ENZYMATIC PROFILES OF SKELETAL MUSCLES FROM HARBOR SEALS (Phoca vitulina) AND FIN WHALES (Balaenoptera physalis).

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ABSTRACT

The enzymatic organization of muscle tissue usually is examined in only a select few muscles of any one animal species. However, because the functional demands placed on individual muscles can vary so widely from muscle to muscle, it is inappropriate to generalize findings from one or two muscles to muscle tissue in general. The differences or similarities in metabolic machinery between skeletal muscles of a wide functional range provides crucial information with respect to a particular animals' whole body metabolism. Nowhere is this understanding more important than in the diving marine mammal which must operate as a closed system (with respect to oxygen supply) while submerged. The goals of this thesis are: 1) to provide a broad body of information on the metabolic organization of a large cross-section of marine mammal muscles, both functionally and with regard to location, 2) to assess the implications of the enzyme differences between muscles to the diving habit, and 3) to compare the metabolic organization of skeletal muscle among several species of marine mammal with different diving abilities and habits.

A series of 13 enzymes were measured in 21 skeletal muscles of the harbor seal, Phoca vitulina. In addition, 23 enzyme activity ratios were calculated and analyzed for these muscles. A similar analysis of 22 muscles from fin whales, Balaenoptera physalis, was conducted -- including 7 key enzymes and 15 activity ratios. Overall, both the maximum activities and the enzyme activity ratios are consistent with

the idea that marine mammal muscle is typical mammalian muscle, exhibiting few significant differences from terrestrial species with respect to catabolic enzymes. The only obvious exception to this in the species examined is observed with fin whale locomotory muscle which has extremely high activities of lactate dehydrogenase (over 2000 units/gm wet wt at 25°C) due to an apparent scaling phenomenon. Tight control of this high potential glycolytic flux is indicated by pyruvate kinase activities that scale downward.

Comparisons of enzyme relationships between muscles of harbor seals seem to indicate a very aerobically poised metabolic make-up. This is especially true with respiratory and locomotory muscles, which also show a high tendency to utilize fat. This pattern of enzyme activities and activity ratios in the locomotory muscles of harbor seal is evidence that muscle contractile activity while diving is powered primarily through oxidative pathways and largely based on fat as fuel. The majority of non-locomotory muscles appear to be more able to function anaerobically utilizing carbohydrate. This pattern may correlate with circulatory redistributions while diving that preferentially fuel the locomotory muscles with oxygen, leaving the inactive muscles significantly more hypoperfused and, therefore, candidates for energy saving O_2 sparing (metabolic depression). Fin whales exhibit an opposite pattern, with enzyme profiles more typical of "white" muscle. Unlike harbor seals, the locomotory muscles of fin whales are consistently the least oxidatively poised of the muscles examined. This apparently more anaerobic nature of

fin whale muscle is possibly complicated by scaling adaptations, but appears to be a real phenomenon.

The examination of three to four skeletal muscles from each of three additional phocid seal species from Antarctica, leopard seals (Hydrurga leptonyx), crab-eater seals (Lobodon carcinophagus), and Weddell seals (Leptonychotes weddelli) confirm that the harbor seal pattern of enzyme profiles is fairly consistent among phocid seals. By these criteria skeletal muscles of phocid seals (particularly the locomotory and respiratory muscles) appear to be designed for sustained aerobic metabolism during diving regardless of the habits or diving capabilities of the seal.

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CHAPTER 1:

GENERAL INTRODUCTION

Historical background. The remarkable diving abilities of certain air-breathing animals has intrigued scientists for well over 200 years (Boyle 1760; Bert 1870). Yet complete understanding of how these divers endure prolonged apneic periods on such a regular basis is still elusive. The pioneering work of Irving (1934; 1938) and Scholander (1940; 1962; 1963) provided the crucial information from which all subsequent investigations of diving in marine mammals and birds have developed. Their discovery of a series of physiological responses to enforced dives in a number of animal species -- apnea, bradycardia, peripheral vasoconstriction, and circulatory redistribution -- elucidated the central mechanisms underlying the enhanced diving tolerance of marine mammals and other expert divers (Irving 1939; Scholander 1962; Butler and Jones 1982; Elsner and Gooden 1983). This series of responses, commonly referred to as the "diving response", appears to be a nearly universal phenomenon among animals when confronted with asphyxia (Scholander 1962, 1963, 1964; Irving 1964; Robin 1966; Andersen 1966, 1969; Jones and Johansen 1972; Ridgway 1972; Kerem and Elsner 1973a; Hochachka 1980; Kooyman et al. 1981; Butler and Jones 1982; Elsner and Gooden 1983; Hochachka and Somero 1984). However, marine mammals in particular seem to have developed these mechanisms to an exceptional degree.

For a time there was controversy between laboratory and field studies with regard to the diving response. While enforced diving in the lab invariably produced a drastic employment of all aspects of the response (Scholander et al. 1942a, 1942b; Elsner et al. 1966a, 1966b, 1969, 1978; Zapol et al. 1979; Kjekshus et al. 1982; Blix et al. 1983), voluntarily diving birds and mammals exhibited a much less pronounced and sometimes absent diving response (Scholander 1962; Kooyman and Campbell 1972; Jones et al. 1973; Woakes and Butler 1975; Butler and Woakes 1979; Kooyman et al. 1980; Kanwisher and Gabrielson 1981; Butler and Jones 1982; Stephenson et al. 1986). This apparent discrepancy resulted from the comparison of breath-hold diving in the presence and absence of exercise (Issekutz et al. 1976; Butler 1982; Castellini et al. 1985; Hochachka 1986a). Recent elegant experiments on the freely diving Weddell seal have clarified this issue (Guppy et al. 1986; Hill et al. 1987). Diving seals do appear to employ aspects of the classical diving response in a graded fashion, depending on the demands of the dive and the habits of that particular individual (Hill et al. 1987; Elsner et al. 1989). This modified diving response is readily apparent in both short (feeding) and long (exploratory) dives (Guppy et al. 1986).

Following the discovery of the diving response, the search began for the biochemical adjustments that might accompany such profound physiological alterations. Marine mammals were found to possess an unusually large oxygen carrying capacity, which is positively correlated with diving ability (Kooyman et al. 1980). This capacity results from a greatly increased weight specific blood volume (Andersen 1966; Lane et al. 1972), as well as high hematocrit and hemoglobin percentages (Lenfant

1969; Lenfant et al. 1969, 1970; Ronald et al. 1969; Vallyathan et al. 1969; Zapol et al. 1989). The release of oxygenated blood into the general circulation is even regulated by a spleen that functions as a "scuba tank", gradually releasing stored red blood cells as the dive progresses (Qvist et al. 1986; Zapol et al. 1989). In addition, total oxygen stores in marine mammals are benefited by high muscle myoglobin levels (Robinson 1939; Scholander 1942a; Blessing and Hartschen-Niemeyer 1969; Lenfant et al. 1970; George et al. 1971; Kooyman et al. 1980). However, by definition, this large total oxygen store relative to body size is insufficient to allow aerobic metabolism to supply the energy needs of the diving animal beyond the "aerobic dive limit" (ADL) (Kooyman et al. 1980, 1983). Yet a number of marine mammals (Weddell seals, elephant seals, and sperm whales, in particular) are renowned for their ability to regularly and repeatedly surpass the ADL for extended periods (Irving 1939; Kooyman et al. 1983; LeBoeuf 1988, 1989).

It was originally believed that the capacity for such prolonged dives is the result of an enhanced ability for anaerobic glycolysis to supplement the inadequate oxygen stores (Scholander 1940; George et al. 1971; Hochachka 1981). The high buffering capacity of marine mammal muscle (Castellini and Somero 1981), and the high glycogen content of heart and brain (Kerem et al. 1973) would help support such increased anaerobic metabolism. However, enzyme data on a number of tissues was conflicting (see Kooyman et al. 1981 for review). Several studies indicated enhanced levels of glycolytic enzymes (George et al. 1971; Simon et al. 1974; Hochachka and Storey 1975; Murphy et al. 1980), while others found normal or even low levels (Castellini et al. 1981; Behrisch and Elsner

1984a). Rapid recovery from dives (Olsen et al. 1969; Denison and Kooyman 1973; Kooyman et al. 1980; LeBoeuf 1988, 1989) up to and even beyond the ADL would seem to deny the existence of excessive anaerobic activity. Of course there is, undoubtedly, a certain amount of anaerobic metabolism occurring in hypoperfused tissues as a distinct, albeit lower than expected, lactate washout profile attests (Scholander 1940; Scholander et al. 1942a, 1942b; Hochachka and Murphy 1979; Zapol et al. 1979; Kooyman et al. 1980; Hochachka 1986b).

The apparent lack of sufficient oxygen to fully support the longer dives of marine mammals (Irving et al. 1935; Scholander 1940; Kooyman et al. 1983; Guppy et al. 1986; Qvist et al. 1986; Kooyman 1989), coupled with the relatively low contribution of anaerobic metabolic pathways to overall metabolism during the dive (above) seems to leave the diving animal with a distinct "energy gap". The solution to this energetic shortfall likely involves some type of metabolic depression in selected tissues of the diving marine mammal (Hochachka 1986b; Guppy et al. 1986; Qvist et al. 1986; LeBoeuf 1988, 1989). If the combined metabolism of these metabolically depressed tissues (ie. kidney, inactive muscle) and the necessarily active tissues, such as brain and locomotory muscle, were lower than the resting metabolic rate (RMR), then available oxygen stores in the diving animal would be able to "stretch" over a much longer time period. Since all estimates of the ADL assume a metabolic rate while diving at least equal to the RMR, a lower overall metabolic rate during the dive would greatly increase the time prior to the ADL. Such an extended ADL in marine mammals would help explain their lack of exceptional

anaerobic capacity, low lactate washout, and absence of a significant oxygen debt upon resurfacing.

Hypoperfusion and metabolic depression. It is clear that a large portion of peripheral circulation is shut down to varying degrees in enforced dives of harbor seals (Elsner et al. 1966a; Elsner 1969), penguins (Millard et al. 1973), ducks (Johansen 1964), and Weddell seals (Zapol et al. 1979; Murphy et al. 1980). However, this decrease in perfusion is much less pronounced and quite variable from tissue to tissue during voluntary dives (Elsner et al. 1966a; Kooyman and Campbell 1972; Jones et al. 1973; Stephenson et al. 1986; LeBoeuf 1988). This difference between the laboratory and field settings greatly confounds the determination of what type of perfusion patterns each tissue is experiencing in the wild; as a result, the specific tissues and organs that are hypoperfused and, therefore, candidates for metabolic depression during the dive is still very much in question.

The marine mammal brain is clearly an organ that must remain active during diving. Its high requirement for oxygen is confirmed by the nearly complete and continuous perfusion of blood it receives throughout the dive (Elsner 1966; Kerem and Elsner 1973b; Dormer et al. 1977; Elsner et al. 1978; Zapol et al. 1979). However, it does have a fairly high capacity for utilizing anaerobic glycolysis and regularly releases small amounts of lactate (Kerem et al. 1971; Kerem and Elsner 1973b; Murphy et al. 1980) -- a feat that brain tissue of terrestrial mammals is incapable of accomplishing (Lekven et al. 1973). Of course, an exception to this inability in terrestrial species is the unusual hypoxia tolerance of fetal and

newborn animals (Dower et al. 1959), and hibernators (Bullard et al. 1960). The capacity for anaerobic glycolysis in the brains of marine mammals is potentially important at the end of extremely long dives when oxygen partial pressures may go as low as 8 - 10 torr (Ridgway et al. 1969; Elsner et al. 1970; Kerem and Elsner 1973b). Arterial oxygen levels this low are incapable of sustaining normal brain function in terrestrial mammals (Meyer et al. 1962).

The hearts of marine mammals also retain varying degrees of perfusion during dives (Blix et al. 1976; Kjekshus et al. 1982). However, the drop in cardiac output that accompanies diving bradycardia (Elsner et al. 1964; Andersen 1966; Murdaugh et al. 1966; Zapol et al. 1979; Kjekshus et al. 1982) greatly reduces the energy requirements of this tissue. In fact, coronary blood flow in restrained grey seals may drop as low as 10% of the pre-dive values (Blix et al. 1976); and periodic interruptions of blood flow through the coronary arteries of marine mammals (Elsner et al. 1981; Elsner et al. 1985) indicates a partial dependence by cardiac muscle on anaerobic metabolism. Lactate is regularly released by the heart, although the overall metabolism of the heart remains primarily aerobic (Kerem and Elsner 1973b; Murphy et al. 1980; Kjekshus et al. 1982). Similar situations in the heart of terrestrial mammals result in irreversible damage (see Katz 1977).

Other major organs of marine mammals such as the kidneys, liver and lungs suffer from extreme hypoperfusion in experimentally dived animals (Schmidt-Nielsen et al. 1959; Murdaugh et al. 1961; Andersen 1966; Elsner et al. 1966a, 1970, 1978; Blix 1976; Blix et al. 1983; Hochachka et

al. 1977, Zapol et al. 1979; Behrisch and Elsner 1984; Guppy et al. 1986; Hochachka and Guppy 1987). Seal kidney seems particularly resistant to lack of oxygen; isolated kidneys recover full function following 1 hour of anoxia (Halasz et al. 1974), a condition which dog kidneys are incapable of withstanding. Other than the adrenal glands, which retain almost normal perfusion levels (Johansen 1964; Elsner et al. 1978; Jones et al. 1979), the remainder of tissues in marine mammals (ie. muscle, skin, blubber, gut) also appear to be significantly hypoperfused, at least occasionally, during dives (Elsner et al. 1966a, 1978; Zapol et al. 1979; Blix et al. 1983).

Unlike the above experiments on restrained animals which seem to maximize the perfusion changes, voluntarily diving animals exhibit a much more complex pattern. In addition to the brain, heart, and adrenals, some skeletal muscle is clearly being perfused during free dives (Murdaugh et al. 1961; Halasz et al. 1974; Zapol et al. 1979; Butler and Jones 1982; Elsner and Gooden 1983); and during long bouts of repeated diving with very little surface time, Weddell (Kooyman et al. 1980; Castellini et al. 1981; Davis et al. 1983; Hill et al. 1987; Castellini et al. 1988) and elephant seals (LeBoeuf 1988, 1989) both continue to metabolize food, have good renal and hepatic function, and maintain core temperature and normal blood chemistry -- indicating that these tissues are not as hypoperfused as laboratory dives would indicate. This has led Kramer (1988) to suggest that these seals might be more appropriately termed surfacers than divers.

The large amount of metabolic activity that seems to be occurring during diving bouts makes the determination of which tissues are available for

metabolic depression even more difficult. Since the oxygen consumption of a tissue is closely tied to oxygen delivery (Pappenheimer 1941; Whalen et al. 1973; Idstrom et al. 1986), a low blood flow should result in a lower metabolic rate (see Butler and Jones 1982; Elsner and Gooden 1983; Hochachka 1986b). This is, in fact, observed in ischemic peripheral organs of several animal species (Meren et al. 1986; Hochachka and Guppy 1987), and there are hints of this in diving birds and marine mammals (Zapol et al. 1979; Butler and Jones 1982). However, details on which tissues are actually experiencing the hypoperfusion and, therefore, available for this metabolic depression are scarce.

A crucial factor in the metabolic makeup of such tissues is the presence of a reversed Pasteur effect (Hochachka 1986b; Hochachka and Guppy 1987). Hypoxia sensitive tissues and animals exhibit a large Pasteur effect, attempting to make-up for oxygen lack by greatly increasing glycolytic flux (Hue 1982; Hochachka 1986b; Hochachka and Guppy 1987; Suarez 1988; Suarez et al. 1989); while hypoxia tolerant species are able to reduce glycolytic flux in the face of arrested oxygen metabolism (Storey 1985; Harris et al. 1986; Hochachka and Guppy 1987). Seal liver is clearly possessed of this capability (Hochachka et al. 1988). Other tissues of marine mammals that exhibit this ability, except for the brain which typically is incapable of a reversed Pasteur effect (Hochachka and Guppy 1987), are not known at present; although there must be others that are metabolically depressed during dives, based on the apparent reduction in whole body metabolic rate that occurs during the dives of a number of marine mammals (discussed below).

The resting metabolic rates of marine mammals are slightly higher than comparably sized terrestrial mammals (Irving 1969, 1972; Elsner et al. 1977; Blix and Steen 1979). And even though capable of increasing their metabolic rate 6 - 8 times resting levels (Elsner and Ashwell-Erickson 1982; Castellini et al. 1985; Davis et al. 1985), actively diving Weddell seals have metabolic rates below RMR (Kooyman 1981; Guppy et al. 1986; Hochachka 1986b; Hochachka and Guppy 1987). In fact, the longer the dive, the lower the metabolic rate (Castellini and Kooyman 1989). Decreased metabolic rates as a survival strategy are a widespread phenomenon in the animal kingdom (see Elsner and Gooden 1983; Hochachka and Somero 1984; Hochachka and Guppy 1987 for literature). However, rather than couple metabolic depression to inactivity, diving animals are involved in extensive swimming and feeding activity in the midst of their lowered overall metabolic rate.

The ability of diving marine mammals to participate in such large amounts of muscular activity while still maintaining a lower than resting metabolic rate is at the heart of the diving problem. Muscle tissue is responsible for approximately 30 % of the RMR (McGilvery 1979); and when active, muscle can consume up to 90% of the available oxygen, as well as producing significant energy via anaerobic pathways. This is readily apparent within marine mammals and diving birds -- where high performance species (dolphins, penguins, and seal lions) regularly overwhelm the diving response with high metabolic rates as a result of exercise demands (Hochachka 1986a, 1989b). Other species of marine mammal (ie. Weddell and elephant seals) are less active and succeed in

maintaining a metabolic rate lower than RMR when diving (Hochachka and Guppy 1987; Hochachka 1989b).

Relatively low average swimming speeds are typical of the freely diving Weddell seal (Littlepage 1963; Hochachka 1989a), although occasional bursts would likely be required for prey capture. Most recent evidence points to this muscular activity being powered by aerobic metabolism. The fact that heart rate increases in diving seals when there is an increase in activity (Guppy et al. 1986; Hill et al. 1987) indicates a need for increased perfusion to working muscle to supply oxygen for catabolic processes, as well as to remove the end products of metabolism. Kooyman and coworkers (1980) were the first to seriously consider fat as the major fuel for diving seals. Since then, both harbor seals (Davis 1983) and Weddell seals (Castellini et al. 1985) have been indicated to greatly depend on the aerobic catabolism of fatty acids and/or triglycerides while diving, as well as during rest and exercise. Such aerobic utilization of fats would make sense for diving marine mammals in part because of their diet. Animals with a diet low in carbohydrates, like marine mammals, tend to have a decreased ability to transport and metabolize glucose in many tissues (Kettlehut et al. 1980). The oxidation of fats rather than carbohydrate would also be advantageous in sparing substrate for the central nervous system which has an obligatory dependence on glucose (Murphy et al. 1980). In addition, since lactate is the major end product of anaerobic glycolysis in mammals (Hochachka et al. 1975), the low lactate washout upon resurfacing would be largely due to a low rate of flux through glycolysis in skeletal muscle. Although lactate oxidation by lung (Murphy et al. 1980), muscle, and liver (Hochachka 1986b) may partially explain

the low lactate washout, Davis' (1983) data on harbor seals indicates a strong tendency to recycle rather than oxidize lactate due to the high fat/protein, low carbohydrate diet.

So it would appear that the perfused, aerobically powered, active muscle is an unlikely candidate for metabolic depression. However, a large portion of the muscle mass of diving animals is probably inactive for much of the dive. Such non-working muscle could very well be hypoperfused and, consequently, a significant contributor to lowered metabolic rates.

Although the mechanisms that cause decreased metabolism under hypoxic conditions are not fully understood (Aw et al. 1987; Hochachka and Guppy 1987), there is one very straightforward factor that is causing part of the metabolic depression of diving animals. A simple Q_{10} effect due to decreased body temperature is partially responsible for the lowered overall metabolic rates observed during dives. Decreased body temperatures have been noted in diving ducks (Andersen 1959) and seals (Scholander et al. 1942b; Elsner et al. 1975; Hammel et al. 1977; Kooyman et al. 1980). Hill and coworkers (1987) have even noted an anticipatory drop in the body temperature of freely diving Weddell seals -- the size of the temperature drop being directly related to the length of the ensuing dive. Such temperature effects would be especially prevalent in the hypoperfused tissues.

Other than the obvious temperature effects on metabolic rate, one likely candidate for reducing the energy demand of various tissues of marine mammals during dives is channel arrest (Hochachka 1989b). A decrease in

the "wasteful" pumping of ions through the ion channels of cell membranes has been shown to be important to the hypoxia tolerance of lower vertebrates (Sick et al. 1982a, 1982b; Hochachka 1986b; Rosenthal et al. 1988). A similar strategy, although probably insufficient for the needs of more active marine mammals, could play a major role in the diving strategies of the less active divers (ie. Weddell seals). In fact, the few investigations of this ability in marine mammal tissues indicate that it could be an important adaptation. The livers of phocid seals do appear to be less "leaky" than those of other mammals, with the better divers exhibiting the more significant ability (Hochachka et al. 1988). Rat liver, for example, is incapable of invoking this adaptation to a great extent when confronted with hypoxic conditions (Aw et al. 1987). Hong (1989) has also demonstrated channel arrest mechanisms in harbor seal kidney. To what extent this, or other mechanisms for decreasing energy demand, exists in other marine mammal tissues remains to be seen.

Although evidence is beginning to accumulate in support of metabolic depression as part of the solution to the apparent energetic shortfall during diving bouts of marine mammals, most investigations of diving have overlooked the potential impact of metabolic arrest mechanisms (Hochachka and Somero 1984). In spite of the clearly lowered overall metabolic rate -- comparison of energy requirements to available oxygen stores (Hochachka 1986b) indicates that a much higher lactate production is necessary to compensate for oxygen lack (assuming a metabolic rate = RMR) than is ever observed for such dives -- the specific tissues that are experiencing metabolic depression are unknown. However, it is probable that some muscle tissue (inactive, hypoperfused muscle) is contributing a

large portion of the lowered metabolic rates; while other skeletal muscles (active, perfused, locomotory muscles) are at the opposite end of the spectrum, highly active in comparison to the resting state and driving overall metabolism up. To minimize the impact of these muscles on the oxygen consumption of the diving animal another strategy has been proposed -- maximizing the efficiency of their ongoing metabolic processes.

Increased efficiency of metabolic activity. Increased efficiency can take many forms, but it is always with one purpose: to increase the amount of work accomplished per ATP consumed. Muscle work efficiency can be increased by minimizing the cost of swimming. For example, harbor seals have accomplished this by both attaining a particular body shape (spindle-like), and swimming at certain speeds, that each have the effect of minimizing drag (Williams and Kooyman 1985). And cetaceans, as well as pinnipeds, tend to avoid an energy draining pattern of surface swimming as much as possible, thereby avoiding the extra propulsive costs due to surface effects (Blake 1983). On a smaller scale, the work done by cardiac muscle in the pumping of blood during dives is minimized by specific properties of marine mammal blood (Wickham et al. 1989) which allow easy flow of the very viscous blood at low flow rates. This, in effect, couples the increased hematocrit and low (often intermittent) flow of blood while diving, with blood properties that permit easy flow at low speeds with a greatly lowered metabolic requirement by the heart.

Another large scale method of increasing efficiency involves participating in feeding dives that are of short, rather than long, duration. Dolphin (1987) found that humpback whales feeding on prey located at shallow

depths had a lower relative energy cost than long dives to depth. Normal diving behavior of seals appears to involve the maximization of time spent underwater while at sea (Kooyman et al. 1980; LeBoeuf et al. 1986, 1988, 1989) and, therefore, time actually spent feeding. Long dives become counterproductive when they begin to require excessively long recovery periods (Kooyman 1985). So it is advantageous for the diving mammal to extend the length of the dive only if it doesn't increase the time necessary for recovery between dives. This is where the dive response, oxygen storage capacity, and metabolic depression are beneficial. Also beneficial in this regard would be any metabolic efficiency increases in the diving animal.

Metabolic efficiency can be increased by utilizing certain fuels and pathways for catabolic processes. Oxidizing glucose rather than fat results in slightly higher ATP/O_2 consumed (Hochachka 1985). However, as discussed above, marine mammals appear not to be taking advantage of this particular efficiency mechanism. Alternatively, fermentation of glycogen rather than glucose also yields a net advantage, more ATP/mole of substrate used. Glycogen is commonly the fuel of choice in hypoxic tissues, for reason of this efficiency advantage, as well as substrate availability. But these types of metabolic efficiency advantages are minor in a quantitative sense.

A much larger impact on overall ATP turnover could be supplied by decreasing the "wastefulness" of ATP requiring processes, that is, increasing the amount of cell work/ATP consumed. This is because the major use of ATP during all activity states involves the functioning of

ATPases (Hulbert and Else 1984; Rappaport 1985). If the various ATPases (Ca^{++}ATPase, $Na^{+}K^{+}$ ATPase or myosin ATPase) were modified to increase efficiency in some way (ie. utilizing of efficient isoforms), it could greatly increase the amount of cell work accomplished per ATP consumed. Evidence for increased efficiency is apparent in muscle with reduced sensitivity to thyroid hormones and in hypothyroid animals (Nwoye et al. 1982). Such muscles are capable of more work per oxygen consumed. Since seals appear to have low levels of T3 and T4 (Leatherland et al. 1982), increased efficiency of the working musculature is a distinct possibility. However, experiments to test this hypothesis are only now being formulated (Hochachka, personal communication).

Similar efficiency strategies are apparent in native Quechua Indians from the Andes (Hochachka et al., in press). As a response to decreased oxygen availability at high altitude these Indians have developed a metabolic organization that is more efficient (more work/ATP or O_2 consumed) than lowlanders. In addition to low lactate output at a given work rate ("the lactate paradox", see West 1986; Hochachka 1988) and preferential aerobic utilization of carbohydrate rather than fat (more ATP/O_2 consumed), the Quechuas seem to have some additional mechanism which allows them to get more power output per metabolic power input. This advantage over lowlanders appears to be due to a closer matching of oxygen and fuel fluxes with energy needs, and is genetically or developmentally fixed. It would not be at all surprising to find this type of efficiency adaptation to high altitude hypoxic stress in an animal subjected to diving hypoxic stress. The tight coupling of energy supply to energy demand is characteristic of hypoxia tolerant species (Hochachka 1988).

The underlying mechanisms for these apparent efficiency advantages are unknown.

Goals. The current state of understanding of diving in marine mammals is as follows: 1) the diving reflex is employed in the vast majority of dives regardless of length, although the extent of the physiological responses comprising this reflex are adjusted to meet demand, 2) oxygen stores are maximized, but insufficient to maintain RMR for the length of a large percentage of dives, 3) anaerobic metabolic pathways do not appear to completely make up the energy shortfall, and 4) a combination of metabolic depression in hypoperfused tissues and/or the maximization of efficiency of metabolic activity are the current suspects in the search for an answer to the diving riddle.

This thesis is an attempt to gain greater understanding of one of the most extensive and metabolically active tissues in the body of a mammal, the muscular system. Little is known about the different conditions experienced by muscles of different function, or in different areas of the body in marine mammals. It is assumed that the enzymatic pattern exhibited by an individual muscle reflects the demands placed on that muscle. Therefore, by examining a spectrum of enzymes in a wide range of skeletal muscles a better idea of overall metabolism, as well as individual muscle usage patterns, will be obtained. Particular emphasis will be placed on metabolism as it relates to the diving habit. This knowledge should have great relevance to future investigations on metabolic depression and/or metabolic efficiency strategies -- both of which must apply to muscle to a large degree to be effective in extending

the aerobic dive limit. Metabolic depression would be particularly important to less active muscles experiencing hypoperfusion during dives, while metabolic efficiency strategies would primarily be of importance to the more active muscles.

CHAPTER 2:

ENZYME ACTIVITY PROFILES OF HARBOR SEAL MUSCLES

INTRODUCTION

Investigations on the metabolic machinery of different tissues in marine mammals have generally focused on major organs such as the heart, brain, kidney, and liver (Kerem and Elsner 1973b; Simon et al. 1974; Murphy et al. 1980; Behrisch and Elsner 1984; Hochachka et al. 1988; Hong 1989). Other than histochemical studies (George et al. 1971; George and Ronald 1973, 1975; Man'kovskaya 1975; Solokov and Rodinov 1978; Suzuki et al. 1983), those that have been applied to a widely dispersed tissue like skeletal muscle generally are very limited in scope. In spite of the fact that very distinct muscles are located in every area of the body and have widely varying functions and usage patterns, most studies on marine mammals have examined only a few muscles (George et al. 1971; Kerem and Elsner 1973b; Simon et al. 1974; Ponganis and Pierce 1978; Castellini and Somero 1981) -- and rather than include a series of muscles with different functional characteristics, the muscles chosen for study have invariably been locomotory muscles, and the occasional diaphragm. Unfortunately, the exception to this (Castellini et al. 1981) averaged all the muscle values together.

Since skeletal muscle constitutes, on average, 30% of the body weight of mammals (McGilvery 1979), the metabolic status of skeletal muscle during a dive should have considerable impact on the overall metabolic rate of the animal and, therefore, its ability to remain submerged. However, as certain skeletal muscles must remain quite active during the dive (ie. locomotory muscle), while others are relatively quiescent (ie. respiratory muscle), the impact of individual muscles on overall metabolism should be extremely variable. Consequently, data obtained from locomotory muscle should not be applied to skeletal muscle in general.

It is clear that muscles of different function must endure different types of stress -- exercise, rapid vs slow contractions, chronic hypoxia exposure, etc. -- and, as a result, have different metabolic requirements and make-up (McGilvery 1975; Gollnick 1983; Hochachka and Somero 1984; Hochachka 1985). The metabolic demands on a tissue are best reflected in its enzymes. So to gain a greater understanding of the conditions encountered by various marine mammal muscles, a detailed examination of the catabolic enzyme activities of a wide functional range of skeletal muscles would be useful. Such information on skeletal muscle should be particularly important since the two mechanisms currently regarded as the most probable biochemical adjustments allowing extended diving time in marine mammals, metabolic depression and increased efficiency (see Chapter 1), could both effect skeletal muscle -- but in very different ways. Metabolic depression should impact inactive muscles during a dive, but would be counterproductive in active muscles, such as locomotory muscle, which must remain functional throughout the dive. Increased metabolic efficiency, on the other hand, would be particularly vital to active muscle

in "squeezing out" the maximum amount of work possible for each O_2 molecule during every contraction, thereby greatly extending the aerobic diving limit.

To this end, an examination of 13 key enzymes of carbohydrate, fatty acid and amino acid metabolism was undertaken on 21 different skeletal muscles of the harbor seal, Phoca vitulina. This species of marine mammal was chosen for study for several reasons. First, it is a readily obtained animal in relatively close proximity to the laboratory. Second, it is known to be a fairly capable diver in comparison with other readily available species such as sea lions (Robin et al. 1963; Kerem and Elsner 1973b; Harrison and Kooyman 1981). Finally, it is a close relative of one of the champion divers of marine mammals, the Weddell seal (Harrison and Kooyman 1981; Zapol 1987; Castellini and Kooyman 1989); and, as such, it may have evolved very similar, though less developed, metabolic strategies for diving.

The 21 muscles included in the study were chosen to cover a wide range of function and location within the body. Samples were removed from muscles of the head (m. masseter), torso (ie. m. obliquus abdominis externus), dorsal areas (ie. m. longissimus), ventral areas (ie. m. pectoralis), neck (ie. m. atlantoscapularis), shoulder (m. deltoideus) and internally (m. diaphragm). In addition, separate superficial vs deep portions of the larger muscles were sampled. The precise function of each muscle could only be inferred from anatomical studies of phocid seal musculature (Howell 1928; Humphrey 1868; Miller 1887) in combination with the biomechanics of the swimming stroke in these animals (Backhouse 1961;

Fish et al. 1988); and by analogy to equivalent muscles in terrestrial mammals due to a distinct similarity in the origins and insertions of the muscles despite obviously different body shapes (Howell 1928).

The primary focus to the analysis of the enzyme activities in these muscles will be on relative differences between muscles, although interspecies comparisons will also be made. Based on the difference in enzyme levels of different harbor seal muscles, individual muscle functions will be defined and clarified. The relative extent of the hypoxic stress encountered by individual muscles will also be estimated. Differences between locomotory and non-locomotory muscles will be incorporated into overall diving strategies as currently thought. The implication of these data to circulatory redistribution during dives, and therefore potential metabolic depression, as well as apparent fuel preference as it relates to metabolic efficiency, will also be discussed.

Materials and Methods

Experimental animals. Harbor seals (Phoca vitulina) were obtained from the Georgia Straits off the southeastern coast of Vancouver Island, British Columbia, Canada. In an attempt to ensure as much consistency in the animals sampled as possible, the following restrictions applied: 1) only adult males weighing approximately 250-300 pounds were used, 2) all sampling was conducted within a single 10 day period in August 1984, and

3) all the seals used came from one of two small rock formations located just north of Sidney Island and within a few hundred yards of each other.

The animals were sacrificed by a single 22 caliber rifle shot to the head. Death was immediate in all cases. The first muscle samples were removed at about 30 minutes post mortem and the dissection was completed within the next 2 hours. Preliminary studies on two muscles (m. longissimus and m. iliocostalis) indicated no significant loss of enzyme activity over the two and one-half hour post mortem time period.

Muscles sampled. Portions of 21 different skeletal muscles were collected from each of 4 seals. The muscles sampled and the location of each sample within the muscle are described in the following list. Three letter abbreviations used throughout the remainder of the study to describe each muscle sample are given in parentheses. Muscle nomenclature and locations are based on Howell (1928). The muscles are listed in the order they were sampled during dissection.

1. m. latissimus dorsi 2 (LAT) -- one sample from the center of this thin triangularly shaped muscle.

2. m. longissimus dorsi (LDS - superficial portion, LDD - deep portion) -- samples were removed from this long, large, cylindrical muscle, midway between the anterior and posterior limbs.

N.B.--In all instances superficial samples included only the outermost 1 cm of muscle at each location. The deep samples were taken from the

innermost one-half of the muscle, directly below the superficial samples' locations.

3. m. (ilio)costalis (ILS - superficial portion, ILD - deep portion) -- same description as m. longissimus dorsi.

4. m. biceps femoris 1 (BFM) -- sampled from the middle of the thickest part of the muscle.

5. m. gluteus maximus (GMS - superficial portion , GMD - deep portion) -- two samples were removed from the center of this thick triangular muscle.

6. m. masseter (MAS) -- sampled from the center of the thickest portion of the muscle.

7. m. psoas minor (PSO) -- one sample, the entire thickness of this short, thin muscle, just over the point of its insertion on the last lumbar vertebra.

8. m. extensor digitorum communis (EDC) -- one sample was taken from the distal one-third of this muscle, encompassing its entire thickness at that point.

9. m. pectoralis (PMS - superficial portion, PMD - deep portion = pars profundus) -- samples were taken from midway between the mid-

ventral line and the mid-lateral line of the seal, at the level of the anterior limbs.

10. m. deltoideus (DLT) -- sampled from the center of the muscle, midway along its length.

11. m. triceps lateralis (TLT) -- same description as m. deltoideus.

12. m. triceps longus (TLG) -- one sample was taken from the distal one third of this muscle, encompassing the entire thickness of the muscle at that point.

13. m. depressor scapulae (=subscapularis) (DEP) -- one sample was removed from the portion of this muscle directly over the center of the scapula -- this sample included only the innermost 2 cm of the muscle at that point.

14. m. pectoralis abdominis lateralis (PAL) -- sampled from the lateral edge of the muscle midway between the anterior and posterior limbs.

15. m. obliquus abdominis externus (OBL) -- one sample of this large, thin, "sheet-like" muscle was taken from along the mid-lateral line of the seal, at a level midway between the anterior and posterior limbs -- only the outermost 1 cm of muscle was sampled.

16. m. semitendinosus 2 (SEM) -- sampled from the middle of the thickest part of the muscle.

17. m. spinotrapezius (SPT) -- this slender, flat muscle was sampled midway along its length, the sample encompassed the entire thickness of the muscle at that point.

18. m. atlantoscapularis superior (ATL) -- this long thin muscle was sampled midway along its length, the sample encompassed the entire thickness of the muscle.

19. m. intercostales externi (EXT) -- sampled along the mid-lateral line of the seal, at a level somewhere between the fifth and eighth ribs, only the outermost 1 cm of muscle was included.

20. m. intercostales interni (INT) -- these samples were taken directly below the samples of m. intercostales externi, only the innermost 1 cm of muscle was sampled in this case.

21. m. diaphragm (DIA) -- one sample was taken from a lateral side of the diaphragm about midway between the costal cartilages and the central tendon, the sample encompassed the entire thickness of the muscle.

Tissue manipulations. Muscle samples were dissected out and immediately freeze-clamped in liquid nitrogen. The frozen muscle was then sealed in an air tight plastic bag. All samples were kept on dry ice until transport to a freezer maintained at -80°C.

Homogenization for enzyme assays. Portions of each sample were dissected away, weighed, thawed, minced with scissors, and homogenized with a Polytron homogenizer (Brinkman Instruments). Homogenization was done at 80% of maximum speed for three 20-second bursts. The samples were kept on ice (0 - 4°C) throughout the procedure.

Two different homogenization buffers were used, depending on the enzymes to be assayed. Buffer 1 consisted of 50 mM Imidazole-Cl (pH 7.0), 1mM ethylenediaminetetraacetic acid (EDTA), 10 mM B-mercaptoethanol and 0.1% Triton X-100. Buffer 2 was made up of 50 mM Tris(hydroxymethyl) aminomethane chloride (Tris - Cl) (pH 8.0) and 0.1% Triton X-100.

Samples homogenized in Buffer 1 were spun at 12,100g for 15 minutes at 4°C. Samples homogenized in Buffer 2 were spun at 600g for 15 minutes at 4°C. All centrifugation was done in a Sorvall RC5C refrigerated ultracentrifuge. Supernatant fractions were taken and kept on ice.

Enzyme assays. All enzymes were assayed in an SP1800 Unicam spectrophotometer with a linear chart recorder and water-jacketed cuvette

holders. Assay temperatures were maintained at 25°C with a Lauda K-2/R constant temperature water bath circulator.

Preliminary experiments were conducted to verify saturating levels of all substrates and co-factors, and to rule out any inhibitory effects of buffer or assay constituents.

Samples homogenized in buffer 1 were assayed in 50 mM Imidazole - Cl (pH 7.0) under the following conditions:

Lactate dehydrogenase (LDH). 2.5 mM pyruvate (omitted for control) and 0.2 mM NADH.

Pyruvate kinase (PK). 5 mM phospho(enol)pyruvate (omitted for control), 5 mM ADP, 0.2 mM NADH, 10 mM $MgCl_2$, 100 mM KCl, and excess lactate dehydrogenase.

α-glycerophosphate dehydrogenase (α-GPDH). 0.7 mM dihydroxyacetone phosphate (omitted for control) and 0.2 mM NADH.

Glucose-6-phosphate dehydrogenase (G6PDH). 5 mM glucose-6-phosphate (omitted for control), and 1 mM $NADP^+$.

Creatine kinase (CPK). 70 mM creatine phosphate (omitted for control), 1 mM ADP, 20 mM glucose, 1 mM $NADP^+$, 10 mM AMP, 10 mM $MgCl_2$, 10 mM B-mercaptoethanol, excess hexokinase and glucose-6-phosphate dehydrogenase.

Phosphofructokinase (PFK). 5 mM fructose-6-phosphate (omitted for control), 1 mM ATP, 0.2 mM NADH, 2 mM AMP, 10 mM $MgCl_2$, 100 mM KCl, 10 mM B-mercaptoethanol, and excess aldolase, triose-phosphate isomerase, and α-glycerolphosphate dehydrogenase.

3-hydroxyacyl-CoA dehydrogenase (HOAD). 0.1 mM acetoacetyl CoA (omitted for control), 0.2 mM NADH and 1 mM EDTA.

Glutamate-pyruvate transaminase (GPT). 200 mM alanine (omitted for control) 7 mM α-ketoglutarate (α-KG), 0.2 mM NADH, and excess LDH.

Glutamate-oxaloacetate transaminase (GOT). 40 mM aspartate (omitted for control), 7 mM α-KG, 0.2 NADH, and excess malate dehydrogenase.

Glutamate dehydrogenase (GDH). 7 mM α-KG (omitted for control), 1 mM ADP, 100 mM ammonium chloride, and 0.2 mM NADH.

