from the unstimulated muscles. This discussion is rooted in the premise that the metabolic integrity of the muscle prior to stimulation may influence the muscle during stimulation. Lactate levels are pertinent because lactate build-up usually describes either a heightened glycolysis and diminished oxidative phosphorylation or simply a diminished oxidative phosphorylation (357). Although lower lactate levels were observed in the unstimulated EDL (p<0.01) (Table 6.4.B), no abnormality in PFK activity was detected during the entire protocol. This is illustrated by the undisturbed ratio of F,1,6-diP: F,6-P (Table 6.4.B). In the absence of a substrate like glucose, due to caloric restrictions, the fall in lactate may simply be the reflection of a diminished substrate flux through glycolysis.

In addition, α-ketoglutarate concentrations fell with malnutrition and remained persistently low during refeeding. A fall in this metabolite, however, cannot be used with any reasonable certainty as an indication of a decline in oxidative phosphorylation. Nevertheless, using reported values in the soleus (Table 6.4.A), it can be stated that this metabolite reacted dissimilarly in the two types of unstimulated muscle. It is important to consider that the unchanged values in the soleus are likely the result of a continued feeding of the TCA cycle via β-oxidation of fat, whereas no such reliance on fat oxidation is likely taking place in the EDL. This is confirmed by the heightened glycogenolysis in the HYPO rats as previously reported (Table 6.3.B). Hence, the fall in α-ketoglutarate in the EDL assessed against the concentrations found in the soleus can more convincingly support the notion of a diminished flux of substrate through the TCA cycle.
6.4.6.4 Metabolites and function in the EDL.

In the hypocaloric rats, the metabolites in the EDL muscle, rather than support abnormalities and/or aberrations in metabolic activity during underfeeding and refeeding, as was the case in the soleus muscle, appear to describe an increase in metabolic activity in the HYPO and first-day refed rats. This heightened metabolism is demonstrated in five ways: first, there is a significant rise in glycogenolysis in the HYPO and RE-1 rats. This was previously discussed. Second, there is no visible change in ΔG,6P, ΔF,6-P, ΔF,1,6-diP in the HYPO rats (Table 6.7.B), thereby indicating a maintained glycolytic flux. Third, the F1,6-diP:F,6-P ratio is not altered (Table 6.5.B). Fourth, there is a significant rise in muscle lactate as well as in the Δ lactates (Tables 6.5.B & 7.B). Fifth, as expected, there was no evidence that glycogenolysis was relied on more heavily than oxidative metabolism, as illustrated by the unchanged Δα-ketoglutarate:Δglycogen ratio (Table 6.8.B). Similarly, there is no change in muscle fatigue with hypocaloric feeding.

In the refed rats, the first day of refeeding significantly improves the endurance of the EDL muscle (Table 6.1.B) as compared to the CN and HYPO rats. This improved performance of the muscle occurs concurrently with high muscle lactates and an elevated glycogenolysis (similar to the HYPO rats). This improved endurance can, however, be attributed to two other factors: (a) The supersaturation of glycogen may prevent abundant K⁺ loss, which normally occurs with high intensity exercise (75). The glycogen provides a buffering role that ultimately prevents a significant depolarization of the sarcolemma during repeated stimuli. Hence, while no correlation could be established between glycogen and performance, perhaps there is a significant association with intracellular K⁺. (b) There is evidence that there may be an increased access of glucose to the muscle cell as evidenced by the
normalised glucose: insulin ratio (Table 5.7, Chapter 5). This is also supported by a two-fold increase in G,6-P in the stimulated EDL (Table 6.5.B). The increased access of glucose would either supplement the glycogenolysis and eventually heighten the rate of ATP generation, or allosterically stimulate glycogen synthase (251). The data seems to support the latter.

Although it has been argued that glucose, rather than supplementing, should spare glycogen (202, 81) and increase endurance. Coyle et al. (91) found that glucose infusion did not spare glycogen, but enhanced endurance by ensuring a continued carbohydrate oxidation. The data presented in this thesis do not support a decline in glycogenolysis with what appears to be a rise in the access of blood glucose to the muscle cell. Hence, glucose appears to supplement glycogenolysis (91) and to increase energy production. Glucose appears to do this very well, as there is no evidence that the sudden increase in glucose exceeds either glycolytic and/or oxidative capabilities of the muscle; an undisturbed F,1 6-diP:F,6-P ratio and a normalization of the G,6-P:lactate ratio confirm this belief (Tables 6.5.B & 6.6.B). Hence, the rise in the EDL endurance on the first day of refeeding appears to be related to a significant glucose flux into the cell and an increased glycogenolysis.

By the 4th day of refeeding, fatigue significantly rose in this muscle. In fact, over the four recovery days, refeeding caused a significant worsening of fatigue (r=0.40; p<0.005). The reason for this increasing fatigue is not clear, as muscle biochemistry was not done in RE-2 and RE-3 rats. However, based on muscle metabolites measured on day 4, there are three contributory factors involved in the process: first, glycogenolysis normalizes on day 4. Second, glucose flux into the cell may have significantly declined, as evidenced by the fall in G,6-P values with refeeding (Table 6.5.B). Hence, refeeding may cause fatigue
to set in, on the second day, by causing glycogenolysis to decline from supernormal to normal. Third, glycogenesis may be more significant than in the CNs as supported by the more elevated G,6-P concentrations.

These results seem, from the outset, to contradict previous work with NMR, in which a rise in FADP levels was detected in the gastrocnemius of the HYPO rats. This was interpreted as a deficient aerobic and anaerobic metabolism (338). Pichard et al. (338) did not distinguish between fast-twitch oxidative fibers (red) and fast-twitch glycolytic fibers (white) that make up this particular muscle. This is an important differentiation because oxidative fast-twitch fibers have elevated mitochondrial enzymes, even greater than those of the soleus (9), and may be susceptible to malnutrition. Furthermore, Pichard et al.'s muscles were unstimulated.

The findings reported here do agree with those reported by Russell et al. (357) in that, although they found evidence that both oxidative and anaerobic metabolism were altered with 21 days of hypocaloric feeding, only the glycolytic enzymes were affected by 5 days of fasting. The results presented herein suggests that glycolytic alterations are taking place in the slow-twitch, but not in the fast-twitch glycolytic muscle, and that no aberration to oxidative metabolism appears to have taken place during underfeeding.

While fatiguability has been previously found in patients fasting for 2 weeks (354) using muscle groups with a mixture of muscle fiber types, these results successfully identify the slow-twitch fibers as possibly being responsible for the observed decline in performance. These findings suggest, therefore, that the slow-twitch fibers may be particularly sensitive to nutritional status.

6.4.7 Serum Insulin and Muscle Fatigue

In the soleus muscle, the involvement of insulin in fatiguability is not of any consequence, as demonstrated by the weak individual
correlations with insulin in the CN (r=0.25; df=1,17; p=N.S), HYPO (r=-0.62; df=1,7; p=N.S) and refeed rats (RE-1 r=0.011; df=1,13; p=N.S) (RE-4, r=-0.12; df=1,16 p=N.S). This dissociation between insulin and soleus fatigue is supported by the previous work of Paulus and Grossie (330), who found tetanic force generation in slow-twitch fibers not to be compromised by chronic hypo-insulinemia. In fact, no correlation could be made between insulin and soleus fatigue (F=3.11; df=1,67; p=N.S) of all rat groups.

There could, however, be a dependency of the soleus on an alternative fuel, namely free fatty acids. The rise in the ratios of G,6-P:lactate (demonstrating a slower glycolysis) and α-ketoglutarate:glycogen provide some credence to this belief. It has been shown, however, that energy needs of a high-intensity exercise cannot be met by the uptake of FFA (365). Therefore, the soleus’ reliance on FFA during repeated tetanic contractions cannot result in enough energy to sustain repeated tetanic contractions; the functional data presented here describe a significantly greater fatigue in the HYPO rats.

In the EDL muscle, contrary to the soleus, fatigue correlates well with insulin levels, particularly in RE-1 (r=-0.60; df=1,10; p<0.05) and RE-4 rats (r=-0.67; df=1,13; p<0.01). The regression slopes in this muscle were homogeneous, indicating that the relationship between insulin and fatigue in the EDL was the same in the CN, HYPO, and refeed animals. This means that the fatiguability of this muscle is no longer different between the feeding groups when adjusted for the covariate, insulin. This finding suggests that given the uniform impact of insulin on this muscle, no other factors or influences appear to affect directly the fatiguability of this muscle during underfeeding and refeeding. This finding supports the notion that the EDL is internally reliant on glucose access to the muscle, perhaps because it supplements glycogenolysis.
6.4.8 Blood Biochemistry and Liver Metabolism.