Samples homogenized in buffer 2 were assayed in 50 mM Tris-Cl (pH 8.0) under the following conditions:

Citrate synthase (CS). 0.5 mM oxaloacetate (omitted for control), 0.2 mM acetyl CoA, and 0.1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB).

Carnitine palmitoyltransferase (CPT). 0.03 mM palmitoyl-CoA, (omitted for control), 2 mM L-carnitine and 0.1 mM DTNB.

Carnitine acetyltransferase (CAT). 0.2 mM acetyl CoA (omitted for control), 2 mM L-carnitine, and 0.1 mM DTNB.

Literature comparisons. When comparing literature enzyme activities expressed in different units, the unit conversions of Scrutton and Utter (1968) were applied (activity per gram wet weight = activity per gram dry weight x 0.28; activity per gram wet weight = activity per milligram protein x 200). In addition, temperature effects (Q_{10}) were applied as follows: 1) activity at 25°C = activity at 30°C - 1.5, and 2) activity at 25°C = activity at 37°C - 2 (Scrutton and Utter 1968). The only exception to this Q_{10} conversion was with citrate synthase (CS). This enzyme's Q_{10} effects appear to be much lower (Alp, Newsholme, and Zammit 1976): 1) activity at 25°C = activity at 30°C - 1.15, and 2) activity at 25°C = activity at 37°C - 1.30.

Comparison enzyme activity ratios, when unavailable in the literature, were calculated from existing data. Whenever possible, the calculation of individual ratios involved only enzyme activity values from the same study. Ratio calculations were the combined data of different studies only when absolutely necessary, and generally using only data from a single, established laboratory (ie. Newsholme).

Chemicals. All biochemicals and enzymes were from Sigma and Boehringer-Mannheim. Other chemicals were from various commercial sources and were of reagent grade.

Statistics. SAS (Statistical Analysis System), a computer system of software products for data analysis, was used for all statistical analyses. SAS manuals (SAS Institute 1985a, 1985b) contain detailed explanations of the procedures used and a wide selection of references on the statistical bases for the procedures.

Means were compared using two-way ANOVA's (by muscle and by animal). Significant differences between individual means were determined exclusively by Student-Newman-Keuls (SNK) multiple range tests. Correlations between variables refer to Spearman correlations in all cases. Due to the non-parametric nature of some of the variables, ANOVA'S and correlations were conducted on ranks. The remainder of the analyses utilized the real values of the data. Cluster analysis was conducted on the means and correlation matrices of the data. Ward's method was used for determination of the heirarchial clusters, with output in the style of Johnson (1967) with the root at the top. The number of clusters to be used in each analysis was determined by plotting the cubic clustering criterion vs the number of clusters.

Results

Maximum Enzyme Activities. The maximum activites of all 13 enzymes in each of the 21 skeletal muscles sampled are listed in Table 1. All values are the mean $\pm$ 1 S.E. (at 25^{o}C) of 4 animals, except for the GDH activities. GDH was determined on the muscle samples of only 1 seal and therefore excluded from further analysis. The correlations between enzymes are

listed in Table 2. Two-way analysis of variance (not shown) exhibits significant muscle differences in all enzymes studied. It also demonstrates significant animal effects with all enzymes but PK, G6PDH, GPT, and GOT. The animal effects, however, are small and appear to be an artifact of the way the assays were conducted (each seal's muscles having been analyzed on a separate day from the others' for each series of enzymes), rather than an indication of important animal differences.

The mean LDH activity of the seal skeletal muscles is relatively high -- but well within the range reported for skeletal muscle from a number of other vertebrate species, both marine and terrestrial (Crabtree and Newsholme 1972a; Storey and Hochachka 1974; Ponganis and Pierce 1978; Castellini and Somero 1981; Castellini et al. 1981; Emmett and Hochachka 1981). It is readily apparent that the locomotory muscles have the highest LDH activities, (Table 1). The more "unusual" and less-used muscles predominate in the low end of the range of activities. Also evident is the fact that, although close in value, the superficial portions of muscles tend to have higher LDH activity than the deep portions. This trend should follow due to the differences in fiber type and capillary distribution generally found between superficial vs deep skeletal muscle (Guth and Samaha 1969; Yellin 1969; Baldwin et al. 1972; Gonyea and Ericson 1977; Gunn 1978; Armstrong 1980; Armstrong et al. 1982). Most muscles are not significantly different from those muscles they are functionally associated with (ie. all swimming muscles group together, muscles of the shoulder and upper arm are not significantly different, etc.) Significant differences between muscles are detailed in Appendix 1. The only potentially unusual grouping of muscle is observed with the

TABLE 1. MAXIMUM ENZYME ACTIVITIES IN HARBOR SEAL MUSCLES (CONT'D ON THE NEXT 2 PAGES).

MUSCLE	LDH		PK		PFK		a-GPDH	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	1021.25	20.24	895.38	68.76	63.18	3.61	29.81	0.63
LDD	927.21	34.63	765.29	53.89	64.19	5.09	30.07	2.51
ILS	943.66	38.95	886.08	56.45	64.67	1.19	29.41	2.84
ILD	967.86	54.98	752.54	33.24	57.94	6.05	28.95	1.33
GMS	1081.01	56.08	1061.34	104.67	71.41	7.36	26.75	0.89
GMD	958.29	43.25	760.18	60.65	67.72	5.21	26.99	2.35
SEM	1062.78	78.89	828.61	30.43	66.01	4.23	28.93	4.37
OBL	917.11	33.15	887.86	114.55	60.56	6.04	28.72	3.09
PSO	870.51	79.94	946.66	76.62	62.91	6.27	27.85	3.42
PMS	1080.81	45.35	1020.26	61.31	75.87	4.41	31.24	1.04
PMO	939.51	22.52	838.42	45.06	68.46	5.85	32.01	3.07
PAL	886.22	67.63	754.37	40.48	49.53	8.23	26.08	0.72
DEP	826.64	71.32	744.82	73.05	47.56	3.58	26.57	5.15
DLT	843.74	42.31	766.32	99.68	58.58	7.45	27.83	2.34
TLT	911.51	47.69	917.41	17.08	55.81	7.78	26.63	0.87
TLG	909.72	53.82	765.97	31.49	70.21	3.83	34.46	1.82
BFM	733.19	45.78	775.62	35.46	58.72	7.48	19.86	0.71
LAT	670.83	28.52	665.21	5.38	44.08	5.01	19.19	1.84
SPT	659.55	49.98	650.06	17.38	31.02	5.72	21.57	4.22
ATL	638.47	34.36	514.62	34.81	21.58	6.15	21.51	4.52
INT	709.45	14.02	565.21	39.85	25.96	6.78	22.62	1.54
EXT	636.79	21.68	538.97	29.35	39.32	2.16	20.54	1.93
EDC	696.65	74.06	637.65	73.11	47.61	3.68	16.48	2.15
MAS	450.31	31.46	395.51	47.86	29.49	1.55	24.37	2.93
DIA	433.57	17.29	282.35	15.05	16.04	3.33	10.78	0.96
ALL	831.06	19.67	744.27	20.81	52.74	1.92	25.57	0.71

TABLE 1. (CONT'D).

MUSCLE	CPK		G6PDH		CS		HOAD	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	2421.05	83.42	0.00	0.00	22.61	0.55	31.51	5.66
LDD	2600.22	101.07	0.00	0.00	21.58	1.54	26.19	6.12
ILS	2635.21	120.37	0.00	0.00	18.89	0.29	24.33	6.99
ILD	2535.92	79.75	0.00	0.00	22.78	0.91	27.41	6.26
GMS	2746.72	58.35	0.00	0.00	19.11	1.13	26.56	8.01
GMD	2506.35	379.52	0.00	0.00	25.22	2.01	32.53	7.36
SEM	2825.21	95.07	0.00	0.00	15.46	2.57	17.84	4.49
OBL	2622.15	122.43	0.00	0.00	17.52	1.25	23.32	6.02
PSO	2917.02	48.54	0.00	0.00	16.39	2.35	18.16	2.35
PMS	3120.81	172.04	0.00	0.00	19.12	1.74	18.21	4.31
PMO	2840.81	222.66	0.00	0.00	19.05	1.51	23.23	6.04
PAL	2505.55	171.55	0.00	0.00	15.66	1.65	19.76	4.84
DEP	2565.91	93.08	0.00	0.00	16.26	3.05	19.94	5.89
DLT	3153.42	57.04	0.00	0.00	18.81	1.49	16.89	3.57
TLT	3023.61	184.65	0.00	0.00	18.63	2.15	17.65	2.89
TLG	3381.85	224.83	0.00	0.00	12.81	2.71	10.15	2.41
BFM	2863.47	241.85	0.00	0.00	12.31	2.12	19.55	5.05
LAT	2519.63	72.32	0.00	0.00	16.01	2.11	20.31	4.02
SPT	2426.41	117.91	0.00	0.00	14.46	1.92	19.36	4.68
ATL	2244.02	289.96	0.00	0.00	10.76	0.42	15.15	3.67
INT	2211.67	228.19	0.00	0.00	15.77	1.45	26.05	6.03
EXT	2361.85	103.26	0.00	0.00	18.33	1.51	27.48	6.52
EDC	2203.47	229.31	0.00	0.00	15.55	0.61	18.39	2.51
MAS	2713.52	150.38	0.00	0.00	16.81	1.99	10.04	2.21
DIA	1302.11	124.73	0.09	0.03	19.48	2.49	35.43	9.78
ALL	2609.92	49.83	0.00	0.00	17.57	0.46	21.82	1.14

TABLE 1. (CONT'D).

MUSCLE	CPT		CAT		GPT		GOT		GDH	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	0.29	0.04	3.21	0.24	7.92	0.53	63.64	1.93	1.81	N.A.
LDD	0.37	0.07	3.48	0.29	9.64	0.99	70.16	4.45	2.08	N.A.
ILS	0.31	0.03	2.78	0.18	7.11	0.93	61.63	3.91	1.42	N.A.
ILD	0.35	0.04	3.61	0.29	8.21	0.53	68.44	3.37	1.96	N.A.
GMS	0.29	0.04	2.84	0.26	5.61	0.47	61.28	2.76	1.11	N.A.
GMD	0.41	0.06	3.96	0.53	6.46	1.11	69.76	10.25	1.77	N.A.
SEM	0.22	0.05	2.23	0.33	7.36	0.43	62.96	3.06	0.74	N.A.
OBL	0.23	0.02	2.86	0.35	5.87	0.72	60.34	4.56	1.49	N.A.
PSO	0.25	0.03	2.06	0.21	6.23	0.75	57.89	4.42	1.75	N.A.
PMS	0.24	0.07	2.82	0.41	8.65	0.81	68.12	4.31	1.28	N.A.
PMO	0.34	0.06	3.18	0.54	8.31	0.64	70.97	4.11	1.42	N.A.
PAL	0.25	0.05	2.15	0.38	5.02	0.23	54.03	3.95	0.94	N.A.
DEP	0.31	0.07	2.81	0.81	8.68	1.18	63.98	6.83	0.27	N.A.
DLT	0.26	0.04	2.34	0.38	6.58	0.39	61.15	2.15	1.01	N.A.
TLT	0.31	0.04	2.38	0.44	6.65	0.78	61.66	1.37	1.72	N.A.
TLG	0.13	0.06	2.26	0.65	5.41	0.59	47.19	5.45	1.01	N.A.
BFM	0.16	0.04	0.97	0.18	2.97	0.15	40.01	2.21	1.51	N.A.
LAT	0.28	0.06	1.55	0.34	3.51	0.17	47.67	3.07	1.72	N.A.
SPT	0.22	0.05	1.21	0.23	4.02	0.00	46.47	1.22	1.33	N.A.
ATL	0.22	0.06	1.54	0.28	6.61	1.02	46.86	8.22	0.62	N.A.
INT	0.26	0.07	2.15	0.37	6.27	0.89	47.81	4.54	0.42	N.A.
EXT	0.29	0.05	2.65	0.38	6.75	0.26	52.38	2.42	1.12	N.A.
EDC	0.25	0.06	1.45	0.18	5.13	0.11	55.93	4.65	2.11	N.A.
MAS	0.24	0.07	2.95	0.61	5.35	0.41	53.81	4.83	0.85	N.A.
DIA	0.46	0.14	2.08	0.05	6.21	0.65	51.91	4.22	2.68	N.A.
ALL	0.28	0.01	2.46	0.11	6.42	0.21	57.84	1.17	1.36	N.A.

Assay temperature = 25°C.
Activities are expressed as units/gm wet wt.
S.E. = 1 standard error of the mean.
n = 4.
See Materials and Methods for assay conditions and abbreviations.

TABLE 2. CORRELATION MATRIX BETWEEN ENZYME ACTIVITIES OF HARBOR SEAL MUSCLE.

	LDH	PK	a-GPDH	G6PDH	CPK	PFK	HOAD	CS	CPT	CAT	GPT	GOT
LDH	* 1	*0.79	*0.54	*-0.33	*0.38	*0.76	0.07	*0.36	0.06	*0.35	*0.37	*0.46
PK	*0.79	* 1	*0.54	*-0.34	*0.46	*0.77	0.13	*0.26	-0.03	*0.25	*0.25	*0.42
a-GPDH	*0.54	*0.54	* 1	*-0.33	*0.44	*0.52	*0.23	0.10	-0.10	*0.39	*0.49	*0.40
G6PDH	*-0.33	*-0.34	*-0.33	* 1	*-0.33	*-0.31	0.16	0.05	0.17	-0.08	-0.01	-0.12
CPK	*0.38	*0.46	*0.44	*-0.33	* 1	*0.48	*-0.46	-0.04	*-0.21	0.04	0.07	0.18
PFK	*0.75	*0.77	*0.53	*-0.32	*0.48	* 1	0.08	*0.31	0.12	*0.38	*0.27	*0.43
HOAD	0.07	0.13	*0.23	0.16	*-0.46	0.08	* 1	0.15	0.14	*0.23	*0.26	0.14
CS	*0.36	*0.25	0.11	0.05	-0.04	*0.31	0.15	* 1	*0.69	*0.77	*0.36	*0.62
CPT	0.06	-0.03	-0.10	0.17	*-0.21	0.12	0.14	*0.69	* 1	*0.55	0.19	*0.38
CAT	*0.35	*0.25	*0.39	-0.08	0.04	*0.38	*0.23	*0.77	*0.55	* 1	*0.57	*0.71
GPT	*0.37	*0.25	*0.49	-0.01	0.08	*0.27	*0.26	*0.36	0.19	*0.57	* 1	*0.73
GOT	*0.46	*0.43	*0.40	-0.12	0.18	*0.43	0.14	*0.62	*0.38	*0.71	*0.73	* 1

* = statistically significant correlation at the 95% confidence level.
All correlations are between data ranks (Spearman correlations).
See Materials and Methods for abbreviations.

respiratory muscles; DIA has significantly lower LDH activity than the intercostales (INT and EXT). The number of significant differences between muscles (Appendix 1) seems to indicate that LDH activity is a useful variable in functionally discriminating the skeletal muscles of harbor seals.

PK activity closely parallels the LDH activity pattern. This is indicated by the high correlation between the two enzymes (Table 2) and the similarity in histograms (Figure 1). The mean PK activity of seal skeletal muscle (Table 1) also falls at the upper end of the range of values reported for skeletal muscle from other animal species (Zammit et al. 1978; Castellini et al. 1981; Emmett and Hochachka 1981). The differences in PK activity between muscles (Appendix 1) mirror the LDH pattern, including the separation of DIA from the intercostales. PK activity therefore, also appears to be a good variable to use in the functional discrimination of skeletal muscle.

Another glycolytic enzyme, PFK, exhibited a mean activity in harbor seal skeletal muscle (Table 1) that is about average for muscle from a number of species of animal (Crabtree and Newsholme 1972a; Newsholme and Start 1973; Zammit et al. 1978). PFK correlates very highly with the other glycolytic enzymes (LDH and PK) (Table 2) and exhibits a similar enzyme pattern between muscles (Appendix 1). The only obvious difference is that, in this case, the DIA is not significantly different from the INT.

An enzyme of the Krebs cycle, CS, has significant positive correlations to the 3 glycolytic enzymes discussed above (Table 2). Although low, the

correlations are significant at the 5% level. Surprisingly, the highest correlation of the 3 is between LDH and CS -- an anaerobic glycolytic indicator and an enzyme of aerobic metabolism. The mean CS activity of the seal skeletal muscles examined approaches the activities reported previously for marine and terrestrial mammals (Castellini and Somero 1981; Emmett and Hochachka 1981). These activities are, however, an order of magnitude lower than what is found in hummingbird flight muscle (Suarez 1986), catbird pectoral muscle (Marsh 1981), and a wide range of insect, invertebrate, and bird muscles (Newsholme and Start 1973; Alp et al. 1976). In contrast to the enzymes of glycolysis, CS activity tends to be slightly lower (or about equal) in the superficial portions of muscles than in the deeper areas (Table 1). Again, this activity pattern would be expected due to differences in fiber type and capillary distribution (above). Swimming muscles have the highest CS values, while the other muscles examined are found in the lower end of the range of values (Table 1). Exceptions to this are relatively high activity in the MAS and the respiratory muscles, particularly the DIA. No significant differences are observed between muscles of similar function (Appendix 1). CS appear to be slightly less effective in discriminating between muscles of the seal than the glycolytic enzymes.

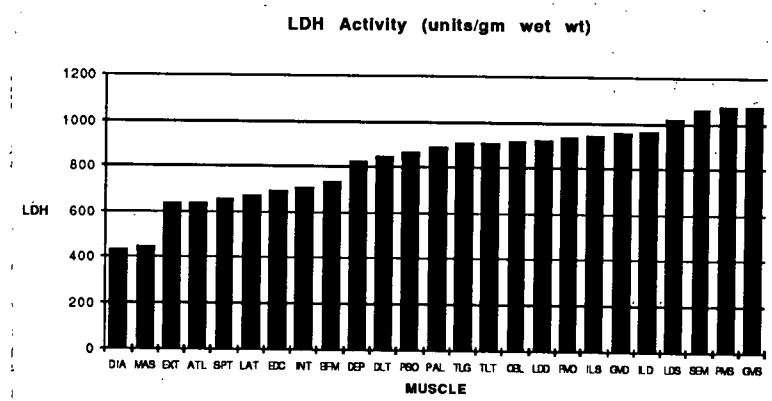
HOAD, an enzyme of B-oxidation, is found in activity levels comparable to other marine mammals (Ponganis and Pierce 1978), but much less than half the values reported for high fat-utilizing muscle such as bird flight muscle (Marsh 1981; Suarez 1986). Surprisingly, HOAD is not significantly correlated with CS activity, or with any of the glycolytic enzymes (Table 2). The pattern of activity of HOAD between individual muscles is,

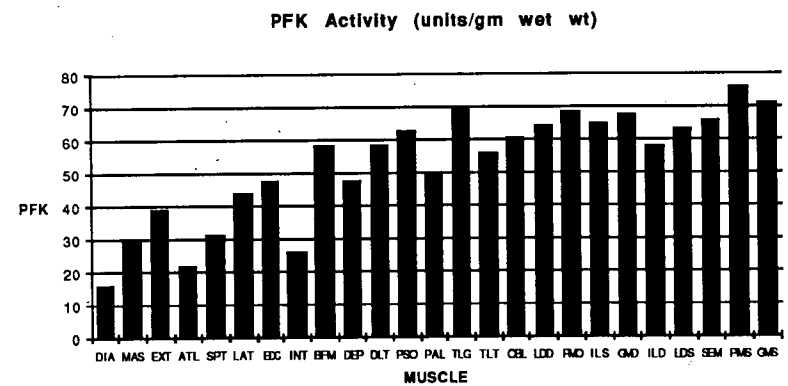
nonetheless, very similar to that found with CS (Appendix 1). Two obvious differences are a very low activity of HOAD in MAS muscle, and a trend toward even higher levels of aerobic enzymes in respiratory muscles than is evidenced by the CS activity pattern.

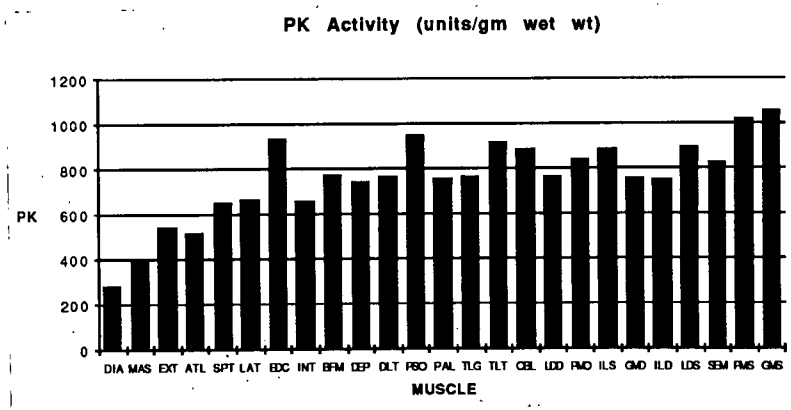
Two other enzymes of fat metabolism, CAT and CPT, exhibit low activity in harbor seal skeletal muscle (Table 1). The mean activity of CPT is comparable to a number of other vertebrate muscles (Crabtree and Newsholme 1972b; Newsholme and Crabtree 1986), but is 1-2 orders of magnitude lower than levels found in the flight muscles of some birds (Crabtree and Newsholme 1972b; Suarez 1986) and insects (Crabtree and Newsholme 1972b; Newsholme and Start 1973). CAT activity, although low, is 6-fold higher that that reported for red and white skeletal muscle of rabbits (Henriksson et al. 1986), and about 4-fold higher than the skeletal muscle of rats (Choi et al. 1977; Negrao et al. 1987). CAT and CPT have a significant positive correlation with one another. However, their correlations with HOAD are slightly different (Table 2). CAT and HOAD have a low, but significant positive correlation. CPT is not significantly correlated with HOAD. This difference is likely due to the fact that CPT activities are so low that differences between muscles are difficult to distinguish (Appendix 1) and, as a result, correlations are not as apparent. In fact, comparing relative enzyme activities, the CPT pattern appears to be more similar to the HOAD pattern than is CAT. Particularly evident are the high DIA values and low MAS activites in both CPT and HOAD. With CAT these muscles' relative values are reversed. The remainder of the

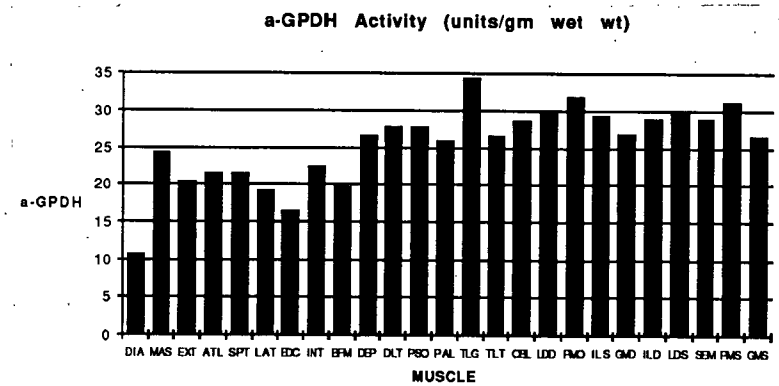
FIGURE 1. HISTOGRAM PROFILES OF MAXIMUM ENZYME ACTIVITY IN HARBOR SEAL MUSCLES (3 PAGES).

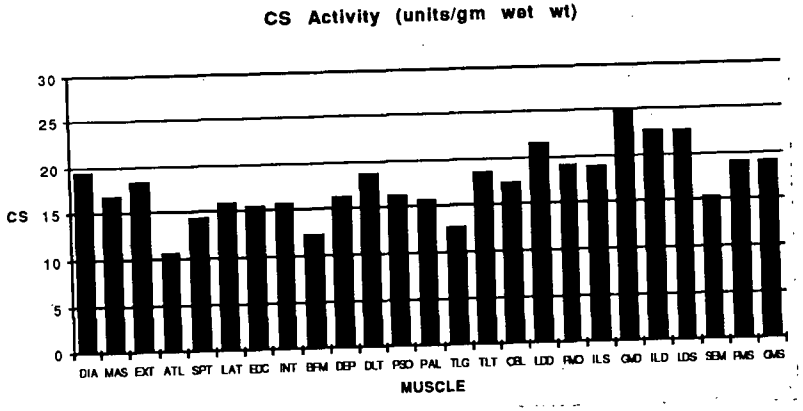
Muscles are listed in order of increasing LDH actvity.
See Materials and Methods for abbreviations.

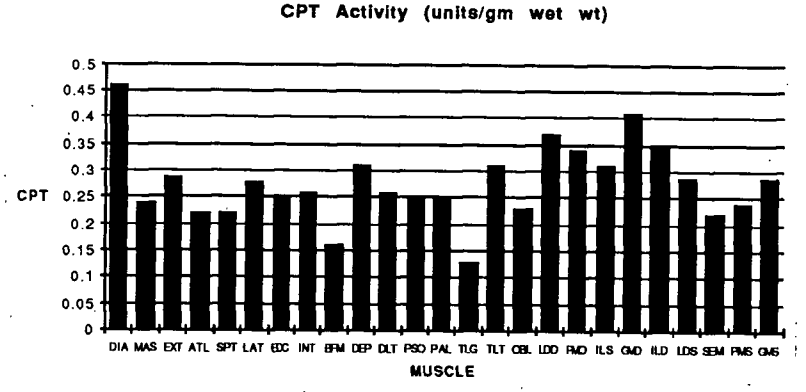

LDH Activity (units/gm wet wt)
LDH
1200
1000
800
600
400
200
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD PMO ILS GMD ILD LDS SEM PMS GMS
MUSCLE

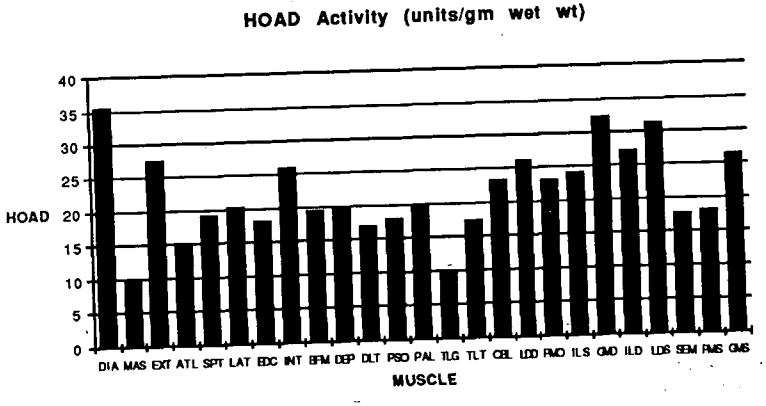

PFK Activity (units/gm wet wt)
PFK
80
70
60
50
40
30
20
10
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD PMO ILS GMD ILD LDS SEM PMS GMS
MUSCLE

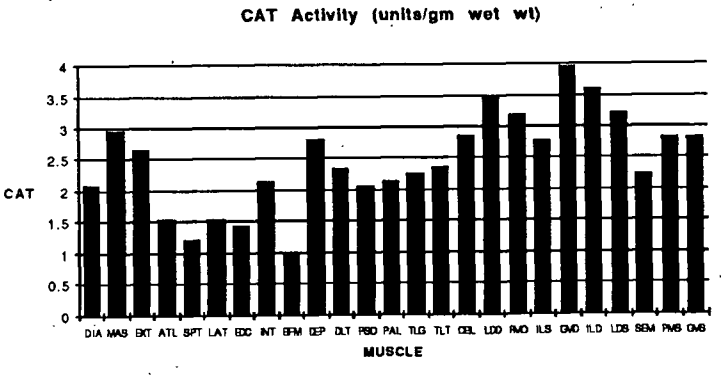

PK Activity (units/gm wet wt)
PK
1200
1000
800
600
400
200
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD PMO ILS GMD ILD LDS SEM PMS GMS
MUSCLE

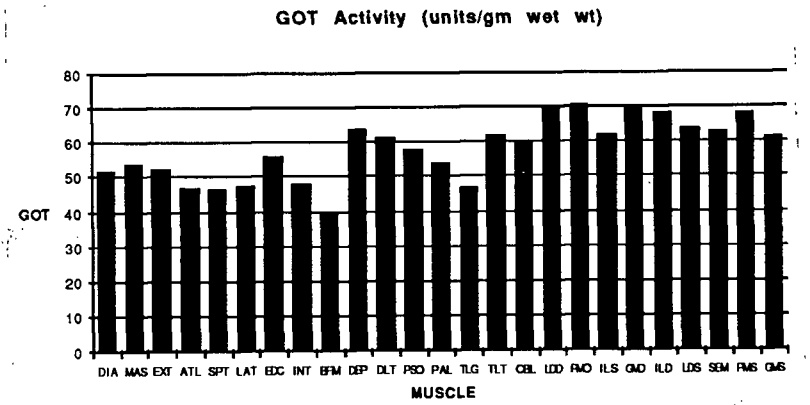

a-GPDH Activity (units/gm wet wt)
a-GPDH
35
30
25
20
15
10
5
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD PMO ILS GMD ILD LDS SEM PMS GMS
MUSCLE

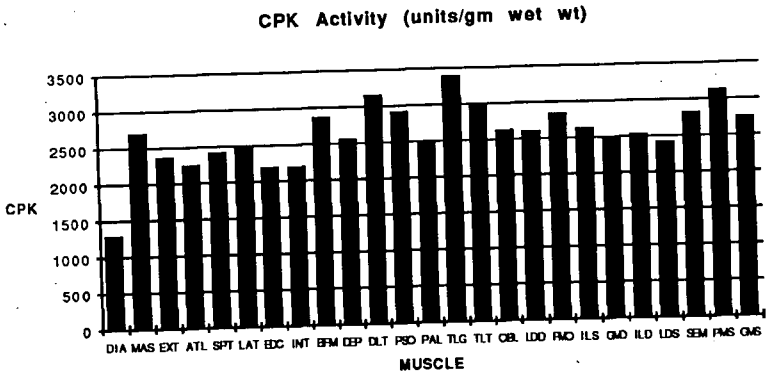

CS Activity (units/gm wet wt)
CS
30
25
20
15
10
5
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMO ILS GMD ILD LDS SEM RMS GMS
MUSCLE

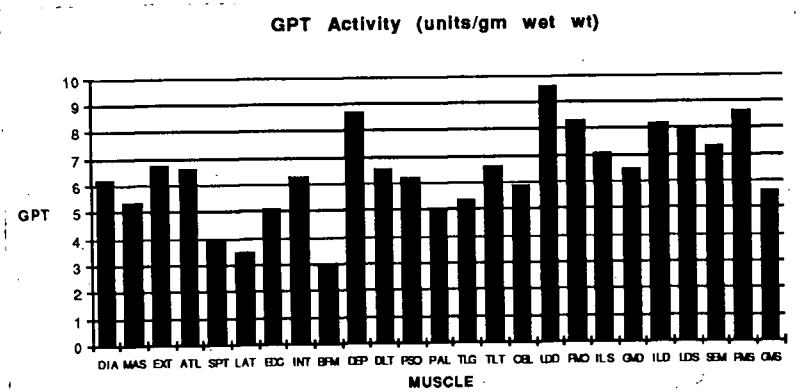

CPT Activity (units/gm wet wt)
CPT
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMO ILS GMD ILD LDS SEM RMS GMS
MUSCLE

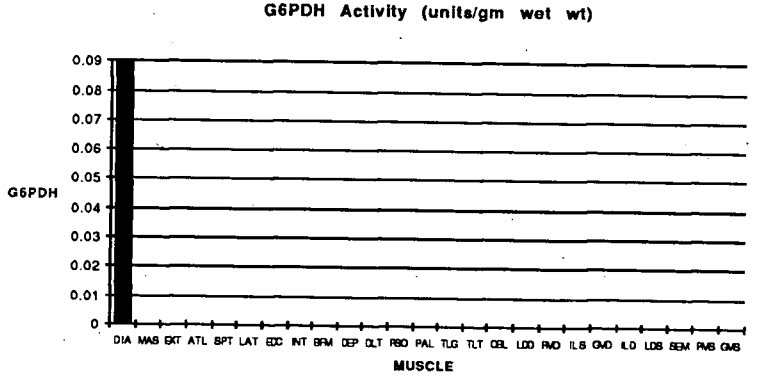

HOAD Activity (units/gm wet wt)
HOAD
40
35
30
25
20
15
10
5
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMO ILS GMD ILD LDS SEM RMS GMS
MUSCLE


CAT Activity (units/gm wet wt)
CAT
4
3.5
3
2.5
2
1.5
1
0.5
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMO ILS GMD ILD LDS SEM RMS GMS
MUSCLE


GOT Activity (units/gm wet wt)
GOT
80
70
60
50
40
30
20
10
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RVO ILS GVD ILD LDS SEM RVS GVS
MUSCLE


CPK Activity (units/gm wet wt)
CPK
3500
3000
2500
2000
1500
1000
500
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RVO ILS GVD ILD LDS SEM RVS GVS
MUSCLE


GPT Activity (units/gm wet wt)
GPT
10
9
8
7
6
5
4
3
2
1
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RVO ILS GVD ILD LDS SEM RVS GVS
MUSCLE


G6PDH Activity (units/gm wet wt)
G6PDH
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RVO ILS GVD ILD LDS SEM RVS GVS
MUSCLE

muscle pattern is fairly similar with all 3 enzymes of fat metabolism (Appendix 1). Of the 3 enzymes, HOAD appears to differentiate between muscles the best.

CPK activities in harbor seal skeletal muscle (Table 1) all fall within the normal range reported for a number of vertebrate species (Newsholme et al. 1978; Suarez 1986). Very few significant differences between the seal muscles are differentiated by this enzyme (Appendix 1). One striking exception to this is the significantly lower level of CPK found in the DIA compared to all other muscles examined. The remainder of the skeletal muscles show no clear pattern with regard to function, except that the muscles of the shoulder and upper arm (DLT, TLT, TLG) have very high values. CPK exhibits significant positive correlations with glycolytic enzymes and significant negative correlations with the enzymes of fat metabolism (Table 2). This type of correlation pattern would be expected according to the proposed role of phosphagen kinases (Newsholme et al. 1978).

The activity of α-GPDH, a branch enzyme of the glycolytic pathway, appears to be relatively low in harbor seal skeletal muscle (Table 1) compared with muscle from a number of other animal species (Crabtree and Newsholme 1978). This enzyme shows significant positive correlations with all but 3 of the enzymes examined (Table 2). CS and CPT show no correlation to α-GPDH, while G6PDH exhibits a significant negative correlation -- but due to the unusual pattern of G6PDH activity (Table 1) this correlation has very little meaning. An examination of the differences

in α-GPDH between muscles demonstrates a pattern roughly similar to the other glycolytic enzymes examined (Appendix 1).

The activity of the Hexose Mono-Phosphate Shunt in harbor seal skeletal muscle was examined by measuring the G6PDH levels. G6PDH is not found in measurable quantities in any muscle except the DIA (Table 1). The extremely low level of this enzyme in skeletal muscle is a common finding (Scrutton and Utter 1968; Storey and Hochachka 1974; Ferreira et al. 1989)

GPT and GOT activities were measured to examine the relative importance of amino acid metabolism in harbor seal muscle. GPT is found in low levels (Table 1) compared with other animal species (Scrutton and Utter 1968; Suarez 1986). GOT values, on the other hand, are relatively high (Table 1). The activity of this enzyme falls well within the range of values reported for muscles from a number of animal species (Scrutton and Utter 1968; Henriksson et al. 1986), but is at least 1 order of magnitude lower than the activity found in hummingbird flight muscle (Suarez 1986). A very high correlation is observed between GPT and GOT (Table 2); and the pattern of differences between muscles shows no important variation between the two enzymes (Appendix 1). The other enzyme of amino acid metabolism examined, GDH, is found in very low levels in all the muscles studied (Table 1). Similarly low levels of GDH are found in both red and white mammalian muscle (Pette and Bucher 1962; Henriksson et al. 1986).

Multi-muscle comparisons ("adaptation factors"). Table 3 shows, for each enzyme measured, the value of the harbor seal "adaptation factors", along with some values calculated for other species from the literature. The adaptation factor is defined as the greatest mean value of an enzyme divided by the smallest mean of that particular enzyme. [For example: in harbor seal muscle the LDH "adaptation factor" = 1081.00 (the GMS value) - 433.57 (the DIA value) = 2.49]. The number resulting from this division is an indication of the magnitude of enzymatic adaptation across the range of muscles examined. Comparison of these numbers to values calculated from multi-muscle studies in the literature may allow some insights into the nature of functional adaptation of particular muscles and/or animals. The range of "adaptation factors" found for harbor seal skeletal muscle is very narrow, 1.77 - 4.73 (Table 3). The seal muscle "adaptation factors" for every enzyme examined fall at the low/middle part of the range calculated for those enzymes from very red versus very white muscles in a variety of vertebrate species (see Table 3). This trend toward low values in harbor seal skeletal muscle is somewhat surprising considering the potential stresses placed on muscle tissue as a result of their diving lifestyle.

Discussion.

Seal muscle metabolic organization. The enzyme activities of harbor seal muscle are generally within the normal vertebrate range (see results). Previous studies on marine mammals give results similar to what has

TABLE 3. ADAPTATION FACTORS OF HARBOR SEAL SKELETAL MUSCLE, WITH COMPARISON VALUES FROM THE LITERATURE AND FIN WHALE MUSCLE.

ENZYME	HARBOR SEAL	FIN WHALE	1	2	3	4	5	6	7	8	9	10
LDH	2.5	12.3	3.0/6.2	- - - - - -	11.7	- - - - -	5.5	10.7/11.5	11.1	4.6/5.1	- - - - - -	3.9/5.2
PK	3.8	6.6	- - - - - -	2.7/3.9	- - - - -	6.2	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
a-GPDH	3.2	5.7	1.6/7.9	- - - - - -	- - - - -	- - - - -	- - - - -	7.5/8.3	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
G6PDH	- - - - -	- - - - -	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
PFK	4.7	- - - - -	3.2/4.8	2.5/7.3	- - - - -	6.5	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
CPK	2.6	2.4	- - - - - -	- - - - - -	1.5	- - - - -	- - - - -	- - - - - - - - -	2.8	- - - - - - -	1.2/2.9	- - - - - - -
CS	2.3	4.1	- - - - - -	- - - - - -	5.9	2.4	13.2	1.9/5.3	- - - - -	3.3	- - - - - -	1.0/3.0
HOAD	3.5	3.3	- - - - - -	- - - - - -	5.7	5.6	5.6	2.6/15.6	- - - - -	- - - - - - -	- - - - - -	1.1/2.8
CPT	3.5	- - - - -	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
CAT	4.1	- - - - -	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
GOT	1.8	3.2	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	4.7	- - - - - - -	- - - - - -	- - - - - - -
GPT	3.2	- - - - -	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -

Adaptation factor = maximum/minimum enzyme activity.
Numbers separated by a "/" are the minimum and maximum adaptation factors calculated for that particular study.
Literature comparisons:

1) Crabtree and Newsholme 1972a
2) Zammit et al 1978
3) Pette 1966, 1985
4) Helig and Pette 1980
5) Pete and Dolken 1975
6) Bass et al 1969
7) Ansay 1974
8) Laborde et al 1985
9) Newsholme et al 1978
10) Pette et al 1975

been found here (George et al. 1971; Kerem and Elsner 1973b; Simon et al. 1974; Ponganis and Pierce 1978; Austin and Geraci 1981; Castellini et al. 1981; Castellini and Somero 1981). A high capacity for glycolysis exists in harbor seal skeletal muscle -- particularly in the locomotory muscles. This is true for both aerobic glycolysis, as represented by the PK and PFK activities, and anaerobic glycolysis (LDH activity). Only a few bird pectoral muscles appear to have a capacity for aerobic glycolysis greater than harbor seal locomotory muscle (Crabtree and Newsholme 1972a; Zammit et al. 1978; Bloomstrand et al. 1986; Newsholme and Crabtree 1986); and bovine muscle is one of the few muscles studied with equivalent LDH activities (Emmett and Hochachka 1981). However, the amazingly powerful white muscle of both tuna (Guppy et al. 1978) and salmon (Mommsen et al. 1980) have LDH activities 5-fold greater than harbor seal.

The activity of the α-GPDH branch of the Embden-Meyerhof pathway is, on the other hand, fairly low. Rabbit white muscle and a number of bird pectoral muscles have much higher α-GPDH activities (Crabtree and Newsholme 1972a). This observation, coupled with the relatively high GOT activity in the skeletal muscles of harbor seals, indicates that the maintenance of cytosolic redox balance is more dependent on the malate-aspartate shuttle in than the α-glycerophosphate cycle. Of course this activity of the malate-aspartate shuttle in seal is still quite low when compared to the superbly adapted flight muscle of the hummingbird (Suarez 1986). Low levels of α-GPDH have also been observed in Weddell seals (Fried et al. 1967).

Aerobic capacity in harbor seal muscle is about average for vertebrate muscle. The CS activity falls in about the middle of the range of values reported if certain bird flight muscles are excluded (Alp et al. 1976; Marsh 1981; Suarez 1986); while HOAD activities are fairly high, particularly in the respiratory and locomotory muscles of the seal. The relatively high activity of CPT, and the high CAT activities confirm that fat oxidation is an important source of energy in seal muscle. There is, however, quite a discrepancy between the two acyl transferases (CPT and CAT). The capacity for the transfer of long chain acyl groups across the mitochondrial membrane (CPT activity) is an order of magnitude lower than bird flight muscle (Crabtree and Newsholme 1972b; Newsholme and Crabtree 1986; Suarez 1986), and only average for a number of other vertebrates. The capacity for transferring short chain fatty acids (CAT activity) is, however, an order of magnitude higher in harbor seal muscle than the few other muscles examined in the literature: rat skeletal muscle (Choi et al. 1977; Negrao et al. 1987), and rabbit red and white muscle (Henriksson et al. 1987). This difference may be indicative of a greater utilization of short chain fatty acids in B-oxidation than long chain fatty acids such as palmitate. However, the accumulating evidence of other, non-mitochondrial, uses for CAT (Bieber et al. 1982; Bremer 1983) make interpretation of these values difficult. The apparently high capacity for oxidizing fat in seal muscle is somewhat expected on the basis of high levels of fatty acids in harbor seal blood (Davis 1983). Such large amounts of plasma fatty acid inhibit glucose transport across cell membranes, thereby inhibiting glycolytic flux (Randle et al. 1963).

The contribution of amino acids as substrate for catabolism in harbor seal muscle is minimal. Low activities of both GDH and GPT are present in all the muscles examined. Although GPT levels are high enough to suggest some role in the provision of additional metabolite for the Krebs cycle by increasing pyruvate turnover, or a role in the maintenance of cytosolic redox balance similar to the function of GOT (Owen and Hochachka 1974).

The CPK activities in harbor seal skeletal muscle are high, but within the normal vertebrate range (Newsholme et al. 1978). Interestingly, the swimming muscles do not contain the highest activities. The m. pectoralis, m. psoas, m. masseter, along with the hamstring muscles, and muscles of the upper arm and shoulder exhibit higher CPK activity (the significance of these differences is detailed below).

Superficial versus deep muscle differences are readily apparent in the seal muscles examined. As would be expected on the basis of the fiber type distribution normally associated between superficial and deep areas of muscle (Guth and Samaha 1969; Yellin 1969; Baldwin et al. 1972; Gonyea and Ericson 1977; Gunn 1978; Armstrong 1980; Armstrong et al. 1982), the superficial muscle samples of harbor seal exhibit consistently higher glycolytic enzyme activities, as well as higher CPK levels. In contrast, aerobic enzymes, including the transaminases, are regularly greater in the deep portions of muscle than in the superficial areas.

The general pattern of enzyme relationships in harbor seal muscle is interesting. Those muscles with higher glycolytic capacity (both aerobic and anaerobic) also exhibit higher levels of Krebs cycle enzymes. This

close correlation does not, however, extend to the enzymes of fat metabolism, which show very little variation between muscles. As a result, all seal muscles appear capable of utilizing fatty acids for fuel at about the same rate. The relative importance of carbohydrate from muscle to muscle is, on the other hand, quite variable. More active muscles, such as those involved in locomotion, utilize carbohydrate to a great extent, both aerobically and anaerobically. Other muscles, with lower glycolytic capacity exhibit a decreased rate of flux through the Krebs cycle as well. This is different from the more commonly observed pattern in vertebrate muscle where an inverse relationship exists between aerobic enzyme activites and glycolytic enzymes (Bass et al. 1969; Talmant et al. 1982, 1986).

This coadaptation of aerobic enzymes and the enzymes of glycolysis in seal muscle may relate to their diving habit. The muscles required on a regular basis for normal aerobic activity have high levels of CS. These are the same muscles, however, that are required to function on occasions when oxygen supplies may be limited (at the end of a long dive for example), thereby resulting in the high levels of glycolytic enzymes present as well.

An equivalent type of relationship exists between the three metabolic fiber types [slow-oxidative (SO), fast-glycolytic (FG), and fast-oxidative-glycolytic (FOG)] normally present in skeletal muscle. When comparing SO fibers to FG fibers the inverse relationship between glycolytic enzymes and enzymes of aerobic pathways is readily apparent (Bass et al. 1969; Talmant et al. 1986). However, if either of these fiber types is compared with FOG fibers the relationship disappears. FOG fibers are characterized

by high levels of both glycolytic and oxidative enzymes. This indicates that the demands placed on seal muscle are generally similar to a FOG type of recruitment pattern.

Adaptation factors. It was originally expected that if seal muscle is subjected to chronic hypoxic stress due to the diving habit, the enzymatic differences between muscles of widely varying function would be unusually extreme. Consequently "adaptation factors" were calculated for seal muscle, along with comparison values based on the literature (Table 3), to identify any such extremes in enzyme variation.

No unusual values are observed for seal muscle. The calculated "adaptation factors" for seal match what is found in a variety of terrestrial vertebrates for the enzymes examined. If anything, the range of "adaptation factors" in seal is narrower than that found for the other species.

These normal "adaptation factors" in seal may be explained in one of three ways: 1) it could indicate that any additional hypoxic stresses placed on active seal muscles while diving are no greater than the additional demands placed on more active muscles of terrestrial animals during other types of activity (ie. exercise), 2) perhaps diving causes all muscles of seal to experience roughly equal hypoxic stress -- some muscles being preferentially fueled with oxygen but highly active, while although inactive, other muscles are deprived of oxygen to a large extent, or 3) the dives may be completely aerobic except on the rarest of occasions, and therefore the only adaptation in enzyme activity levels are those that are

exercise induced. This analysis does not, of course, take into consideration other biochemical modifications that may take place (isozymes, regulatory aspects, etc.).

In light of the normal maximal activities of the enzymes examined, the lack of any unusual "adaptation factors" seems to indicate that the muscles of harbor seals are not stressed to an unusual degree by diving. The combination of physiological adjustments with the possibility of strong selective metabolic depression and/or increased efficiency (see Chapter 1) appears to adequately "insulate" the seal muscle, thereby allowing sufficient aerobic activity in swimming muscles for exploration and prey capture, and low metabolic demands by other muscles so that available oxygen resources are adequate to meet demands.

Muscle relationships. The relation of their enzymatic profile to the probable function of individual muscles seems to be relatively clear-cut with the harbor seal muscles sampled. The fairly close similarity of phocid seal musculature to the normal mammalian pattern (Howell 1928) allows analogies between muscles to be drawn with only minor modification. General muscle actions discussed below are derived from human muscle anatomy and kinesiology (Travill 1962; Kendall et al. 1971; Gray 1989; MacConaill and Basmajian 1977; Basmajian 1978; McMahon 1984). The following discussion refers only to relative (intraspecies), rather than absolute (interspecies/comparative) enzyme activity differences. That is, a "high" or "low" activity is only "high" or "low" in relation to the other seal muscles examined -- not necessarily with regard to other species unless specifically stated.

As expected, the DIA appears to be quite unique compared with the other skeletal muscles examined. It is characterized by its very low glycolytic capacity, as indicated by low LDH, PK, PFK, and α-GPDH levels, and a very low CPK activity. In contrast, the activities of HOAD and CPT in DIA are extremely elevated for a seal muscle, and its CS level is also relatively high. Clearly, the DIA is the most aerobic of all the seal muscles examined, poised for using both carbohydrates and fats for fuel. As a muscle utilized exclusively for respiration, this type of enzymatic activity pattern would seen to be appropriate (Close 1972; Gollnick and Hermansen 1973; Holloszy 1973; Burke and Edgerton 1975; Armstrong 1980; Macova et al. 1985).

The two accessory muscles of respiration examined (INT and EXT), although generally more similar to the DIA than the other seal skeletal muscles, exhibit some important differences. The intercostales both have significantly higher CPK and glycolytic enzyme activities (including LDH), and marginally lower levels of CS and HOAD than are present in DIA. Thus, the intercostales appear better suited for rapid contraction than the DIA. There are, potentially, two key roles for this apparent difference in metabolism. First, the action of the intercostales may be crucial to the onset of rapid breathing following prolonged dives. Second, there can be action on the part of both sets of intercostales in lateral trunk flexion and, therefore, a locomotory as well as a postural role.

Of the harbor seal skeletal muscles examined, m. longissimus dorsi, m. iliocostalis, and m. gluteus maximus appear to be the primary swimming

muscles. Ignoring superficial vs deep differences, which confound the analysis at this level, these 3 muscles exhibit consistent metabolic patterns which distinguish them from the other seal muscles. They have high activities of all the enzymes examined, except for relatively lower levels of CPK. The high activities are particularly evident with regard to the enzymes of aerobic metabolism (CS, HOAD, CPT, and CAT), and LDH. Thus, the swimming muscles of the harbor seal seem particularly well-suited for continuous aerobic contractile activity on a regular basis, but with the glycolytic machinery necessary for rapid swimming or contraction under hypoxic stress (such as at the end of a prolonged dive). And, as is the case with the respiratory muscles, the swimming muscles are capable of using fats as an energy source (high HOAD activity) to a greater degree than the other skeletal muscles examined. This enzyme pattern seems to confirm that harbor seals probably utilize the large m. longissimus and m. iliocostalis in lateral flexion of the trunk while swimming, as well as the powerful back extension movements that characterize the lurching type of terrestrial locomotion in these seals (Harrison and Kooyman 1981). The use of the m. gluteus maximus as a swimming muscle is less obvious. However, once the condition of perpetual lateral rotation found with the posterior limbs of phocid seals is taken into account (Howell 1928), the action of this muscle in extension of the hind flippers plays an integral part in the swimming stroke. Accordingly, two other functions for m. gluteus maximus would also be important to the swimming seal. First, the action of this muscle as lateral rotator of the "leg" would help maintain the seal's unusual posture. Second, the function of m. gluteus maximus as a stabilizer of the femur at both the hip and knee joints would help maintain the integrity of the extended "leg" during the swimming stroke.

The m. pectoralis profundus appears to be enzymatically similar to the swimming muscles in several respects. It, too, has high capacity for both glycolysis and the Krebs cycle. But the HOAD activity, and therefore its capacity for utilizing fat as fuel, is much lower. The CPK activity, on the other hand, is relatively higher. These differences from the swimming muscles conform to the probable primary uses of m. pectoralis in harbor seals: 1) sudden forceful adductions of the anterior limbs during terrestrial locomotion, and 2) occasional quick movements of the anterior limbs during more rapid turns while swimming. This type of activity is best fueled by the rapid flux of carbohydrate through glycolysis to LDH and the Krebs Cycle, and/or phosphocreatine hydrolysis (Gollnick and Hermansen 1973; Holloszy 1973; McGilvery 1975; Gollnick 1983; Hochachka 1985).

The PSO, although commonly regarded as a swimming muscle in phocid seals (Castellini, personal communication), does not group together with the other swimming muscles. It has low levels of all the enzymes examined except for CPK and the enzymes of glycolysis. As a result, this muscle is primarily anaerobic in nature and appears best suited for occasional rapid contraction. This apparent difference in function between the PSO sample and conventional thoughts on m. psoas function in seals is probably due to a sampling difference in this study. PSO, here, refers to the m. psoas minor. Normally the psoas muscle is probably sampled from the m. psoas magnus, a more robust portion of the iliopsoas complex (resulting in the possibility of some of the sample coming from the m. hypaxialis as well). While the m. psoas magnus probably is, in fact, an

integral part of the swimming musculature as a hip flexor, the m. psoas minor (PSO) is likely only an accessory swimming muscle. The highly glycolytic nature of PSO would make it ideal for recruitment during occasional more forceful (or rapid) swimming strokes, or possibly at the end of a prolonged dive when oxygen supplies may be limiting (Gollnick and Hermansen 1973; Holloszy 1973; McGilvery 1975; Gollnick 1983; Hochachka 1985).

One of the two hamstring muscles examined, the SEM, has a similar enzymatic patten to the PSO, with high CPK and glycolytic enzyme activites. As the more medial of the 2 hamstring muscles examined, the action of SEM in hip extension can play a role in the swimming stroke of harbor seals. Although its glycolytic nature seems to indicate that, like PSO, it is primarily involved only when the movements are rapid, forceful or hypoxic. The other hamstring muscle studied is the BFM. BFM is the more lateral of the two hamstring muscles sampled. Theoretically, it too could have an impact on swimming by its action as a hip extensor. The enzymatic profile of BFM, however, does not support this role. Its relatively low activities of most of the enzymes examined, in comparison with the other seal muscles, indicate that it is more likely a postural muscle (Close 1972; Burke and Edgerton 1975; Armstrong 1980). This function makes sense with regard to the angle, extreme lateral rotation, at which the posterior limbs of phocid seals are maintained at all times (Howell 1928). As the hamstring muscle responsible for lateral rotation of the "leg", BFM would be primarily used for the maintenance of this unusual posture. Although relatively high CPK activity (and slightly high PK and

PFK) may indicate the capacity for occasional recruitment during a powerful swimming stroke.

Four other muscles (SPT, ATL, LAT, and EDC) also seem to be enzymatically geared primarily as postural muscles. Both the ATL and SPT have 2 main functions in terrestrial mammals. They stabilize, elevate and rotate the scapula (for use in weight-bearing by the arm in man, for example), and they incline, rotate, and extend the head and neck. In the case of harbor seals, the weight-bearing actions on the scapula would be minimal, leaving only shoulder stabilization and movements of the head as important actions. The low activities of all the enzymes examined support this type of role for both muscles. The LAT, being a sample of the small m. latissimus dorsi 2 rather than the larger m. latissimus dorsi 1 [as identified by Howell (1928)] appears to have fairly limited use in the harbor seal. The LAT has low activities of all 13 enzymes. This would indicate that the m. latissimus dorsi 2 (LAT) is probably involved only in the retraction of the shoulder girdle (rowing-type movements) -- a very minor, and primarily postural, activity in harbor seal. The more important actions, forceful depression of the anterior limbs and assistance in lateral flexion of the trunk, are left to the m. latissimus dorsi 1 which was not sampled. The other muscle examined that also appears to be postural in nature is the EDC. Again, it has relatively low activities of all the enzymes measured. In mammals the EDC is normally used for extension and powerful gripping movements of the hand. In the seal, the design of the anterior limb as a flipper rather than a "true" hand make these actions fairly obsolete. The low enzyme activites tend to confirm the use of the remaining EDC muscle as primarily postural.

Two muscles originally thought to be potentially important in locomotion are the PAL and OBL. The PAL, as such an unusual extension of the m. pectoralis profundus caudally to the posterior limbs of phocid seals (Howell 1928), appears to be ideally suited for assistance in trunk flexion (either laterally for swimming, or dorso-ventrally for assistance terrestrial locomotion). Its enzymatic profile is, however, quite different from the major locomotory muscles identified above. The activities of all the enzymes measured are about in the middle of the range of values found for the other seal muscles examined. This relatively average metabolic potential in PAL seems to indicate that, although probably more than just a postural muscle, its actions with regard to force generation for locomotion are likely unspectacular. The OBL, by virtue of its function as a lateral flexor of the trunk, could also play an important role in harbor seal swimming movements. However the enzymatic profile of this muscle tends not to support this as being as important as originally thought. Relatively low activities of most of the enzymes measured are present in OBL. The one exception to this is a slightly higher relative level of glycolytic enzymes found in this muscle. This glycolytic capacity may indicate a support role for OBL in the swimming stroke (possibly as a trunk stabilizer during more violent movements), but it appears that it is not one of the primary swimming muscles. An additional possibility exists that the OBL may, rather, play a role in rapid breathing following prolonged dives by assisting with forced expiration. The slightly elevated levels of glycolytic enzymes could also help support this type of activity.

The muscles of the upper arm and shoulder (TLT, TLG and DLT) have enzymatic profiles fairly similar to one another. They all tend to have enzyme activites in about the middle of the range of values observed for harbor seal muscles. Exceptions to this are relatively lower HOAD activities which would indicate more reliance on carbohydrate for fuel than many of the other muscles, and very high CPK activities. The DLT (medial head sampled) is active in nearly all "arm" movements, primarily as an abductor, and the 2 triceps muscles sampled (TLT and TLG) extend the "forearm." Both of these types of movements in harbor seals are relatively limited in comparison to other mammals. The use of the foreflippers during both marine and terrestrial locomotion for occasional rapid thrusts and, at times, for the manipulation of objects are, apparently, the primary uses of these muscles in seals. The tendency of all three muscles to utilize carbohydrate, coupled with very high CPK activities would seem to support these types of uses. One interesting difference between the TLG and the other two muscles (TLT and DLT) is the more highly anaerobic nature of TLG, as evidenced by lower levels of aerobic enzymes as well as slightly higher activities of CPK and glycolytic enzymes. This probably results because the TLG, as the long head of the m. triceps, is recruited last (of the 3 triceps muscles) and only during specific arm movements (such as adduction of the humerus). Consequently the demand for action from the TLG would be even less than the demands upon the DLT and TLT, which are both active in a much larger number of "arm" movements.

The enzyme activities of DEP are similar to what is observed with the upper arm and shoulder muscles (TLT, TLG, and DLT), but with two crucial differences: 1) it has higher activities of the enzymes of fat metabolism

(HOAD, CPT and CAT), and 2) relatively less CPK. These differences result in an enzyme activity profile that, although slightly higher in absolute activites, is reminiscent of the postural muscles. Since the DEP functions primarily in stablilizing the humerus in the glenoid fossa, the enzymatic profile of a postural muscle for DEP is not unexpected. However, the slightly elevated enzyme activities over that found in the other seal postural muscles examined, along with the relatively robust size of the DEP (Howell 1928), indicate greater functional demands on DEP than the other postural muscles. This more "powerful" nature of the DEP is a result of its vital role in preventing displacement of the "arm" at the shoulder joint. This would be important to the seal particularly during the occasional forceful thrusts of the foreflipper during terrestrial locomotion.

The final seal muscle examined, MAS, is also generally similar to the postural muscles with regard to enzyme activities. The levels of all enzymes are relatively low except for slightly elevated CS and CPK activity. The similarity in enzymatic profile to the postural muscles is somewhat surprising, considering the generally accepted role of the MAS as muscle for active force generation in biting and chewing. The low activities of the enzymes examined are only low, however, in relation to the other seal muscles. The actual capacity in MAS for glycolysis and the Krebs cycle (as compared to muscle from other animal species) appears adequate for mastication. And the high CPK activity indicates the ability for rapid closing of the jaw in prey capture.

Cluster analysis. Clustering of the seal muscles, based on the activities of all 12 enzymes, indicates the existence of 4 clearly separated clusters. The

DIA is in a cluster by itself. A second group indicated is primarily composed of postural muscles (ATL, EXT, INT, BFM, EDC, SPT, LAT, PAL, and MAS). The third cluster is made up of locomotory "assistance" muscles and superficial portions of locomotory muscles (PMS, GMS, ILS, TLG, TLT, DLT, PSO, SEM, OBL, and DEP). The final cluster is composed of the primary locomotory muscles, particularly the deeper portions of the muscle (GMD, ILD, LDD, LDS, and PMD).

This clustering pattern statistically confirms the general trends between muscle functions and enzymatic activities as discussed above.

Summary. Clear enzymatic differences have been demonstrated between a large number of seal muscles of widely varying function. The importance of defining specific locations for muscle samples is emphasized by the variable enzyme activities between muscles, as well as superficial vs deep areas of muscle. The functional relationships of the muscles have been clarified by their particular enzyme activity patterns in relation to the other muscles examined. The general pattern of metabolism in harbor seal muscle has been elucidated. A combination of "adaptation factors" and absolute enzyme activities indicate that seal skeletal muscle does not appear to undergo unusual hypoxic stress in comparison with terrestrial species. The well-known physiological responses to diving coupled with a selective metabolic depression is suggested to account for this finding.

CHAPTER 3:

HARBOR SEAL ENZYME ACTIVITY RATIOS

Introduction. Enzyme activity ratios have been shown to be particularly effective in clarifying metabolic organization differences between muscles (Pette et al. 1962a, 1962b; Bass et al. 1969; Flavell and Woodward 1970; Staudte and Pette 1972; Pette and Dolken 1975; Hochachka et al. 1982; Pette 1985). The advantages of utilizing ratios, rather than simple maximal enzyme activities, are several. First, differences in the amount of foreign material (i.e. connective tissue), dilution, or degradation in muscle samples that may affect maximal activity levels does not alter activity ratios, since the series of enzyme assays are commonly conducted on the same sample. Besides the correction and masking of such potential methodological errors, ratios allow accurate comparison of widely varying species whose absolute enzyme activities may vary by orders of magnitude. Even though their metabolism appears to vary a great deal based on maximum activities, the basic metabolic organization of these species may be quite similar when viewing the unitless ratios (Staudte and Pette 1972); while other ratios that vary as much, or more, may clarify the same or other metabolic differences. Also, because ratios are unitless measures they allow easy and accurate comparisons between studies whose enzyme activities are expressed in different units and/or at different temperatures without the need for conversion factors which may or may not be accurate for that enzyme in that particular tissue.

Perhaps the greatest advantage in utilizing ratios is simply because of what the resulting number represents: a measure of the relative capacities between two pathways or portions of the same pathway (Pette and Dolken 1975; Hochachka et al. 1982; Pette 1985). In this way one is able to view the relationships between metabolic pathways clearly -- likely a much better indication of metabolic organization than activities alone. This is possible because of the discovery of "constant proportion groups of enzymes" (Vogel et al. 1959; Pette et al. 1962a, 1962b; Pette and Bucher 1963; Pette 1965, 1966). This observation, that particular segments of energy-supplying metabolism exist as non-variable units within the enzyme activity pattern, allows ratios to fairly represent relative pathway capacities. (Although, clearly, they are not equal to in vivo flux rates). Constant proportion groups of enzymes have been found in glycolysis, B-oxidation, the Krebs cycle and the respiratory chain (see Pette 1985 for literature), such that within each group the relative enzyme activities are the same in spite of wide variation in absolute enzyme activites when different tissues are compared.

Enzyme activity ratios may be variable or constant (discriminative or non-discriminative). Obviously, ratios between enzymes of the same constant proportion group (or metabolic pathway) would vary little between tissues (ie. enzymes of glycolysis) (Pette et al. 1962a, 1962b; Pette 1985). While other ratios, between different metabolic pathways (ie. Krebs cycle/glycolysis) have been found to vary by orders of magnitude (Bass et al. 1969; Staudte and Pette 1972; Pette and Dolken 1975; Hochachka et al. 1982; Hochachka 1985).

Utilizing the enzyme activities determined in Chapter 2 for the 21 harbor seal skeletal muscles, a series of 23 enzyme activity ratios was calculated for these muscles. By comparing the ratios, a much clearer picture of the seals' muscle metabolism develops. This is particularly true concerning interspecific muscle differences. In addition, since all determinations of the constant or variable nature of various enzyme ratios involve between species comparisons, or comparison between muscles (within the same species) that are "pure red" or "pure white" with regard to fiber type, it was of interest to examine the variable or constant nature of these ratios between "mixed" muscles of the same species. All the harbor seal muscles sampled have between 20% and 70% Type IIb (=white or fast glycolytic) fibers (Foreman, unpublished observations).

To a large extent the ratios examined have been studied previously in other species (ie. PK/LDH, HOAD/CS, PK/HOAD). However, several of the ratios (10 in all) have not been reported prior to this (GOT/CS, α-GPDH/CS, CPK/PK, HOAD/CPT, HOAD/CAT, GOT/glycolytic enzymes, α-GPDH/PK, and α-GPDH/PFK); although in the majority of cases similar/equivalent enzyme ratios exist in the literature. Comparison values for these "new" ratios were calculated from existing data and are included in the text of the results section.

Materials and Methods.

--as described in Chapter 2

Results.

Enzyme ratios. Table 4 lists the means ± 1 S.E. of 23 enzyme activity ratios for each of the 21 skeletal muscles studied. The correlations between these ratios and the maximum activities of the 12 enzymes measured are shown in Appendix 2. Correlations between the ratios can be found in Table 5. Two-way analysis of variance (not shown) indicates significant muscle differences in all ratios examined except for HOAD/CPT. Significant animal effects are also evident with all ratios except PK/LDH and PFK/CS. However, the animal effects are, again, small and appear to be an artifact of the way the assays were conducted (each seal's muscles having been analyzed on a separate day from the others' for each series of enzymes), rather than an indication of important animal differences.

The relationship between aerobic and anaerobic glycolysis in skeletal muscle was compared by examining the PK/LDH, PFK/LDH, and α-GPDH/LDH enzyme ratios (Table 4). The PK/LDH ratio is strikingly constant across the 21 muscles examined (Appendix 1). The only exception is the DIA, which is significantly lower than 5 other muscles (PSO, BFM, TLT, SPT, GMS). It is also apparent that, although not statistically significant, the superficial portions of muscles have higher PK/LDH ratios than the deep areas (Table 4).

TABLE 4. ENZYME ACTIVITY RATIOS OF HARBOR SEAL MUSCLES (CONT'D ON THE NEXT 3 PAGES).

MUSCLE	PK/LDH		PFK/LDH		a-GPDH/LDH		PK/PFK		a-GPDH/PK		a-GPDH/PFK	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	0.88	0.06	0.062	0.002	0.029	0.001	14.14	0.42	0.034	0.003	0.48	0.03
LDD	0.83	0.08	0.071	0.007	0.033	0.003	11.98	0.54	0.039	0.002	0.47	0.04
ILS	0.94	0.03	0.069	0.003	0.031	0.002	13.72	0.93	0.033	0.001	0.46	0.05
ILD	0.79	0.06	0.061	0.005	0.031	0.003	13.47	1.61	0.039	0.003	0.51	0.05
GMS	1.00	0.14	0.066	0.008	0.025	0.002	15.82	3.46	0.026	0.002	0.39	0.05
GMD	0.80	0.06	0.071	0.007	0.028	0.003	11.27	0.55	0.036	0.002	0.41	0.01
SEM	0.79	0.05	0.062	0.002	0.028	0.004	12.72	0.98	0.036	0.006	0.44	0.06
OBL	0.96	0.10	0.066	0.004	0.031	0.003	14.64	1.35	0.033	0.001	0.48	0.03
PSO	1.12	0.14	0.073	0.007	0.033	0.006	15.21	0.96	0.031	0.004	0.45	0.06
PMS	0.95	0.06	0.071	0.004	0.029	0.002	13.45	0.22	0.031	0.003	0.42	0.03
PMD	0.89	0.03	0.073	0.005	0.034	0.003	12.45	1.03	0.038	0.004	0.47	0.05
PAL	0.86	0.03	0.058	0.011	0.031	0.002	17.41	4.55	0.035	0.001	0.61	0.15
DEP	0.90	0.05	0.058	0.001	0.032	0.006	15.61	0.87	0.035	0.005	0.56	0.11
DLT	0.90	0.08	0.069	0.006	0.033	0.003	13.26	1.28	0.038	0.005	0.51	0.09
TLT	1.02	0.06	0.061	0.007	0.029	0.001	17.41	2.32	0.029	0.001	0.51	0.07
TLG	0.85	0.04	0.078	0.007	0.038	0.002	11.03	0.83	0.045	0.003	0.51	0.05
BFM	1.06	0.05	0.081	0.011	0.028	0.003	13.58	0.99	0.026	0.002	0.35	0.04
LAT	0.98	0.05	0.066	0.009	0.029	0.003	15.44	1.70	0.029	0.003	0.46	0.09
SPT	1.00	0.09	0.047	0.007	0.032	0.004	22.97	3.74	0.033	0.006	0.72	0.11
ATL	0.81	0.07	0.034	0.009	0.035	0.009	33.43	12.31	0.044	0.011	1.57	0.68
INT	0.80	0.05	0.036	0.009	0.032	0.003	27.32	7.35	0.041	0.005	1.22	0.05
EXT	0.85	0.04	0.062	0.004	0.032	0.003	13.89	1.30	0.038	0.003	0.53	0.07
EDC	0.91	0.02	0.069	0.005	0.024	0.001	13.32	0.92	0.026	0.002	0.35	0.04
MAS	0.87	0.06	0.067	0.008	0.055	0.009	13.71	2.28	0.064	0.011	0.83	0.11
DIA	0.65	0.04	0.037	0.008	0.025	0.003	22.83	8.70	0.038	0.003	0.89	0.36
ALL	0.90	0.02	0.063	0.002	0.031	0.001	16.01	0.84	0.036	0.001	0.58	0.04

TABLE 4. (CONT'D).

MUSCLE	LDH/CS		PK/CS		PFK/CS		a-GPDH/CS		LDH/HOAD		PK/HOAD	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	45.31	1.88	39.79	3.64	2.81	0.21	1.32	0.02	37.31	9.16	32.16	7.02
LDD	43.53	3.19	36.15	3.74	3.01	0.27	1.43	1.19	43.40	11.87	33.47	6.28
ILS	50.07	2.77	47.02	3.47	3.42	0.04	1.56	0.16	48.42	12.15	44.42	9.63
ILD	42.74	3.12	33.09	1.17	2.59	0.39	1.28	0.11	44.45	14.58	33.37	9.09
GMS	57.21	4.34	56.66	8.35	3.71	0.21	1.42	0.11	53.01	14.48	51.43	14.3
GMD	38.36	1.92	30.82	3.58	2.76	0.35	1.11	0.15	36.22	10.36	27.44	6.09
SEM	72.25	7.76	57.99	9.73	4.51	0.51	2.07	0.44	73.67	18.91	58.67	16.4
OBL	53.01	3.63	50.36	4.43	3.48	0.31	1.64	0.13	48.17	11.43	42.98	6.68
PSO	56.15	9.13	62.53	11.19	4.21	0.93	1.88	0.46	52.89	13.08	56.84	13
PMS	57.88	5.53	53.98	3.25	4.02	0.27	1.68	0.19	71.87	17.61	66.09	14.2
PMD	50.57	5.43	45.48	6.39	3.71	0.57	1.74	0.29	50.53	14.45	45.02	12.1
PAL	59.21	9.92	50.09	6.92	3.11	0.29	1.73	0.23	55.11	14.18	47.53	12.8
DEP	55.94	9.57	49.13	6.68	3.22	0.53	1.75	0.37	52.62	12.88	45.66	9.89
DLT	45.46	3.16	40.61	3.32	3.09	0.21	1.52	0.21	56.55	10.69	49.01	6.51
TLT	51.36	7.33	51.63	7.16	3.27	0.83	1.49	0.21	59.87	12.66	61.38	14.6
TLG	86.98	27.45	71.76	19.74	6.27	1.31	3.32	1.04	104.60	21.59	90.61	21.7
BFM	63.29	8.11	67.84	9.97	5.17	1.05	1.75	0.27	48.88	15.44	49.86	13.7
LAT	44.48	7.06	43.53	6.62	2.85	0.34	1.32	0.31	38.21	9.14	36.64	7.45
SPT	47.45	5.82	46.89	4.89	2.14	0.26	1.49	0.16	42.30	12.66	40.82	10.2
ATL	59.97	5.43	48.08	3.85	2.03	0.61	2.01	0.39	51.88	13.56	43.37	13.3
INT	46.22	4.52	36.16	1.49	1.57	0.32	1.51	0.22	33.19	8.68	27.05	7.79
EXT	35.63	3.92	30.25	3.68	2.17	0.13	1.17	0.21	27.94	6.73	23.75	5.91
EDC	44.99	5.26	41.07	4.74	3.07	0.23	1.07	0.17	40.94	7.71	37.71	7.62
MAS	27.58	2.95	23.65	1.39	1.83	0.23	1.53	0.27	52.48	12.08	46.97	12.7
DIA	23.01	2.14	15.31	2.41	0.82	0.17	0.59	0.12	16.53	5.38	10.38	2.98
ALL	50.35	1.89	45.19	1.77	3.15	0.15	1.57	0.07	49.64	2.81	44.11	2.51

TABLE 4. (CONT'D).

MUSCLE	PFK/HOAD		a-GPDH/HOAD		CPK/LDH		CPK/PK		HOAD/CS	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	2.27	0.49	1.12	0.33	2.38	0.13	2.77	0.28	1.41	0.27
LDD	2.82	0.59	1.31	0.23	2.81	0.08	3.45	0.26	1.25	0.31
ILS	3.43	0.98	1.43	0.26	2.82	0.22	3.03	0.33	1.31	0.41
ILD	2.52	0.69	1.25	0.28	2.64	0.16	3.41	0.24	1.23	0.31
GMS	3.29	0.81	1.28	0.31	2.56	0.16	2.65	0.23	1.34	0.33
GMD	2.38	0.45	0.94	0.17	2.63	0.41	3.32	0.44	1.35	0.33
SEM	4.46	1.02	1.88	0.45	2.71	0.24	3.42	0.15	1.23	0.34
OBL	3.06	0.61	1.42	0.25	2.87	0.17	3.14	0.51	1.28	0.26
PSO	3.72	0.75	1.55	0.09	3.44	0.33	3.16	0.34	1.21	0.28
PMS	4.89	1.02	2.09	0.55	2.92	0.27	3.12	0.36	0.97	0.25
PMD	3.69	1.11	1.61	0.31	3.02	0.21	3.38	0.19	1.25	0.32
PAL	3.31	1.11	1.62	0.41	2.85	0.17	3.32	0.11	1.37	0.44
DEP	3.04	0.75	1.47	0.17	3.17	0.28	3.55	0.36	1.23	0.26
DLT	3.92	0.81	1.83	0.33	3.77	0.23	4.31	0.48	0.91	0.19
TLT	3.83	1.19	1.79	0.43	3.34	0.26	3.29	0.17	0.96	0.21
TLG	8.14	1.85	3.95	0.78	3.72	0.16	4.41	0.14	0.95	0.34
BFM	3.62	0.89	1.24	0.31	3.93	0.34	3.68	0.19	1.77	0.52
LAT	2.49	0.61	1.03	0.17	3.76	0.06	3.85	0.13	1.46	0.51
SPT	2.17	0.86	1.48	0.63	3.72	0.24	3.73	0.13	1.47	0.47
ATL	2.08	0.91	1.52	0.29	3.57	0.55	4.38	0.57	1.41	0.33
INT	1.42	0.63	1.01	0.19	3.11	0.29	3.88	0.17	1.79	0.54
EXT	1.76	0.48	0.85	0.16	3.73	0.23	4.45	0.41	1.59	0.46
EDC	2.84	0.62	0.95	0.15	3.19	0.23	3.51	0.25	1.21	0.19
MAS	3.49	0.92	2.71	0.51	6.13	0.61	7.09	0.71	0.66	0.19
DIA	0.66	0.25	0.37	0.08	3.01	0.24	4.71	0.61	2.04	0.71
ALL	3.17	0.21	1.51	0.09	3.27	0.09	3.72	0.11	1.31	0.07

TABLE 4. (CONT'D).

MUSCLE	HOAD/CPT		HOAD/CAT		GOT/CS		GOT/PK		GOT/PFK		GOT/LDH	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LDS	109.05	20.53	10.24	2.15	2.82	0.07	0.073	0.007	1.02	0.09	0.062	0.003
LDD	79.01	20.88	7.36	1.51	3.32	0.35	0.092	0.003	1.10	0.08	0.076	0.005
ILS	83.11	21.35	8.81	2.42	3.26	0.20	0.071	0.003	0.95	0.05	0.065	0.004
ILD	80.21	17.08	7.46	1.48	3.02	0.17	0.091	0.005	1.23	0.16	0.072	0.007
GMS	88.87	18.78	9.16	2.22	3.22	0.05	0.061	0.006	0.88	0.07	0.057	0.005
GMD	83.86	17.45	9.24	2.78	2.80	0.43	0.096	0.018	1.71	0.23	0.073	0.011
SEM	100.92	43.41	8.46	2.58	4.35	0.60	0.076	0.004	0.96	0.04	0.061	0.002
OBL	98.33	18.63	7.76	1.28	3.44	0.05	0.071	0.006	1.01	0.08	0.066	0.004
PSO	76.75	16.16	8.78	0.68	3.70	0.48	0.063	0.008	0.96	0.15	0.069	0.011
PMS	84.64	17.91	6.91	2.19	3.59	0.11	0.067	0.003	0.90	0.05	0.063	0.005
PMD	74.98	18.87	7.54	1.86	3.76	0.19	0.086	0.009	1.06	0.10	0.076	0.005
PAL	101.91	43.41	10.78	3.90	3.49	0.17	0.073	0.009	1.18	0.18	0.063	0.008
DEP	80.45	34.96	7.30	1.42	4.18	0.50	0.086	0.004	1.35	0.13	0.078	0.008
DLT	66.79	12.98	7.66	2.06	3.30	0.23	0.083	0.009	1.08	0.10	0.073	0.002
TLT	60.39	13.83	7.99	2.27	3.45	0.40	0.067	0.002	1.18	0.18	0.068	0.004
TLG	351.74	285.12	6.07	2.68	4.13	0.79	0.063	0.011	0.67	0.05	0.053	0.007
BFM	152.45	43.67	22.19	5.97	3.47	0.48	0.052	0.002	0.71	0.07	0.055	0.001
LAT	100.77	47.79	17.89	8.68	3.09	0.30	0.073	0.004	1.12	0.14	0.071	0.005
SPT	103.61	28.51	19.29	7.32	3.37	0.39	0.072	0.001	1.65	0.27	0.072	0.007
ATL	100.96	49.88	10.10	2.36	4.33	0.66	0.092	0.019	2.90	0.87	0.076	0.017
INT	203.26	133.92	15.35	6.55	3.04	0.11	0.084	0.003	2.25	0.55	0.067	0.005
EXT	107.27	35.13	12.09	4.48	2.89	0.17	0.098	0.007	1.34	0.07	0.083	0.004
EDC	89.26	23.80	13.47	2.53	3.58	0.18	0.091	0.009	1.18	0.09	0.082	0.008
MAS	58.14	25.51	4.35	1.67	3.24	0.12	0.138	0.009	1.85	0.23	0.121	0.009
DIA	145.77	88.39	16.70	4.31	2.78	0.40	0.184	0.012	4.04	1.38	0.121	0.009
ALL	107.31	13.68	10.52	0.78	3.42	0.08	0.084	0.003	1.35	0.10	0.073	0.002

See Materials and Methods for abbreviations.
S.E. = 1 standard error of the mean.
n = 4.

In comparison with the data of Hochachka (1985) the only muscles with comparable PK/LDH ratios to harbor seal skeletal muscle are found in a high-altitude adapted deer (Taruca) and the shrew. (N.B. -- The values of m. gastrocnemius reported by Hochachka have been corrected for the 3 high-altitude adapted species. The published values were inadvertently overestimated because the LDH activity used in the calculation was not the maximal activity reported. The new ratios are as follows: Taruca = 0.89, Llama = 0.65, and Alpaca = 0.68.) The result is that the harbor seal skeletal muscle mean value of 0.90 (Table 4) places it near the top of the normal range of PK/LDH activity ratios reported for other vertebrate species, ranging between 0.44 and 0.93 (Pette and Dolken 1975; Hochachka 1985). One glaring exception to this is a PK/LDH activity ratio of nearly 3 in hummingbird flight muscle, a tissue supremely adapted for carbohydrate oxidation (Suarez 1986). The PFK/LDH activity ratio is significantly correlated to the PK/LDH ratio (Table 5), and the pattern of relationships between skeletal muscles is roughly similar. The magnitude of the PFK/LDH activity ratios observed in seal skeletal muscle (Table 4) is about equal to those calculated for rabbit soleus, 0.07 (Pette and Dolken 1975) and single human muscle fibers, 0.05 (Lowry et al. 1978). α-GPDH/LDH activity ratios of seal muscle are not correlated with either PK/LDH or PFK/LDH ratios (Table 5), nor does the α-GPDH/LDH activity ratio discriminate well among the seal skeletal muscles. The only significant trend is that the MAS has a much higher ratio than all the other muscles (Appendix 1). The α-GPDH/LDH values found in harbor seal skeletal muscle are about the same as that calculated for the data of hummingbird flight muscle (Suarez 1986); with the MAS value of 0.055

slightly higher than, and the remainder of the muscles (0.038 to 0.024) slightly lower than, the hummingbird value of 0.041.