The serum biochemistry and liver metabolites indicate that there are no signs of anoxia due to either anesthesia or malnutrition that could alter rat metabolism. In addition, substrate availability during hypocaloric feeding and refeeding suggests a maintained oxidative metabolism with underfeeding and a normalization of glycolysis with refeeding. This is shown in five ways:

1. Serum lactate levels (Table 5.7. chapter 5) are not altered by the HYPO feeding and refeeding. Serum lactate is derived mostly from erythrocytes and muscle cells, and are mostly metabolized by the liver (168). Therefore, the uniform serum lactates reported herein (Table 5.7. chapter 5) allude to three possibilities: (a) a maintained hepatic metabolism, thereby oxidating serum lactates; (b) well oxygenated tissue; and (c) the absence of systemic disorders (168). Throughout this experiment, hypoxia was avoided and normal aerobic metabolism encouraged. Lactate values presented herein agree with the values of Robinson et al. (348).

2. Liver lactates in the HYPO rats were normal and α-keto-glutarate concentrations (Table 6.9.) were uniform throughout the experiment. This suggests a normal TCA cycle activity.

3. The ratio G,6-P:Lactate in the liver remained normal throughout the study. This confirms a normal glycolysis and oxidative metabolism. The sudden rise in lactate and G,6-P observed on the first day of refeeding are indicators of a sudden influx of glucose into the hepatic tissue. The rise in G,6-P, as in the case of the muscle, can allosterically stimulate glycogen synthase. There is no evidence, however, of a disruption in TCA cycle flux.

4. The serum FFA concentrations in the underfed rats (Table 5.7. Chapter 5) were similar to the CN values, yet were more elevated than the CN values reported by Zorzano (440). This anomaly suggests that the post-prandial period for the CN rats in the present experiment was
shorter than for the HYPO rats. Therefore the more elevated FFA is the direct result of a more elevated sucrose diet (163b) that is in the form of an easily absorbable liquid. Certainly, the amount of fat given to the control rats was not greater than their normal capacity to metabolize fat. The percent fat present in the liquid diet approximates the amount of fat normally present in the chow. Furthermore, there is no indication that the CN rats were oxidizing fat to any significant degree; both glucose and insulin levels were significantly greater than in the HYPO rats (Table 5.7. chapter 5). Hypocalorically fed rats, therefore, demonstrate serum FFA levels that are sufficiently abundant to suggest that β-oxidation of fat is taking place at a greater rate than in the CNs. These elevated concentrations, in comparison to the low insulin levels, appear to describe a milieu capable of promoting a greater oxidative phosphorylation in tissue. Although the FFA were abundant in the HYPO rats in quantities sufficient to be used in oxidative phosphorylation, it is doubtful that the rate of cellular uptake of FFA would be sufficient to meet the energy needs during the course of several high-frequency contractions (337). Furthermore, serum FFA levels fell with refeeding, and remained low for the most part until day 4 of refeeding (Table 5.7. Chapter 5). This suggests that this substrate was not fully available for oxidative metabolism. Moreover, the rise of serum insulin levels in the RE-1 and RE-4 rats (Table 5.6. Chapter 5) does not contribute to a metabolic environment that would support the notion that β-oxidation of fat reserves is taking place to any greater degree than in CN rats.

5. A significant fall in both insulin and glucose occurred in the HYPO rats. However, the fall was disproportionate and implies that there was excessive glucose relative to the insulin concentration in the underfed and refeed states. Glucose, therefore, is not likely the substrate of choice in the underfed state.
This discussion about the role substrates and metabolites play in the performance of the soleus and EDL muscles is intended to provide an interpretation of the data derived during this study. The consistency with and occasional variances from other studies have been pointed out. Based on the interpretations of the data presented earlier, several conclusions have been drawn; these are presented in the next section.

6.5 CONCLUSIONS

The data are consistent with the hypothesis that muscle function responds earlier to refeeding than structure. This is demonstrated by the recovery of the soleus' MRR, Po(N), Po(N)/g by day 4 of refeeding in the soleus. Moreover, these parameters normalized at a time when neither muscle dry mass, muscle cross-sectional area, nor muscle protein improved.

The data also supports the hypothesis that the response of the muscle to underfeeding and refeeding is fiber type specific. This is evidenced by the functional and biochemical data: During hypocaloric feeding, fatigue was observed in only the soleus muscle. Furthermore, while soleus fatigue did not recover by days 1 and 4 of refeeding, there was a marked increase in the EDL's resistance to fatigue with the first day of refeeding, followed by a subsequent increase in fatiguability in the EDL, which persists until day 4.

The high carbohydrate refeeding diet did not normalize the fatigue in either muscle. The results indicate that the macronutrient composition of the diet may not make any difference in the recovery time of function in either muscle, suggesting glucose access to the soleus may be limited because of a disproportionate build up of glucose relative to insulin. This diminished glucose uptake in combination with a readjustment from supernormal to normal glycogenolysis with refeeding, in the EDL, and a slowly recovering glycogenolysis, in the soleus, cause a persistent fatigue in these muscles during refeeding.
The link with insulin is most critical for the EDL during refeeding; there were significant correlations between fatigue and insulin in the RE-1 and RE-4 rats. The EDL, in this setting, relies on glucose to supplement glycogenolysis. In the HYPO rats, characterized by low insulin levels, there is an exclusive reliance on muscle glycogen. Providing this reserve is not depleted, it will sustain performance. By contrast, a continued greater reliance on the oxidation of fat in the soleus, during underfeeding, in combination with a diminished glycolysis prevents the generation of sufficient ATP to sustain endurance during underfeeding. It appears that oxidative phosphorylation in the soleus may, in fact, compensate for the defective glycolysis thereby preventing full recovery.

This compensatory model is justified based on the fact that the α-ketoglutarate levels are maintained despite a diminished glycogenolysis in the soleus. This is further supported by the significant rise in the α-ketoglutarate:glycogen ratio in the HYPO and RE-1 rats, and by the increase in α-ketoglutarate:lactate, which lasts until day 4. The persistence of these elevated ratios with refeeding suggests that, despite the sudden abundance of food, there is still a lingering dependence on FFA on this first day and possibly on day 4. It is this dependence that causes the sustained fatigue in the soleus, rather than a slowdown and/or aberration in oxidative phosphorylation.

Despite the abundant availability of insulin and glucose on the first day of refeeding, fatigue does not normalize in the soleus because of a subnormal glycogenolysis. This confirms the hypothesis that refeeding will not normalize fatigue by merely replenishing serum glucose concentrations. This finding helps identify the performance of the slow-twitch muscle as being particularly reflective of its metabolic integrity.

With refeeding, although glycogenolysis improves significantly (F=6.99; p<0.025), there is no significant improvement in soleus
fatigue. This hints of FFA superseding the role of glucose. The strong negative correlations between the soleus MRR and insulin, in the HYPO (r=-0.81; p<0.005) and RE-4 rats (r=-0.51; p<0.025), further supports the notion of a metabolic etiology of fatigue in the soleus. This negative correlation describes the reliance of MRR on oxidative energy during malnutrition. Any rise in insulin during the underfed state decreases oxidative phosphorylation and shifts substrate dependence in the direction of glucose and glycogen, neither of which can feed the glycolytic pathway because of a diminished glycogenolysis, an unusual build up of glucose relative to insulin, and/or inhibited hexokinase. The lingering sub-optimal insulin concentrations seen at day 4, along with the negative correlation that was seen between insulin and MRR on day 4 suggest that this muscle still metabolizes FFA for energy, albeit in smaller quantities than in HYPOs.

Glycogen concentrations in the muscles do not appear to be tied with performance in either the soleus or the EDL during refeeding. No correlation could be established between fatigue and glycogen, nor was there a link between glycogen concentrations and glycogenolysis during refeeding therapy. This disproves the hypothesis that glycogen concentrations are critical to the performance of muscle in this setting.

There is some evidence, nevertheless, that glycogenolysis plays a significant role in the performance of both muscles during repeated tetanic contractions; for example, the soleus fatigued at a time that glycogenolysis was greatly diminished in the hypocaloric state. Furthermore, evidence of an aberration of glycolysis and of a compensatory reliance on oxidative metabolism appears to be restricted to slow-twitch fibers during malnutrition. A persevering glycolytic disturbance appears to last until day 1, after which a decreased flux through glycolysis is still evident in the soleus. The significantly reduced ΔG,6-P in the RE-1 and RE-4 rats (Table 6.7.A) gives some
credence to the idea that glucose is simply not getting phosphorylated on these days, due to a slow hexokinase activity. This is especially noticeable on the first day of refeeding, when the glucose:insulin ratio is no longer abnormal. It is therefore erroneous to assume that fatigue will normalize with key glycolytic rate-limiting enzymes, like PFK. Furthermore, there is no data supporting an aberration in oxidative phosphorylation.