Anaerobic glycolysis was also compared to two other aerobic pathways using enzyme ratios. The relationship to the Krebs Cycle was examined by LDH/CS activity ratios, and fat metabolism relationships were compared using LDH/HOAD ratios. A significant positive correlation exists between these two sets of ratios (Table 5); and both LDH/CS and LDH/HOAD exhibit the same general trends across the 21 muscles examined (Appendix 1). The swimming muscles tend to be in the middle/lower part of the range of values, and superficial muscle samples have higher ratios than deep ones. An unusual observation is that the TLG is significantly higher in both ratios than most other muscles, including those closely associated with it (TLT and DLT). The respiratory muscles tend to be low in both LDH/CS and LDH/HOAD. This close similarity between the two ratios can be seen clearly in Figure 2. The mean value of the LDH/CS activity ratio in the seal skeletal muscles examined (50.34) is at the low end of a range of values calculated for a number of vertebrate muscles (Bass et al. 1969; Pette and Dolken 1975; Emmett and Hochachka 1981; Mackova et al. 1985). According to the data of Bass and coworkers (1969), red muscles have low values ranging between 16 and 26, while white muscle is much higher, between 200 and 1100. The pattern observed with the LDH/HOAD ratios in vertebrate muscle is similar. The mean value of LDH/HOAD for harbor seal muscle (49.64) is on the low end of a wide range of values found in vertebrate muscles, from 1 to 1241 (Bass et al. 1969; Pette and Dolken 1975; Ponganis and Pierce 1978; Emmett and Hochachka 1981; Mackova et al. 1985). The same relationship between red and white muscle

TABLE 5. CORRELATION MATRIX BETWEEN ENZYME ACTIVITY RATIOS OF HARBOR SEAL MUSCLE (CONT'D ON THE NEXT PAGE).

	PK/LDH	PK/CS	PK/HOAD	LDH/CS	LDH/HOAD	HOAD/CS	a-GPDH/LDH	CPK/LDH	PFK/LDH	a-GPDH/PK	a-GPDH/HOAD	a-GPDH/CS
PK/LDH	* 1	*0.49	0.19	0.07	-0.06	0.07	0.14	*0.28	*0.36	*-0.46	-0.01	0.12
PK/CS	*0.49	* 1	*0.33	*0.87	*0.22	*0.24	0.01	-0.02	0.19	*-0.31	0.19	*0.65
PK/HOAD	0.19	*0.33	* 1	*0.31	*-0.96	*-0.79	*-0.23	*0.21	0.14	*-0.34	*0.87	0.03
LDH/CS	0.07	*0.87	*0.31	* 1	*-0.32	*0.21	-0.03	-0.17	0.06	-0.07	*0.27	*-0.72
LDH/HOAD	-0.06	*0.22	*0.96	.*0.32	* 1	*-0.83	*-0.28	0.12	0.06	*-0.23	*0.89	0.01
HOAD/CS	0.06	*0.24	*-0.79	*0.21	*-0.83	* 1	*0.31	*-0.22	-0.04	*0.24	*-0.73	*0.37
a-GPDH/LDH	0.14	0.01	*-0.23	-0.03	*-0.28	*0.31	* 1	*0.28	0.13	*0.77	0.13	*0.61
CPK/LDH	*0.28	-0.02	*0.21	-0.17	0.12	*-0.22	*0.28	* 1	0.11	0.09	*0.29	0.11
PFK/LDH	*0.36	0.19	0.14	0.06	0.06	-0.04	0.13	0.11	* 1	-0.12	0.12	0.13
a-GPDH/PK	*-0.46	*-0.31	*-0.34	-0.07	*-0.23	*0.24	*0.77	0.09	-0.12	* 1	0.11	*0.45
a-GPDH/HOAD	-0.01	0.19	*0.87	*0.27	*0.89	*-0.73	0.13	*0.29	0.12	0.11	* 1	*0.27
a-GPDH/CS	0.12	*0.65	0.03	*0.72	0.01	*0.37	*0.61	0.11	0.13	*0.45	*0.27	* 1
PK/PFK	*0.26	0.19	-0.08	0.07	-0.16	0.19	0.04	0.01	*-0.73	-0.11	-0.15	0.07
GOT/CS	0.15	*0.61	0.11	*0.62	0.07	*0.28	*0.24	0.13	0.11	0.13	0.18	*0.65
CPK/PK	*-0.39	*-0.37	0.08	*-0.22	0.17	*-0.29	0.14	*0.73	-0.14	*0.41	*0.28	0.01
a-GPDH/PFK	-0.14	-0.11	*-0.31	-0.06	*-0.31	*0.31	*0.66	0.18	*-0.59	*0.68	-0.01	*0.41
PFK/CS	*0.31	*0.74	*0.36	*0.71	*0.31	0.07	0.02	-0.05	*0.66	-0.19	*0.29	*0.52
GOT/PK	*-0.53	*-0.79	*-0.32	*-0.64	*-0.21	-0.14	0.11	0.14	*-0.21	*0.42	-0.11	*-0.39
GOT/PFK	*-0.27	*-0.53	*-0.36	*-0.47	*-0.32	0.05	0.11	0.14	*-0.72	*0.28	*-0.24	*-0.25
GOT/LDH	0.02	*-0.59	*-0.31	*-0.74	*-0.34	-0.06	*0.21	*0.35	-0.04	0.17	*-0.21	*-0.39
PFK/HOAD	0.05	*0.22	*0.93	*0.27	*0.94	*-0.79	*-0.23	0.18	*0.36	*-0.26	*0.85	0.01
HOAD/CPT	0.13	*0.38	*-0.55	*0.34	*-0.61	*0.83	*0.34	-0.18	-0.02	*0.23	*-0.51	*0.47
HOAD/CAT	0.14	*0.25	*-0.69	0.18	*-0.75	*0.87	0.08	-0.14	-0.13	0.01	*-0.75	*0.21

TABLE 5. (CONT'D).

	PK/PFK	GOT/CS	CPK/PK	a-GPDH/PFK	PFK/CS	GOT/PK	GOT/PFK	GOT/LDH	PFK/HOAD	HOAD/CPT	HOAD/CAT
PK/LDH	*0.26	0.15	*-0.39	-0.14	*0.31	*-0.53	*-0.27	0.02	0.05	0.13	0.14
PK/CS	0.19	*0.62	*-0.37	-0.11	*0.74	*-0.79	*-0.51	*-0.59	*0.22	*0.38	*0.25
PK/HOAD	-0.08	0.11	0.08	*-0.31	*0.36	*-0.32	*-0.36	*-0.31	*0.93	*-0.55	*-0.69
LDH/CS	0.07	*0.62	*-0.22	-0.06	*0.71	*-0.64	*-0.47	*-0.74	*0.27	*0.34	0.18
LDH/HOAD	-0.16	0.07	0.17	*-0.31	*0.31	*-0.21	*-0.32	*-0.33	*0.94	*-0.61	*-0.75
HOAD/CS	0.19	*0.28	*-0.29	*0.31	0.07	-0.14	0.05	-0.06	*-0.79	*0.83	*0.87
a-GPDH/LDH	0.04	*0.24	0.14	*0.66	0.02	0.11	0.11	*0.21	*-0.23	*0.34	0.08
CPK/LDH	0.01	0.13	*0.73	0.18	-0.05	0.14	0.15	*0.35	0.18	-0.18	-0.14
PFK/LDH	*-0.73	0.11	-0.14	*-0.59	*0.66	*-0.21	*-0.72	-0.04	*0.36	-0.02	-0.13
a-GPDH/PK	-0.11	0.13	*0.41	*0.68	-0.19	*0.42	*0.28	0.17	*-0.26	*0.22	0.01
a-GPDH/HOAD	-0.15	0.18	*0.28	-0.01	*0.29	-0.11	*-0.24	*-0.21	*0.85	*-0.51	*-0.75
a-GPDH/CS	0.07	*0.65	0.01	*0.41	*0.52	*-0.39	*-0.25	*-0.39	0.01	*0.47	*0.21
PK/PFK	* 1	0.03	-0.17	*0.57	*-0.43	-0.17	*0.55	0.01	*-0.39	*0.21	*0.31
GOT/CS	0.03	* 1	-0.01	0.13	*0.51	-0.09	-0.07	-0.01	0.09	*0.36	0.19
CPK/PK	-0.17	-0.01	* 1	*0.23	*-0.26	*0.49	*0.31	*0.28	0.15	*-0.29	*-0.25
a-GPDH/PFK	*0.57	0.13	*0.23	* 1	*-0.48	*0.22	*0.63	*0.21	*-0.48	*0.32	*0.21
PFK/CS	*-0.43	*0.51	*-0.26	*-0.48	* 1	*-0.58	*-0.86	*-0.51	*0.48	0.16	-0.01
GOT/PK	-0.17	-0.09	*0.49	*0.22	*-0.58	* 1	*0.65	*0.79	*-0.21	*-0.28	*-0.23
GOT/PFK	*0.55	-0.07	*0.31	*0.63	*-0.86	*0.65	* 1	*0.62	*-0.51	-0.04	0.08
GOT/LDH	0.01	-0.01	*0.28	*0.21	*-0.51	*0.79	*0.62	* 1	*-0.29	-0.19	-0.11
PFK/HOAD	*-0.39	0.09	0.15	*-0.48	*0.48	*-0.21	*-0.51	*-0.29	* 1	*-0.58	*-0.74
HOAD/CPT	*0.21	*0.36	*-0.29	*0.32	0.16	*-0.28	-0.04	-0.19	*-0.58	* 1	-0.78
HOAD/CAT	*0.31	0.19	*-0.25	*0.21	-0.01	*-0.23	0.08	-0.11	*-0.74	*0.78	* 1

* = statistically significant correlation at the 95% confidence level. All correlations are between data ranks (Spearman correlations). See Materials and Methods for abbreviations.

as observed with LDH/CS is found with LDH/HOAD (Bass et al. 1969; Ponganis and Pierce 1978).

The activity ratios PK/CS, PFK/CS, and α-GPDH/CS were determined to access the relationship of aerobic glycolysis to the Krebs Cycle in harbor seal skeletal muscle. High positive correlations exist between all 3 ratios (Table 5). The pattern of distribution of the ratio values between muscles is also very similar (Figure 2; Appendix 1). In fact, the pattern of distribution of these 3 enzyme ratios is virtually identical to the LDH/CS pattern (Appendix 1). This closeness is evidenced by the high positive correlations between ratios (Table 5), and the similarity in histogram profiles (Figure 2). The PK/CS ratios (Table 4) observed in seal skeletal muscle fit well within a range of values determined for a number of mammals, between 2 and 200 (Helig and Pette 1980; Emmett and Hochachka 1981). Red muscles tend to have the lower values within this range. The PFK/CS ratios calculated for seal muscle (Table 4) also appear to be normal in comparison with other mammals. Human gastrocnemius muscle has a PFK/CS value of 3.3, which decreases to about 1.9 with chronic ischemia (Bylund-Fellinius et al. 1981), while flight muscle of birds is very low, <0.5 (Marsh 1981; Suarez 1986). α-GPDH/CS values are scarce for comparison purposes, but a ratio of 0.03 in both hummingbird flight muscle (Suarez 1986) and rat soleus muscle (Kubista et al. 1971) is 1-2 orders of magnitude lower than what is observed in the harbor seal skeletal muscles examined. Rat white muscle (rectus femoris) has an α-GPDH/CS ratio of 0.54, much closer to the seal muscle values.

Extremely high positive correlations are found between the PK/HOAD, PFK/HOAD, and α-GPDH/HOAD activity ratios (Table 5). All 3 ratios show nearly identical distribution pattens across the seal muscles examined (Figure 2). These three ratios also exhibit the same muscle distribution pattern as that observed with LDH/HOAD. The PK/HOAD activity ratios of seal muscle 10.38 - 90.60 (Table 3), fall in the lower part of the range of values (2-280) observed in a wide variety of mammals (Helig and Pette 1980; Hochachka et al. 1982; Suarez 1986). Highly aerobic, fat utilizing muscle, such as mouse soleus, hummingbird flight muscle and shrew gastrocnemius tend to have values <15. The mean PFK/HOAD ratio of 3.17 (Table 4) found in seal muscle is also relatively low, similar to mammalian red muscles from what could be determined in the literature. Rabbit soleus muscle has a ratio of 1.6, while the "whiter" extensor digitorum longus muscle of the rabbit has a value of 37.1 (Helig and Pette 1980). Hummingbird flight muscle has a PFK/HOAD ratio of only 1.13 (Suarez 1986). The mean α-GPDH/HOAD ratio in seal muscle, 1.51, is in the center of the range of values calculated from the literature. Ratios as low as 0.10 can be found in hummingbird flight muscle (Suarez 1986), or as high as 3.12 in various human skeletal muscles (Falholt et al. 1974).

The relationship between carbohydrate and fat-based aerobic metabolism in seal skeletal muscle was examined by comparing HOAD/CS activity ratios (Table 4). This activity ratio is relatively constant across a wide range of vertebrate muscles (Bass et al. 1969; Kubista et al. 1971; Staudte and Pette 1972; Helig and Pette 1980; Emmett and Hochachka 1981). Vastly different types of skeletal muscle, from very red to very white, all have HOAD/CS activity ratios between 0.2 and 2.0. The values obtained for

seal skeletal muscle, from 0.66 to 2.04, cover nearly the entire range found in the literature. The only significant differences observed between the seal muscles are that the DIA has a higher HOAD/CS ratio than several muscles, and the MAS has a significantly lower ratio than 3 of the muscles examined (Appendix 1).

Ratios between the enzymes of glycolysis are fairly constant across the 21 seal muscles examined. PK/PFK, α-GPDH/PK, and α-GPDH/PFK activity ratios only exhibit significant differences with 2 muscles (Appendix 1). The α-GPDH/PK ratio of MAS is higher than all the other seal muscles examined; ATL has a significantly higher value for PK/PFK and α-GPDH/PFK than all but 3 other muscles (Appendix 1). Significant positive correlations exist between the 3 ratios (Table 5). The mean value of PK/PFK from seal muscle is well within the range of values observed in other vertebrates, between 8 and 22 (Zammit et al. 1978). However, 2 seal muscles, ATL and INT have PK/PFK activity ratios clearly higher than 22 (Table 4). The α-GPDH/PFK ratios, on the other hand, all fall within the normal vertebrate range of 0.1 to 2.1 (Crabtree and Newsholme 1972a). Seal muscle also has relatively normal α-GPDH/PK ratios. Combining the data of two studies by Newsholme and coworkers (Crabtree and Newsholme 1972a; Zammit et al. 1978) demonstrates a very constant α-GPDH/PK activity ratio of approximately 0.05 across a number of vertebrate skeletal muscles. Seal skeletal muscles are similar, with a mean value of 0.04 (Table 4).

The activity ratios between the enzymes of fat metabolism, HOAD/CAT and HOAD/CPT, appear to be somewhat different in seal skeletal muscle than

what can be determined from the literature. The mean value of HOAD/CPT in seal muscle is 2-fold what is observed in rats (Negrao et al. 1987), and 5-fold the hummingbird flight muscle value (Suarez 1986), while the HOAD/CAT ratio in seal is less than half the rat muscle value of about 40 (Negrao et al. 1987). No other data appropriate for comparing these ratios are available. The range of values for HOAD/CPT across the 21 seal muscles studies appears large; however, there are no significant differences found between the muscles (Appendix 1). The HOAD/CAT activity ratios, on the other hand, do exhibit a few significant differences. BFM is greater than a number of muscles, and MAS and TLG are significantly lower than a few. The HOAD/CAT and HOAD/CPT activity ratios are strongly correlated with one another, and both tend to be higher in the superficial portions of seal muscle than in the deep areas. It is also evident that the swimming muscles fall at the middle/low parts of both ranges (Appendix 1).

Comparison of CPK with the enzymes of glycolysis exhibits some clear trends: 1) Both the CPK/PK and CPK/LDH activity ratios are significantly higher in MAS than all other seal muscles examined. 2) The swimming muscles have lower ratios of CPK/PK and CPK/LDH than most other muscles, and 3) The deep areas of muscles tend to have higher values than the superficial portions of those muscles (Table 4). The magnitude of the CPK/PK ratios in seal muscle all fall within the normal range of values calculated from the literature for vertebrate muscle, of between 1 and 8 (Newsholme et al. 1978; Zammit et al. 1978; Suarez 1986). The CPK/LDH ratios in vertebrate muscle are, on the other hand, much more variable than the CPK/PK activity ratios. Most vertebrate muscles, including the

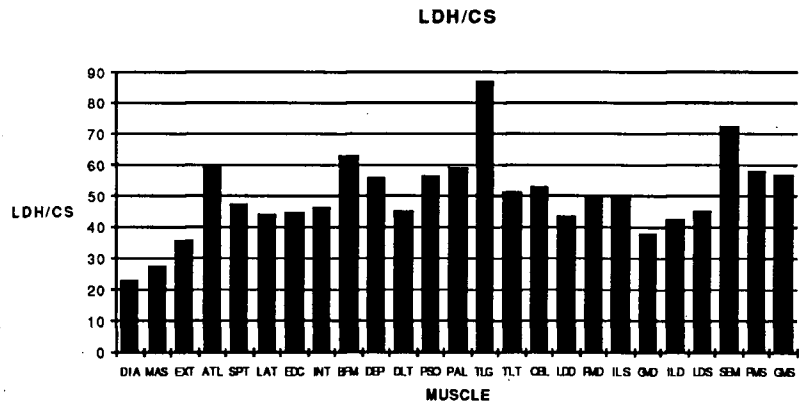
seal muscles examined, appear to fall between 1 and 9 for CPK/LDH (Crabtree and Newsholme 1972a; Newsholme et al. 1978). However, redder muscles have higher values: from 12 to 20 for hummingbird flight muscle and rabbit semitendinosus, to as high as 106 for trout red muscle (Crabtree and Newsholme 1972a; Newsholme et al. 1972; Suarez 1986).

Ratios of GOT with glycolytic enzymes, GOT/PK, GOT/PFK, GOT/LDH, are not very discriminative between the seal muscles examined. The only clear differences are: that MAS and DIA are significantly higher in all 3 ratios than most other muscles examined (Appendix 1); BFM, ATL and GMS are the lowest; and deep portions of muscles have higher ratios than the superficial areas. High correlations exist between all 3 enzyme ratios. The mean values of GOT/PK, GOT/PFK, and GOT/LDH found in the skeletal muscle of harbor seals are about equal to those reported for other species (Scrutton and Utter 1968). A striking exception to the normal range of values reported is, however, found in hummingbird flight muscle (Suarez 1986). This muscle is at least 1 order of magnitude higher in all 3 activity ratios.

GOT activity was also compared with CS. The GOT/CS ratios observed are remarkably constant across all 21 seal muscles examined (Table 4). No significant differences are found between any of the muscles. The only interesting trend apparent is that the swimming and respiratory muscles seem to have relatively low values. The magnitude of the GOT/CS ratios found in seal muscle are about the same as could be calculated for hummingbird (Suarez 1986) and rat (Scrutton and Utter 1968; Emmett and Hochachka 1981).

FIGURE 2. HISTOGRAM PROFILES OF ENZYME ACTIVITY RATIOS IN HARBOR SEAL MUSCLES (6 PAGES).

Muscles are listed in order of increasing LDH activity.
See Materials and Methods for abbreviations.


LDH/CS
LDH/CS
90
80
70
60
50
40
30
20
10
0
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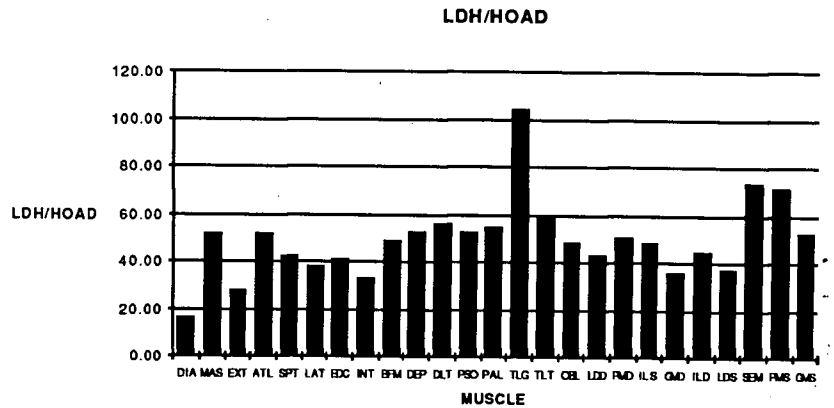
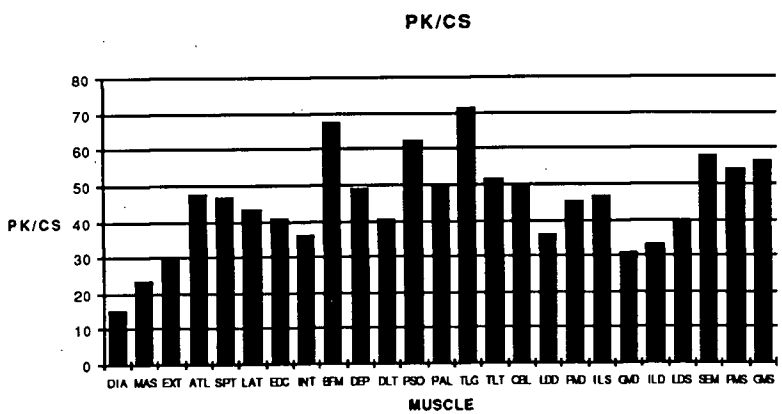
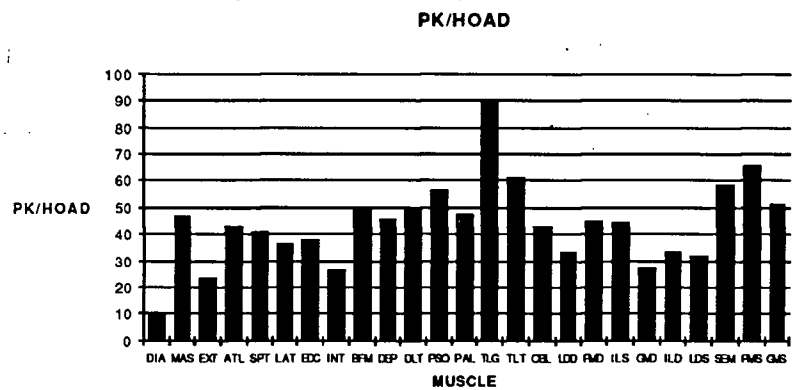
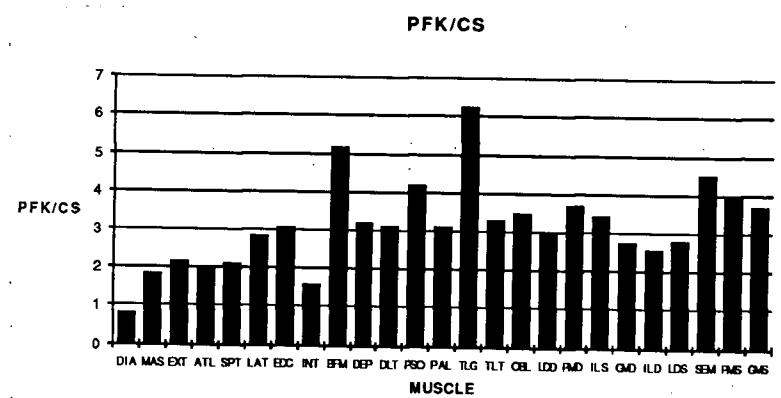
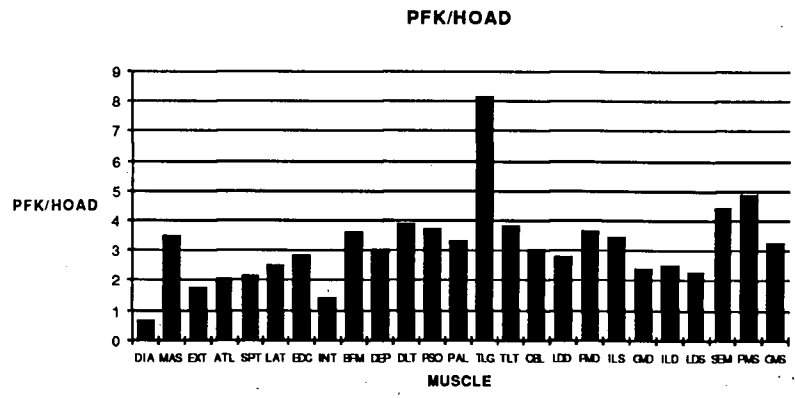
MUSCLE

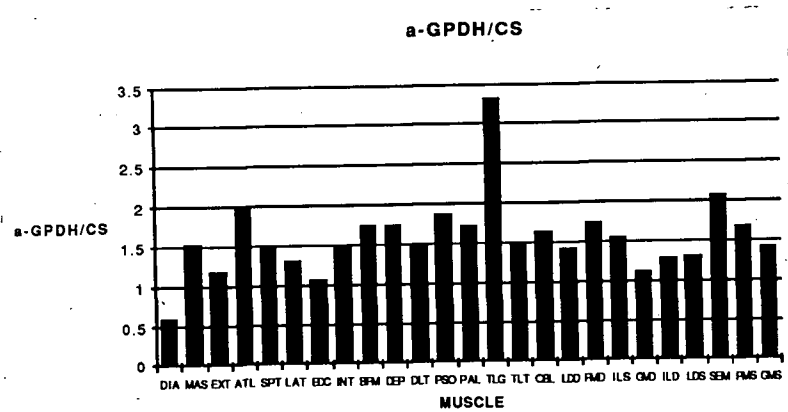

LDH/HOAD
LDH/HOAD
120.00
100.00
80.00
60.00
40.00
20.00
0.00
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

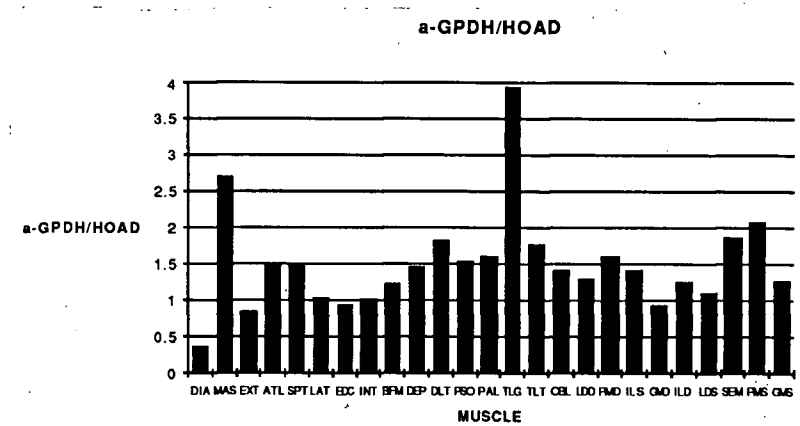

PK/CS
PK/CS
80
70
60
50
40
30
20
10
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

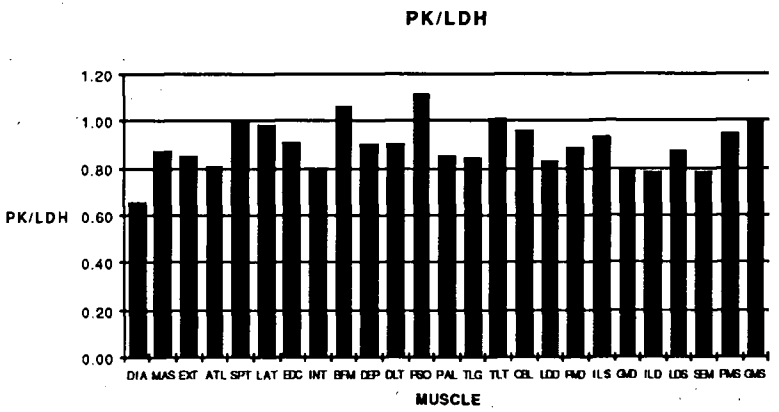

PK/HOAD
PK/HOAD
100
90
80
70
60
50
40
30
20
10
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

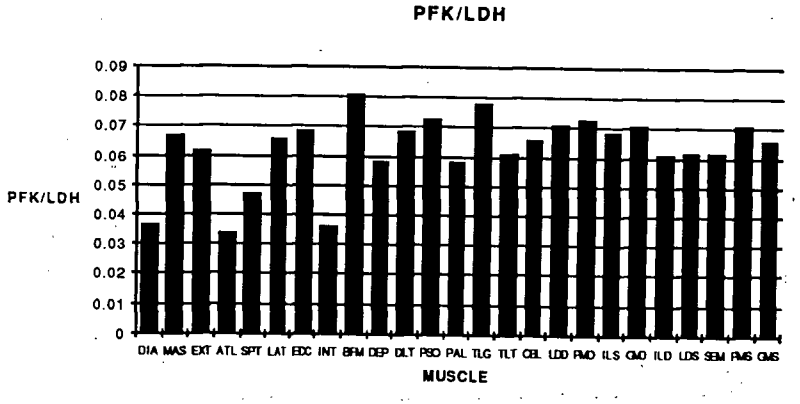

PFK/CS
PFK/CS
0
1
2
3
4
5
6
7
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

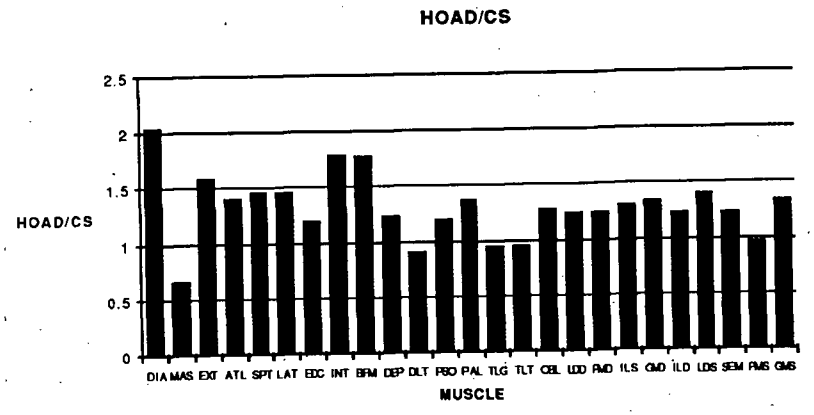

PFK/HOAD
PFK/HOAD
0
1
2
3
4
5
6
7
8
9
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

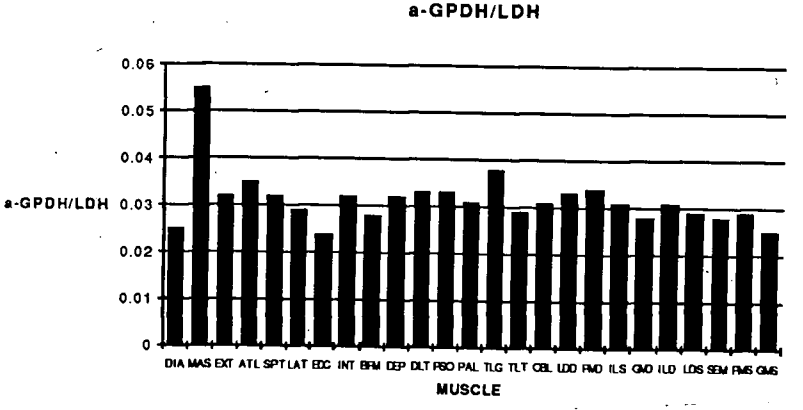

a-GPDH/CS
a-GPDH/CS
0
0.5
1
1.5
2
2.5
3
3.5
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

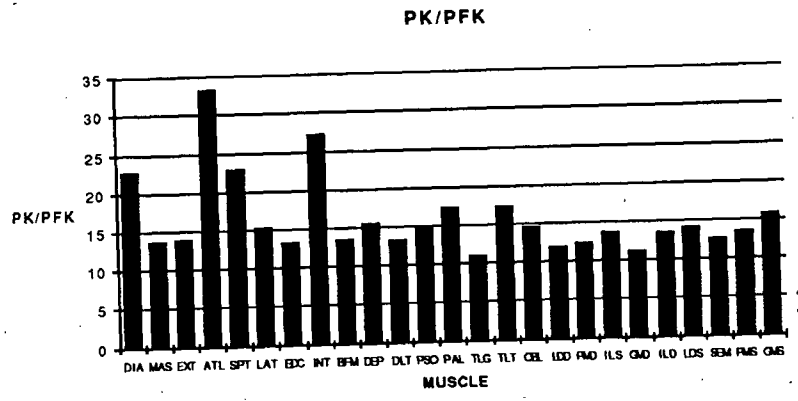

a-GPDH/HOAD
a-GPDH/HOAD
0
0.5
1
1.5
2
2.5
3
3.5
4
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

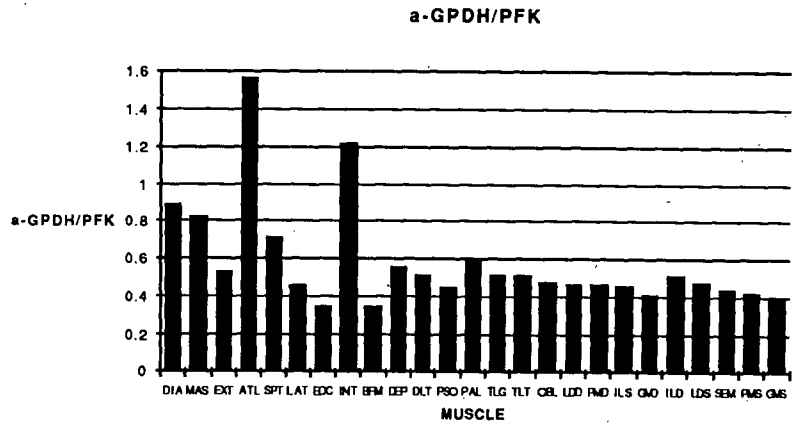

PK/LDH
PK/LDH
1.20
1.00
0.80
0.60
0.40
0.20
0.00
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE

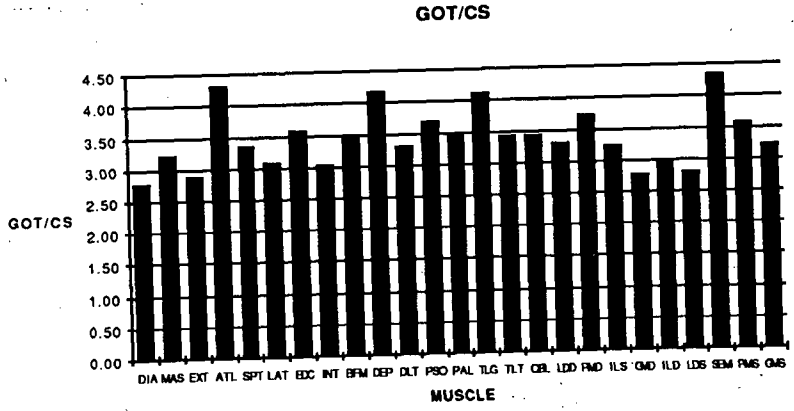

PFK/LDH
PFK/LDH
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE

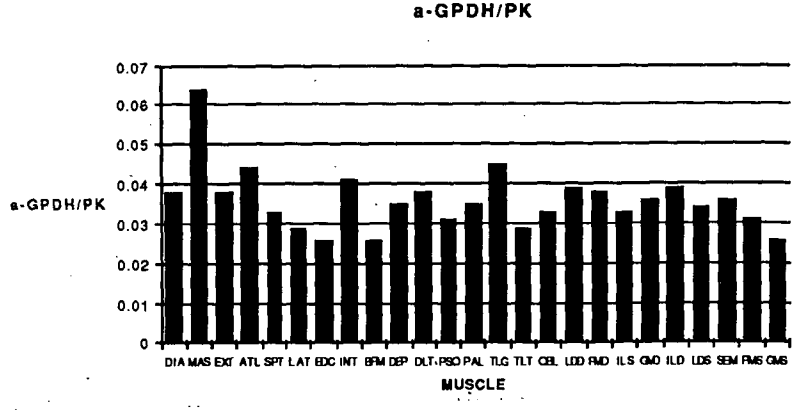

HOAD/CS
HOAD/CS
2.5
2
1.5
1
0.5
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE


a-GPDH/LDH
a-GPDH/LDH
0.06
0.05
0.04
0.03
0.02
0.01
0
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE


PK/PFK
PK/PFK
35
30
25
20
15
10
5
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


a-GPDH/PFK
a-GPDH/PFK
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


GOT/CS
GOT/CS
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSO PAL TLG TLT OBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


a-GPDH/PK
a-GPDH/PK
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
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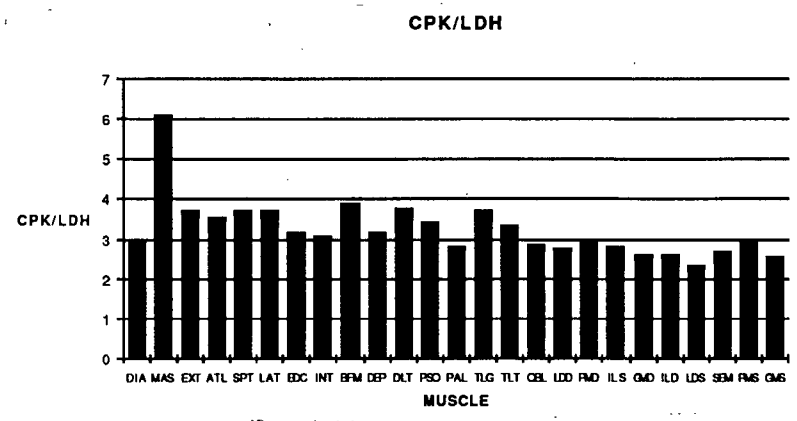
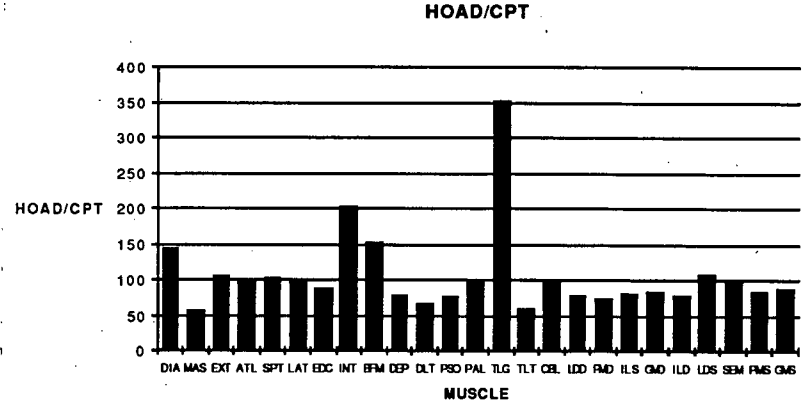
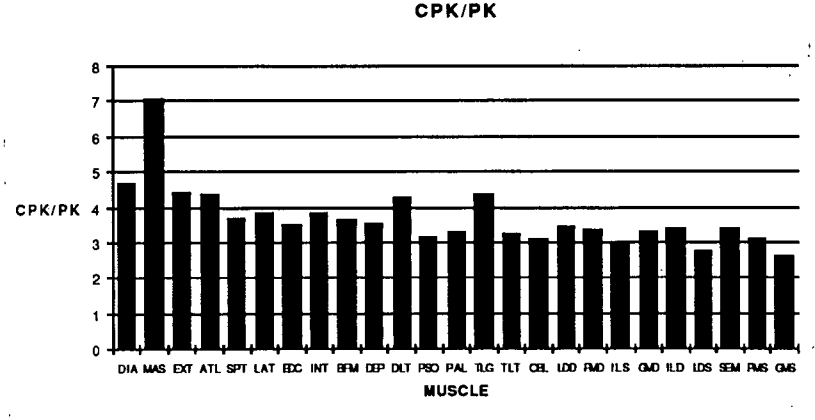
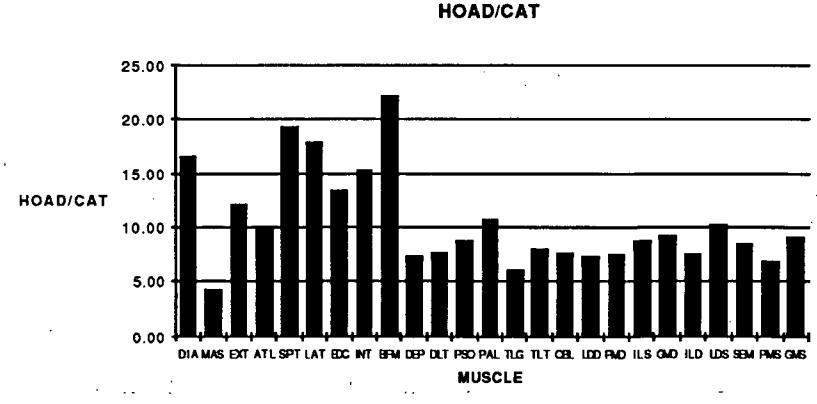
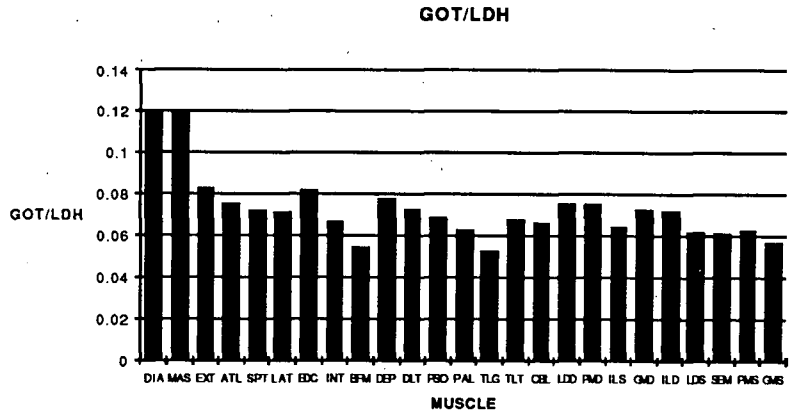
MUSCLE

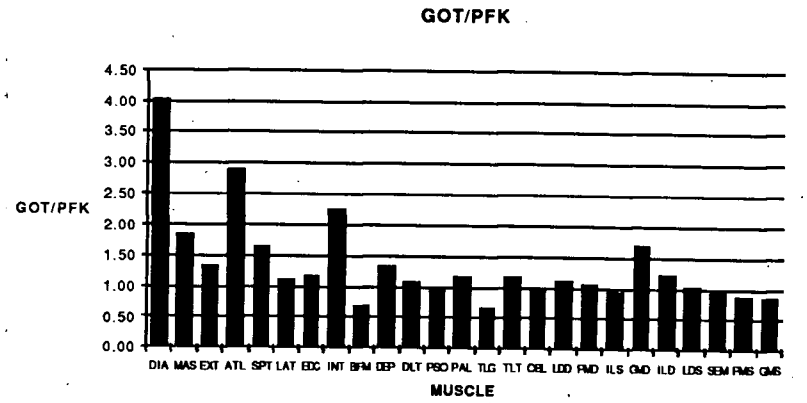

CPK/LDH
CPK/LDH
0
1
2
3
4
5
6
7
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE

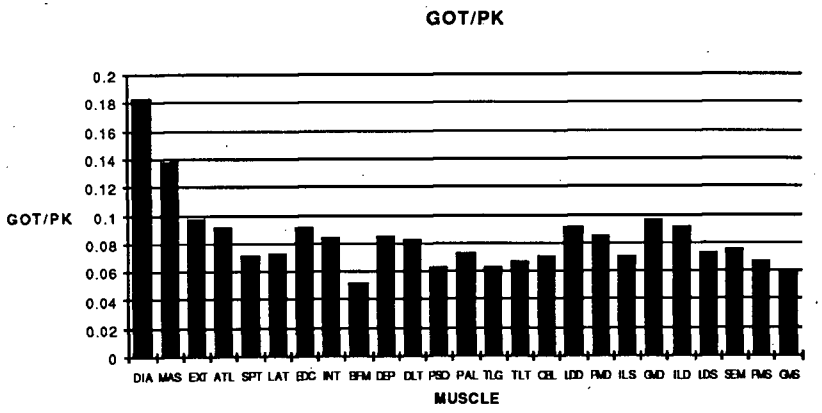

HOAD/CPT
HOAD/CPT
0
50
100
150
200
250
300
350
400
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


CPK/PK
CPK/PK
0
1
2
3
4
5
6
7
8
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


HOAD/CAT
HOAD/CAT
0.00
5.00
10.00
15.00
20.00
25.00
DIA MAS EXT ATL SPT LAT EDC INT BRM DEP DLT PSO PAL TLG TLT CBL LDD RMD ILS GMD ILD LDS SEM RMS GMS
MUSCLE


GOT/LDH
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
GOT/LDH
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSD PAL TLG TLT CBL LDD PMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE


GOT/PFK
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
GOT/PFK
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSD PAL TLG TLT CBL LDD PMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE


GOT/PK
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
GOT/PK
DIA MAS EXT ATL SPT LAT EDC INT BFM DEP DLT PSD PAL TLG TLT CBL LDD PMD ILS GMD ILD LDS SEM PMS GMS
MUSCLE

(BLANK)

Discussion.

Discriminative vs non-discriminative ratios. Of the 23 enzyme ratios examined, several are quite constant across the 21 seal muscles. GOT/CS values have a narrow range and exhibit no significant differences between any of the muscles. This coadaptation of GOT and CS is not unexpected. A similar non-discriminative nature has been observed in other species between GOT and several other Krebs cycle enzymes (Flavell and Woodward 1970), as well as various cytochromes (Pette et al. 1962b). The fact that both GOT and CS are mitochondrial enzymes results in this tight coupling. CS is also closely related to another mitochondrial enzyme (HOAD). The HOAD/CS ratio is relatively constant in all 21 seal muscles. This close relationship between fat catabolism and overall aerobic flux capacity in muscle is commonly observed across a wide range of species. Exceptions are rare and generally involve unusual muscles such as bee flight muscle (Pette and Dolken 1975). The constancy of both of these ratios (GOT/CS and HOAD/CS) is evidence that the metabolic machinery of the mitochondria remains relatively unchanged throughout the 21 seal muscles examined. Any differences between muscles involving these mitochondrial enzymes are maximal activity changes (Chapter 2). Such changes, apparently, are a result of increases in the size and/or number of mitochondria rather than the makeup of the individual mitochondria themselves (Holloszy 1973).

The existence of the constant proportion group of glycolytic enzymes (Pette et al. 1962a, 1962b; Pette 1985) is clearly evident throughout the 21 seal muscles. PK/PFK, α-GPDH/PK, and α-GPDH/PFK ratios are not very discriminative. Only one of two muscles (MAS or ATL) exhibit significant differences in any of the three ratios. This constancy of molar proportions among the glycolytic enzymes is even evident when comparing LDH activities to other glycolytic enzymes. Normally considered a variable ratio (Pette et al. 1962b; Pette and Dolken 1975), α-GPDH/LDH is constant among the seal muscles. The PFK/LDH ratio, although exhibiting several statistically significant differences between muscles, only ranges from 0.03 to 0.08. Considering that truly variable ratios often vary by 4 orders of magnitude or more between species (Staudte and Pette 1972), this variability in PFK/LDH may easily be considered inconsequential. When the sensitivity of PFK to various effectors is taken into account, this low (<3-fold) variability seems even less significant. The PK/LDH ratio also varies by only about 3-fold in seal muscle, and only 1 statistically significant difference is observed between muscles. This ratio, although relatively invariant, does appear to exhibit some interesting trends in seal muscle (discussed below) and among other species (Hochachka et al. 1982; Hochachka 1985). Since all of these enzymes are an integral (or closely associated) part of a single metabolic pathway whose function is to catabolize carbohydrate to pyruvate -- a change in activity of one enzyme should be reflected by a similar change in all of the enzymes of the pathway. Not surprisingly, this does appear, in fact, to be the situation with seal muscle, as has been observed to be in other species (Pette et al. 1962b; Pette and Bucher 1963; Kubista et al. 1971; Pette and Dolken 1975).

A constant proportion group is also evident with the enzymes involved in fatty acid oxidation. The HOAD/CPT ratio exhibits no significant differences between muscles. This coupling between the transfer of long chain fatty acids across the mitochondrial membrane (CPT) and B-oxidation (HOAD) makes sense since both enzymes are mitochondrial and each has been suggested as a regulatory step in fatty acid oxidation (Newsholme and Start 1973; Atkinson 1977). The relationship does not, however, stand up when HOAD is compared with CAT. The HOAD/CAT ratio does exhibit significant differences between muscles. This discrepancy is indicative of the fact that CAT is involved in functions other than the transfer of short chain acyl groups into the mitochondria. Exactly what these other functions may be in muscle is very much in doubt , however, CAT is clearly associated with cell structures other than mitochondria (Markwell et al. 1973; Bieber et al. 1982; Bremer 1983). CPT, on the other hand is strictly a mitochondrial enzyme. As a result of this difference, the molar proportions of HOAD and CPT vary proportionately with any increase or decrease in size or number of mitochondria. CAT, being associated with other cellular structures (ie. peroxisomes and microsomes) has activity levels that are fairly independent of the mitochondrial enzyme activities.

The remainder of the ratios examined appear to be somewhat discriminative. This variability is particularly evident when comparing anaerobic to aerobic pathways (LDH/CS). This ratio has been shown, clearly, to be variable in a number of animals (Pette and Dolken 1975; Hochachka et al. 1982; Hochachka 1985). Different seal muscles also

exhibit several differences in LDH/CS. Three other ratios comparing glycolytic enzymes to CS activity are highly correlated to the LDH/CS activity ratio in seal muscle and, as a result, are also quite variable between muscles. Both the PK/CS and PFK/CS ratios have been demonstrated to be discriminative in other species (Helig and Pette 1980). The variability in the α-GPDH/CS ratio is expected, by analogy to the constant proportion groups of glycolytic enzymes, as well as by the variable nature of α-GPDH/cytochrome a ratio (Pette et al. 1962b; Pette and Bucher 1963; Kubista et al. 1971).

The comparison of anaerobic metabolic capacity (LDH activity) to B-oxidation (HOAD activity) is also variable between seal muscles. The between species discriminative nature of LDH/HOAD has been shown previously (Hochachka et al. 1982; Hochachka 1985). Similar discriminative patterns are observed within the seal when other glycolytic enzymes are compared with HOAD. The PK/HOAD and PFK/HOAD activity ratios' variability has been observed in other species (Helig and Pette 1980; Pette and Spamer 1986). However, the α-GPDH/HOAD ratio does not appear to be quite as variable, varying <6x in man (Falholt et al. 1974).

The relationship of GOT with glycolytic enzymes is also discriminative in seal muscle. The pattern of discrimination observed with these ratios (GOT/PK, GOT/PFK, and GOT/LDH) is nearly identical to the patterns seen with CS and each of these glycolytic enzymes. This results from the highly coadaptive nature of GOT with CS as observed in the constant GOT/CS ratio. The variability of GOT/PK, GOT/PFK, and GOT/LDH are somewhat expected due to analogy with the equivalent ratios involving CS, and by the

discriminative nature observed with the GOT/aldolase activity ratio (Schimassek 1961).

The variable ratios discussed above all involve mitochondrial vs non-mitochondrial enzymes. While, constant ratios seem to be observed when they are between 2 mitochondrial enzymes or 2 cytosolic enzymes. However, the final two ratios examined, CPK/PK and CPK/LDH, are quite variable in spite of the fact they are both between pairs of cytosolic enzymes. Both ratios exhibit statistically significant differences between muscles and vary over 1-2 orders of magnitude. The discriminative nature of CPK/LDH has been observed before (Lowry et al. 1978), but the widely different CPK/PK ratios in seal muscle are somewhat unusual in light of the constant ratios of CPK/triose-phosphate dehydrogenase previously seen in other species (Staudte and Pette 1972). This difference likely results from the fact that the seal muscles examined are so different in their functional demands. Muscles of similar usage patterns (even in different species) may be expected to have relatively constant (CPK/PK) ratios, however, vastly different muscles (even within the same species) appear to have molar proportions of CPK and PK that do not coadapt. The variable nature of the CPK/LDH and CPK/PK ratios indicates that, in spite of the close relationship between CPK and glycolysis (Newsholme et al. 1978), muscular adaptations can occur that effect each pathway very differently.

Muscle metabolism. The harbor seal muscles appear to be metabolically "typical" vertebrate muscle based on the enzyme ratios examined. The majority of the ratios are about average compared with ratios from other vertebrate species (see results for details). The exceptions to this

"average" nature are generally indicative of a more highly aerobic muscle tissue in seal. High values for PK/LDH, and low LDH/CS, LDH/HOAD, and associated ratios (PK/CS, PK/HOAD, PFK/CS, etc.) are present in the harbor seal muscles. The values of these ratios are typical of what is observed in very "red" muscles. In addition, a normal relationship between the dependence of muscle on both fat and carbohydrate as fuel, as evidenced by the HOAD/CS ratio, is present in seal muscle.

These ratio patterns confirm the aerobic nature of harbor seal skeletal muscle suggested by the maximal enzyme activities (Chapter 2). Again, since this aerobic nature is particularly evident in the major swimming muscles of the seal, it suggests that hypoxic conditions are rarely experienced by these muscles. Swimming muscles of harp seals also appear to be more aerobically poised than non-swimming muscles, as evidenced by a higher percentage of Type 1 muscle fibers (George et al. 1971; George and Ronald 1973, 1975). Consequently, the behavioral and/or physiological adaptations to diving in seals appear to be successfully structured to avoid the vast majority of muscle tissue hypoxic stress. An additional possibility of severely reduced metabolic rates in "unused" tissues, and increased efficiency in all tissues may also be crucial factors in the avoidance of muscular hypoxia (see Chapter 1). An interesting fact is the relatively normal dependence of seal muscle on fat. It has been suggested that, due to greater energetic efficiency, carbohydrate should be the preferred fuel for animals, including man, working under potentially hypoxic conditions (Hochachka 1985). This does not appear to be the case for harbor seals, although specific fuel usage patterns may, in fact, result in a predominance of carbohydrate utilization

during dives. The energetic advantages of carbohydrate catabolism may be offset by the greater supply of fats for use as fuel. This increased supply may be of great benefit during the incredible feats of consecutive diving sometimes seen in phocids, such as those observed with elephant seals (LeBoeuf 1986, 1988, 1989). Of course, the solution of the fuel supply problem does not explain how the "on-board" oxygen supply is able to support the activity over those periods of time. In fact, it makes the problem greater, since utilizing fat requires more oxygen per ATP produced than when carbohydrate is the fuel (Hochachka 1985). As a result, selective metabolic depression in "unused" tissues and/or increased efficiencies would be even more crucial to a diver utilizing fat.

Enzyme activity ratios. In comparing aerobic to anaerobic glycolysis the most instructive activity ratio is PK/LDH. In spite of the fact that few statistically significant differences exist between muscles, the range of values found for PK/LDH in the 21 harbor seal muscles is approximately equal to the range of values determined for the 13 different mammalian species examined by Hochachka (1985). So, although generally quite high, the PK/LDH ratios of various seal muscles can differ substantially from muscle to muscle. The higher ratios tend to occur in superficial portions of swimming muscles, and a variety of postural and "auxiliary" locomotory muscles. The lowest values observed are in the respiratory muscles and deep areas of the primary swimming muscles.

High values of PK/LDH seem to be found in animals that must utilize available oxygen supplies with maximum efficiency due to either limited availability [high altitude adapted species (Hochachka 1985; Hochachka

1986b)], or extremely high metabolic rates [shrews (Emmett and Hochachka 1981), and hummingbirds (Suarez 1986)]. As seal muscle does not possess a high metabolic rate (Hochachka 1986b), the high PK/LDH ratios are possibly evidence of adaptation to limited oxygen supply. The fact that the respiratory and swimming muscles appear to have lower values than the other seal muscles is significant. Higher values would be unnecessary for respiratory muscles since they are required to function only when oxygen is readily available. The low values for the deep portions of swimming muscles are consistant with the idea that the well known circulatory redistributions made by diving mammals (see Chapter 1) are directed towards these muscles more so than other seal muscles. As a result, the swimming muscles of the diving seal are allowed to function aerobically to a greater extent than might otherwise be possible. The higher values of PK/LDH in the superficial portions of these swimming muscles are a result of the generally lower blood supply to superficial muscle (Guth and Samaha 1969; Yellin 1969; Baldwin et al. 1972; Gonyea and Ericson 1977; Gunn 1978; Armstrong 1980; Armstrong et al. 1982).

The LDH/CS activity ratio is commonly regarded as a measure of anaerobic vs aerobic metabolic capacity (Hochachka et al. 1982). Vertebrate red muscle has low values (<30), while white muscle values are between 200 and 1100 (Bass et al. 1969). Seal muscle, with a mean LDH/CS ratio of 50.34, tends to be much closer to the red muscle values. Respiratory and deep areas of swimming muscles of the harbor seal have low LDH/CS ratios compared with the other seal muscles examined. This "redder" nature of these muscles reinforces what was observed with the PK/LDH ratios (above). Swimming, as well as respiratory, muscles appear to be geared

primarily for aerobic contractile activity. The other seal muscles examined, although generally aerobic in nature appear likely to utilize anaerobic metabolic pathways to a greater extent.

The relative importance of fat metabolism compared to anaerobic pathways is viewed via the LDH/HOAD activity ratio. This ratio exhibits the same general trends as the LDH/CS ratio, indicating that total aerobic capacity is closely related to the ability to utilize fat as substrate in harbor seal skeletal muscle. The magnitude of the LDH/HOAD ratios in seal muscle is similar to values calculated for red skeletal muscle (Bass et al. 1969; Ponganis and Pierce 1978).

These relationships between glycolytic capacity and aerobic pathways are also evident in ratios comparing the enzymes of aerobic glycolysis (PK, PFK, and α-GPDH) to either HOAD or CS. Examination of any of these ratios results in the conclusion that seal muscle, on the whole, is quite aerobic in nature. The most aerobic muscles (based on these ratios) invariably include the respiratory muscles and deep portions of swimming muscles.

The coadaptation of B-oxidation and the Krebs cycle, as measured by HOAD/CS is strikingly constant across a wide range of vertebrate skeletal muscles (Bass et al. 1969; Emmett and Hochachka 1981). The range of 0.66 to 2.04 in the 21 seal muscles examined spans nearly the entire range of values found in other animal species. Although few significant differences are evident, the respiratory and postural muscles appear to have the highest dependence on fat while the masseter and "auxiliary" locomotory muscles have a higher tendency to utilize a carbohydrate for

catabolic processes. The swimming muscles fall in about the middle of the range of values. The greater dependence of postural and respiratory muscle on fat is appropriate for the slow, "low level" activity required by these muscles. The "auxiliary" muscles of locomotion have a higher relative capacity for carbohydrate utilization in order to fuel the quick and/or powerful movements for which they generally are recruited. The codependence of the swimming muscles on both fat and carbohydrate results from the need for both regular, long-term aerobic activity as well as the rapid/powerful and possibly anaerobic contractions sometimes necessary during locomotion and diving.

The mixed demands placed upon locomotory muscles of the seal are further evidenced by their low CPK/PK and CPK/LDH activity ratios. Although these muscles appear to be generally aerobic in nature (as discussed above), these particular ratios in the locomotory muscles of the seal are more typical vertebrate white muscle (Crabtree and Newsholme 1972a; Newsholme et al. 1978). This similarity to white muscle is, again, likely due to the need for rapid powerful contractions during locomotion, and possibly even rare hypoxic function at times while diving. The implication of a relationship to oxygen availability is reinforced by the especially low levels of these ratios in the superficial portions of muscle, which routinely are less saturated with oxygen due to capillary distribution (Guth and Samaha 1969; Yellin 1969; Baldwin et al. 1972; Gonyea and Ericson 1977; Gunn 1978; Armstrong 1980; Armstrong et al. 1982). The highest values for CPK/PK and CPK/LDH are observed in the respiratory muscles which, like the swimming muscles, are aerobic in nature, but are not required to function either powerfully of anaerobically.

Muscle relationships. The relationship between muscle function and metabolic machinery determined on the basis of maximum enzyme activities (Chapter 2) are generally confirmed and clarified by the enzyme ratios.

The DIA clearly remains the most aerobic of the seal muscles examined, with the lowest ratios of glycolytic enzymes to LDH, and glycolytic enzymes (including LDH) to CS and HOAD -- along with the highest GOT/glycolytic enzyme ratios. Its dependence on fat is confirmed by a very high HOAD/CS ratio. High ratios of CPK to glycolytic enzymes indicate any rapid or anaerobic contraction by the DIA to be more dependent on creatine phosphate hydrolysis for energy than is the case with the other skeletal muscles. This finding is significant, elucidating a similarity (greater dependence on creating phosphate) to the whale diaphragm (Chapters 4 and 5) that is not evident when comparing absolute enzyme activities. These relatively higher CPK/PK and CPK/LDH ratios are also a characteristic of more aerobic muscle (Crabtree and Newsholme 1972a; Newsholme et al. 1978). The enzyme ratio pattern of the other two muscles of respiration (INT and EXT) is in accordance with the findings based on maximum enzyme activities (Chapter 2). These muscles are similar to the DIA, but appear to be slightly more "powerful" in nature.

The swimming muscles of the seal are also generally aerobic, with low levels of glycolytic (including LDH) to aerobic (HOAD and CS) and glycolytic/LDH ratios, and high ratios of GOT/glycolytic enzymes. The

codependence of the muscles on both fat and carbohydrate is confirmed by their average HOAD/CS values. However, differences from highly aerobic DIA are evident in ratios of CPK/PK and CPK/LDH. The values of these two ratios are much lower in the swimming muscles. This trend is primarily due to the much higher glycolytic capacity of the swimming muscles (Chapter 2). These ratio patterns confirm the mixed aerobic/anaerobic nature of the swimming muscles observed with maximum activities.

The remainder of the muscles (postural, auxiliary, locomotory muscles, etc) exhibits a wide range of ratio patterns that generally conform to their function as described in Chapter 2. Some differences between muscles are highlighted when viewing the enzyme ratios rather than maximal activities (ie. the anaerobic nature of PSO and TLG is striking with the ratios). Other muscles, such as m. pectoralis profundus, appear about the same in relation to the other seal muscles examined as when maximum activities are utilized. However, a third group of muscles exhibit widely varying and complex patterns of enzyme ratios (ie. SEM and ATL). These differences are likely due to subtle usage patterns that are well beyond the scope of present knowledge of seal musculature. Muscle specific patterns of circulatory redistribution while diving, and patterns of recruitment of individual muscles during specific types of movement would need to be thoroughly understood before such sensitive analyses could be undertaken. In addition, the majority of the activity ratio differences in question are not statistically significant, so the apparent variations in ratio pattern between the muscles may not represent real differences. Therefore, any further analysis of the ratios at this level will not be undertaken at present.

Cluster analysis. Cluster analysis of the seal muscles, based on all 23 enzyme activity ratios, results in 5 clusters. The clustering pattern is slightly different than that observed in Chapter 2. The differences seem to primarily involve those muscles (as mentioned above) whose specific functions and usage patterns are difficult to assess.

The first cluster includes the DIA, INT, and ATL. Although not surprising to see an accessory inspiratory muscle (INT) cluster with the DIA, the presence of ATL is unusual. Examination of the individual enzyme ratios gives a clear indication of why this muscle is so closely associated with the 2 respiratory muscles. The cause of this strange cluster seems to involve unusually high PK/PFK, α-GPDH/PK, and α-GPDH/PFK ratios in ATL. The only 2 other muscles with high values of all 3 of these ratios are the INT and DIA. The metabolic importance, if any, of these differences in the constant proportion group of glycolysis is unknown. However, it does explain why the metabolically similar INT and EXT cluster separately, since this is the only apparent difference between the two muscles. It further explains how ATL, a muscle with a very unique profile of enzyme ratios, clusters with any of the other muscles.

The especially anaerobic nature of TLG that is suggested by maximum enzyme activities (Chapter 2) becomes very clear when observing the enzyme ratio pattern. TLG appears in a second cluster by itself. This isolation is due to its consistantly high glycolytic (including LDH) to aerobic (HOAD and CS) activity ratios.

The third cluster is the MAS by itself. This muscle has a very unique pattern of activity ratios. The principal difference between MAS and the other muscles examined is that, although it appears to be highly aerobic (like DIA is most respects), its relative dependence on fat is very low. It seems to rely on carbohydrate (low HOAD/CS ratio) for fuel to a much greater extent than any of the other muscles that have a relatively low glycolytic capacity. This dependence on carbohydrates is likely a result of the fact that masticatory activity in the seal is of a comparatively short-term powerful nature, requiring the occasional more rapid catabolism of carbohydrate when it functions. The use of fat for fuel would be of little use to MAS except, possibly in holding the mouth closed -- but since MAS is not generally considered to be an important postural muscle this activity would be very minimal.

A fourth cluster contains GMD, ILD, LDD, LDS, EXT, SPT, LAT, and EDC. The inclusion of 1 accessory respiratory muscle (EXT) and 3 postural muscles (SPT, LAT and EDC) in a group of swimming muscles is surprising. Although seemingly different in general, the ratio pattern of these 4 non-swimming muscles is similar to the primary swimming muscles in one significant area. The ratios of glycolytic (including LDH) to aerobic enzymes (HOAD and CS) are very similar in all 8 muscles. It is this similarity that causes these functionally very different muscles to cluster together. It is interesting that, although the absolute enzyme activities of these widely different muscles differs markedly (Chapter 2), they exhibit such similar relationships between glycolysis, B-oxidation, and the Krebs cycle. The postural muscles appear to be simply "tuned down" versions of

the major swimming muscles; pathway dependences are similar, only the flux through the pathway is different.

The final cluster separated is large, including GMS, ILS, PMS, PMD, PSO, OBL, PAL, SEM, BFM, DEP, DLT, and TLT. It is less that all these muscles have ratio patterns extremely similar to one another (except for a generally more anaerobic nature) than that the unusual combinations of differences between the muscles are too complex for clear differentiation between them. The differences result from aspects of the muscles, such as recruitment patterns, capillary distribution, and circulatory redistribution patterns while diving that are relatively unknown in seal muscle and therefore impossible to elucidate at this time.

Summary. The intra-specific discriminative or non-discriminative nature of the 23 ratios examined is discussed in comparison to literature findings in this regard. The generally aerobic metabolism of seal skeletal muscle (particularly swimming and respiratory muscles) observed in Chapter 2 is confirmed by the enzyme activity ratios. The specific functional relationships between muscles indicated by maximum enzyme activities (Chapter 2) are also confirmed, in general, by the ratio patterns. Certain aspects of the metabolic differences appear clearer using ratios, others seem about the same or become overly complex. Interesting similarities are found between ratios of the primary swimming muscles and several postural muscles, indicating a metabolic machinery that appears to vary only in flux rates, rather than pathway relationships, between some functionally very different seal skeletal muscles.

CHAPTER 4:

ENZYME ACTIVITY PROFILES OF FIN WHALE MUSCLES

Introduction. Non-anatomical or histochemical investigations on more than two or three muscles of a particular animal species are rare. This is particularly true with studies of enzyme activity. Briand and coworkers have examined 12 sheep skeletal muscles for a series of glycolytic and mitochondrial enzymes (Briand et al. 1981a, 1981b; Talmont et al. 1982); and a number of muscles from cows and pigs have been analyzed for various enzyme activities, primarily with regard to meat quality/food science (Ansay 1974; Monin 1980; Talmant et al. 1986; Monin et al. 1987). Other than these few studies little else exists. This is unfortunate in light of the useful information gained from the examination of the 23 harbor seal skeletal muscles (Chapters 2 and 3).

The clarification and elucidation of several aspects of muscle metabolism with regard to the diving habit in phocid seals (Chapters 2 and 3) led us to question how similar cetacean adaptations to diving might be. As a result, 22 skeletal muscles from the fin whale, Balaenoptera physalus, were examined in a similar manner to the harbor seal muscles (Chapter 2). A broad cross-section of muscles with widely varying functional demands and locations throughout the body of the fin whale were sampled, and a series of 7 catabolic enzymes were measured in each sample. Particular attention was paid to the selection of locomotory muscle. A number of trunk flexors and extensors were sampled to see if there is any metabolic

indication of the asymmetry between upstroke and downstroke in cetacean locomotion. The controversy as to which is the powerstroke has gone on for decades (see Parry 1949). Recent kinematic investigations on dolphins indicate that the downstroke produces greater propulsive force on average than the upstroke (Videler and Kammermans 1985). In spite of the propulsive asymmetry, both the dorsal and ventral muscles may have to work equally hard, since the difference appears to be due to increased drag on the animal during the upstroke (probably due to a less efficient angle of attack of the fin and the resulting less forward propulsive force). In fact, similar demands on both flexors and extensors are indicated by the approximately equal size (cross sectional area) of hypaxial and epaxial musculature, and the caudal tendons of shortfin pilot whales (Arkowitz and Rommell 1985). Therefore, equal metabolic demands may very well be placed on both sets of muscles.

As was the case with harbor seal (Chapter 2), specific muscle functions are based on anatomical studies (Schulte and Smith 1918; Howell 1927, 1930; Slijper 1936; Pilleri et al. 1976; Pabst 1990) and by analogy to equivalent muscles in terrestrial vertebrates, although the highly specialized cetacean body plan makes the establishment of homologies for some muscles much more difficult than was the case for harbor seal. Like Chapter 2, fin whale muscle enzyme activites will be examined with particular emphasis placed on the differences between muscles of varying function. The implications of these differences to the physiological and potential biochemical adaptations to diving will be discussed, and similarities or differences to the metabolic pattern found in phocid seals (Chapters 2 and 3) will be pointed out.

Materials and Methods

Experimental animals. Fin whales (Balaenoptera physalus) were collected commercially off the west coast of Iceland by the whaling company Hvalur H.F., Iceland. Muscle samples were obtained upon return of the whaling ships to the coastal whaling station in Hvalfiordur during June of 1985. The majority of samples were collected 15 to 17 hours post mortem. Samples of m. extensor digitorum and m. triceps were obtained from different specimens, 18 to 25 hours post mortem. Preliminary studies of muscle samples obtained at sea and placed in a freezer, and those frozen approximately 20 hours post mortem showed no decrease in enzyme activity (Table 6). This somewhat surprising result is probably due to the low metabolic rate characteristic of such a large animal (Hemmingsen 1960; Siesjo and Nordstrom 1977; Emmett and Hochachka 1981; Schmidt-Nielsen 1979, 1984; Hochachka and Somero 1984). Another contributing factor to the lack of enzyme degradation may involve diving adaptations which cause the muscle tissues to be more "tolerant" of general asphyxia. In addition, the Icelandic practice of cutting open the carcass and infusing it with sea water may successfully lower the temperature of the muscles sufficiently to prevent excessive degradation. A striking feature of the at sea vs on shore comparisons (Table 6) is the even higher levels of enzymes observed 20 hours post mortem (particularly mitochondrial enzymes). This unusual result, although of questionable validity due to the small sample size, could be a consequence of the more "violent" freezing conducted on shore (freeze-clamping in liquid nitrogen).

Lower freezing temperatures have been found to be positively correlated with increased enzyme activity levels when assayed, due to a greater release of mitochondrial or membrane-bound enzymes from their associated structures (Hamm and Kormendy 1969). Consequently, the samples taken at sea (which were allowed to freeze "naturally" in a standard -20°C freezer) may have lost some enzyme activity with the pellet during the centrifugation procedure.

The majority of the muscle samples were obtained from whales ranging in size from 57-67 feet long and including 3 females and 1 male. However, the m. extensor digitorum and m. triceps samples were obtained from 2 males and 2 females ranging in size from 55-65 feet long.

Muscles sampled. Portions of 20 different skeletal muscles were collected from each of 4 whales. The muscles sampled and the location of the sample within the muscle are described in the following list. Two or three letter abbreviations used throughout the remainder of the study to describe each muscle sample are given in parentheses. The muscles are listed in the approximate order they were sampled during the flensing procedure.

1. m. nasorostralis superficialis (BH) -- a superficial portion of muscle along the mid-dorsal line in the anterior lip of the blow-hole.

2. m. mylohyoideus (MYL) -- one sample from along the mid-ventral line, midway along the length of the mandible, only the outer 1 cm of muscle was included.

TABLE 6. COMPARISON OF ENZYME ACTIVITY BETWEEN FIN WHALE SKELETAL MUSCLE SAMPLES TAKEN AT SEA AND EQUIVALENT SAMPLES FROZEN 20 HOURS POST MORTEM (ON DECK).

	LDH	PK	a-GPDH	PFK	CPK	GDH
AT SEA	985.31	212.21	5.52	32.49	1736.9	0.15
ON DECK	1065.82	212.21	9.41	35.81	1879.1	0.21

	CS	HOAD	CPT	CAT	GOT	GPT
AT SEA	2.87	2.31	0.21	0.12	13.79	0.79
ON DECK	4.11	3.87	0.28	0.19	17.68	1.25

Values are the mean of 2 samples taken from an extreme posterior portion of m. hypaxialis.
Assay conditions and abbreviations are explained in Materials and Methods.
Additional enzymes not measured in the other fin whale muscles were assayed as described in Chapter 2 for harbor seal muscle.

3. m. panniculus carnosus (PNC) -- sampled from the lateral portion of the head just anterior to the anterior limbs, the entire thickness of the muscle was included.

4. m. deltoideus (DLT) -- a superficial portion of muscle (outermost 2 cm) sampled midway along its length.

5. m. masseter (MAS) -- one sample taken from the middle of the thickest portion of the muscle.

6. m. latissimus dorsi (LD) -- taken from the center of the muscle, midway along its length.

7. m. extensor caudae medialis (ECM) -- sampled along the mid-dorsal line, just posterior to the dorsal fin in the middle of the muscle.

8. m. pectoralis major (PM) -- sampled midway between the mid-ventral and mid-lateral lines, at the level of the anterior limbs -- only the center portion of muscle was included.

9. m. rectus abdominus (RAM -- mid-body portion, RAP-- posterior portion) -- sampled from the mid-ventral line in the center of the muscle; RAM taken midway between the anterior limbs and the dorsal fin, RAP sampled just anterior to the urogenital openings.

10. m. hypaxialis (HYP) -- sampled about 6 inches lateral to the mid-ventral line and 6 inches deep, just posterior to the dorsal fin.

11. m. obliquus abdominis externus (OBL) -- a superficial sample (outer 2 cm) along the mid-lateral line, at a level midway between the anterior limbs and the dorsal fin.

12. m. intertransversarius caudae dorsalis (ICD) -- one sample from the center of the muscle, from midway between the dorsal fin and the tail flukes.

13. m. intertransversarius caudae ventralis (ICV) -- same description as ICD.

14. m. longissimus dorsi (LD) -- sampled just anterior to the dorsal fin at a depth of about 6 inches.

15. m. (ilio)costalis (IL) -- same description as LD.

16. m. multifundus (=spinalis dorsi) (SPD) -- superficial sample (outer 2 cm) taken from along the mid-dorsal line just anterior to the dorsal fin.

17. m. interspinales (IS) -- the entire center portion of muscle from between the neural spines of 2 lumbar vertebrae just posterior to the dorsal fin.

18. m. diaphragm (DIA) -- these samples were taken at random locations (due to the nature of the flensing process), they included the entire thickness of the muscle.

19. m. intercostales externi (EXT) -- precise locations were again difficult, however samples were generally obtained close to the mid-lateral line from between the middle ribs, only the outer 2 cm of muscle was included.

20. m. intercostales interni (INT) -- sampled from directly below the EXT samples, only the inner 2 cm of muscle was included.

Another 2 muscles were sampled from each of 4 additional whales. This was necessitated by the difficulty in locating and excising the small amount of muscle available in the large anterior limbs (foreflippers).

21. m. extensor digitorum (EDC) -- any muscle tissue that could be found in the center of the lateral aspect of the anterior limb was included in these samples.

22. m. triceps (TRI) -- the distal portion of this muscle was removed from the caudal border/medial aspect of the anterior limb.

Muscle location and nomenclature is based on the work of several different authors. The axial musculature is named as suggested by Pabst (1990) in her attempt to create a consistent nomenclature for cetacean axial muscles. The muscles of the flipper are based on Howell (1930). The names and locations of the remaining muscles are derived from Schulte and Smith (1918), Howell (1927, 1930), Slijper (1936), and Pilleri et al. (1976).

Tissue manipulations. As described in harbor seal materials and methods.

Homogenization for enzyme assays. As described in harbor seal materials and methods except that the homogenization was conducted with an Ultra-Turrax homogenizer rather than a Polytron.

Enzyme assays. Buffer conditions and equipment used were the same as described in the harbor seal materials and methods. The following enzymes were assayed at 25°C:

Lactate dehydrogenase (LDH). -- same concentrations as harbor seal

Pyruvate kinase (PK). -- same as harbor seal

α-glycerophosphate dehydrogenase (α-GPDH). -- same as harbor seal

Glucose-6-phosphate dehydrogenase (G6PDH). -- 10 mM glucose-6-phosphate (omitted for control), and 2 mM NADP.

Creatine kinase (CPK). -- 70 mM creatine phosphate (omitted for control), 2 mM ADP, 20 mM glucose, 2 mM $NADP^+$, 10 mM AMP, 10 mM $MgCl_2$, 10 mM B-mercaptoethanol, excess hexokinase and G6PDH

3-hydroxyacyl-CoA dehydrogenase (HOAD). -- same as harbor seal

Citrate synthase (CS). -- same as harbor seal

Glutamate-oxaloacetate transaminase (GOT). -- same as harbor seal

Chemicals.

As described in harbor seal materials and methods.

Results

Maximum Enzyme Activities. Maximum activities of the 8 enzymes measured in each of the 22 fin whale muscles examined are listed in Table 7. All values are the mean ± 1 S.E. of 4 animals measured at 25°C (except for GDH, which was conducted on only one whale and, therefore, excluded from further analysis). The correlations between enzymes are listed in Table 8. Two-way analysis of variance (not shown) exhibits significant muscle differences in all enzymes studied except for HOAD. Animal differences are found only in LDH, CPK, and HOAD. The animal effects are, however, small and appear to be an artifact of the way the assays were conducted (each whale's muscles having been analyzed on a separate day from the others' for each series of enzymes), rather than an indication of important animal differences.

The mean LDH activity of fin whale skeletal muscle (Table 7) is virtually identical to that found in harbor seal (Table 1). However, the range of values is much greater in the fin whale. The large swimming muscles of the whale have LDH activities over 2x the magnitude reported for skeletal muscle from nearly any other animal (Scrutton and Utter 1968; Crabtree

TABLE 7. MAXIMUM ENZYME ACTIVITIES IN FIN WHALE MUSCLES.

MUSCLE	LDH		PK		a-GPDH		CPK		HOAD		CS		GOT		GDH	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
BH	203.56	35.45	91.53	14.82	2.31	1.01	2040.24	214.48	3.84	0.71	2.24	0.22	15.18	1.98	0.01	N.A.
DIA	305.81	31.48	119.62	25.27	4.58	0.87	2305.24	181.35	5.26	0.54	2.95	0.41	18.51	2.04	0.28	N.A.
DLT	380.54	71.57	137.58	9.56	3.12	0.46	1844.46	108.99	3.45	0.33	2.27	0.21	12.23	0.64	0.12	N.A.
ECM	1630.82	369.75	329.22	50.67	7.38	2.12	2196.81	258.23	3.45	0.88	3.17	0.39	12.68	0.99	0.01	N.A.
EDC	215.53	18.88	85.66	9.17	3.13	0.44	1290.34	85.81	4.97	0.55	3.61	0.31	14.56	2.08	0.57	N.A.
EXT	646.99	102.81	175.27	17.54	5.36	1.93	2062.74	108.57	4.28	0.94	3.69	0.78	10.35	1.17	0.01	N.A.
HYP	945.03	70.71	242.51	16.61	7.51	1.56	1556.87	285.87	4.23	1.55	4.18	1.01	15.59	3.63	0.21	N.A.
ICD	1027.28	369.72	192.55	20.13	11.19	3.61	1570.25	216.78	5.11	1.19	3.02	0.43	10.77	1.93	0.01	N.A.
ICV	1267.15	267.53	298.63	65.93	4.16	1.01	1522.81	117.93	7.02	2.52	5.78	1.24	22.56	2.83	0.11	N.A.
IL	1089.51	142.11	328.92	67.74	11.73	3.52	2314.31	103.58	3.94	0.84	2.96	0.24	12.88	1.52	0.12	N.A.
INT	361.99	58.03	88.79	5.01	3.81	0.19	1868.34	88.41	3.92	0.95	1.81	0.19	10.91	1.81	0.03	N.A.
IS	340.02	47.81	127.81	19.93	2.83	0.51	1669.62	145.72	3.49	0.43	1.65	0.11	7.42	0.52	0.11	N.A.
LAT	701.68	65.28	190.39	18.38	3.32	0.82	2218.79	190.64	3.71	0.65	2.61	0.21	12.44	1.31	0.01	N.A.
LD	2062.64	98.11	492.48	37.75	13.11	3.06	2207.65	113.82	4.08	0.64	4.73	0.39	11.79	0.81	0.23	N.A.
MAS	167.68	22.97	74.81	8.74	3.45	0.85	2192.51	145.73	4.95	0.91	3.19	0.61	13.58	2.07	0.21	N.A.
MYL	195.61	65.84	105.53	23.83	3.24	0.67	1183.72	204.05	4.28	0.86	2.27	0.46	8.84	1.56	0.14	N.A.
OBL	785.21	292.35	190.63	41.76	3.81	0.42	1599.26	105.28	5.01	0.81	4.07	1.23	15.82	2.36	0.14	N.A.
PM	337.51	53.07	120.41	15.58	3.61	0.61	1952.56	81.31	4.34	0.56	2.17	0.11	11.78	0.69	0.21	N.A.
PNC	212.64	31.21	114.78	10.65	2.85	0.55	1122.59	43.43	3.52	0.35	2.13	0.11	10.44	0.16	0.21	N.A.
RAM	1713.22	74.83	442.13	22.56	7.44	1.04	2723.57	317.21	6.36	1.03	6.17	0.46	18.57	0.94	0.28	N.A.
RAP	1696.64	185.71	330.11	55.07	2.94	1.05	2399.82	192.36	2.36	0.11	2.53	0.21	7.12	0.44	0.01	N.A.
SPD	1738.34	371.33	351.86	87.01	6.57	1.47	2441.74	239.27	7.85	2.57	6.59	1.26	17.11	3.36	0.28	N.A.
TRI	310.65	32.29	117.64	16.39	4.57	0.81	1706.04	117.71	4.97	0.79	3.11	0.53	17.31	2.58	0.23	N.A.
ALL	797.22	70.28	206.47	14.11	5.31	0.43	1912.64	54.05	4.54	0.23	3.34	0.18	13.41	0.52	0.14	N.A.