In the EDL, no correlation could be made between fatigue and glycogenolysis when a regression was established vs. all four refeeding days. It appears that glucose access to the muscles may be an important factor for the EDL, thereby relating the issue of performance, in this muscle, to a lack of substrate. The MRR in this muscle contrasts with that of the soleus, in that it does not appear to reflect the state of muscle energetics. Despite a significant rise in glycogenolysis in this muscle, the MRR is significantly reduced. The MRR may be more reflective of myofibrillar erosion, which may damage the S.R. This is supported not only by the fall in MRR, which reflects sarcoplasmic reticulum calcium uptake, but also by the Po(N) and Po(N)/g, which remain suboptimal by day 4. The diminished tetanus could be the result of a compromised sarcoplasmic reticulum calcium release channel. The work done on the hearts of these rats confirms that despite a normalized Ca-ATPase, there still remains a persistent aberration in calcium ion uptake and calcium release channels (319). The full recovery of only the soleus MRR would reflect recovery from two perspectives: (a) the return to a normal balance between glycogenolysis/glycolysis and oxidative phosphorylation in the soleus; and (b) a recovery of calcium kinetics within the cell.

The MRR and the percentage of force lost over repeated tetany of both muscle types have been identified as key parameters of muscle function;
these functions appear to reflect the nutritional status in that they did not readily recover with the normalization of serum glucose, insulin and glycogen concentrations.

Although the Po(N) did decline in the soleus and EDL of the HYPO rats, the soleus' sub-optimal performance appeared to be precipitated, in part, by an insulin-induced membrane depolarization. This needs to be further investigated.

The EDL's Po(N), in contrast, was unaffected by insulin, and therefore the decline was representative of muscle atrophy which may have affected the sarcoplasmic reticulum.

There is also an apparent need to control for insulin concentrations when measuring the percent force lost in the fast-twitch muscles. A great deal of inter-rat variability could be attributed to the varying levels of insulin during refeeding. Postprandial times should be very specific.

The conclusions drawn in this study have much broader applications, even though a rat model was used to study muscle function. Because fast-twitch and slow-twitch muscles were specifically studied here, it is possible to functionally and biochemically differentiate what is occurring in fast-twitch and slow-twitch muscles during malnutrition and early refeeding. The findings presented here suggest that the slow-twitch muscle's glycolytic pathway is compromised with malnutrition and that glycolysis does not automatically recover with the sudden availability of substrate that occurs with immediate refeeding. This is important knowledge, because the muscle that is often assessed in the clinical setting is the adductor pollicis muscle; it is made up of 80% slow-twitch fibers. In addition, half the muscle fibers in the diaphragm are slow-twitch fibers (358). Therefore, our knowledge relevant to how these muscle types react can further enhance the nutritional management of patients.
A conventional nutritional assessment process generally attempts to answer four basic questions, when used in a clinical setting (42): (a) Is the patient severely or mildly malnourished? (b) Does the patient require special nutritional management? and if so, how much? (c) What are the risks of morbidity and mortality? And, (d) how is the patient responding to nutritional therapy? In light of the findings presented here and those of other researchers, it can be stated that muscle function would be capable of answering, early on, three of these critical questions.

(a) With respect to the detection of malnutrition, it appears that the detection of poor nutritional status is possible by measuring the MRR and endurance. Both of these parameters fell in the soleus. In addition, the MRR significantly fell in the EDL. Muscular wasting takes place in both type of muscles (315, 314), and therefore, compromised function is not unexpected. The reasons for muscle fatigue have generally been related to decreases in neuromuscular transmission, in excitation-contraction-coupling, in membrane potential, and in substrate; or because of metabolic aberrations and fiber atrophy (337, 158, 62, 105, 121). Within the context of malnutrition, evidence is provided here suggesting that an aberration in glycolysis and a slowdown in glycolytic flux are likely responsible for muscle fatigue. These are valid determinants of an individual’s health and nutritional status, as they reflect the individual’s ability to be ambulatory.

(b) With respect to establishing the risks of morbidity and deciding whether a patient requires special nutritional intervention, the literature has shown that muscle function has a predictive index of outcome of 78% in preoperative patients (435). Malnutrition causing muscle wasting of significant importance will likely produce an anomalous MRR. This has been demonstrated in this thesis. Nishio and Jeejeebhoy (315) have shown that a 2-day fast in rats, a protocol known to produce insignificant losses of muscle dry mass (314), did not
produce a rise in the relaxation time. Consequently, a disturbance of the MRR is likely the result of moderate to severe malnutrition, and therefore, can serve as an index that justifies nutritional support. The extent of the support given is dependent on energy needs, and on the capacity of the body to handle the food (368, 235).

(c) With respect to determining if the rat is responding to nutritional therapy, both the MRR and the endurance have been shown here to be reflective of metabolic abnormalities. The endurance of the slow-twitch muscle remained diminished after 4 days of refeeding; this occurred concurrently with a persistently slow rate of glycolytic flux (Table 6.7.A). The endurance of the EDL muscle appears to be compromised by an insulin resistance during refeeding; this is inferred from the fatigue still observed in the RE-4 rats despite the normalized glycogenolysis and glycolytic flux in these rats. Furthermore, insulin resistance has previously been associated with refeeding high sucrose and high fat diets to rats (161). The work was focused on the very early period of refeeding, demonstrating that the functional changes occurring with refeeding are not strictly dependent on the availability of substrates like glucose and glycogen or on muscle mass. Therefore, recovery of function, as was reported by Russell et al. (355) and Lopes et al. (257), in the clinical setting, are likely linked with the recovery of glycolysis and insulin resistance. This needs to be verified with more extensive studies. Furthermore, Christie and Hill (73) have shown, in the clinical setting, a rapid improvement in muscle function despite the absence of protein gain. This group observed a maximal effect of intravenous nutrition on all physiological measurement within 4 days of refeeding; function had not normalized, but did improve dramatically in that period of time. They looked at respiratory and skeletal muscle function using, among other parameters, maximal voluntary grip strength, maximal involuntary force and force expiratory volume. Although maximal tetanic force did improve, this measure has
been shown to correlate strongly with insulin in the malnourished state, raising the possibility that improvements in this parameter may be attributed to simply attaining a maximal depolarization of the membrane with insulin.

Observations made from this work, lead to the propositions that both the endurance and MRR of muscle should be used in assessing the nutritional status of individuals as these functional parameters appear to reflect best the integrity of glycolysis and insulin sensitivity in the slow-twitch muscles. This is especially significant since the muscles most important in ambulatory patients are, in fact, slow-twitch muscles.
CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

In the three preceding chapters, the results derived from the investigations into the research objectives have been presented for the study. In each of these chapters, the results have been discussed and compared to the relevant literature. Based on this, conclusions germane to the original hypotheses have been drawn. In this chapter a broader picture of how this study fits into current clinical nutrition research is examined, and the relevance of the findings in the process of recovery from undernutrition are presented.

From the outset, several controversial positions were taken for the nutritional assessment model that was developed in this thesis.

First, the primary hypothesis of this thesis (3.1.1) describes muscle function as more sensitive, and therefore, more capable of responding earlier to refeeding than changes in body composition. It is a position that puts into question the value of relying solely on body composition data for nutritional assessment. This is far from being a novel position; the validity of using anthropometric and biochemical data has been criticised for some time (263, 79, 185, 183, 173) because of the inaccuracy and poor sensitivity of such data in early detection. Collins et al. (79) conclude that only gross changes can be detected using the parameters of weight, height and skinfolds. This is generally accepted on the basis of the wide inter-observed variations and on the insensitive nature of the measurements.

Second, it was hypothesized that the response of muscle to undernutrition and prompt refeeding would depend on muscle fiber type. Physiological and endocrine differences have been previously pointed (9, 212) out, and in doing so, the reasons for the fatiguable and non-fatiguable characteristics of the fast-twitch and slow-twitch muscles
(270) were emphasized. However, undernutrition can threaten the integrity of the contractile machinery, which consequently affects function similarly in both muscles. The two muscle types likely react dissimilarly because of the distinctive-ness of their metabolic properties.

Third, in this thesis it was proposed that neither substrate availability, glycolysis, nor glycogenolysis can fully explain the fatigueability of muscle in the malnourished state and during recovery. In order to demonstrate this, it was the intent of this thesis to explore the question of muscle fatigue in relation to glycolytic aberrations in undernutrition and sequential refeeding. Such an explanation required a study of muscle performance from two main perspectives. The first dealt with measuring parameters of function. The second perspective considered the function of muscle concurrently with metabolic markers of glycolysis and oxidative phosphorylation as major determinants of fatigue and recovery from under-nutrition. The first issue to examine is the adequacy of the control and HYPO rats.