Assay temperature = 25°C.
Activities are expressed as units/gm wet wt.
S.E. = 1 standard error of the mean.
n = 4.
N.A.= not applicable.
See Materials and Methods for assay conditions and abbreviations.

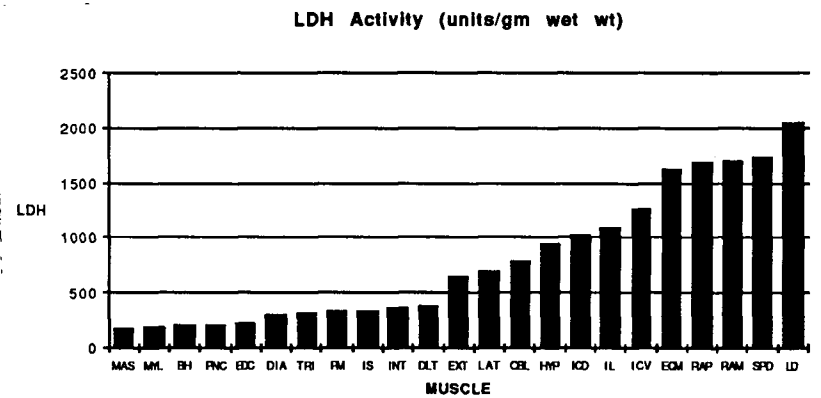
TABLE 8. CORRELATION MATRIX BETWEEN ENZYME ACTIVITIES OF FIN WHALE MUSCLE.

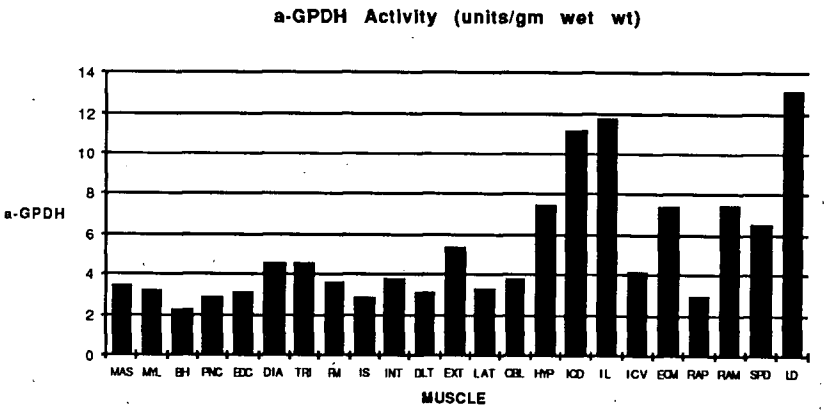
	LDH	PK	a-GPDH	CPK	HOAD	CS	GOT
LDH	* 1	*0.91	*0.52	*0.35	-0.01	*0.49	0.12
PK	*0.91	* 1	*0.57	*0.34	0.03	*0.51	0.14
a-GPDH	*0.51	*0.57	* 1	*0.25	0.20	*0.41	*0.23
CPK	*0.35	*0.34	*0.25	* 1	0.08	*0.24	0.17
HOAD	-0.01	0.04	0.20	0.08	* 1	*0.57	*0.57
CS	*0.49	*0.51	*0.41	*0.24	*0.57	* 1	*0.56
GOT	0.12	0.14	*0.23	0.17	*0.57	*0.56	* 1

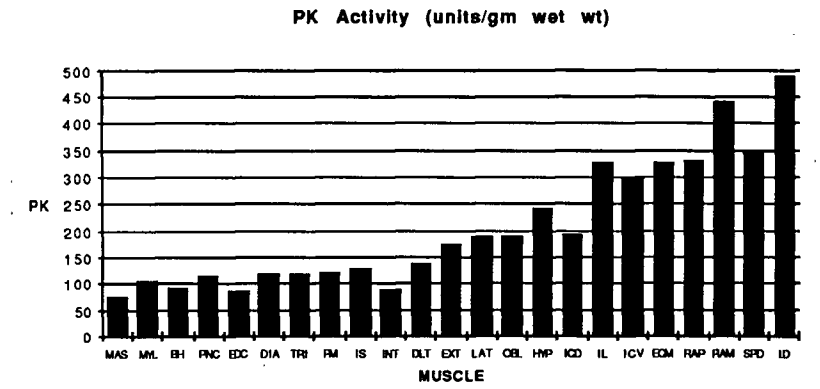
* = statistically significant correlation at the 95% confidence level. All correlations are between data ranks (Spearman correlations). See Materials and Methods for abbreviations.

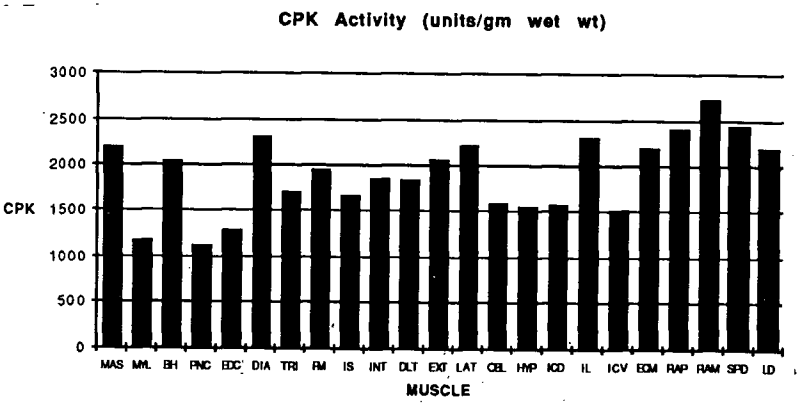
FIGURE 3. HISTOGRAM PROFILES OF MAXIMUM ENZYME ACTIVITY IN FIN WHALE MUSCLES (2 PAGES).

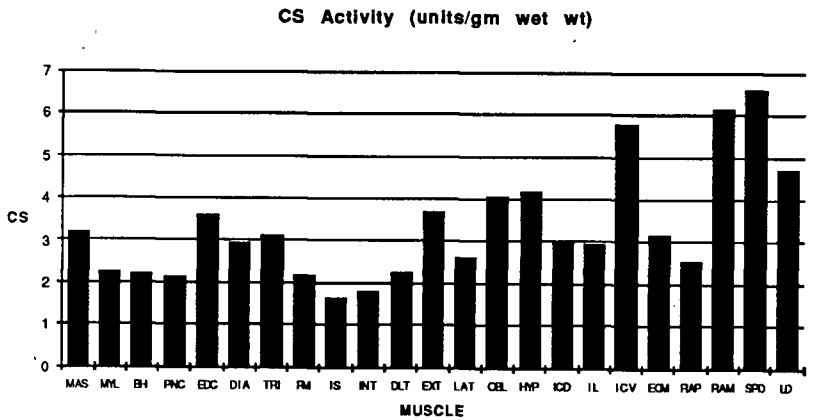
Muscles are listed in order of increasing LDH activity. See Materials and Methods for abbreviations.

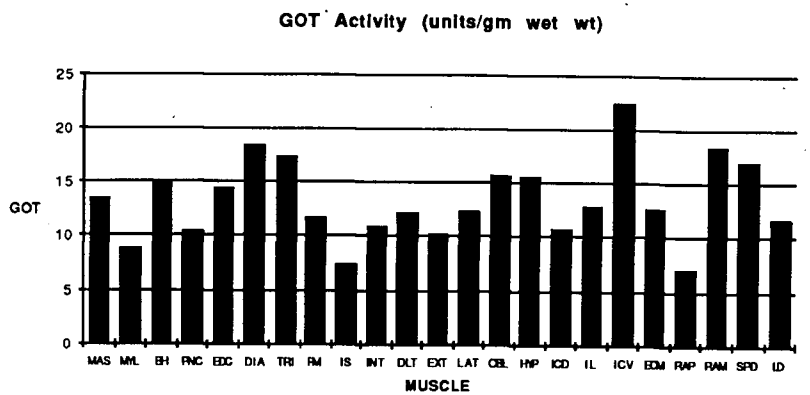

LDH Activity (units/gm wet wt)
LDH
2500
2000
1500
1000
500
0
MAS MVL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE

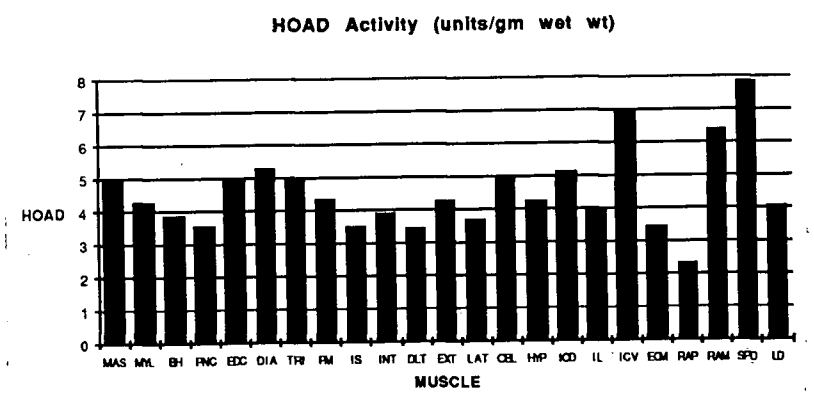

a-GPDH Activity (units/gm wet wt)
a-GPDH
14
12
10
8
6
4
2
0
MAS MVL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


PK Activity (units/gm wet wt)
PK
500
450
400
350
300
250
200
150
100
50
0
MAS MVL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


CPK Activity (units/gm wet wt)
CPK
3000
2500
2000
1500
1000
500
0
MAS MVL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


CS Activity (units/gm wet wt)
CS
0
1
2
3
4
5
6
7
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


GOT Activity (units/gm wet wt)
GOT
0
5
10
15
20
25
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


HOAD Activity (units/gm wet wt)
HOAD
0
1
2
3
4
5
6
7
8
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE

and Newsholme 1972a; Emmett and Hochachka 1981), including other marine mammals (Storey and Hochachka 1974; Ponganis and Pierce 1978; Castellini et al. 1981). Two exceptions to this are the white muscle of tuna (Guppy et al. 1979) and salmon (Mommsen et al. 1980) which both have LDH activity over 2x the fin whale values. The pattern of LDH activity across the 22 muscles examined is very similar to that found in the harbor seal. The swimming muscles have exceptionally high values, while the MAS, DIA and other "unusual" muscles have very low LDH activity (Table 7). Except for a few statistically significant differences between the swimming muscles, most functionally associated muscles group together (Appendix 3).

PK activity in fin whale muscle (Table 7) is, unlike LDH, quite low in comparison with the harbor seal values reported (Table 1). Activity levels are about what is commonly observed in a number of marine and terrestrial mammals (Storey and Hochachka 1974; Ponganis and Pierce 1978; Castellini et al. 1981; Emmett and Hochachka 1981). The distribution of PK activity across the 22 whale muscles is nearly the same as the LDH pattern (Appendix 3). This closeness between the 2 enzymes is evidenced by the very high positive correlation of PK with LDH (Table 8), and the similarity in histograms (Figure 3).

A third glycolytic enzyme measured in whale muscle is α-GPDH. This enzyme is found in very low amounts (Table 7) compared to what is observed in a number of vertebrate species (Crabtree and Newsholme 1972a); and it is only one-fifth the activity of α-GPDH found in harbor seal muscle (Table 1). Significant positive correlations (Table 8) exist between

all 3 glycolytic enzymes examined (LDH, PK, α-GPDH), and the distribution pattern of α-GPDH among the whale muscles in similar to that observed for both LDH and PK (Appendix 3). The primary exception to this similarity is a greater lack of cohesiveness between the swimming muscles with α-GPDH, than is observed with the activities of LDH and PK.

A Krebs Cycle enzyme, CS, exhibited significant positive correlations with all 3 enzymes of glycolysis (Table 8). The distribution pattern among the muscles is accordingly, somewhat similar to that found with LDH, PK, and α-GPDH (Appendix 3). One interesting observation is that the CS activity of the posterior portion of a particular whale muscle is significantly lower than its more anterior part (RAM>RAP; SPD>ECM). The activities of CS observed among the 22 whale muscles (Table 7) are very low in comparison to what has been reported for a number of vertebrate species (Castellini and Somero 1981; Emmett and Hochachka 1981; Marsh 1981; Suarez 1986) including harbor seal (Table 1).

HOAD, an enzyme of B-oxidation, is found in extremely low levels in fin whale muscle (Table 7), only one-fifth the activity observed in the harbor seal (Table 1). Most other vertebrates also have HOAD activites higher than that found in whale muscle (Ponganis and Pierce 1978; Emmett and Hochachka 1981; Marsh 1981; Suarez 1986). Significant positive correlations exist between HOAD and only 2 of the other enzymes examined (CS and GOT). No significant differences in HOAD activity could be detected between the whale muscles (Appendix 3), but the pattern of activity across the muscle samples is similar to that observed with CS.

GOT activity in whale muscle has strong positive correlations to both CS and HOAD (Table 8). The pattern of distribution of muscles with all 3 enzymes is very similar (Appendix 3). Even the differences between the anterior and posterior areas of muscle observed with CS are readily apparent with GOT. RAM has significantly higher GOT activity than RAP, and the amount of GOT in SPD is much greater than ECM (Appendix 3). The GOT activities found in whale muscle (Table 7) are very low in comparison with a number of other animals (Scrutton and Utter 1968; Suarez 1986), including harbor seal skeletal muscle (Table 1). In fact, the highest GOT activity observed in whale muscle is only about one half the lowest seal muscle value.

The CPK activity of whale muscle (Table 7) falls within the normal range of values reported for a number of vertebrate species (Newsholme et al. 1978; Suarez 1986), however, the level of CPK activity in whales is slightly lower than what is observed in seal muscle (Table 1). Whale muscle CPK does not correlate highly with any of the other enzymes measured (Table 8). The pattern of activity of CPK across the 22 muscles is, nonetheless, somewhat similar to the pattern observed with the glycolytic enzymes (Appendix 3). The primary differences involve unusually high levels of CPK in DIA and BH, and less cohesion in the grouping of the swimming muscles with CPK.

Multi-muscle comparisons ("adaptation factors"). Table 9 shows, for each enzyme measured, the value of the "adaptation factors" for whale muscle, along with some values calculated from the literature. ("Adaptation

TABLE 9. ADAPTATION FACTORS OF FIN WHALE SKELETAL MUSCLE, WITH COMPARISON VALUES FROM THE LITERATURE AND HARBOR SEAL MUSCLE.

ENZYME	FIN WHALE	HARBOR SEAL	1	2	3	4	5	6	7	8	9	10
LDH	12.3	2.5	3.0/6.2	- - - - - -	11.7	- - - - -	5.5	10.7/11.5	11.1	4.6/5.1	- - - - - -	3.9/5.2
PK	6.6	3.8	- - - - - -	2.7/3.9	- - - - -	6.2	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
a-GPDH	5.7	3.2	1.6/7.9	- - - - - -	- - - - -	- - - - -	- - - - -	7.5/8.3	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
G6PDH	- - - - -	- - - - -	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
PFK	- - - - -	4.7	3.2/4.8	2.5/7.3	- - - - -	6.5	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
CPK	2.4	2.6	- - - - - -	- - - - - -	1.5	- - - - -	- - - - -	- - - - - - - - -	2.8	- - - - - - -	1.2/2.9	- - - - - - -
CS	4.1	2.3	- - - - - -	- - - - - -	5.9	2.4	13.2	1.9/5.3	- - - - -	3.3	- - - - - -	1.0/3.0
HOAD	3.3	3.5	- - - - - -	- - - - - -	5.7	5.6	5.6	2.6/15.6	- - - - -	- - - - - - -	- - - - - -	1.1/2.8
CPT	- - - - -	3.5	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
CAT	- - - - -	4.1	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -
GOT	3.2	1.8	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	4.7	- - - - - - -	- - - - - -	- - - - - - -
GPT	- - - - -	3.2	- - - - - -	- - - - - -	- - - - -	- - - - -	- - - - -	- - - - - - - - -	- - - - -	- - - - - - -	- - - - - -	- - - - - - -

Adaptation factor = maximum/minimum enzyme activity.
Numbers separated by a "/" are the minimum and maximum adaptation factors calculated for that particular study.
Literature comparisons:

1) Crabtree and Newsholme 1972a
2) Zammit et al 1978
3) Pette 1966, 1985
4) Helig and Pette 1980
5) Pette and Dolken 1975
6) Bass et al 1969
7) Ansay 1974
8) Laborde et al 1985
9) Newsholme et al 1978
10) Pette et al 1975

factor" is defined in Chapter 3: results section) As is the case with seal muscle, most "adaptation factors" calculated for whale muscle fall within the normal range (Table 9). Excluding LDH, whale muscle "adaptation factors" are between 2.43 and 6.58. LDH, however, has a high value of 12.30. This factor is higher than any of the literature values for this or the other enzymes examined (Table 9), indicating an unusual degree of adaptation between certain whale muscles with regard to LDH activity. Some of the larger swimming muscles have higher levels of LDH than might be expected on the basis of "normal" muscle adaptation, in comparison with a few of the more "unusual" whale muscles (Table 7). One exception to this, not listed in the table, is between salmon red and white muscle (Mommsen et al. 1980), which has a value over 18. However, even tuna muscle only has an adaptation factor of 10 between its red and white muscle (Guppy et al. 1979).

Discussion

Whale muscle metabolic organization. The enzyme activities of fin whale skeletal muscle exhibit some interesting differences from what is observed in harbor seal (Chapter 2). On average, LDH activity in the whale muscle is high, but within the normal vertebrate range (Scrutton and Utter 1968; Crabtree and Newsholme 1972a; Emmett and Hochachka 1981). However, extremely high LDH activities are evident in whale swimming muscles. These activities are up to twice the level of activity found in other vertebrates -- except for tuna and salmon white muscle (Guppy et al. 1979; Mommsen et al. 1980) -- including other marine mammals

(Ponganis and Pierce 1978; Castellini and Somero 1981). The enormous size of the fin whales (60 - 70 feet in length) would suggest that these very high LDH activities are a result of scaling (Emmett and Hochachka 1981; Schmidt-Nielsen 1977, 1984). However, the other glycolytic enzymes do not scale upwards like the LDH activity. In fact, the activities of PK and α-GPDH are both low in comparison with other vertebrate species (Scrutton and Utter 1968; Crabtree and Newsholme 1972a; Zammit et al. 1978; Emmett and Hochachka 1981). If the high LDH activities were a simple scaling phenomenon, all the glycolytic enzymes would be expected to rise in activity. Consequently, the high LDH activity may represent a more truly anaerobic nature to fin whale swimming muscles than is evident in harbor seal (Chapter 2).

The unusually low PK activities (in comparison with LDH) have been observed in another cetacean, the pacific white-striped dolphin (Storey and Hochachka 1974). The enzyme kinetics of the dolphin PK suggested unusually tight regulatory control as a way of sparing carbohydrate for aerobic-anaerobic transitions. PK has long been considered a key control point in the glycolytic pathway (Scrutton and Utter 1968; Simon and Robin 1972). This type of tight control would be particularly important in the fin whale due to its exceptionally high LDH activities. In addition, tight regulation of PK has been suggested to be a crucial aspect of metabolic arrest in turtles and fishes (Storey 1988). Therefore, the low PK activities in fin whale skeletal muscle may be a key adaptation for extending the ADL (Aerobic Dive Limit) through metabolic depression (see Chapter 1).

The low activities of aerobic enzymes in all fin whale muscles imply a generally low oxidative capacity. Overall low levels of oxidative enzymes have also been observed in another large cetacean, the blue whale (Lawrie 1953). This trend is, again, reminiscent of what would be expected from the scaling strategy of a large mammal (Simon and Robin 1971; Emmett and Hochachka 1981; Schmidt-Nielsen 1979, 1984). As is the case with harbor seal (Chapter 2), the HOAD activity in fin whale is relatively constant across a wide range of whale skeletal muscles. Thus, the dependence on fat as fuel for muscular work is similar throughout the muscles examined; although swimming muscles, along with the DIA, MAS, and foreflipper muscles, tend to be relatively higher in HOAD activity. The capacity of the Krebs cycle (CS activity), although low, is clearly higher in the swimming muscles.

GOT activity is also quite low compared to other vertebrates (Chapter 2; Scrutton and Utter 1968). This fact, coupled with the even lower activities of α-GPDH, indicates that the capacity for maintaining cytosolic redox balance in whale muscle is limited, in both the α-glycerophosphate cycle and the malate-aspartate shuttle. It is likely that the high LDH activity makes up for the lack of flux through both these systems. Of course the resulting high levels of aerobic lactate formation (see Brooks 1986 for review) must be dealt with by the whale, either through recycling or oxidation.

CPK activity in fin whale, although high, is within the normal vertebrate range (Newsholme et al. 1978). The activities approximate those observed in harbor seal (Chapter 2). In general, the swimming muscles, along with

MAS, DIA, and LAT, have the highest levels of CPK. The high CPK activity in the MAS of harbor seal (Chapter 2) is mirrored by the fin whale MAS, in spite of their vastly different methods of prey capture (harbor seals feeding on fish and crustaceans; fin whales filter-feeding primarily krill). However, it is advantageous for fin whale, as well as the harbor seal, to have the ability for rapid mouth closure to prevent the escape of significant numbers of prey (the muscle differences are detailed further below).

The general pattern of pathway relationships in fin whale muscle is similar to what is found in harbor seal. The positive correlation between glycolytic enzymes and CS is evident, as is the independence of fat metabolism from the trends of overall carbohydrate utilization between muscles. There is, however, some evidence of higher fat utilization (HOAD activity) in muscles that exhibit high CS activity. This relationship between HOAD and CS is much less evident in seal muscle (Chapter 2). Its importance in whale muscle is probably minor, since the HOAD activities between muscles are not significantly different from one another.

This pattern of enzyme relationships is (like the harbor seal) different from the more commonly observed pattern in vertebrate muscle where an inverse relationship exists between aerobic enzymes and the enzymes of glycolysis (Bass et al. 1969; Talmant et al. 1982; Talmant and Monin 1986). Instead of the typical case, where a muscle with high levels of glycolytic enzymes has correspondingly low aerobic enzyme activity, whale muscles' glycolytic and aerobic pathway enzymes coadapt.

This type of relationship is also seen in the fast-oxidative-glycolytic (FOG) fibers of a number of vertebrate muscles. When comparing slow-oxidative (SO) to fast-glycolytic (FG) fibers the inverse relationship between glycolytic and aerobic pathway enzymes is readily apparent (Bass et al. 1969; Talmant et al. 1986). However, if either fiber type is compared with FOG, the relationship disappears. FOG fibers are characterized by higher levels of both glycolytic and oxidative enzymes. In this respect, fin whale muscle (like harbor seal) is reminiscent of a FOG pattern of enzyme activities.

The coadapting glycolytic and aerobic enzyme activities in whale muscle may be a byproduct of their diving lifestyle. Aerobically active muscles which have, accordingly, high CS activity, might occasionally be required to work proportionately more under hypoxic conditions as well (ie. during long dives). As a result, the activity levels of glycolytic enzymes would also be high in these muscles. The same possibility holds true for scaling adaptations, with chronically active swimming muscles (high CS) also required to generate huge amounts of power during burst activity (high LDH activity).

Adaptation factors. The "adaptation factors" determined for fin whale muscle are generally within the range of values determined for terrestrial vertebrates (Table 9). An exception to this is observed with the LDH "adaptation factor" of whale muscle, which has the highest value calculated (other than salmon red vs white muscle, not shown). This extreme variation in LDH activity is between the muscles involved with locomotion and some less active skeletal muscles. The unusual difference could be

due to hypoxic stress as a result of diving. However, taking into consideration the enormous size of fin whales, the more likely explanation is a scaling effect (Emmett and Hochachka 1981; Schmidt-Nielsen 1979, 1984).

The much higher LDH activities observed may be required by the fin whale locomotory muscles to help to generate the power necessary for burst type activity by greatly increasing the maximum rate of glycolysis -- this may, in part, be a compensation for the lowered maximal flux rates of aerobic metabolic pathways typically observed in these and other large animals. However, a detailed model to explain the need for such a higher weight specific power output capacity is currently unavaliable (R. Blake, personal communication). Attempts to explain the higher glycolytic flux capacities of larger animals' muscles have invariably been based on steady state, drag dominated, models (e.g. Somero and Childress 1980). A model based on unsteady state hydrodynamics would be required to account for strenuous burst swimming activity. However, the high levels of glycolytic enzymes observed in large animals -- such as the high LDH activity in fin whale locomotory muscle -- would likely underpin any such unsteady state model, whatever it's nature (R. Blake, personal communication).

The significance of the normal values for the remainder of the "adaptation factors" in fin whale muscle is as discussed in detail in Chapter 2 for harbor seals, and the conclusion reached is the same. The diving habit does not appear to significantly effect the enzymatic activities of the whale muscle. Again, the combination of physiological adjustments and the possibility of strong selective metabolic depression and/or increased

efficiency appear to adequately "insulate" the muscle from the effects of diving. An additional factor in fin whale is the fact that it probably pushes its dives up to, and beyond, the aerobic limit to a much lesser extent than do phocid seals (Harrison and Kooyman 1981).

Muscle relationships. Relating enzyme profiles to specific muscle functions is much more difficult to accomplish in fin whales than was the case for the harbor seal (Chapter 2). This problem arises from two central facts. The animals, and therefore the muscles, are so large that anterior vs posterior, and superficial vs deep variance in enzyme activity levels of the muscle samples can blur any differences between muscles to a large degree. Second, the specific demands placed on individual muscles are much harder to interpret, due to greater difficulty in establishing homologies between cetacean musculature and the typical mammalian pattern. Cetaceans are the most modified of marine mammals as a result of an entire life spent at sea (Harrison and Kooyman 1981). Nevertheless, the whale muscles examined did separate into at least two clearly defined groups: 1) swimming muscles, and 2) miscellaneous, non-swimming muscles. Both of these groups can be further broken down, however the differences in enzyme pattern responsible for any subsequent divisions becomes increasingly difficult to establish. The general muscle actions discussed below are based on human muscle anatomy and kinesiology (Cunningham and Basmajian 1969; Kendall et al. 1971; Hrycyshyn and Basmajian 1972; Gray 1989; MacConaill and Basmajian 1977; Basmajian 1978; McMahon 1984; The following discussion refers only to relative (intraspecies), rather than absolute (interspecific/comparative) enzyme

activites. That is a "high" or "low" activity is "high" or "low" only in relation to the other whale muscles examined -- not necessarily with regard to other species unless specifically stated.

The swimming muscles of the whale exhibit a fairly clear enzymatic pattern in comparison with the other muscles examined. In general, they have a very high glycolytic potential, as evidenced by high activities of LDH, PK and α-GPDH, coupled with high CPK activities. They also exhibit a relatively high level of aerobic enzyme activity (although low in absolute terms). The muscles that are identified by this enzyme pattern as being an integral part of the swimming stroke are the 7 muscles (LD, ECM, SPD, IL, HYP, ICD, and ICV) previously identified by anatomical studies as being important to cetacean swimming (Pabst 1990), and the m. rectus abdominus.

Three of the swimming muscles (SPD, RAM, and ICV) are distinguished by their especially high levels (for fin whale) of aerobic enzymes, both CS and HOAD. In all three instances this difference, along with slightly higher levels of glycolytic enzymes, clearly differentiates them from a closely associated swimming muscle or a different area of the same muscle. The ECM, a caudal extension of the m. multifundus (SPD), which is perhaps the most important back extensor, has lower levels of aerobic enzymes than the SPD. This is somewhat surprising, considering that the posterior one third of the whale is where the majority of motion takes place during the swimming stroke. This activity pattern could be merely a sampling effect; the area from which the ECM samples were taken may have been more of a superficial part of the muscle, in terms of capillary distribution and fiber

type (Guth and Samaha 1969; Yellin 1969; Baldwin et al. 1972; Gonyea and Ericson 1977; Gunn 1978; Armstrong 1980; Armstrong et al. 1982), than the SPD samples. If this is not the case, the enzyme activities suggest that the more "central" portion of the m. multifundus (SPD) is utilized for steady and burst type swimming activity to a greater extent than its more posterior area (ECM). The ECM, by virtue of its slightly more glycolytic nature would be better suited to recruitment when swimming rates are increased (Holloszy 1973; Gollnick and Hermansen 1973).

The high activities of aerobic enzymes in RAM, in relation to the more posterior portion of the m. rectus abdominis (RAP), tend to indicate that the differences between SPD and ECM are, in fact, due to different recruitment patterns. Again, the more "central" part of the muscle seems slightly better suited to aerobic activity than its more posteriorly located counterpart. The fact that the m. rectus abdominis appears to be an important swimming muscle in fin whale is not surprising. Its typical actions in actively flexing the trunk, as well as stabilizing the torso during hyperextension should be crucial to the dorso-ventral swimming motion of cetaceans.

The differences observed between the ICV and ICD are interesting in another respect. Rather than an anterior versus posterior distinction, these muscles are related dorso-ventrally. The ICD functions to extend the caudal peduncle in conjunction with the LD, while the ICV aids the HYP in flexing the tail flukes. The relatively higher activities of aerobic and anaerobic enzymes in ICV, if truly indicative of higher demands on this muscle than the ICD, may be important with regard to the upstroke vs

downstroke asymmetry in cetaceans. The higher enzyme activities in ICV seem to support the idea that the downstroke is the power stroke in cetacean locomotion (Videler and Kammermans 1985). The higher level of aerobic enzymes suggests that the ICV is required for active tail flexion on a regular basis to a slightly greater extent than the ICD is needed for extension, and is capable of generating higher forces (higher glycolytic and CPK activity). The high activities of glycolytic enzymes in ICD would, however, allow it to actively contribute to quickly resetting the tail to the top of the powerstroke when higher swimming speeds are required (Holloszy 1973; Gollnick and Hermansen 1973).

Unfortunately, this relationship with regard to the powerstroke does not appear to hold for the larger swimming muscles. The enzyme patterns of dorsal and ventral pairs of muscle samples (extensors vs flexors) located approximately opposite to one another are quite similar. The SPD and RAM activity patterns are virtually identical, while the ECM and RAP patterns are also very similar to each other. On the other hand, the HYP (a large flexor of the caudal peduncle) and the LD (a powerful back extensor), although high in the activity of glycolytic enzymes and CS like the other swimming muscles, show quite a large discrepancy between their activity patterns. LD has about twice the LDH, PK, and α-GPDH and CPK levels of HYP. The similarity in aerobic enzyme levels, along with this difference in glycolytic capacity seems to run counter to what is observed between the ICV and ICD. However, a large part of this discrepancy again may involve anterior versus posterior differences. The HYP sample, having been taken from a more posterior location than the LD sample would be expected (based on the above observations of anterior/posterior muscle differences)

to have lower levels of anaerobic enzymes, although perhaps not to this great extent. And, of course, the concomitantly higher activities of aerobic enzymes that would also be expected are not observed in the LD.

The other muscle that is involved in the swimming stroke is the IL. The IL is also an important back extensor, although to a lesser extent than the other extensor muscles sampled. It is characterized by high glycolytic capacity and CPK levels as in the other swimming muscles. The enzymes of aerobic metabolism are, however, relatively low -- similar to the activities found in samples of the posterior portions of m. multifundus and m. rectus abdominis. This type of relationship is, again, suggestive that this muscle is recruited more for powerful strokes than it is on a regular basis for swimming (Holloszy 1973; Gollnick and Hermansen 1973).

Based on the above relationships, it would appear that the SPD and LD muscles are the primary extensors of the swimming stroke, while the m. rectus abdominis seems to be as important a tail flexor as the HYP. The enzymatic pattern of these muscles does not indicate any difference in metabolic potential that would account for the apparent differences observed between upstroke and downstroke in cetaceans. However, because of the variation in enzyme profiles found in the other powerful swimming muscles (ECM, IL), and the differences observed between ICV and ICD, a potential metabolic difference between hypaxial and epaxial musculature can not be ruled out.

The OBL, originally thought to be a potentially important swimming muscle, due to its action as a trunk flexor, appears not to be a significant

factor in power generation. Its average glycolytic capacity and low CPK activity, coupled with its relatively high aerobic enzyme activities, suggest a somewhat different role. Its lateral flexion capabilities are very likely used regularly in stabilization of the spinal column and torso throughout the swimming stroke. This type of continuous, low power, activity would require the relatively more aerobic nature exhibited by this muscle (Holloszy 1973; Gollnick and Hermansen 1973).

Two other muscles that have relatively high levels of aerobic enzymes coupled with low CPK are the TRI and EDC. However, the activities of the glycolytic enzymes in these muscles are much lower than found in OBL -- and both TRI and EDC are extremely reduced in size in the fin whale compared to other mammals (Howell 1930). The inflexible nature of the whale foreflippers makes the normal actions of these muscles ("hand" and "forearm" movements) quite obsolete. Their smaller size and the low levels (in absolute terms) of all the enzymes in TRI and EDC attest to their lack of use for other than structural/postural purposes.

The BH also appears to be structural in nature as was suggested by Howell (1927), with the drawing forward of the anterior lip of the blow hole as a secondary function. The low levels of all enzymes, except for relatively average CPK activity, would support such a role for BH.

The MAS is similarly adapted to BH with regard to enzyme activities, except for a slightly higher aerobic enzyme level. This low metabolic potential would normally be considered somewhat surprising for the MAS of a mammal. However, lack of masticatory activity by the fin whale jaws

(as it is a baleen whale), makes this type of enzymatic machinery perfectly adequate for the low power opening and closing of its jaw.

The DIA of the fin whale appears to be similar to harbor seal DIA in enzymatic profile, with one exception. The pattern of low glycolytic enzyme and relatively high HOAD, along with average CS levels, is the same. The difference is found in CPK activities which are higher in fin whale DIA. Since seals are obviously much more at home out of the water than cetaceans (Harrison and Kooyman 1981), perhaps this higher CPK activity is advantageous to the whale in helping it obtain a very rapid breath upon surfacing so that it can submerge quickly.

The accessory muscles of respiration, INT and EXT, have fairly low levels of all the enzymes examined. This type of enzyme pattern is more conducive to a postural and passive respiratory role for these muscles, rather than any active use during trunk flexion (Holloszy 1973; Gollnick and Hermansen 1973). The slightly higher activity of all enzymes in EXT suggests a more active role for this muscle, possibly as an aid to forced inspiration.

The PNC appears to be a very diffuse structural/postural muscle, investing the blubber layers. The very low levels of all the enzymes measured demonstrate the very inactive nature of this muscle. Another postural muscle sampled is the IS. This muscle, although involved in back extension like the swimming muscles, is not primarily used in the generation of force for movement of the tail flukes. Rather, the function of the IS is to adjust the motion between individual vertebrae. The low

activities of all the enzymes measured tend to confirm this type of "low demand" role.

The LAT, on the other hand, appears to be more than postural in function. Although low in enzymes of aerobic catabolism, there is a relatively high glycolytic capacity and CPK activity in LAT. Its actions on the anterior limbs (adduction and rotation), and ability to assist in lateral flexion would make this an important maneuvering muscle for changing direction while swimming. The glycolytic nature of the LAT would support the occasional type of movement required in this regard (Holloszy 1973; Gollnick and Hermansen 1973). The DLT and PM have similar, although somewhat lower, activities of enzymes in comparison to LAT. These two muscles are probably also used by the whale to manoeuvre via their actions on the anterior limbs (DLT - abduction; PM - adduction and rotation).

The remaining muscle sampled in fin whale was the MYL. This intraramal muscle functions to elevate the hyoid and tongue as the first stage in swallowing. The extremely low levels of all the enzymes measured, except for HOAD which is relatively high for whale muscle, would indicate this muscular activity to be slow, and probably based largely on fat as well as carbohydrate oxidation.

Cluster analysis. Clustering of the whale muscles, based on the activities of all 12 enzymes, indicates the existence of three clearly defined groups. One cluster is composed of several swimming muscles (ECM, IL, LD, HYP, RAP and ICD). The second group is made up of the remaining three

swimming muscles (ICV, RAM, and SPD). The final group is composed of the rest of the muscles, all non-swimming in nature.

This clustering pattern statistically confirms the general trends between muscle function and enzyme activities as discussed above.

Summary. Enzymatic differences have been demonstrated between a large number of fin whale skeletal muscles of widely varying function. These differences are not as clear as with harbor seal muscle (Chapter 2), except in comparison of the swimming musculature to the non-swimming muscles. The importance of defining specific locations for muscle samples is again emphasized by the widely varying enzyme activities between muscles, and anterior vs posterior portions of the same muscle. The functional relationships of the muscles have been clarified by their particular enzyme pattern in relation to the other muscles examined. The general pattern of metabolism in fin whale muscle has been elucidated, and differences between the fin whale pattern and that of the harbor seal have been noted. A combination of "adaptation factors" and absolute enzyme activities indicate that whale skeletal muscle does not appear to be subjected to unusual hypoxic stress in comparison with the muscles from terrestrial species. As is the case with harbor seal, physiological adjustments to diving and possibly a selective metabolic depression of inactive tissues is suggested as sufficient to prevent severe hypoxia in the muscles.

CHAPTER 5:

FIN WHALE ENZYME ACTIVITY RATIOS

Introduction. The apparent differences observed between fin whale muscle (Chapter 4) and harbor seal muscle (Chapter 2) are greatly complicated by the size differential between the two species. The potential scaling effects (Schmidt-Nielsen 1979, 1984; Emmett and Hochachka 1981; Hochachka and Somero 1984) in an animal as large as a fin whale, make maximum enzyme activities a difficult way to conduct interspecific comparisons. However, enzyme activity ratios, by their very nature (Chapter 3), allow clear comparisons of metabolic organization to be conducted -- in spite of huge size differentials.

Therefore, to confirm and clarify the differences between cetacean and phocid seal muscle metabolism suggested by the maximum enzyme activities (Chapter 4), a series of 15 enzyme activity ratios was examined in the 22 fin whale skeletal muscles. The same 15 ratios were also analyzed previously in harbor seal (Chapter 3). Although between muscle differences are verified as well, the primary focus of this Chapter is on interspecific comparisons. The differences are discussed in terms of the fin whales' size and lifestyle, which does not appear to include as great a degree of extended diving times as phocid seals (Ridgway 1972; Martin 1977; Harrison and Kooyman 1981).

Materials and methods.

--as described in Chapter 4

Results.

Enzyme ratios. Table 10 lists the means ± 1 S.E. of 15 enzyme activity ratios for each of the 23 skeletal muscles examined. The correlations of these ratios with the maximum activities of the 7 enzymes measured are shown in Appendix 4. Correlations between the ratios can be found in Table 11. Two-way analysis of variance (not shown) indicates significant muscle differences in all ratios except α-GPDH/CS and α-GPDH/HOAD. Significant animal effects are also evident in all but 6 of the ratios (PK/HOAD, α-GPDH/PK, α-GPDH/HOAD, α-GPDH/CS, GOT/CS, GOT/PK). These effects are, again, small and appear to be an artifact of the way the assays were conducted (each seal's muscles having been analyzed on a separate day from the others' for each series of enzymes), rather than an indication of important animal differences.