7.1 ADEQUACY OF CONTROLS AND HYPO RATS

The general discussion on the relevancy of the present findings depends on the assumption that the malnourished rat model presented herein is truly representative of an uncomplicated form of malnutrition. The accuracy of this interpretation is of course conditional on demonstrating that the control (CN) animals were well nourished, and therefore, maintained their physiological and metabolic integrity. This was clearly demonstrated in several ways within chapter 5.

First, the mean weight gain observed in the CN animals (3.74 g/day), following a brief adaptation period, suggests that nutritional requirements were sufficient to ensure normal growth. This average weight gain is particularly noteworthy since it has been demonstrated (172, 248) that rats, when fed a liquid elemental diet, have a tendency
to gain less weight than chow fed rats. Furthermore, the rate of weight gain observed over 10 days in these adult rats comply with growth rates estimated from age-for-weight tables made available by the supplier (Charles River, Montreal) (data not provided).

Correspondingly the muscle force, MRR, and fatigue were not different over the 10 days of study. Therefore, the control data, over the 10 days, were stable and were therefore pooled into one reference group of CN rats.

Adult male rats were specifically chosen, because of the slower growth rates of well fed controls, which for the purpose of this study, would help minimize differential growth changes in lean mass over the duration of the study.

Second, the HYPO rats lost 20-25 percent of their initial body weight. This amount of weight loss has been often observed in the clinical setting in cases of anorexia nervosa, and therefore, represents a substantive loss, but one clearly insufficient to precipitate morbidity.

7.2 CONFOUNDERS AFFECTING MUSCLE FATIGUE IN MALNUTRITION

It became evident when reviewing the literature, that the physical wasting observed in undernutrition, whether it be detected by the significant weight loss or the dramatic changes in skinfolds measurements, is relied on heavily as a clinical marker of malnutrition. Therefore, it would be expected that if muscle function is an accurate index of nutritional status, it would follow the wasting and then the restoration of body structure. This is in fact what is reported over long-term recovery. In this thesis, however, the early period of refeeding was investigated, in part, to determine the extent which tissue deposition and energy metabolism contribute to recovery.
There are, however, important confounders that must be considered in this scenario of malnutrition and refeeding. The first possible confounder concerns the diminished blood and oxygen supply to the muscle. Excessive fluid loss due to diuresis in the early stages of weight loss could cause a decrease in blood volume and a capillary collapse. In this thesis, no attempt was made to measure either muscle blood flow or oxygenation. The assumption that blood flow or oxygenation was not compromised in the rats is made on the basis of three key findings: (a) the Po(N) values in CNs and HYPO rats resemble those of other studies (316); (b) Russell et al. (358) have found, in malnourished patients, no aberrations in interstitial capillaries, despite a histochemical muscle analysis that suggested advanced fiber degeneration, suggesting that the capillary-to-fiber interface was increased to ensure that oxygen needs are met; and (c) Pichard et al. (338) have previously shown blood gases (PCO₂ and HCO₃⁻) and blood pH to be unaffected by the anesthetic or diet treatment.

The second confounder relates to the diet and the issue of substrate availability, the degree of caloric restriction, macronutrient and micronutrient composition, feeding route and feeding time. The third confounder is associated with a possible central nervous system defect in action potential generation or in an anomaly in excitation-contraction coupling.

In the following sections, the impact of these three confounders on the onset of muscle fatigue and recovery during malnutrition will be explored, and in the process, the reliability of function as an assessor of malnutrition and of the recovery process will be assessed.

7.2.1 Body Composition Changes

The structural erosion ensuing from muscle wasting would likely be, from the outset, the most visible determinant in muscle fatigue. This could be argued physiologically in terms of myofibrillar erosion and
damage of the contractile machinery. The cross-sectional area (CSA) of the muscle, representative of fiber size, and therefore strength, is also indicative of myofibrillar integrity. The erosion of any one of these would contribute to a fall in CSA.

In this work, data were corrected for wet weight and, (in a subset of the population) for CSA, yet the results demonstrated a persistent tension depression in the fast-twitch muscles. It was also obvious that, despite refeeding for 4 days, neither body weight, muscle CSA, muscle weight and protein in the soleus and EDL, nor organ protein concentrations normalized, except as noted for the liver. In this early period of refeeding, however, functional improvement was observed in both fiber types during refeeding (using Po(N)); in the case of soleus, full recovery was observed while measuring the Po(N)/g and the MRR. The fact that fatigue did not improve with glycogen deposition in either muscle eliminates the possibility that muscle fatigue was merely a reflection of muscle glycogen depletion. These observations raise the likelihood that muscle function may in fact be a more sensitive tool for assessing recovery from malnutrition than the more traditional parameters of composition.

The data presented in this thesis are consistent with the hypothesis that muscle performance in the slow-twitch muscle is, in reality, a sensitive index of protein-calorie malnutrition and refeeding, because it recovered with refeeding, while parameters of body composition such as body weight, muscle weight, protein, cross-sectional area and serum proteins, continued to be affected by malnutrition. Body composition was in fact particularly insensitive to early refeeding.

7.2.2 Multidimensional Influences of Diet

7.2.2.1 Substrate availability. The diet is of particular interest, since its energy and macronutrient composition are thought to influence recovery significantly as it influences the relative
availability of carbohydrate, fat and protein to the biological system. This, in turn, determines the protein and fat depositions in the body as well as substrate availability in the form of muscle and hepatic glycogen, muscle phosphocreatine, serum glucose, and free fatty acids. The availability of substrate can be seen as critical, as muscle is fuelled by substrate. The latter is, in fact, the basis of the energy reserve of the muscle. It can be manipulated by the diet and made to be more or less abundant despite severe physical wasting. The question that is asked here is whether the performance of muscle is simply a reflection of the availability of the substrate. If this is so, then the function of muscle would rely on the fuel from the last meal eaten. In contrast to this assertion, the performance of the muscle was shown, in this thesis, to be unaffected by glycogen concentrations measured in the muscle prior to stimulation, giving further support to the notion that function is a significant index of protein-calorie malnutrition rather than glycogen content. Furthermore, the serum glucose was most abundant on the first day of refeeding, yet the soleus remained highly fatiguable. By day 4, despite a continued feeding regimen, fatiguability still persisted even though glycogenolysis had normalized in the soleus. However, serum glucose concentrations remained sub-optimal, and the glucose:insulin ratio was still significantly elevated. Hence, the infusion of substrate, in itself, does not automatically cause muscle to recover.

7.2.2.2 Rat eating pattern. Worthy also of consideration is the time at which the meal was eaten prior to any functional or compositional observations. With this in mind, the eating pattern of the rat was investigated when fed a HYPO diet. The data shown in this thesis demonstrates that the HYPO rats adopt a diurnal eating behavior. Insulin was also found to remain consistently low throughout the day in
HYPO rats that are fed early on the previous day. Insulin, therefore, is not likely to be a major confounder as long as rats consume their diet in the morning the day before the investigation.

7.2.2.3 Severity of caloric restriction. The degree of caloric restriction prior to refeeding is a confounder that most likely affects compositional changes and metabolism. The studies in this thesis used hypocaloric diets that were isovolemic with control diets, which permitted the implementation of 7 days of weight loss that simulated the chronic nature of undernutrition, often found in the human population. Generally, total fasting is not found among those that are chronically malnourished, rather a persistently low nutrient and caloric intake is more commonly observed. Fasting also increases the likelihood of metabolic lagtime with refeeding, hence a hypocaloric feeding model was adopted.

7.2.2.4 Diet composition. An important confounder is the composition of the diet. The likelihood of a micronutrient deficiency was corrected for in order to focus more specifically on the implications of macro-nutrient composition. This was possible because the hypocaloric diets contained the same micronutrient density as controls. Moreover, the micronutrient composition of the diets favoured a maintenance of serum electrolyte concentrations in the HYPO rats that were similar to CN values. A high carbohydrate refeeding diet was chosen as the most representative of current nutritional support therapies aimed at maximizing recovery in the clinical setting.

7.2.2.5 Refeeding method. Ad libitum ingestion of a liquid diet was chosen to avert physiological problems associated with elevated infusion rates (typically found in TPN). For example, some electrolyte alterations caused by elevated infusion rates could be life threatening, and hyperglycemia (encountered in enteral feeding) can alter normal metabolic activity. The idea here was to present the simplest model
possible that simulated the least aggressive refeeding protocol. Therefore, the changes in function and metabolism cannot be attributed to the changes precipitated by invasive and continuous feeding practices.

7.2.3 Centrally Derived Action Potential, Neuro-Muscular Transmission, and Excitation-Contraction Coupling

The argument that fatigue was derived from an abated action potential arising from a defect of the central nervous system has been corrected for in this thesis, by electrically stimulating the isolated sciatic nerve. An action potential, sufficiently large to incite a tetanus, is thereby generated. Whether this action potential reaches the muscle, however, is dependent on the integrity of the neuromuscular transmission. In fact, a defect at this step is critical, for there would not be a depolarization of muscle membrane, and therefore, a propagation of the action potential. The normalization of the soleus' tetanus, after correction for muscle weight in the HYPO rats, confirms that the neurological transmission at the junction with muscle was preserved in the underfed state.

Under normal unstarved conditions, the likelihood that fatigue does not originate from post-synaptic changes has been advanced by Jones et al. (220). They were successful at directly stimulating isolated muscle and showing a pattern of force loss during high frequency stimulation that was similar to muscles stimulated via the nerve. In keeping with the idea of a post-synaptic defect, other investigators have looked at the integrity of the membrane potential. An abnormal membrane potential arising from a fall in intracellular $K^+$ ($[K^+]_i$) should be considered a probable cause for the post-synaptic etiology of fatigue, even though such values were not calculated in this thesis. There are several reasons for such an assumption: (a) Fong et al. (130) have specifically shown the soleus to be more susceptible to $K^+$ loss than the fast-twitch
gastrocnemius. (b) Offerijns et al. (321) and Otsuka et al. (412) have shown that potassium depleted muscles are more susceptible to depolarization by insulin. And, (c) the negative relationship found between insulin and Po(N)/g in the HYPO rats describes a possible depolarization of the sarcolemma. The tension (Po(N)) was corrected for weight, thereby ruling out an important confounder. Nevertheless, the association between insulin and tension remains simply correlational because intracellular potassium levels were not measured. This unsubstantiated potassium loss, however, is not likely to be of great importance in comparing the HYPO rats with the CN’s or refed rats, since the tetanic tension normalized in this muscle once it was corrected for weight. Furthermore, multiple regression analysis demonstrated that in the soleus muscle, insulin did not contribute significantly to the prediction of Po(N), rather muscle wet weight seemed to be mildly important. Hence, the depolarization of the membrane is not likely a significant factor in the soleus fatigue observed in the HYPO and RE-1 rats.

This is further supported by the absence of a correlation between insulin and fatigue, in the soleus, and by the similarity in regression slopes that was calculated between fatigue and insulin in the HYPO and CNs. The covariate insulin was significant, indicating that other factors were contributing to the fatigue. The force depression in the EDL is not likely arising from a fall in membrane potential because it has been previously shown that the fast-twitch muscle is not susceptible to declines in [K'], (130) during malnutrition. This is further supported by the absence of a correlation between Po(N) and insulin in the HYPO rats. Furthermore, the Po(N)/g wet weight was positively correlated, albeit moderately (r=0.63), with insulin in the HYPO rats specifically. The positive correlation simply confirms that insulin increases the action potential of muscle when cellular potassium concentrations are normal (437).
A multiple regression of Po(N) vs. insulin and muscle wet weight over all rat groups, revealed that insulin did not contribute as a significant predictor of Po(N). The wet weight of the muscles, however, explained = 50% of the Po(N) response (r=0.69). Even though both muscles lost about the same percentage of their initial dry weight (10% loss for the soleus and 16.7% for the EDL) as well as similar protein loss (16% for the soleus and 13% for the EDL), the significance of the loss appears to be more pronounced in the EDL muscle, as this muscle did not normalize once corrected for weight. This suggests a disturbance of some intracellular mechanism. Hain et al. (166b) found that phosphorylation by the kinase proteins modulated the release of Ca\(^{2+}\) from the S.R. Many of these studies were done in vitro. Gechtman et al. (140b) observed an increase in Ca\(^{2+}\) efflux from the S.R. with phosphorylation of the junctional channel found in the S.R.-T.tubule system. There may be some argument that malnutrition could cause a diminished phosphorylation because of a less active kinase, resulting in the closure of some releasing channels and a decrease in tension.

The last step from which an aberration in function may take place is the excitation-contraction coupling. Should there be a failure in this mechanism, there would conceivably be an indication of low frequency fatigue (121). There was, however, no such observation in either the soleus or the EDL, as represented by the unchanged F10/Fmax ratio. This was likely indicative of a uniform loss of tension across all stimulation frequencies, giving further credence to the idea that low-frequency fatigue was not taking place in the malnourished state.

The slower MRR in both the soleus and EDL muscles during HYPO feeding is a finding that suggests several possible mechanisms. First, there may be decreased Ca\(^{2+}\) uptake into the S.R., thereby resulting in cytosolic Ca\(^{2+}\) accumulation, which would interfere with mitochondrial activity if the excessive calcium was, in turn, pumped into the mitochondrial matrix by a Ca\(^{2+}\) pump (206). The intracellular accumulation could also result
from a diminished efflux from the cell, the consequence of a diminished Ca\(^2+\)-Mg\(^2+\) ATPase system and of a less active ATP stimulated Na\(^+\)-Ca\(^2+\) exchange in the sarcolemma (214). Ca\(^2+\)-Mg\(^2+\) ATPase also acts as a Ca\(^2+\) pump ensuring Ca\(^2+\) uptake by the S.R. (222b). Kabbara and Stephenson (222b) report maximal accumulation of Ca\(^2+\) in the S.R. at Mg\(^2+\) concentrations varying between 1 and 10 mM in both the EDL and soleus muscles. Significant changes in myoplasmic Mg\(^2+\) levels can occur in many physiological conditions (discussed in 222b), and there is some question whether malnutrition could cause a significant efflux of Mg\(^2+\) from the muscle and thus alter MRR. Pichard et al. (338), however, using a feeding protocol similar to that used here, measured free muscle Mg\(^2+\) levels in control, 2 day fasted, hypocalorically fed and refed rats. They found no difference in free Mg\(^2+\) levels between all groups using an ANOVA. In this thesis, Mg\(^2+\) is not a likely problem. In addition, the intracellular accumulation of calcium could be derived from a decline in energy generation resulting from mitochondrial abnormalities or hypothyroidism (422). A lack of free available energy arising from mitochondrial abnormalities could result, in turn, in an impaired energy supply to the biological system, and thereby decreasing Ca\(^2+\) taken up by the S.R.

Second, there is evidence that a slower MRR may arise from a membrane associated Ca\(^2+\)/Calmodulin-dependent protein kinase that can modulate the influx and efflux of Ca\(^2+\) from the S.R.. This is conducted through direct phosphorylation of the Ca\(^2+\)-ATPase in slow-twitch, but not in fast-twitch, muscles (174b). The phosphorylation causes a 2-fold increase in ATP hydrolysis and Ca\(^2+\) transport into the S.R. Most of the work on phosphorylation has been conducted thus far in vitro. Hain et al. (166b) argue that while there is some evidence for phosphorylation of the transverse tubules and ryanodine receptors, there is very little in situ evidence for phosphorylation in the excitation-contraction
coupling process in skeletal muscle. Furthermore, there have been no publications documenting the impact of underfeeding on phosphorylation, although there is some suggestion that malnutrition may decrease calmodulin levels (214). There is by contrast stronger evidence relating the fall in MRR to an energy deficit; Russell et al. (357) in discussing the literature, reports that free energy of ATP has been correlated with the muscle relaxation rate. This group provides ample evidence supporting a fall in $\Delta G_{\text{ATP}}$ during malnutrition (295, 338).

Fourth, structural alterations of the S.R. membrane could also contribute to a slower S.R. calcium uptake (417). This, however, was not verified in this study. Fifth, a possible reduction could occur in cross-bridge detachment (417), making less free calcium available for entry into the S.R. A reduction in available calcium could also arise from less phosphorylation of myosin light-chain kinase by cyclic AMP-dependent protein kinase. Miller et al. (296) have shown that such phosphorylation in tracheal smooth muscle is important in reducing the affinity of the kinase for Ca$^{2+}$. The activation of myosin-light-chain-kinase is critical, however, only in smooth muscle (296b). Finally, the sixth possibility is the cooling of the muscle, which is also related to a fall in MRR (422); this would also precipitate fatigue. The latter, however, was not likely as the internal temperature of the rat, and the muscle temperature were maintained within an acceptable range with the use of a heat lamp.

In summary, the biochemical data reported in this thesis, does not support the notion of a mitochondrial defect being of any significance in either muscle. The increase in the $\alpha$-ketogl: lactate ratio can only suggest the possibility of a slowdown in $\alpha$-ketoglutarate dehydrogenase (7). In fact, no evidence of a glycolytic or a glycogenolytic slowdown was found in the soleus. The loss of energy resulting from the slowdown in these pathways could support the use of the "energy generating deficit model" in explaining the fall of MRR in the soleus; the membrane
pump systems would be compromised in this instance. The possibility that both the calmodulin and calsequestrin calcium binding protein levels had fallen in the case of the EDL cannot be ruled out either, nor can the notion of an altered phosphorylation of Ca$^{2+}$-Mg$^{2+}$ ATPase or releasing channels by protein kinase. In addition, there may be a reduced cross-bridge detachment, thereby reducing the availability of free calcium. Another possibility could be a damaged S.R. membrane that would contribute to a decline in calcium movement into the S.R. (417, 148). It is also possible that such membrane damage, caused by prolonged undernutrition, could also affect the calcium release from the S.R. and therefore the contractions of the muscles.