PK/LDH, and α-GPDH/LDH activity ratios were calculated for whale skeletal muscles (Table 10) in order to compare aerobic with anaerobic glycolysis in this tissue. A strong positive correlation exists between the two ratios (Table 11). The pattern of muscle distribution across these enzyme ratios is also very similar (Appendix 3). The swimming muscles have low values while the more "unique" muscles (ie. MYL, MAS) have much higher ratios with PK/LDH and α-GPDH/LDH. Neither ratio is particularly effective at discriminating statistically significant differences between the whale muscles examined (Appendix 3). This distribution pattern and lack of discrimination between muscles is very similar to that found with harbor seal skeletal muscle when comparing these ratios (Appendix 1). There is, however, a striking difference in the magnitude of the PK/LDH

TABLE 10. ENZYME ACTIVITY RATIOS OF FIN WHALE MUSCLES (CONT'D ON THE NEXT 2 PAGES).

MUSCLE	PK/LDH		a-GPDH/LDH		a-GPDH/PK		HOAD/CS		a-GPDH/CS	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
BH	0.47	0.07	0.013	0.006	0.027	0.011	1.71	0.26	1.04	0.43
DIA	0.38	0.05	0.015	0.001	0.042	0.009	1.86	0.22	1.61	0.26
DLT	0.40	0.08	0.009	0.001	0.023	0.003	1.52	0.06	1.36	0.15
ECM	0.25	0.10	0.006	0.003	0.023	0.006	1.02	0.20	2.48	0.68
EDC	0.41	0.08	0.015	0.003	0.036	0.004	1.44	0.25	0.90	0.17
EXT	0.28	0.04	0.008	0.001	0.031	0.011	1.16	0.08	1.82	0.92
HYP	0.26	0.02	0.008	0.002	0.032	0.008	0.92	0.14	2.31	0.94
ICD	0.24	0.05	0.013	0.004	0.055	0.014	1.79	0.54	3.92	1.48
ICV	0.24	0.03	0.003	0.001	0.013	0.002	1.17	0.23	0.86	0.26
IL	0.32	0.08	0.012	0.004	0.043	0.016	1.35	0.33	4.00	1.16
INT	0.26	0.04	0.012	0.002	0.044	0.005	2.14	0.35	2.22	0.34
IS	0.37	0.03	0.009	0.002	0.024	0.006	2.12	0.24	1.76	0.38
LAT	0.27	0.02	0.005	0.001	0.017	0.003	1.39	0.15	1.24	0.26
LD	0.24	0.01	0.006	0.001	0.026	0.004	0.85	0.08	2.86	0.74
MAS	0.45	0.01	0.021	0.004	0.044	0.008	1.67	0.28	1.24	0.37
MYL	0.66	0.16	0.021	0.004	0.032	0.003	1.90	0.17	1.56	0.43
OBL	0.32	0.07	0.007	0.002	0.022	0.003	1.48	0.30	1.20	0.31
PM	0.40	0.09	0.013	0.005	0.031	0.005	1.99	0.22	1.64	0.21
PNC	0.56	0.04	0.014	0.003	0.025	0.005	1.65	0.12	1.37	0.32
RAM	0.26	0.01	0.004	0.001	0.017	0.002	1.02	0.13	1.25	0.24
RAP	0.20	0.03	0.002	0.001	0.009	0.003	0.96	0.11	1.26	0.53
SPD	0.20	0.03	0.004	0.001	0.023	0.007	1.14	0.16	1.17	0.39
TRI	0.38	0.04	0.015	0.003	0.039	0.004	1.97	0.78	1.61	0.36
ALL	0.34	0.02	0.011	0.001	0.029	0.002	1.49	0.07	1.77	0.14

TABLE 10. (CONT'D).

MUSCLE	LDH/CS		PK/CS		LDH/HOAD		PK/HOAD		a-GPDH/HOAD	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
BH	94.23	18.86	40.51	3.59	61.01	17.25	25.37	4.36	0.56	0.14
DIA	109.96	17.06	43.05	9.44	58.28	2.32	22.29	3.26	0.86	0.09
DLT	167.18	29.71	61.94	6.51	111.42	21.84	41.11	5.25	0.91	0.14
ECM	581.78	216.71	109.32	20.63	938.82	636.45	145.18	65.41	3.19	1.40
EDC	60.61	5.61	24.96	5.11	45.01	6.01	18.01	2.87	0.68	0.16
EXT	206.49	64.23	52.49	9.44	188.41	71.96	46.61	10.84	1.72	0.99
HYP	260.36	50.28	66.45	11.91	330.75	103.91	82.98	24.28	2.93	1.45
ICD	376.24	154.72	68.13	12.09	267.28	146.49	46.48	13.96	3.05	1.37
ICV	245.32	55.35	59.32	14.78	224.81	66.65	52.92	15.12	0.78	0.28
IL	365.83	28.90	112.32	21.44	310.41	57.31	109.78	41.07	3.91	1.97
INT	211.74	50.95	50.24	3.32	112.07	33.75	25.91	5.11	1.14	0.24
IS	210.55	39.39	78.33	14.04	103.16	19.81	39.01	8.03	0.81	0.13
LAT	271.99	24.17	73.52	5.71	206.81	36.72	55.31	8.66	0.91	0.21
LD	445.51	43.71	106.67	13.18	541.86	84.92	129.82	22.71	3.44	0.96
MAS	60.13	14.57	27.28	7.16	37.11	8.09	16.61	3.47	0.74	0.18
MYL	99.91	40.94	49.24	13.34	49.96	19.78	25.31	6.34	0.79	0.19
OBL	177.58	20.24	53.38	8.04	146.31	42.89	38.27	6.39	0.81	0.12
PM	159.66	29.88	55.71	7.24	85.51	23.34	28.81	4.15	0.83	0.07
PNC	102.19	17.81	54.83	6.96	64.51	14.03	34.31	5.74	0.82	0.15
RAM	283.36	29.24	73.07	7.43	299.84	64.25	76.71	15.02	1.33	0.35
RAP	667.09	25.21	130.24	18.61	730.88	102.71	140.48	24.89	1.21	0.37
SPD	291.15	71.57	62.68	19.05	286.45	81.33	61.81	18.90	1.09	0.37
TRI	106.12	12.06	40.59	6.57	69.51	15.38	25.11	3.88	0.94	0.14
ALL	241.52	20.26	64.97	3.53	229.14	35.41	56.01	5.46	1.45	0.17

TABLE 10. (CONT'D).

MUSCLE	CPK/LDH		CPK/PK		GOT/CS		GOT/PK		GOT/LDH	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
BH	11.23	2.77	23.22	2.73	6.73	0.32	0.17	0.01	0.081	0.02
DIA	7.68	0.74	21.37	3.32	6.46	0.54	0.19	0.07	0.062	0.01
DLT	5.38	0.95	13.51	0.84	5.47	0.45	0.09	0.01	0.035	0.01
ECM	1.64	0.46	7.25	1.68	4.21	0.56	0.04	0.01	0.011	0.00
EDC	6.05	0.41	15.63	1.88	4.23	0.88	0.18	0.04	0.071	0.01
EXT	3.46	0.61	12.16	1.52	3.01	0.41	0.06	0.00	0.017	0.00
HYP	1.66	0.32	6.31	0.83	3.74	0.07	0.06	0.01	0.016	0.00
ICD	2.06	0.65	8.23	1.02	3.54	0.14	0.06	0.02	0.015	0.01
ICV	1.36	0.31	6.13	1.85	4.12	0.43	0.09	0.03	0.021	0.01
IL	2.21	0.25	7.94	1.61	4.53	0.81	0.04	0.01	0.013	0.00
INT	5.72	1.26	21.39	2.16	6.36	1.42	0.12	0.02	0.033	0.01
IS	5.14	0.77	14.53	3.53	4.49	0.13	0.06	0.01	0.023	0.00
LAT	3.28	0.46	12.07	1.81	4.86	0.61	0.07	0.01	0.019	0.00
LD	1.08	0.11	4.61	0.54	2.52	0.14	0.02	0.00	0.006	0.00
MAS	13.69	1.64	30.23	3.02	4.57	0.64	0.18	0.03	0.083	0.01
MYL	10.79	4.78	15.05	5.83	4.41	1.11	0.11	0.03	0.065	0.02
OBL	3.48	1.31	9.66	2.02	4.67	0.87	0.09	0.01	0.029	0.01
PM	6.31	1.14	17.11	2.36	5.44	0.26	0.11	0.01	0.039	0.01
PNC	5.89	1.42	10.22	1.61	4.93	0.23	0.09	0.01	0.054	0.01
RAM	1.59	0.17	6.13	0.52	3.03	0.11	0.04	0.00	0.011	0.00
RAP	1.46	0.18	8.17	2.07	2.84	0.18	0.02	0.01	0.004	0.00
SPD	1.72	0.49	9.65	3.73	2.87	0.61	0.06	0.01	0.011	0.00
TRI	5.72	0.79	15.43	2.29	6.41	1.72	0.16	0.03	0.059	0.01
ALL	4.72	0.44	12.87	0.81	4.51	0.18	0.09	0.01	0.034	0.00

See Materials and Methods for abbreviations.
S.E. = 1 standard error of the mean.
n = 4.

activity ratios found in the whale (Table 10) as compared with the harbor seal values (Table 4). The highest PK/LDH ratio observed in fin whale muscle (0.66) is only as high as the lowest harbor seal value (0.65). The mean whale muscle PK/LDH ratio of 0.34 places it at the low end of the range of values observed in other vertebrate muscles (Pette and Dolken 1975; Hochachka 1985; Suarez 1986), just the opposite of the harbor seal. α-GPDH/LDH ratios in whale muscle are also low (Table 10). The mean value of 0.01 in whale muscle is less than the seal muscle mean of 0.03 (Table 4) or the 0.05 ratio found in hummingbird flight muscle (Suarez 1986).

The relationship between anaerobic glycolysis and aerobic metabolism in fin whale skeletal muscle is observed in the ratios of LDH/CS and LDH/HOAD (Table 10). The correlation between these two activity ratios is extremely high (Table 11). The pattern of muscle distribution with both LDH/CS and LDH/HOAD is, accordingly, nearly identical (Figure 4). The distribution patterns with these ratios are also very similar to what is observed with PK/LDH and α-GPDH/LDH ratios (Table 11; Appendix 3). The LDH/CS activity ratios found in whale muscle (60-667) are relatively high for vertebrate skeletal muscle (Bass et al. 1969; Pette and Dolken 1975; Emmett and Hochachka 1981; Mackova et al. 1985). These whale muscle values place it more in the line with white muscle, while the harbor seal values (Table 4) are more like what is observed with red vertebrate skeletal muscle (Bass et al. 1969). This same relationship exists with regard to the LDH/HOAD ratios. Whale muscle LDH/HOAD (Table 10) is much higher than the level found in harbor seal (Table 4), and much more toward the middle of the range of values, 1-1241, calculated for other

vertebrate skeletal muscles (Bass et al. 1969; Ponganis and Pierce 1978; Mackova et al. 1985).

The activity ratios, PK/CS and α-GPDH/CS were determined to assess the relationship of aerobic glycolysis to the Krebs Cycle in the 22 whale muscles examined (Table 10). The correlation between these two enzyme ratios, although not very high, is statistically significant (Table 11). The PK/CS ratios exhibit a muscle distribution pattern virtually identical with that found for LDH/CS (Appendix 3). The α-GPDH/CS pattern, on the other hand, is completely non-discriminative -- no significant differences are observed between whale muscles (Appendix 3). Whale muscle PK/CS activity ratios (Table 10) are higher than what is observed in harbor seal muscle (Table 4). This is particularly evident when comparing swimming muscles: a range of 60 to 130 in whale and only 31-47 in seal. Fin whale muscle PK/CS ratios are near the center of a range of values determined for mammalian muscles, of between 2 and 200 (Helig and Pette 1980; Emmett and Hochachka 1981). Data from which to calculate comparison α-GPDH/CS activity ratios are scarce. However, the mean whale muscle value of 1.77 is much higher than the 0.03 ratio determined for both hummingbird flight muscle (Suarez 1986) and rat soleus (Kubista et al. 1971), or the 0.54 value found in rat femoris. The mean α-GPDH/CS ratio in harbor seal muscle (1.57), however, is about the same.

A strong positive correlation exists between PK/HOAD and α-GPDH/HOAD, two ratios comparing glycolysis with B-oxidation (Table 11). As is the case with α-GPDH/CS, the α-GPDH/HOAD activity ratio is non-discriminative between whale muscles (Appendix 3). Although no significant differences

are observed between muscles, the distribution pattern is roughly similar to that found with PK/HOAD. The PK/HOAD ratios in whale swimming muscles are clearly higher than in the other muscles examined (Appendix 3), while "unusual" muscles such as the DIA, MAS, TRI and EDC are particularly low. The PK/HOAD activity ratios (mean=56.01) in whale muscle (Table 10) are slightly higher than, but comparable to, seal muscle values (Table 4). The range of values calculated for a number of vertebrates is between 2 and 280 (Helig and Pette 1980; Hochachka 1985; Suarez 1986), with highly aerobic, fat-utilizing muscle generally <15. The mean α-GPDH/HOAD ratio in whale muscle (1.45) is about equal to the harbor seal value of 1.51 (Table 4). The range of activities determined from the literature for this ratio is 0.10 - 3.12 (Falholt et al. 1974; Suarez 1986).

The relationship between carbohydrate and fat-based aerobic metabolism in whale skeletal muscle was examined by comparing HOAD/CS activity ratios (Table 10). No statistically significant differences between muscles are observed (Appendix 3). However, high correlations exist with LDH/HOAD and PK/HOAD (Table 11), and the general trends are similar, with high values for swimming muscles, etc. (Figure 4). The mean HOAD/CS ratio of whale muscle (1.49) is about the same as that found in seal muscle (Table 4). This value falls comfortably within the narrow range, 0.2-2.0, commonly observed in vertebrate muscle (Bass et al. 1969; Kubista et al. 1971; Staudte and Pette 1972; Helig and Pette 1980; Emmett and Hochachka 1981).

TABLE 11. CORRELATION MATRIX BETWEEN ENZYME ACTIVITY RATIOS OF FIN WHALE MUSCLE (CONT'D ON NEXT PAGE).

	PK/LDH	PK/CS	PK/HOAD	LDH/CS	LDH/HOAD	HOAD/CS	a-GPDH/LDH	CPK/LDH
PK/LDH	* 1	*-0.24	*-0.39	*-0.69	*-0.71	*0.47	*0.67	*0.74
PK/CS	*-0.24	* 1	*0.87	*0.84	*0.77	*-0.33	*-0.38	*-0.62
PK/HOAD	*-0.39	*0.87	* 1	*0.84	*0.92	*-0.69	*-0.53	*-0.75
LDH/CS	*-0.69	*0.84	*0.84	* 1	*0.93	*-0.46	*-0.61	*-0.83
LDH/HOAD	*-0.71	*0.77	*0.92	*0.93	* 1	*-0.73	*-0.67	*-0.88
HOAD/CS	*0.47	*-0.33	*-0.69	*-0.46	*-0.73	* 1	*0.49	*0.62
a-GPDH/LDH	*0.67	*-0.38	*-0.53	*-0.61	*-0.67	*0.49	* 1	*0.69
CPK/LDH	*0.74	*-0.62	*-0.75	*-0.83	*-0.88	*0.62	*0.69	* 1
a-GPDH/PK	0.18	*-0.33	*-0.42	*-0.31	*-0.38	*0.32	*0.83	*0.38
a-GPDH/HOAD	*-0.22	*0.58	*0.63	*0.55	*0.58	*-0.41	0.13	*-0.41
a-GPDH/CS	0.02	*0.46	*0.27	*0.35	*0.21	0.14	*0.45	-0.11
GOT/CS	*0.39	-0.16	*-0.36	*-0.32	*-0.43	*0.54	*0.37	*0.54
CPK/PK	*0.32	*-0.69	*-.76	*-0.66	*-0.71	*0.53	*0.47	*0.85
GOT/PK	*0.39	*-0.84	*-.86	*-0.81	*-0.81	*0.51	*0.47	*0.76
GOT/LDH	*0.74	*-0.72	*-.81	*-0.91	*-0.91	*0.57	*0.66	*0.91

TABLE 11. (CONT'D).

	a-GPDH/PK	a-GPDH/HOAD	a-GPDH/CS	GOT/CS	CPK/PK	GOT/PK	GOT/LDH
PK/LDH	0.18	*-0.22	0.02	*0.39	*0.32	*0.39	*0.73
PK/CS	*-0.33	*0.58	*0.46	-0.16	*-0.69	*-0.83	*-0.73
PK/HOAD	*-0.42	*0.63	*0.27	*-0.36	*-0.76	*-0.86	*-0.81
LDH/CS	*-0.31	*0.55	*0.35	*-0.32	*-0.66	*-0.81	*-0.91
LDH/HOAD	*-0.38	*0.58	*0.21	*-0.43	*-0.71	*-0.81	*-0.91
HOAD/CS	*0.32	*-0.41	0.14	*0.54	*0.53	*0.51	*0.57
a-GPDH/LDH	*0.83	0.13	*0.45	*0.37	*0.47	*0.47	*0.66
CPK/LDH	*0.38	*-0.41	-0.11	*0.54	*0.85	*0.76	*0.91
a-GPDH/PK	* 1	*0.36	*0.61	*0.24	*0.41	*0.37	*0.34
a-GPDH/HOAD	*0.36	* 1	*0.81	-0.14	*-0.41	*-0.52	*-0.49
a-GPDH/CS	*0.61	*0.81	* 1	0.19	-0.16	*-0.27	-0.21
GOT/CS	*0.24	-0.14	0.19	* 1	*0.47	*0.62	*0.62
CPK/PK	*0.41	*-0.41	-0.16	*0.47	* 1	*0.78	*0.72
GOT/PK	*0.37	*-0.52	*-0.27	*0.62	*0.78	* 1	*0.89
GOT/LDH	*0.34	*-0.49	-0.21	*0.62	*0.71	*0.89	* 1

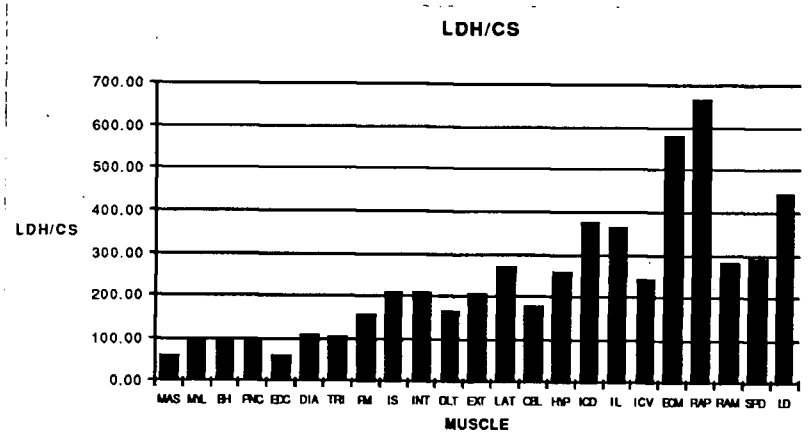
* = statistically significant correlation at the 95% confidence level. All correlations are between data ranks (Spearman correlations). See Materials and Methods for abbreviations.

The ratio between the glycolytic enzymes measured (α-GPDH/PK) is relatively constant among the various whale muscles (Table 10; Figure 4). However, one difference exists between the muscles that is quite unusual. The ICD is significantly higher than ICV and RAP (Appendix 3). This difference appears to be nothing more than an extreme example of the dispersed distribution of the swimming muscles (also observed in harbor seal muscles) with regard to the α-GPDH/PK ratio, and is probably unimportant to the interpretation of the data. The level of α-GPDH/PK in whale muscle (0.03) is slightly lower than the harbor seal value of 0.04 (Table 4), and the very constant ratio of 0.05 from a number of vertebrate skeletal muscles (Crabtree and Newsholme 1972a; Zammit et al. 1978).

As is the case with harbor seal skeletal muscle (Table 4), the CPK/LDH and CPK/PK activity ratios exhibit some clear trends among the whale muscles examined. Swimming muscles have the lowest levels of both ratios (Table 10), while the more "unusual" muscles cluster at the top of the range. The CPK/PK ratios of the whale muscles (5-30) are high compared with other vertebrate muscles, which range between 1 and 8 (Table 4; Zammit et al. 1978; Suarez 1986). The CPK/LDH ratios in whale are, in contrast, low. The mean CPK/LDH ratio in whale muscle of 4.74 (Table 10) is about the same as observed in harbor seal (Table 4), but at the low end of the range of values (1-106) found in other vertebrate skeletal muscles (Crabtree and Newsholme 1972a; Newsholme et al. 1978; Suarez 1986).

FIGURE 4. HISTOGRAM PROFILES OF ENZYME ACTIVITY RATIOS IN FIN WHALE MUSCLES (4 PAGES).

Muscles are listed in order of increasing LDH activity. See Materials and Methods for abbreviations.


LDH/CS
LDH/CS
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600.00
500.00
400.00
300.00
200.00
100.00
0.00
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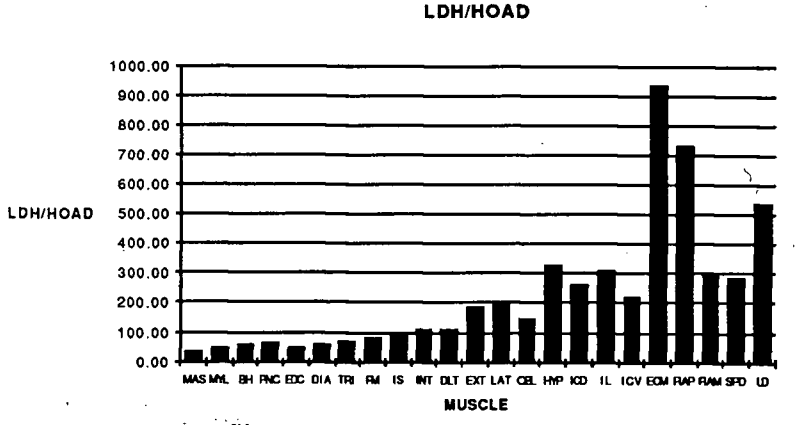
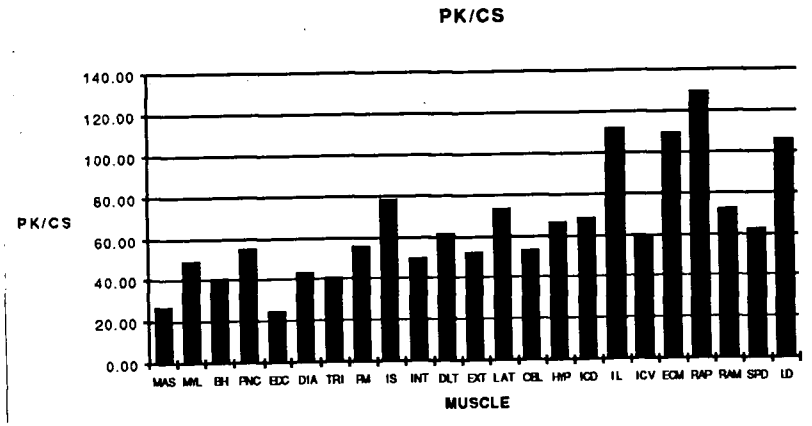
MUSCLE


LDH/HOAD
LDH/HOAD
1000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
MAS MVL BH FNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


PK/CS
PK/CS
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120.00
100.00
80.00
60.00
40.00
20.00
0.00
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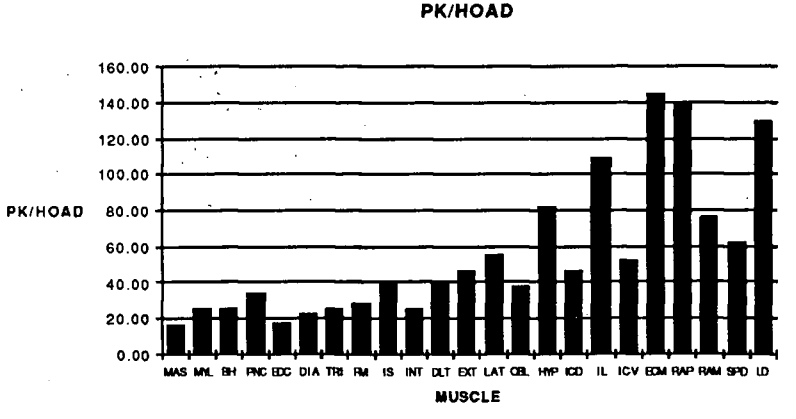
MUSCLE


PK/HOAD
PK/HOAD
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140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
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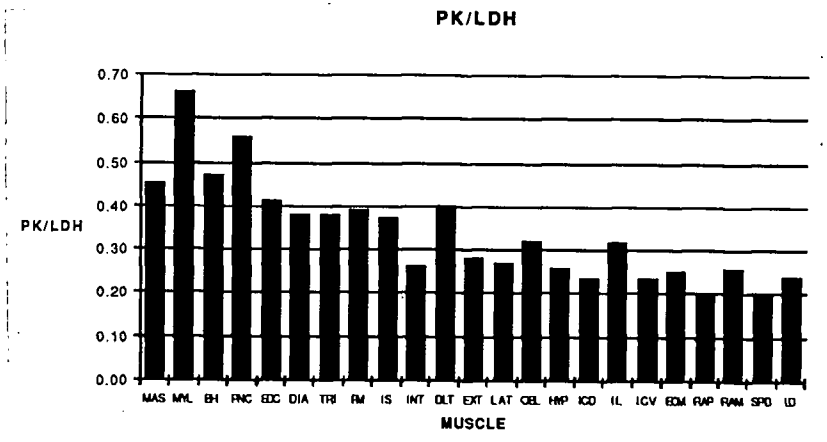
MUSCLE


PK/LDH
PK/LDH
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
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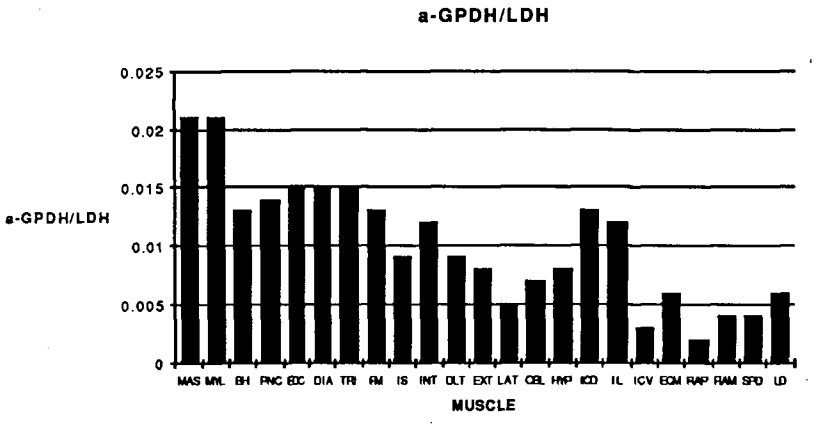
MUSCLE


a-GPDH/LDH
a-GPDH/LDH
0.025
0.02
0.015
0.01
0.005
0
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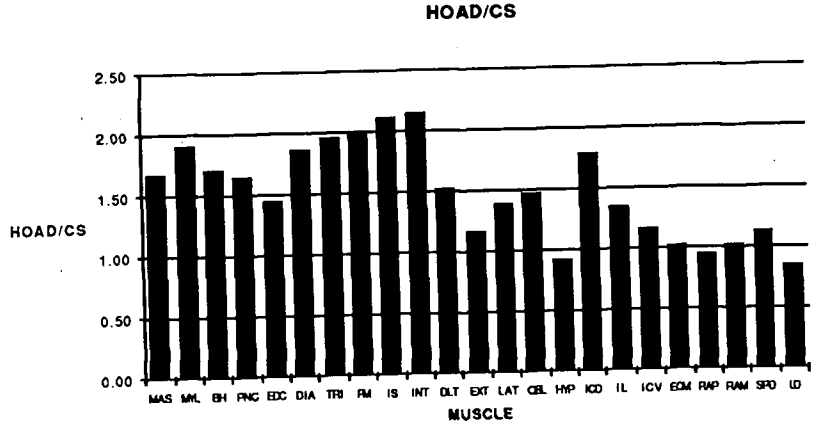
MUSCLE


HOAD/CS
HOAD/CS
2.50
2.00
1.50
1.00
0.50
0.00
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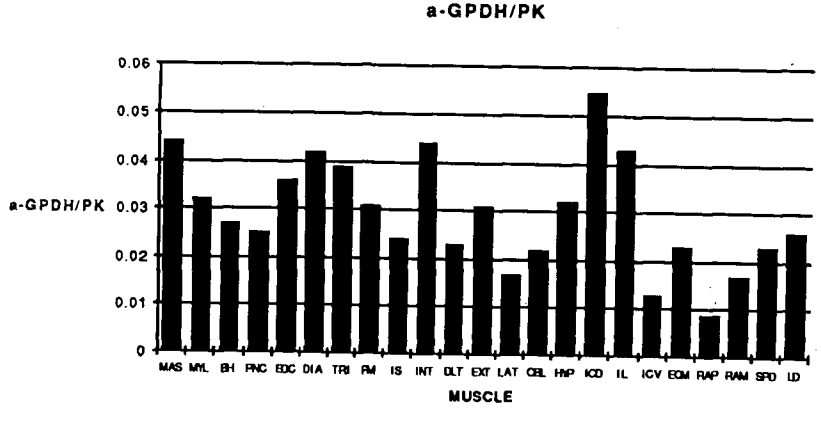
MUSCLE


a-GPDH/PK
a-GPDH/PK
0.06
0.05
0.04
0.03
0.02
0.01
0
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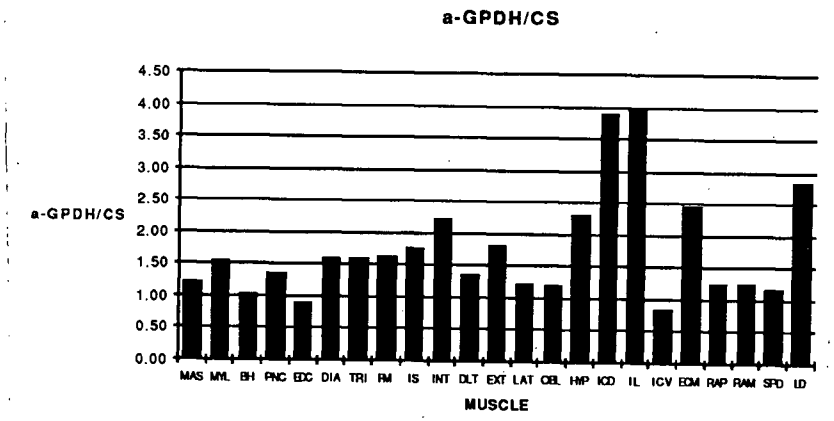
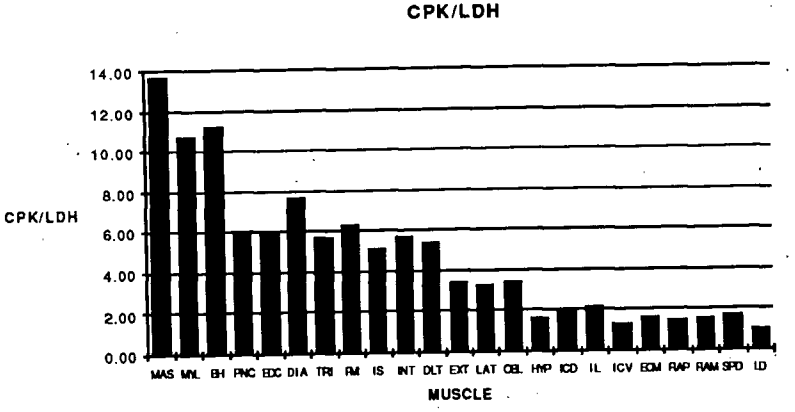
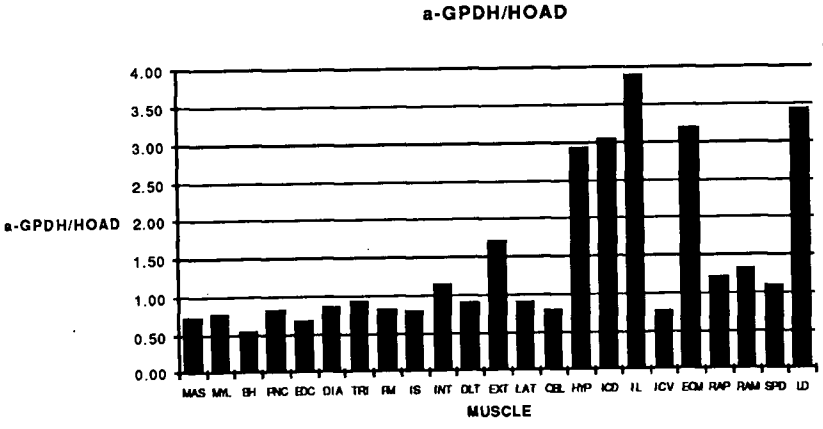
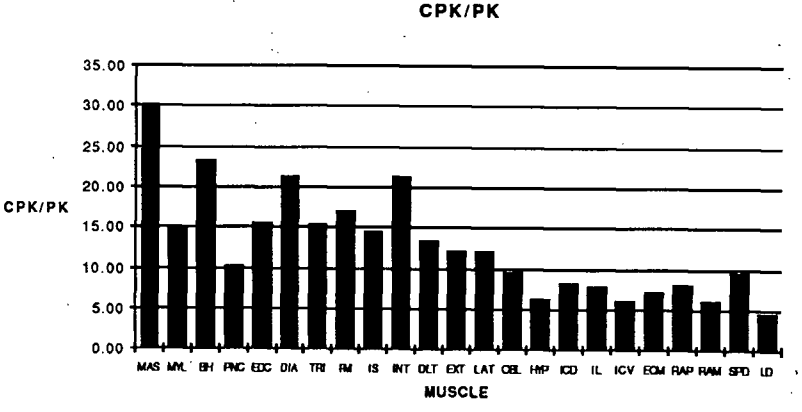
MUSCLE


a-GPDH/CS
a-GPDH/CS
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


CPK/LDH
CPK/LDH
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


a-GPDH/HOAD
a-GPDH/HOAD
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


CPK/PK
CPK/PK
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30.00
25.00
20.00
15.00
10.00
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0.00
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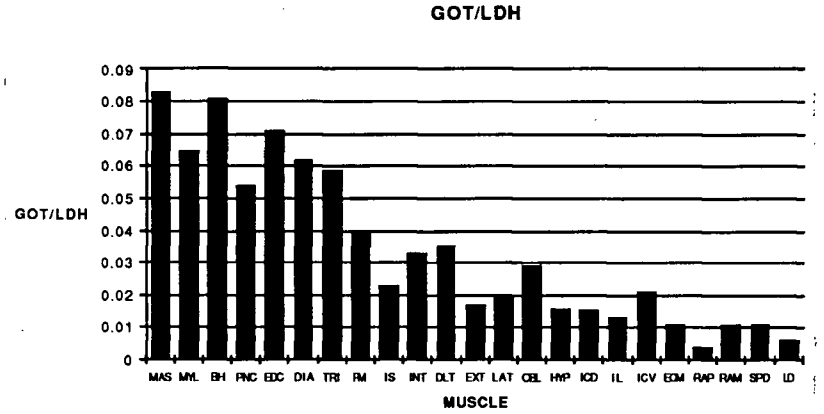
MUSCLE


GOT/LDH
GOT/LDH
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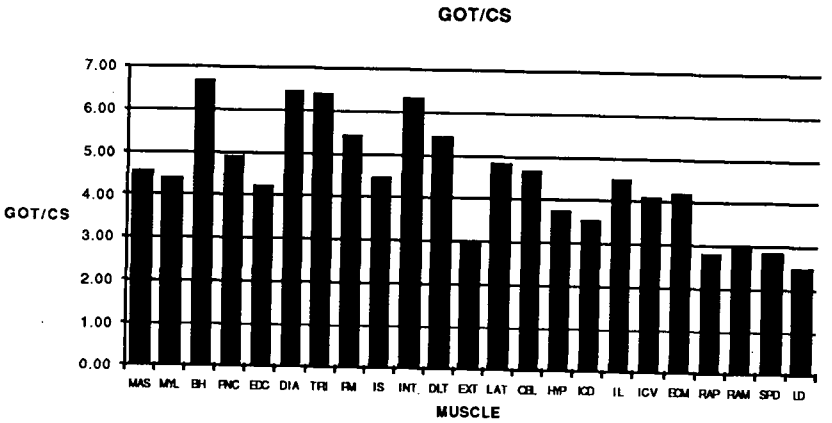
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE


GOT/CS
GOT/CS
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6.00
5.00
4.00
3.00
2.00
1.00
0.00
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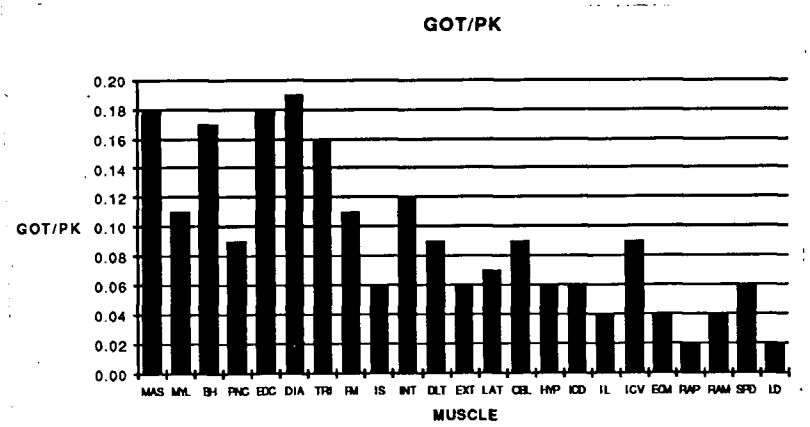
MUSCLE


GOT/PK
GOT/PK
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0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
MAS MYL BH PNC EDC DIA TRI RM IS INT DLT EXT LAT OBL HYP ICD IL ICV EOM RAP RAM SPD LD
MUSCLE

Ratios of GOT with glycolytic enzymes, GOT/PK and GOT/LDH, are very highly correlated with one another (Table 11). Swimming muscles of the whale tend to have low values of both ratios, while the most "unusual" muscles (DIA, MAS, BH, EDC, TRI) have the highest values (Table 10). The mean ratios of GOT/PK and GOT/LDH in whale muscle are both about equal to those reported for other species (Table 4; Scrutton and Utter 1968). An exception to the normal range of values is found with hummingbird flight muscle which is one order of magnitude higher in both ratios (Suarez 1986).

The GOT/CS activity ratio has strong positive correlations to both GOT/PK and GOT/LDH (Table 11). The pattern of distribution of this ratio across the whale muscles is very similar as well (Figure 4). The mean GOT/CS ratio for whale muscle (4.5) is about the same as calculated for seal (Table 4), hummingbird (Suarez 1986), and rat (Scrutton and Utter 1968; Emmett and Hochachka 1981).

Discussion.

Discriminative vs non-discriminative ratios. As is the case with harbor seal, several of the 15 enzyme activity ratios examined in fin whale are non-discriminative. The ratios comparing pairs of mitochondrial enzymes (GOT/CS and HOAD/CS) are, as expected, very constant across all 22 whale muscles. Both GOT/CS and HOAD/CS only vary by about 2-fold. This

confirms what is observed in harbor seal; the metabolic machinery of the mitochondria is very much the same in all the skeletal muscles. Any differences in mitochondrial enzyme activity between muscles appears to be due to changes in the size and/or number of mitochondria, rather than the makeup of the individual mitochondria themselves (Holloszy 1973).