Work done on the hearts of the rats used in this thesis, has shown that the sarcoplasmic reticulum Ca$^{2+}$-uptake was significantly diminished (319) in the HYPO rats. Furthermore, a diminished Ca$^{2+}$ dependent ATPase was observed in the HYPO rats and recovered in the RE-1 rats. In this thesis, no attempt was made to determine if in fact there was such a problem in Ca$^{2+}$ movement in the S.R. of the skeletal muscle, since these same measures were not done on the fast-twitch or slow-twitch muscles; however, these results suggest that the diminished MRR may originate from energy dependent enzymes and pumps. There is also some indirect evidence presented herein which suggests that the abated MRR in the EDL may not simply be the result of poor energy generation; rather, it might result from decreased calcium-binding protein concentrations. This would have to be verified.

### 7.3 Major Determinants of Fatigue and Recovery in Malnutrition

The role of diet in recovery is not limited to the deposition of proteins or to the replenishment of substrate reserves. In fact, no body composition parameters normalized with refeeding despite a visible recovery of function, except for the liver. As for substrate, the
relationship with fatigue is not a simple one, in that it is not a matter of substrate simply being present in large enough concentrations, nor is it a matter even of substrate accessing the cell. This is shown by the disassociation between glycogen concentrations and fatiguability, and between insulin and fatiguability in the soleus (the regression of FFA vs. soleus fatigue could not be done). The soleus is a slow-twitch muscle, and therefore, relies on aerobic oxidation for energy. During malnutrition, this reliance appears to be heightened because of a slowdown in PFK, glycogenolysis and a rise in the glucose:insulin ratio. On the first day of refeeding, despite the abundance of macronutrients, the shift to glycolysis is not immediate, therefore causing a persistent fatigue.

However, a strong relationship was established between insulin and EDL fatigue. This muscle is not greatly dependent on aerobic oxidation, but rather on glycogenolysis. This is exemplified by the heightened dependency on glycogenolysis observed in the HYPO and reford rats. Substrate from diet appears quite important because serum glucose appears to be able to supplement glycogenolysis and to improve fatigue. However, within the context of the entire four day recovery period, the unrelenting glucose build-up relative to insulin appears to contribute to fatigue even though refeeding has been occurring for four days. In this thesis, the issue of whether an improvement in this resistance and in function is related in a dose dependent fashion to the carbohydrate content of the diet has not been resolved. It has been demonstrated, however, that even a high carbohydrate diet does not normalize function, glycolysis (in soleus) and the glucose:insulin ratio within 4 days of refeeding. Hence, a complete picture of fatigue is not given by merely considering the concentration of insulin or the glucose:insulin ratio.

Insulin’s action is in fact regulated, first, by the availability of membrane-anchored receptors (93, 254). Second, the affinity of these receptors to insulin is variable; the affinity decreases in instances of
acute hyper-insulinemia and hyperglycemia (208), and has been found to increase in obese patients following a fast. Others have found the affinity to decline upon re-feeding (20) and with the ingestion of an oral glucose load (302), a high sucrose diet (102, 427a) or a high fat diet (47, 162). Camps et al. (59) investigated the dose response relationship of insulin's action and concluded that there may be a need to distinguish between the maximal response to insulin, which is dependent on receptor numbers, and insulin sensitivity. The latter is more complex in that it involves three mechanisms: (a) the binding affinity, which can change according to differences in equilibrium constants (161); (b) the coupling between receptor and tyrosine kinase activation (161, 59); and (c) changes in processes occurring at the post-receptor level.

James et al. (211) found important differences in how glucose uptake and glycogen synthesis responded to insulin; the response of red muscle was 3- to 4-fold greater than in white muscle, suggesting a greater number of receptors. James et al. (212) later concluded that the differences related also to a more significant tyrosine kinase receptor activity in the red muscles. On a purely speculative basis, this difference in insulin receptor density and sensitivity can partly explain the non-fatiguable and fatiguable nature of red and white muscle, respectively. In the hypocalorically fed rats, the sensitivity of the receptors is likely elevated (208), suggesting that any decreased access of glucose to the cell is likely related to insufficient insulin. Upon refeeding a high glucose liquid diet, there is some evidence that the sensitivity to insulin receptors may have declined (302), thereby encouraging a continued fatigue in the soleus and precipitating fatigue in the EDL on the first day of refeeding, despite normalized insulin concentrations. The persistent fatigue by day 4 may be arising from a combination of low circulating insulin relative to glucose concentrations and a persistent insulin receptor insensitivity.
The role of diet in the short term refeeding period appears to be limited to that of ensuring, in the case of the soleus, a shift from oxidative phosphorylation to glycolysis and, in the case of the EDL, a reduction of the rate of glycogenolysis back to normal levels, and in the process increasing its fatiguability. Hence, refeeding does not produce a linear recovery of function, but can precipitate, as in the case of the fast-twitch glycolytic muscle, a temporary recovery on day 1 followed by a lasting depression in tension.

The recovery of the MRR in the soleus indicates a normalization of both S.R. activity and the function of Ca-ATPase and other energy base pumps and enzymes. This is a valid conclusion, since MRR has been shown to be linked to energetics (96) and to remain independent of contraction mechanics. Based on the measurements of the heart muscle in the hypocaloric rats, it was clear that the capacity for ATP production and utilization were compromised as depicted by a slower creatine kinase, as well as a reduced Ca\(^{2+}\) ATPase and myofibrillar ATPase activity (319). It was also clear that recovery of these enzymes occurred with refeeding. Although these enzymatic measurements were not done in the skeletal muscles, the information collected from the heart is suggestive of a defect and of a subsequent recovery, in energetics with refeeding. The MRR in the EDL, however, did not recover nor did the Po(N). This difference compared to the soleus suggests that the EDL may be more sensitive to damage of the Z band of the sarcomere, as has been shown by Russell et al. (358). This finding emphasizes the point that MRR in the EDL of the malnourished rat may not be a reliable index of energetics, but one indicative of structural recovery.

In conclusion, these findings carry several repercussions in the clinical setting. First, the use of both fatigue and MRR in assessing muscle function during malnutrition and recovery appear to be very sensitive assessment tools. More specifically, the repercussion of using both the MRR and fatigue in the assessment of slow-twitch muscle, is
that they seem to reflect the recovery of glycolysis, and therefore, of a greater energy generating ability. The persistent fatigue, however reflects a possible insulin resistance and perhaps insulin receptor insensitivity. The MRR of the EDL suggests a sensitivity to the structural integrity of the muscle. The second clinical repercussion of these findings concerns the metabolic lag-time taking place in the soleus on the first day of refeeding. Fatigue and MRR remain persistently subnormal upon introducing a normal calorically dense diet. This may also relate to insulin receptor insensitivity, which in turn may be the consequence of a high glucose load. The findings do not support aggressive nutrition rehabilitation, especially since there is in addition a significant depression in EDL resistance following the first day of refeeding and a persistent fatigue in the soleus.

These findings, in the clinical setting, point to the relevancy of measuring various parameters of muscle function as a nutritional assessment tool. Important confounders for this study have been taken into account, and the findings presented here are consistent with the literature. In the next chapter, the main features of the study will be summarized and highlighted.
CHAPTER 8

SUMMARY OF THESIS

Traditionally, the effects of a hypocaloric intake and refeeding have been defined in terms of wasting and restoration of total lean body mass. However, recent studies have suggested that changes in muscle performance occur earlier, and correlate better with risk of postoperative complications than changes in body composition. However, in these previous studies, changes in muscle performance have not been controlled for muscle wasting, fiber type, and electrolyte-micronutrient deficiency. In addition, in these previous studies, the effect of hypocaloric feeding on glycogen levels and glycolysis have not been correlated with performance, especially in a muscle composed of white fast-twitch glycolytic muscle fibers, nor were studies performed on the internal organ mass and diaphragm mass concomitantly with function.

It was first hypothesized that changes in muscle performance during malnutrition and refeeding are independent of muscle size, weight, cross-sectional area and body composition, including serum proteins, visceral mass. Second, it was also hypothesized that the muscle’s performance in relation to underfeeding and refeeding is muscle type specific. Third, it was hypothesized that muscle fatigue was dependent on altered glycolytic and glycogenolytic activity. Fourth, it was hypothesized that the fatiguability of the muscle was independent of substrate availability. Hence, glycogen concentrations and glucose concentration did not significantly influence whether a muscle would regain normal endurance during refeeding. Rather, it was the fiber type-specificity of the muscle in relation to the abundance of glycolytic and oxidative pathways in the specific fibers that influenced the muscles’ response to underfeeding and sudden refeeding. The soleus (oxidative slow-twitch red fibers) and the EDL (glycolytic fast-twitch
white fibers) in adult male Wistar rats were studied in the control state, after a week of hypocaloric feeding, and during refeeding for 4 days. The feeding protocol caused a 20% decline in body weight in the HYPO rats, and a significant weight recovery during the first 4 days of refeeding; yet, muscle dry mass, and muscle protein levels did not change with refeeding, nor did the dry weights and protein levels of the internal organs change, except for the liver.

The maximal relaxation rate of both muscles were slowed by hypocaloric feeding. The tension of a single tetanic contraction (Po(N)) significantly improved with refeeding and remained independent of muscle mass. When the tension was normalized for muscle weight (Po(N)/g), it was evident that hypocaloric feeding did not affect the tension of the single tetanus in the soleus, whereas it did in the EDL. The performance of both muscles is summarized in Table 8.1.

**TABLE 8.1 Summary of muscle function data, glycogen status & net glycogenolysis of the soleus and EDL in HYPO and refed rats.**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Po(N) SOL</th>
<th>Po(N) EDL</th>
<th>MRR SOL</th>
<th>MRR EDL</th>
<th>Fatigue SOL</th>
<th>Fatigue EDL</th>
<th>Glycogen SOL</th>
<th>Glycogen EDL</th>
<th>△ Glycogen SOL</th>
<th>△ Glycogen EDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYPO</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>△</td>
<td>↑</td>
</tr>
<tr>
<td>RFED DAY1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>△ N</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>RFED DAY4</td>
<td>↑ N</td>
<td>↓</td>
<td>↑ N</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓ N</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

↑ - Function, concentration or flux has increased compared to controls
↓ - Function, concentration or flux has decreased compared to controls
→ - Function, concentration or flux has not changed compared to controls
↓↓ - Value is lower than control and previous diet treatment
↑N - Values have increased to normal values
↓N - Values have decreased to normal values
Hypocaloric feeding also caused an increased fatigue in the soleus, but did not in the EDL. Fatigue occurred in the soleus even though glycogen was still present in the muscle, although the concentration was significantly lower than in controls. Replenishing glycogen reserves in this muscle on the first day of refeeding did not cause the soleus fatigue to recover. The EDL, by contrast, significantly fatigued during refeeding, even though glycogen concentrations had recovered in this muscle.

In the soleus, the ratio F1,6-diP:F,6-P significantly fell with malnutrition, indicating a slower glycolysis. Although refeeding normalized this ratio and glycogenolysis, fatigue still persisted in this muscle. The EDL did not show any signs of glycolysis being compromised by underfeeding, rather there was evidence of a heightened glycogenolysis which appeared to sustain tension. Refeeding caused the glycogenolysis to return to normal, and in so doing, precipitated fatigue, which did not recover by day 4.

The impact of malnutrition and refeeding on muscle performance was dissimilar between muscles, and affected function independently of mass, protein, cross-sectional area of muscle, and glycogen concentrations. The assessment of the slow-twitch muscle fatigue during malnutrition appears to be reflective of muscle metabolic anomalies that far exceed the mere importance of substrate availability.

The issues of insulin sensitivity and S.R. function need to be further explored in order to gain a more complete understanding of the muscle’s response to underfeeding and refeeding. In this thesis, the understanding of the role of muscle in nutritional assessment has been highlighted, and a clear association with muscle metabolism and substrate utilization has been defined. These results represent an important step in validating muscle function as a tool for nutritional assessments.
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Phosphatase, phosphofructokinase and phosphoenol-pyruvate carboxykinase in white muscle and red muscle. Biochem.J. 103:391-399


ANNEX-1
# Standardized Recipe of Rat Diet

## High CHO Diet:

<table>
<thead>
<tr>
<th>1 Day</th>
<th>3 Days</th>
<th>4 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAVASOL 10% - 25.69</td>
<td>TRAVASOL 10% - 77.07 ML</td>
<td>TRAVASOL-102.76</td>
</tr>
<tr>
<td>DEXTROSE 50% - 36.56 ML</td>
<td>DEXTROSE 50% - 109.7 ML</td>
<td>DEXTRSE-146.24</td>
</tr>
<tr>
<td>INTRALIPID 20% - 5.46 ML</td>
<td>INTRALIPID 20% - 16.4 ML</td>
<td>INTRLPID-21.84</td>
</tr>
<tr>
<td>D.WATER - 0 ML</td>
<td>D.WATER - 0 ML</td>
<td>D.WATER - 0 ML</td>
</tr>
<tr>
<td>TOTAL VOLUME: - 69.25 ML</td>
<td>TOTAL VOLUME: - 207.75 ML</td>
<td>TTL VOL-277.0</td>
</tr>
</tbody>
</table>

### Electrolytes X 1

<table>
<thead>
<tr>
<th>Electrolytes</th>
<th>Dose Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>410.60 uL</td>
</tr>
<tr>
<td>Mg</td>
<td>58.96 uL</td>
</tr>
<tr>
<td>P</td>
<td>137.52 uL</td>
</tr>
<tr>
<td>Cu</td>
<td>36.35 uL</td>
</tr>
<tr>
<td>Zn</td>
<td>47.50 uL</td>
</tr>
<tr>
<td>Cr</td>
<td>339.70 uL</td>
</tr>
<tr>
<td>Mn</td>
<td>29.00 uL</td>
</tr>
<tr>
<td>Fe</td>
<td>0.975 uL</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.00</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.1216 g</td>
</tr>
<tr>
<td>K KAcet</td>
<td>0.3485 g</td>
</tr>
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</table>

**Electrolytes: 596.38 uL**

**Osmolality:**

### Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Dose Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-1000</td>
<td>5.48 uL</td>
</tr>
<tr>
<td>Folate</td>
<td>5.48 uL</td>
</tr>
<tr>
<td>Berocca-C</td>
<td>1.65 uL</td>
</tr>
<tr>
<td>Vit-E</td>
<td>0.40 uL</td>
</tr>
<tr>
<td>Vit-B12</td>
<td>0.11 uL</td>
</tr>
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</table>

**Total Vol:** 13.12 uL

### Electrolytes X 3

<table>
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<tr>
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<th>Dose Volume</th>
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<tr>
<td>Ca</td>
<td>1.23 ML</td>
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<td>Mg</td>
<td>164.88 uL</td>
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<td>P</td>
<td>412.59 uL</td>
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<tr>
<td>Cu</td>
<td>109.05 uL</td>
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<tr>
<td>Zn</td>
<td>87.60 uL</td>
</tr>
<tr>
<td>Cr</td>
<td>142.50 uL</td>
</tr>
<tr>
<td>Mn</td>
<td>1019.10 uL</td>
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<tr>
<td>Se</td>
<td>87.00 uL</td>
</tr>
<tr>
<td>Fe</td>
<td>2.925 ul</td>
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<tr>
<td>NaOH</td>
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<tr>
<td>NaCl</td>
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<tr>
<td>K KCl</td>
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</tr>
<tr>
<td>NaAcet</td>
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**Electrolytes: 1789.14 uL**

### Electrolytes X 4

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<th>Electrolytes</th>
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</thead>
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<tr>
<td>Ca</td>
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<td>Mg</td>
<td>219.84 uL</td>
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<td>P</td>
<td>550.12 uL</td>
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<tr>
<td>Cu</td>
<td>145.40 uL</td>
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<tr>
<td>Zn</td>
<td>116.80 uL</td>
</tr>
<tr>
<td>Cr</td>
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<tr>
<td>Mn</td>
<td>1358.80 uL</td>
</tr>
<tr>
<td>Se</td>
<td>116.00 uL</td>
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<tr>
<td>Fe</td>
<td>3.90 uL</td>
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<tr>
<td>NaOH</td>
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<tr>
<td>NaCl</td>
<td>0.4864 uL</td>
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<tr>
<td>KCl</td>
<td>1.3940 uL</td>
</tr>
<tr>
<td>NaAcet</td>
<td>1418 uL</td>
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</table>

**Electrolytes: 2385.52 uL**

### Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Dose Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-1000</td>
<td>16.44 uL</td>
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<tr>
<td>Folate</td>
<td>16.44 uL</td>
</tr>
<tr>
<td>Berocca-C</td>
<td>4.95 uL</td>
</tr>
<tr>
<td>Vit-E</td>
<td>1.20 uL</td>
</tr>
<tr>
<td>Vit-B12</td>
<td>0.33 uL</td>
</tr>
</tbody>
</table>

**Total Vol:** 39.36 uL

### Protein:

- **13%**

### CHO:

- **73%**

### Fat:

- **13%**
**1 DAY**

TRAVASOL 10% - 49.40 mL
DEXTROSE 50% - 31.62 mL
INTRALIPID 20% - 4.62 mL
D.WATER - 0 mL
TOTAL VOLUME: - 86.80 mL

**ELECTROLYTES X 1**

<table>
<thead>
<tr>
<th>DOSE VOLUME</th>
</tr>
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<tbody>
<tr>
<td>Ca 410.60 uL</td>
</tr>
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<td>Mg 54.96 uL</td>
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<tr>
<td>P 58.70 uL</td>
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<tr>
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<tr>
<td>Cr 47.50 uL</td>
</tr>
<tr>
<td>Mn 339.70 uL</td>
</tr>
<tr>
<td>Fe 0.975 uL</td>
</tr>
<tr>
<td>NaCl 0.4004 g</td>
</tr>
<tr>
<td>NaAcet 0.0000 g</td>
</tr>
</tbody>
</table>

ELECTROSOL: 596.38 uL
PH: 7.38
Osmolality: 305 mOsm/kg

**VITAMINS**

<table>
<thead>
<tr>
<th>Multi-1000</th>
<th>Folate</th>
<th>Berocca-C</th>
<th>Vit-E</th>
<th>Vit-B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.48 uL</td>
<td>5.48 uL</td>
<td>1.65 uL</td>
<td>0.40 uL</td>
<td>0.11 uL</td>
</tr>
</tbody>
</table>

TOTAL VOL 13.12 uL

**ELECTROLYTES X 3**

<table>
<thead>
<tr>
<th>DOSE VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca 1.23 mL</td>
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<tr>
<td>Mg 164.88 uL</td>
</tr>
<tr>
<td>P 412.69 uL</td>
</tr>
<tr>
<td>Cu 109.05 uL</td>
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<tr>
<td>Zn 87.60 uL</td>
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<tr>
<td>Cr 147.10 uL</td>
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<tr>
<td>Mn 1019.00 uL</td>
</tr>
<tr>
<td>Se 87.00 uL</td>
</tr>
<tr>
<td>Fe 2.925 uL</td>
</tr>
<tr>
<td>NaCl 0.6012 g</td>
</tr>
<tr>
<td>NaAcet 1.0455 g</td>
</tr>
</tbody>
</table>

ELECTROSOL: 1789.14 uL

**VITAMINS**

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TOTAL VOL 39.36 uL

**ELECTROLYTES X 4**

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<thead>
<tr>
<th>DOSE VOLUME</th>
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<tbody>
<tr>
<td>Ca 1.642 mL</td>
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<tr>
<td>Mg 219.84 uL</td>
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<tr>
<td>NaCl 0.8016 uL</td>
</tr>
<tr>
<td>KCl 1.3940 uL</td>
</tr>
<tr>
<td>NaAcet 0.0000 uL</td>
</tr>
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ELECTROSOL: 2385.52 uL

**VITAMINS**

<table>
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<tr>
<th>MVI-1000</th>
<th>Folate</th>
<th>Berocca-C</th>
<th>Vit-E</th>
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</tr>
</thead>
<tbody>
<tr>
<td>21.92 uL</td>
<td>21.92 uL</td>
<td>6.60 uL</td>
<td>1.60 uL</td>
<td>0.44 uL</td>
</tr>
</tbody>
</table>

TOTAL VOL 52.48 uL

**DIET - LIQUID (CN)**

1 DAY

TRAVASOL 10% - 49.40 mL
DEXTROSE 50% - 31.62 mL
INTRALIPID 20% - 4.62 mL
D.WATER - 0 mL
TOTAL VOLUME: - 86.80 mL

**ELECTROLYTES X 1**

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ELECTROSOL: 596.38 uL
PH: 7.38
Osmolality: 305 mOsm/kg

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TOTAL VOL 13.12 uL

**ELECTROLYTES X 3**

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ELECTROSOL: 1789.14 uL

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<tbody>
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TOTAL VOL 39.36 uL

**ELECTROLYTES X 4**

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ELECTROSOL: 2385.52 uL

**VITAMINS**

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</tr>
</thead>
<tbody>
<tr>
<td>21.92 uL</td>
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<td>6.60 uL</td>
<td>1.60 uL</td>
<td>0.44 uL</td>
</tr>
</tbody>
</table>

TOTAL VOL 52.48 uL

**PROTEIN:** 25%
**CHO:** 64%
**FAT:** 11%
### HIGH CHO LOW ENERGY (HYPO)

<table>
<thead>
<tr>
<th>1 DAY</th>
<th>3 DAYS</th>
<th>4 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAVASOL 10% - 6.42 ML</td>
<td>TRAVASOL 10% - 19.26 ML</td>
<td>TRAVASOL - 25.68</td>
</tr>
<tr>
<td>DEXTROSE 50% - 9.14 ML</td>
<td>DEXTROSE 50% - 27.42 ML</td>
<td>DEXTROSE- 36.56</td>
</tr>
<tr>
<td>INTRALIPID 20% - 1.36 ML</td>
<td>INTRALIPID 20% - 4.08 ML</td>
<td>INTRALIPID- 5.44</td>
</tr>
<tr>
<td>D.WATER - 0 ML</td>
<td>D.WATER - 0 ML</td>
<td>TTL VOL- 75.44</td>
</tr>
<tr>
<td>TOTAL VOLUME: - 18.86 ML</td>
<td>TOTAL VOLUME - 56.58 ML</td>
<td>TOTAL VOLUME - 56.58 ML</td>
</tr>
</tbody>
</table>

#### ELECTROLYTES X 1

<table>
<thead>
<tr>
<th>DOSE VOLUME</th>
<th>Ca 410.60 uL</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Mg</td>
<td>54.96 uL</td>
</tr>
<tr>
<td>*P</td>
<td>137.53 uL</td>
</tr>
<tr>
<td>*Cu</td>
<td>36.25 uL</td>
</tr>
<tr>
<td>*Zn</td>
<td>29.20 uL</td>
</tr>
<tr>
<td>*Cr</td>
<td>47.50 uL</td>
</tr>
<tr>
<td>*Mn</td>
<td>339.70 uL</td>
</tr>
<tr>
<td>*Se</td>
<td>29.00 uL</td>
</tr>
<tr>
<td>*Fe</td>
<td>0.975 uL</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0270 g</td>
</tr>
<tr>
<td>NaAcet</td>
<td>773.67 uL</td>
</tr>
</tbody>
</table>

#### ELECTROLYTES X 3

<table>
<thead>
<tr>
<th>DOSE VOLUME</th>
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<tbody>
<tr>
<td>*Mg</td>
<td>164.88 uL</td>
</tr>
<tr>
<td>*P</td>
<td>176.10 uL</td>
</tr>
<tr>
<td>*Cu</td>
<td>412.59 uL</td>
</tr>
<tr>
<td>*Zn</td>
<td>109.05 uL</td>
</tr>
<tr>
<td>*Cr</td>
<td>87.60 uL</td>
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<tr>
<td>*Mn</td>
<td>29.25 uL</td>
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<tr>
<td>*Se</td>
<td>87.00 uL</td>
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<tr>
<td>*Fe</td>
<td>0.975 uL</td>
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<tr>
<td>NaCl</td>
<td>0.0270 g</td>
</tr>
<tr>
<td>NaAcet</td>
<td>2321.01 uL</td>
</tr>
</tbody>
</table>

#### ELECTROLYTES X 4

<table>
<thead>
<tr>
<th>OSE VOLUME</th>
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<tbody>
<tr>
<td>*Mg</td>
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</tr>
<tr>
<td>*P</td>
<td>234.80 uL</td>
</tr>
<tr>
<td>*Cu</td>
<td>550.12 uL</td>
</tr>
<tr>
<td>*Zn</td>
<td>142.50 uL</td>
</tr>
<tr>
<td>*Cr</td>
<td>190.00 uL</td>
</tr>
<tr>
<td>*Mn</td>
<td>1358.80 uL</td>
</tr>
<tr>
<td>*Se</td>
<td>116.00 uL</td>
</tr>
<tr>
<td>*Fe</td>
<td>3.90 uL</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0270 g</td>
</tr>
<tr>
<td>NaAcet</td>
<td>3094.68 uL</td>
</tr>
</tbody>
</table>

#### ELECTROSOL: 596.38 uL

<table>
<thead>
<tr>
<th>PH=</th>
<th>OSMOLALITY=</th>
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<tbody>
<tr>
<td></td>
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<td>1.60 uL</td>
<td>0.44 uL</td>
<td>52.48 uL</td>
</tr>
</tbody>
</table>

**PROTEIN: 13%**  
**CHO: 74%**  
**FAT: 13%**