The existence of the constant proportion group of glycolytic enzymes (Pette et al. 1962a, 1962b; Pette 1985) is also evident in the whale muscles. As is observed in the harbor seal, the α-GPDH/PK ratio is somewhat non-discriminative, varying about 5-fold, but with only a single significant difference (ICD>RAP and ICV). This constancy of molar proportions among the glycolytic enzymes is more evident in the PK/LDH ratio. This ratio varies by only about 3-fold across the whale muscles, with just 2 muscles exhibiting statistically significant differences. However, as is the case with harbor seal (Chapter 3) and several other mammalian species (Hochachka 1985), this ratio does appear to exhibit interesting trends among muscles (discussed below). Because these enzymes are part of the same metabolic pathway (Embden-Meyerhoff), a change in the activity of one enzyme should generally be reflected by a similar change in the other enzymes of the pathway. The above ratios demonstrate this relationship in whale muscle, as it has been observed with seal muscle (Chapter 3) and other animal species (Crabtree and Newsholme 1972a; Zammit et al. 1978). However, the glycolytic constant proportion group is more complicated when α-GPDH is involved, particularly if compared with LDH. Although few significant differences are found between the whale muscles with α-GPDH/LDH, they vary by an order of magnitude in value. The variability in this ratio is not unexpected. While seal muscle appears somewhat

unique in having a constant α-GPDH/LDH ratio (Chapter 3), other studies have shown the ratio to be discriminative (Pette et al. 1962b; Falholt et al. 1974; Pette and Dolken 1975). Of any 2 enzymes in the glycolytic pathway, below the level of glucose phosphate isomerase, LDH and α-GPDH are probably the most likely to adapt differently from one another. Even though they are part of the same pathway, LDH, as a completely anaerobic branch of the pathway, and α-GPDH, closely tied to mitochondria, have very different relationships with the other parts of the metabolic machinery. As a result, a variable nature of α-GPDH/LDH is generally the rule.

An association between α-GPDH and mitochondria is much more evident in fin whale muscle than is the case with harbor seal (Chapter 3). While α-GPDH appears to adapt somewhat differently from the mitochondrial enzymes (HOAD and CS) in seal, the α-GPDH/CS and α-GPDH/HOAD ratios in whale are less variable. Both ratios vary by about 4-fold among the whale muscles, and no significant differences between muscles are evident with either ratio. This fairly non-discriminative nature of these ratios is a little surprising in light of the more variable ratio values found with seal muscle (Chapter 3) and other species (Pette et al. 1962b; Pette and Bucher 1963; Kubista et al. 1971; Falholt et al. 1974). The α-GPDH/PK ratio varies about the same amount (5-fold) as these two ratios in whale; while seal α-GPDH/PK only varies abut 2-fold, compared with 6-fold for α-GPDH/CS and 10-fold α-GPDH/HOAD (Chapter 3).

The remainder of the ratios examined appear to be quite discriminative across the 22 whale muscles. This variability is particularly evident when

comparing LDH to mitochondrial enzymes. The LDH/CS ratio is discriminative between seal muscles (Chapter 2) as well as a number of other species (Hochachka et al. 1982; Hochachka 1985). Whale muscles are also clearly differentiated by this ratio. The PK/CS ratio also varies in a similar manner, but only by about half as much. Comparing LDH with another mitochondrial enzyme (HOAD), although exhibiting few statistically significant differences between muscles, gives a ratio (LDH/HOAD) that varies over two and one half orders of magnitude. The LDH/HOAD ratio is variable in seal muscle (Chapter 3) and between several other mammals (Hochachka et al. 1982; Hochachka 1985). Again, inserting PK for LDH gives a ratio (PK/HOAD) whose variability is similar among whale muscles, but is only about half the magnitude of the LDH/HOAD variation. PK/HOAD is also found to be variable in a number of other species (Helig and Pette 1980).

GOT/PK and GOT/LDH, both ratios comparing a mitochondrial to a non-mitochondrial enzyme are clearly discriminative between whale muscles. This same variability is found in seal muscle (Chapter 3), and results from the highly coadaptive nature of GOT and other mitochondrial enzymes, as exhibited by the constant GOT/CS ratio.

The variable ratios discussed above, again (like the harbor seal), all involve a mitochondrial vs non-mitochondrial enzyme. Non-discriminative ratios, in contrast, are composed of pairs of mitochondrial or non-mitochondrial enzymes. However, cytosolic enzymes such as α-GPDH, which are closely associated with mitochondria, can confound this relationship. But two other enzyme ratios examined in whale, CPK/LDH and CPK/PK, are

discriminative even though they are composed of pairs of cytosolic enzymes. Both ratios exhibit statistically significant differences between muscles and vary over 1 - 2 orders of magnitude. CPK/LDH ratios are variable in other species (Lowry et al. 1978) including harbor seal (Chapter 3). The variable CPK/PK ratios in whale muscle are also observed with harbor seal (Chapter 3). These discriminative ratios demonstrate that despite the close relationship between CPK and glycolysis (Newsholme et al. 1978), muscular adaptations occur that effect each pathway very differently.

Muscle metabolism. The fin whale muscles examined appear to be metabolically, fairly "typical" vertebrate muscle based on the enzyme ratios. The majority of the ratios fall within the normal vertebrate range (see results for details). However, the whale muscle differs from harbor seal muscle (Chapter 3) in a number of crucial respects.

Rather than exhibiting an aerobic nature, several ratios in the whale muscles are much more typical of white skeletal muscle. Low PK/LDH and CPK/LDH values, and high LDH/CS, PK/CS, LDH/HOAD, and PK/HOAD are all evidence of a generally more anaerobic metabolism. Complicating this trend are a couple of ratios (CPK/PK and PK/HOAD) that are in the range of values normally associated with red muscle. However, the PK/HOAD values are much higher in whale swimming muscles than the more "unusual" muscles, which is exactly the opposite trend observed with the harbor seal muscles. Thus, the locomotory muscles of the whale do, in fact, appear to be more anaerobic in nature even with the generally aerobic

PK/HOAD ratio. The unusually "red" CPK/PK ratios in whale muscle are similarly distributed. The extremely high values are observed only in muscles such as MAS, BH, DIA, etc. The swimming muscles all fall near the normal range of values (between 1 and 8) found for vertebrate skeletal muscle (Newsholme et al. 1978; Zammit et al. 1978; Suarez 1986), including harbor seal (Chapter 3). This dichotomy in the enzyme ratio patterns is just as clear with the ratios that exhibit generally anaerobic metabolic values (above). The most anaerobic of the muscles invariably are the swimming muscles, while the other muscles appear to be much more aerobic in nature. All the muscles have a normal relationship between fat and carbohydrate utilization (HOAD/CS ratios).

These ratio patterns confirm and clarify the observations made in Chapter 4 on the basis of maximum enzyme activities. Whale muscle is much more anaerobic than the muscle of harbor seal, particularly with regard to the propulsive muscles of the swimming stroke. Since the more anaerobic metabolic make-up is found with the swimming muscles of the whale, it suggests quite a different type of adaptation than is observed with the seal. This pattern may result from several things: 1) less efficient diving adaptations in the whale, 2) less "stressful" diving activity, and/or 3) metabolic adjustments as a result of the great size of the whales.

If the physiological adaptations to diving in fin whales are less effective in "insulating" the muscles of locomotion from hypoxic conditions, the anaerobic type of metabolism they possess would be expected. However, the more aerobic nature of the non-swimming muscles is somewhat contradictory to this idea. A more favorable adaptation should involve

reduced circulation to these "non-working" muscles, preferentially supplying the swimming muscles with oxygen (see Chapter 1) as seems to be indicated by the harbor seal metabolic profiles (Chapters 2 and 3). The result of this would be an even more anaerobically poised metabolism in the non-swimming muscles than is found in the swimming muscles. Of course several other factors complicate this idea, such as, any degree of metabolic depression encountered by the individual muscles, functional demands placed on individual muscles, and the impact of the size of the animal on the metabolic machinery necessary in the locomotory muscles (below).

The second possibility involves the behavior of the fin whale. Perhaps its on-board oxygen supplies are always sufficient to supply the metabolic needs of the muscle tissue due to the fact that it severely restricts the length of its dives to well within the aerobic limit. Therefore, the more aerobic metabolism indicated for its non-swimming musculature would be appropriate. Occasional power needs, or the potentially rare hypoxia experienced by the whale would then be dealt with by the anaerobic pathways evident in locomotory muscles.

The third option is simply an extension of the second. It assumes that whale muscle is metabolically no different from typical vertebrate skeletal muscle with regard to hypoxic adaptations. The similarity of the large swimming muscles to white muscle results strictly from the need of these muscles to overcome the large drag on an animal this size accelerating in a liquid medium (see Chapter 4).

The situation in fin whale is likely a combination of all the above. They appear much less likely to dive for extended periods, compared with phocid seals (Harrison and Kooyman 1981). This lack of extended diving times would result in less need for efficient physiological and/or biochemical adaptations to diving in the whale, as evidenced by the enzyme ratio patterns. The unusual anaerobic power available in the large locomotory muscles is clearly due, to a large extent, to the need for extremely high propulsive forces necessary, at times, in an animal of such large mass.

Enzyme activity ratios. The comparison of aerobic to anaerobic glycolysis via the PK/LDH activity ratio is very useful to the understanding of metabolism in fin whale muscle. The PK/LDH ratio, although relatively constant between muscles, clearly exhibits a crucial difference between locomotory and non-swimming whale muscles. High values of this ratio are regularly observed in the muscle of species that must utilize available oxygen supplies with maximum efficiency. High altitude adapted animals (Hochachka et al. 1982; Hochachka 1985), species with extremely high metabolic rates (Emmett and Hochachka 1981; Suarez 1986), and harbor seals (Chapter 3) all have very high PK/LDH ratios. The low values of this ratio in whales, particularly in the swimming muscles, seem to indicate that they are not required to maximize their efficiency of oxygen use to any unusual extent. This suggests that fin whale dives are very aerobic, at least as far as muscle tissue conditions are concerned. The lower PK/LDH ratios in swimming muscles of the whale, compared to the more "unusual" muscles, are assumed to be due to higher power requirements rather than

preferential circulatory redistribution to these muscles. This conclusion is reached because the pattern of glycolytic/aerobic enzymes (below) is also taken into consideration. A preferential circulatory redistribution to these muscles should also result in more aerobic values in the glycolytic/aerobic enzyme ratios, as is observed in harbor seal (Chapter 3). The opposite is true in the whale.

The LDH/CS activity ratio, a measure of aerobic vs anaerobic metabolic capacity (Hochachka et al. 1982), exhibits a pattern of values in whale muscle that is quite unlike that found in harbor seal (Chapter 3). It is generally similar to white rather that red muscle values (Bass et al. 1969), with the swimming muscles having the most anaerobic metabolism of any of the whale muscles examined. The DIA, as would be expected of a respiratory muscle, has a much more aerobic nature, along with the rest of the non-swimming muscles. This pattern of enzyme ratios is also observed when comparing aerobic glycolysis to CS (PK/CS and α-GPDH/CS). The relative importance of fat metabolism to glycolysis is viewed via the LDH/HOAD, PK/HOAD, and α-GPDH/HOAD activity ratios. The patterns of all 3 of these ratios are very similar to their counterpart ratios involving CS rather than HOAD. This consistency indicates that total aerobic capacity is closely related to the ability to utilize fat as a substrate in whale muscle, as is the case in harbor seal. Examination of most of these ratios results in the conclusion that whale muscle metabolism is similar to that of white vertebrate muscle (see results). All of the ratios clearly show the locomotory muscles to be the least aerobic muscles examined.

The coadaptation of B-oxidation and the Krebs cycle, as measured by HOAD/CS, is strikingly constant in all 22 whale muscles. This lack of variability is similar to what is observed in the seal muscles (Chapter 3), and a wide range of vertebrate skeletal muscles (Bass et al. 1969; Emmett and Hochachka 1981). Although relatively constant, this ratio does indicate that the swimming muscles are relatively more dependent on carbohydrate for fuel, while the respiratory and postural muscles are more likely to utilize fat. The swimming muscles' higher requirement for carbohydrate utilization stems from the need for occasional high power output from these muscles due to the whales' size (as discussed in Chapter 4). The greater reliance of the "non-swimming" muscles on fat is appropriate for their more "low level" activities.

The CPK/LDH ratios in whale muscle are, on the whole, suggestive of white muscle. The fact that the more anaerobic swimming muscles have lower values of this ratio than the non-swimming muscles indicates that any rapid contractions in these non-locomotory muscles would be due to creatine phosphate hydrolysis to a much larger extent. The swimming muscles appear to be capable of sustaining such rapid/powerful contractions for a longer time period because of their greater reliance on rapid glycolytic flux for the generation of the ATP. The CPK/PK ratio mirrors this trend between muscles, with very high values of this ratio present in the non-swimming muscles. The much lower values, characteristic of white muscle, are found in the swimming muscles. These ratios (CPK/LDH and CPK/PK) exhibit similar trends in seal muscle (Chapter 3). This similarity is believed to be due to the need for high power output

in the locomotory muscles of both whales and seals, rather than a similar relationship to diving hypoxic stress, if any.

Muscle relationships. The relationships between muscle function and metabolic machinery determined on the basis of maximum enzyme activities (Chapter 4) are generally confirmed and clarified by the enzyme ratios. However it is still impossible to clearly differentiate between the whale muscles to any greater extent than swimming or non-swimming muscles. The dorso-ventral relationship if ICD and ICV, and the anterior/posterior differences of RAM with RAP, and SPD with ECM observed in Chapter 4 are slightly less apparent using the ratios. However, all nine swimming muscle samples (RAM, RAP, LD, ECM, SPD, IL, HYP, ICD, and ICV) are clearly differentiated from the other muscles by their enzyme ratio patterns. The nature of these differences is detailed throughout the preceding sections and will, therefore, not be repeated here.

There is some indication of a separation within the non-swimming muscles when comparing ratios. One group of these muscles (PNC, BH, EDC, MYL, MAS, and DIA) seems to be particularly similar to one another in certain ratio patterns. All glycolytic/mitochondrial enzyme ratios are consistently low. While ratios of GOT/glycolytic enzymes, and aerobic/anaerobic glycolytic enzyme ratios are consistently high. This type of ratio pattern (Chapter 3) is expected in postural, and similar low activity muscles (ie. MAS and DIA). However, the other ratios have quite different

relationships in these muscles, making it difficult to conclude with confidence that these muscles belong together metabolically.

The remainder of the muscles have quite variable ratio patterns, but they generally seem to fall somewhere between the swimming muscles and the "postural group" in the magnitude of the ratios. These muscles (EXT, INT, LAT, OBL, DLT, PM, IS and TRI) are typically more active than the muscles of the "postural group", but less active than the swimming muscles. So these "average" values of the ratios are appropriate to their general level of activity. However, the specific ratio pattern of these muscles is extremely variable.

This complex nature of enzyme ratio patterns is also observed in seal muscle (Chapter 3). The ratios are generally consistent with the whale muscle functions as described in Chapter 4. Some differences between muscles are highlighted when viewing the enzyme ratios. For example, TRI appears to be a more active muscle than EDC, which is likely due to the highly active flipper use (which may involve TRI), but complete lack of a hand and, therefore, EDC use. Other muscles seem about the same (ie. BH, PNC, MYL) in relation to the other muscles examined as when maximum activities are utilized. However, as is the case with seal muscle, a third group of muscles exhibit widely varying and complex patterns of enzyme ratios, such as INT, EXT, and PM. These differences are due to specific muscle usage and circulatory redistribution patterns that are not well understood. As a result, no attempt will be made to differentiate ratio patterns on a muscle by muscle basis.

Cluster analysis. Cluster analysis of the whale muscles, based on the 15 enzyme activity ratios, results in only 2 clusters. The clustering pattern is slightly different from that observed in Chapter 4. The differences involve 2 muscles (EXT and LAT) whose specific usage patterns are difficult to assess (above).

The first cluster is made up entirely of non-swimming muscles (BH, MAS, DIA, TRI, INT, EDC, MYL, PNC, DLT, OBL, IS, and PM). The ratio pattern resulting in this cluster has been discussed in detail above. The second cluster is made up of the 9 samples from swimming muscles (SPD, ECM, LD, RAP, RAM, IL, ICD, ICV, and ICD) and 2 non-swimming muscles (LAT and EXT). The inclusion of the LAT with the swimming muscles is not that surprising, since LAT is likely a very important maneuvering muscle (Chapter 4). As a result, it would have a usage pattern fairly similar to the swimming muscles -- and, therefore, a similar pattern of enzyme ratios. The presence of EXT in this cluster is more difficult to explain. It appears to be primarily due to low ratios involving GOT, or CPK. The remainder of the ratios are similar to the non-swimming muscle pattern. This mixed pattern in EXT is curious. It seems to suggest that EXT is less dependent on CPK for rapid contraction than other non-swimming muscles and has a lower relative capacity for the maintenance of redox balance via the malate-aspartate shuttle as opposed to lactate formation. This seemingly high use of the glycolytic pathway in EXT may result from its use in forced inspiration (Chapter 4). This extra function for occasional rapid contractions is best fueled by the increased flux of carbohydrate through

the glycolytic pathway (Gollnick and Hermansen 1973) as indicated by the unusual ratios involving CPK and GOT.

Summary. The intra-specific discriminative or non-discriminative nature of the 15 ratios examined is discussed in comparison to the harbor seal (Chapter 3), and other studies of enzyme ratios. The generally anaerobic metabolism of whale skeletal muscle (particularly swimming muscles) observed in Chapter 4 is confirmed by the enzyme activity ratios. The specific functional relationships between muscles indicated by maximum enzyme activities (Chapter 4) are also confirmed, in general, by the ratio patterns. A lack of lengthy diving activity, and a great need for high power output in locomotory muscles is suggested as the reason for the differences in ratio patterns between the fin whales and harbor seals (Chapters 3 and 5).

CHAPTER 6:

ANTARCTIC SEAL MUSCLE METABOLISM

Introduction. The apparent differences in metabolic organization between fin whales and harbor seals (Chapter 2-5) seem to suggest that harbor seals are possibly the more capable diver of the two species (in terms of diving time, not depth). Whether or not this "superior" metabolic organization is characteristic of phocid seals led to the examination of muscles of 3 additional species of phocid from Antarctica: leopard seals (active predators), crab-eater seals (krill feeders), and Weddell seals (benthic feeding, champion divers)(Martin 1977).

Samples of 3 or 4 skeletal muscles were obtained from each seal species. The same 7 enzymes and 15 ratios as were examined in fin whale muscle are analyzed in these antarctic seals. A superficial examination of differences in absolute enzyme activities among muscles of the same species, and among the 3 antarctic species is conducted in terms of lifestyle differences. However, the principal focus of this Chapter is on interspecies comparisons of the activity ratios. The surprising similarities in metabolic organization among all the phocids (including harbor seal, Chapters 2 and 3), are contrasted with the distinctive fin whale pattern. Similar data should be obtained from a related, but smaller, cetacean (ie. minke whales) before the apparent differences in metabolism can be confirmed.

Materials and Methods

Experimental animals. Weddell seals (Leptonychotes weddelli), leopard seals (Hydrurga leptonyx), and crab-eater seals (Lobodon carcinophagus) were collected in the Antarctic Peninsula region of Antarctica during February and March of 1986. Seals used in this study were captured either by bagging or by darting with intramuscular Ketamine or Sufentanil, and sacrificed by anesthetic overdose and intracardiac KCl. Some of them (1 Weddell, 3 crab-eater, 3 leopard) were used for cortisol turnover experiments and maintained under intravenous Ketamine anesthesia for up to 3 hours before being sacrificed. Muscle samples were obtained within 5-10 minutes post-mortem.

Muscles sampled. Portions of m. longissimus dorsi (LD), m. masseter (MAS), m. diaphragm (DIA), and m. psoas (PSO) were collected from 5 Weddell seals, 4 leopard seals and 5 crab-eater seals (all muscles were not collected from each seal). Samples were taken from the center of the muscle, midway along its length.

Tissue manipulations. Immediately following dissection the muscle samples were placed in a -80°C freezer. These samples were kept stored at -80°C until assayed.

Homogenization for enzyme assays. As described in harbor seals materials and methods except that the homogenization was conducted with an Ultra-Turrax homogenizer rather than a Polytron.

Enzyme assays. As described in harbor seal materials and methods, however only the following enzymes were assayed: lactate dehydrogenase (LDH), pyruvate kinase (PK), α-glycerophosphate dehydrogenase (α-GPDH), creatine kinase (CPK), phosphofructokinase (PFK), 3-hydroxyacyl CoA dehydrogenase (HOAD), citrate synthase (CS), and glutamate-oxaloacetate transaminase (GOT).

In addition, assay conditions of PFK varied slightly; twice the substrate concentration was required (10 mM fructose-6-phosphate, and 2 mM ATP). All other conditions were the same.

Chemicals. As described in harbor seal materials and methods.

Results.

Maximum enzyme activities. The maximum enzyme activities of 10 muscles from the 3 antarctic phocid seal species, along with the mean values for these enzymes in skeletal muscle of harbor seals (Chapter 2) and fin whales (Chapter 4) are listed in Table 12. The correlations between enzymes are illustrated in Table 13. Two-way analysis of variance (not shown) exhibits significant muscle differences with all

TABLE 12. MAXIMUM ENZYME ACTIVITIES IN SKELETAL MUSCLES FROM 3 SPECIES OF PHOCID SEAL FROM ANTARCTICA.

ANIMAL	MUSCLE	LDH		PK		CPK		CS		HOAD		a-GPDH		GOT	
		MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LEOPARD	LD	466.52	57.86	300.73	36.04	1369.91	99.41	5.99	0.28	12.39	0.78	6.59	1.03	32.15	2.84
SEAL	MAS	387.57	14.35	308.31	6.85	1297.92	31.71	5.77	0.32	3.75	0.69	8.53	1.21	31.92	1.13
	DIA	308.35	45.83	205.27	30.69	1308.65	122.75	9.21	1.22	19.71	2.65	3.36	0.41	42.27	5.35
CRAB-EATER	LD	812.47	144.38	536.84	55.61	1295.61	87.51	9.24	1.09	11.15	1.25	9.69	1.68	35.04	4.05
SEAL	MAS	442.12	23.21	372.99	35.95	1378.02	60.91	7.87	0.44	5.81	1.15	23.15	2.11	54.26	6.02
	DIA	564.24	55.21	379.32	31.87	1028.25	112.56	14.24	2.42	19.81	2.77	13.01	1.46	55.89	6.87
WEDDELL	LD	351.69	29.01	280.63	33.98	1210.82	37.04	4.64	0.87	8.17	2.38	7.41	1.66	16.18	3.17
SEAL	MAS	371.13	37.24	250.56	21.81	1448.61	127.81	6.46	0.79	4.11	0.81	18.84	2.57	21.05	1.06
	PSO	334.94	33.49	247.86	29.21	1269.61	38.81	4.98	0.32	12.06	0.01	4.77	0.42	17.15	1.07
	DIA	281.35	40.19	221.06	20.11	1284.75	21.55	12.87	9.19	16.08	4.02	3.11	1.91	18.63	5.48
ALL		442.81	30.41	316.87	18.31	1289.98	31.02	7.95	0.69	11.03	1.08	10.35	1.14	33.62	2.61
LEOPARD SEAL		387.48	29.89	271.43	20.19	1325.49	49.51	6.98	0.61	11.95	2.14	6.16	0.81	35.45	2.36
CRAB-EATER SEAL		606.28	66.18	429.72	31.83	1233.96	64.91	10.45	1.16	12.26	2.01	15.28	1.96	48.41	4.15
WEDDELL SEAL		342.99	17.53	254.65	14.02	1308.92	47.01	6.55	1.36	9.04	1.46	9.66	2.02	18.28	1.27
HARBOR SEAL		831.06	19.67	744.27	20.81	2609.92	49.83	17.57	0.46	21.82	1.14	25.57	0.71	57.84	1.17
FIN WHALE		797.22	70.28	206.47	14.11	1912.64	54.05	3.34	0.18	4.54	0.23	5.31	0.43	13.41	0.52

Assay temperature = 25°C.
Activities are expressed as units/gm wet wt.
S.E. = 1 standard error of the mean.
n = 4, except for Weddell seal DIA (n=2) and Weddell seal PSO (n=3).
See Materials and Methods for assay conditions and abbreviations.
In addition to individual muscle values, mean enzyme activities for all muscles are included for each species.

TABLE 13. CORRELATION MATRIX BETWEEN ENZYME ACTIVITES OF ANTARCTIC SEAL MUSCLE.

	LDH	PK	CPK	CS	HOAD	a-GPDH	GOT
LDH	* 1	*0.87	-0.29	*0.37	0.03	*0.46	*0.44
PK	*0.87	* 1	-0.18	0.27	-0.15	*0.51	*0.37
CPK	-0.31	-0.18	* 1	-0.06	-0.31	0.05	-0.03
CS	*0.36	0.27	-0.06	* 1	*0.54	0.28	*0.66
HOAD	0.03	-0.15	-0.31	*0.54	* 1	*-0.43	*0.34
a-GPDH	*0.46	*0.51	0.05	0.28	*-0.43	* 1	*0.34
GOT	*0.43	*0.37	-0.03	*0.67	*0.34	*0.34	* 1

* = statistically significant correlation at the 95% confidence level.
All correlations are between data ranks (Spearman correlations).
See Materials and Methods for abbreviations.
All muscles from all 3 Antarctic phocid seal species were included in the calculations.

enzymes but CPK. Significant animal effects are also apparent, except with CPK and HOAD. These animal differences refer to between species comparisons and are due to real differences in enzyme activities between the leopard, Weddell, and crab-eater seals (see Appendix 5).

Crabeater seals have significantly higher glycolytic capacity than either of the 2 other species, as demonstrated by their high LDH, PK and α-GPDH activities. They also exhibit higher activities of aerobic enzymes CS, HOAD, and GOT). CPK activities are roughly equal in all 3 species. The leopard seals tend to have marginally higher activities of all enzymes (except α-GPDH) than the Weddell, which has the lowest metabolic capacity of all 3 species.

There is some question as to the validity of the enzyme activities reported here. Although they still are, generally, within the normal range of values found in other marine mammals (Ponganis and Pierce 1978; Castellini and Somero 1981; Chapter 2; Chapter 4) they appear unusually low compared to the other phocid seal examined (Phoca vitulina, Chapter 2). If the means from all 3 antarctic species are averaged together (see Table 12), the resulting activity values are approximately one half the mean harbor seal muscle activities. This indicates the strong possibility of a systematic (x2) error. Due to the number of outside personnel involved in the collection, transport and assay of the antarctic seal samples, and the fact that all the assays of these samples were conducted together in a single day, the error most likely involves the antarctic seal muscle. Because of this possibility, detailed comparisons with other animal species will not be conducted. But the between muscle differences, and differences between

these 3 phocid seal species, remain valid and will be analyzed. Detailed interspecies comparisons will, however, be done on the enzyme ratios (below) -- which are unaffected by any such systematic errors.

Enzyme ratios. Table 14 lists the means $\pm$ 1 S.E. of 15 enzyme activity ratios for each of the 10 skeletal muscles from leopard, crab-eater and Weddell seals, along with the mean muscle values from harbor seals (Chapter 3) and fin whales (Chapter 5). The correlations between the ratios are listed in Table 15; correlations between the ratios and maximum enzyme activities are shown in Appendix 6. Two-way analysis of variance (not shown) indicates significant muscle differences in all ratios except PK/LDH. Significant animal effects (between species) are evident with all ratios except PK/LDH, PK/CS, PK/HOAD, LDH/CS, and LDH/HOAD.

The most important observation in the enzyme activity ratios is the striking similarity between the 3 antarctic seal species and the harbor seal (Table 14). The pattern of ratios is virtually identical to harbor seal, and quite dissimilar to the fin whale in crucial ratios. All 4 species of seals exhibit much higher PK/LDH, and very much lower LDH/CS, LDH/HOAD and CPK/PK ratios than the fin whale. The similarity to harbor seal metabolism is even evident in more subtly different ratio patterns, such as with PK/CS, PK/HOAD, and CPK/LDH. So, even though all 4 phocids exhibit minor variations in their enzyme ratios, they clearly demonstrate very similar metabolic pathway relationships. This ratio pattern is

TABLE 14. ENZYME ACTIVITY RATIOS OF MUSCLES FROM THE 3 ANTARCTIC PHOCID SEAL SPECIES (CONT'D ON THE NEXT 2 PAGES).

ANIMAL	MUSCLE	PK/LDH		a-GPDH/LDH		a-GPDH/PK		HOAD/CS		a-GPDH/CS	
		MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LEOPARD	LD	0.66	0.07	0.014	0.002	0.022	0.002	2.08	0.14	1.09	0.14
SEAL	MAS	0.81	0.03	0.022	0.003	0.028	0.004	0.64	0.09	1.48	0.19
	DIA	0.67	0.07	0.012	0.003	0.019	0.005	2.15	0.15	0.37	0.03
CRAB-EATER	LD	0.69	0.07	0.013	0.003	0.019	0.004	1.22	0.12	1.12	0.25
SEAL	MAS	0.84	0.08	0.053	0.007	0.065	0.013	0.73	0.13	2.96	0.29
	DIA	0.68	0.03	0.023	0.001	0.034	0.002	1.46	0.17	0.96	0.11
WEDDELL	LD	0.79	0.05	0.021	0.004	0.026	0.005	1.72	0.32	1.95	0.75
SEAL	MAS	0.68	0.04	0.052	0.008	0.078	0.016	0.62	0.05	2.91	0.18
	PSO	0.74	0.04	0.015	0.002	0.021	0.004	2.44	0.16	0.96	0.09
	DIA	0.79	0.04	0.012	0.009	0.015	0.011	2.09	1.18	0.28	0.05
ALL		0.73	0.02	0.025	0.003	0.034	0.004	1.46	0.13	1.48	0.17
LEOPARD SEAL		0.71	0.04	0.016	0.002	0.023	0.002	1.62	0.22	0.98	0.16
CRAB-EATER SEAL		0.74	0.04	0.031	0.006	0.041	0.007	1.14	0.12	1.68	0.30
WEDDELL SEAL		0.75	0.03	0.028	0.005	0.039	0.009	1.61	0.26	1.76	0.35
HARBOR SEAL		0.90	0.02	0.031	0.001	0.036	0.001	1.31	0.07	1.57	0.07
FIN WHALE		0.34	0.02	0.011	0.001	0.029	0.002	1.49	0.07	1.77	0.14

TABLE 14. (CONT'D).

ANIMAL	MUSCLE	LDH/CS		PK/CS		LDH/HOAD		PK/HOAD		a-GPDH/HOAD	
		MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LEOPARD	LD	78.76	11.39	50.03	4.63	37.93	4.49	24.48	3.11	0.53	0.08
SEAL	MAS	68.08	5.68	54.11	4.18	115.54	22.51	90.86	16.1	2.45	0.39
	DIA	36.69	8.45	25.01	6.73	17.08	4.22	11.74	3.48	0.18	0.02
CRAB-EATER	LD	86.94	8.05	58.47	1.33	72.66	8.93	48.94	4.11	0.89	0.15
SEAL	MAS	56.45	3.09	47.74	5.14	86.24	17.23	75.74	19.5	4.38	0.81
	DIA	41.93	4.61	28.84	4.44	29.34	3.11	20.07	2.66	0.68	0.09
WEDDELL	LD	85.72	20.81	67.62	15.49	55.77	16.51	44.66	12.6	1.32	0.53
SEAL	MAS	59.08	6.23	40.75	6.31	97.75	14.72	68.14	14.1	4.81	0.52
	PSO	68.67	11.34	50.99	9.31	27.77	2.78	20.55	2.42	0.41	0.04
	DIA	49.15	38.22	37.32	28.21	19.33	7.33	14.99	5.01	0.17	0.07
ALL		63.75	4.24	46.43	3.14	58.68	6.61	44.06	5.42	1.69	0.31
LEOPARD SEAL		61.18	7.09	43.05	4.77	56.85	14.58	42.36	11.6	1.05	0.32
CRAB-EATER SEAL		61.77	6.38	45.01	4.24	62.75	9.42	48.25	9.16	1.98	0.57
WEDDELL SEAL		67.96	8.64	50.85	6.87	52.62	10.76	41.76	7.99	2.01	0.59
HARBOR SEAL		50.35	1.89	45.19	1.77	49.64	2.81	44.11	2.51	1.51	0.09
FIN WHALE		241.52	20.26	64.97	3.53	229.14	35.41	56.01	5.46	1.45	0.17

TABLE 14. (CONT'D).

ANIMAL	MUSCLE	CPK/LDH		CPK/PK		GOT/CS		GOT/PK		GOT/LDH	
		MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
LEOPARD	LD	3.16	0.61	4.78	0.73	5.35	0.33	0.11	0.01	0.072	0.011
SEAL	MAS	3.36	0.16	4.21	0.11	5.56	0.19	0.10	0.01	0.083	0.005
	DIA	4.83	1.34	7.24	2.05	4.79	0.70	0.22	0.03	0.145	0.023
CRAB-EATER	LD	1.74	0.33	2.51	0.35	4.11	0.90	0.07	0.01	0.049	0.012
SEAL	MAS	3.13	0.13	3.77	0.27	6.98	0.91	0.16	0.04	0.125	0.019
	DIA	1.84	0.17	2.75	0.34	4.08	0.32	0.15	0.01	0.098	0.003
WEDDELL	LD	3.53	0.37	4.53	0.61	3.52	0.34	0.06	0.02	0.048	0.012
SEAL	MAS	4.09	0.63	6.01	0.92	3.40	0.41	0.09	0.01	0.059	0.007
	PSO	3.86	0.38	5.29	0.73	3.45	0.13	0.07	0.01	0.052	0.007
	DIA	4.67	0.74	5.87	0.63	2.33	1.24	0.09	0.03	0.071	0.031
ALL		3.34	0.23	4.62	0.34	4.49	0.26	0.11	0.01	0.081	0.007
LEOPARD SEAL		3.78	0.51	5.41	0.77	5.24	0.26	0.14	0.02	0.101	0.012
CRAB-EATER SEAL		2.24	0.22	3.01	0.23	5.06	0.57	0.12	0.02	0.091	0.012
WEDDELL SEAL		3.96	0.26	5.37	0.39	3.29	0.24	0.08	0.01	0.056	0.006
HARBOR SEAL		3.27	0.09	3.72	0.11	3.42	0.08	0.08	0.00	0.073	0.002
FIN WHALE		4.72	0.44	12.87	0.81	4.50	0.18	0.09	0.01	0.034	0.003

See Materials and Methods for abbreviations.
S.E. = 1 standard error of the mean.
n = 4, except for Weddell seal DIA (n=2) and Weddell seal PSO (n=3).
In addition to individual muscle values, mean enzyme activity ratios for all muscles are included for each species.

TABLE 15. CORRELATION MATRIX BETWEEN ENZYME ACTIVITY RATIOS (CONT'D ON NEXT PAGE).

	PK/LDH	PK/CS	PK/HOAD	LDH/CS	LDH/HOAD	HOAD/CS	a-GPDH/LDH	a-GPDH/CS
PK/LDH	* 1	*0.46	*0.43	0.08	0.28	-0.31	0.21	0.22
PK/CS	*0.46	* 1	*0.62	*0.89	*0.58	-0.06	-0.16	*0.38
PK/HOAD	*0.43	*0.62	* 1	*0.51	*0.98	*-0.78	*0.43	*0.74
LDH/CS	0.08	*0.89	*0.51	* 1	*0.52	0.06	-0.31	0.29
LDH/HOAD	0.28	*0.58	*0.98	*0.52	* 1	*-0.77	*0.42	*0.75
HOAD/CS	-0.31	-0.06	*-0.78	0.06	*-0.77	* 1	*-0.69	*-0.61
a-GPDH/LDH	0.21	-0.16	*0.43	-0.31	*0.42	*-0.69	* 1	*0.75
a-GPDH/CS	0.22	*0.38	*0.74	0.29	*0.75	*-0.61	*0.75	* 1
a-GPDH/HOAD	0.28	0.27	*0.84	0.16	*0.85	*-0.84	*0.81	*0.92
a-GPDH/PK	0.01	-0.22	*0.36	-0.29	*0.38	*-0.63	*0.97	*0.74
CPK/PK	-0.18	*-0.35	*-0.36	-0.31	*-0.35	0.21	-0.02	-0.21
CPK/LDH	0.22	-0.19	-0.17	-0.31	-0.22	0.01	0.13	-0.09
GOT/PK	-0.29	*-0.77	*-0.51	*-0.81	*-0.48	0.07	0.15	-0.28
GOT/LDH	-0.01	*-0.64	*-0.33	*-0.81	*-0.36	-0.08	0.26	*-0.17
GOT/CS	0.17	0.21	0.25	0.07	0.25	-0.11	0.05	0.29

TABLE 15. (CONT'D).

	a-GPDH/HOAD	a-GPDH/PK	CPK/PK	CPK/LDH	GOT/PK	GOT/LDH	GOT/CS
PK/LDH	0.28	0.01	-0.18	0.22	-0.29	-0.01	0.17
PK/CS	0.27	-0.22	*-0.35	-0.19	*-0.77	*-0.64	0.21
PK/HOAD	*0.84	*0.36	*-0.36	-0.17	*-0.51	*-0.33	0.26
LDH/CS	0.16	-0.29	-0.31	-0.31	*-0.81	*-0.81	0.07
LDH/HOAD	*0.85	*0.38	*-0.35	-0.22	*-0.48	*-0.36	0.25
HOAD/CS	*-0.84	*-0.63	0.21	0.01	0.07	-0.08	-0.11
a-GPDH/LDH	*0.81	*0.97	-0.02	0.13	0.15	0.26	0.05
a-GPDH/CS	*0.92	*0.74	-0.21	-0.09	-0.28	-0.17	0.29
a-GPDH/HOAD	* 1	*0.76	-0.26	-0.11	-0.21	-0.09	0.23
a-GPDH/PK	*0.76	* 1	0.01	0.08	0.21	0.27	0.08
CPK/PK	-0.26	0.01	* 1	*0.88	0.25	0.19	-0.11
CPK/LDH	-0.11	0.08	*0.88	* 1	0.15	0.21	-0.07
GOT/PK	-0.21	0.21	0.25	0.15	* 1	*0.93	*0.36
GOT/LDH	-0.09	0.27	0.19	0.21	*0.93	* 1	*0.46
GOT/CS	0.23	0.08	-0.11	-0.07	*0.36	*0.46	* 1

* = statistically significant correlation at the 95% confidence level.
All correlations are between data ranks (Spearman correlations).
See Materials and Methods for abbreviations.
All muscles from all 3 Antarctic phocid seals species were included in the calculation.

indicative of generally aerobic muscle (Chapter 3). The fin whale muscle, on the other hand, exhibits a very different pattern of ratios, much more characteristic of white muscle (Chapter 5).

Discussion.

Seal muscle metabolism. The enzyme activities of muscle from all 3 antarctic seal species fall within the normal vertebrate range (see Chapters 2 and 4 for details). Previous studies on marine mammals give results similar to what is observed in these seals (George et al. 1971; Kerem et al. 1973; Simon et al. 1974; Ponganis and Pierce 1978; Austin and Geraci 1981; Castellini and Somero 1981), although these values appear a bit low in comparison with harbor seals (Chapter 2). The reason for this "low" activity is probably a systematic error at some stage of the analysis (see results). However, the basic pattern of the enzyme activities, between species and between muscles, mirrors the harbor seal pattern (Table 12). A fairly high capacity for glycolysis exists in the muscle of these seal species, particularly the locomotory muscles. Weddell and leopard seal muscles are about equal, while glycolytic enzyme activities in crab-eater seals are significantly higher.

In addition to having higher activities of glycolytic enzymes than leopard or Weddell seals, crab-eaters also have higher levels of aerobic enzymes (CS, GOT and HOAD). This high metabolic potential in the crab-eater seal is quite surprising in view of its "grazing" type of feeding activity. The collection of krill, although time-consuming, should require little in the

way of "high-demand" muscular work. Consequently, the fact that this species has consistently higher levels of catabolic enzymes in its skeletal muscles than an expert diver (the Weddell) or an active predator (the leopard seal) is completely unexpected. One explanation of this need for high enzyme activities in crab-eaters is to allow escape from predation. The continuous type of feeding required by the crab-eater seal leaves it continually subjected to potential predation by killer whales and leopard seals. This highly-geared metabolism would allow these seals to escape at "the last-minute", thereby maximizing feeding time. These high enzyme levels are perhaps most evident during terrestrial locomotion in these seals. This species is unusually fast over the Antarctic snow, reputedly reaching speeds of 15 mph (Martin 1977). This ability to escape predation, coupled with the unusual feeding style of the crab-eater, (and therefore no competition for food with other seals), probably accounts for its abundance.

Of the 3 seal species examined, the Weddell has the lowest enzyme activities. As a marathon diver feeding on benthic fish, crustaceans and cephalopods, the Weddell seal appears to have little need for high flux rates in its catabolic pathways; and predation is avoided in this seal more by its isolation (deep diving or location at far off breathing holes in the ice, than by active escape on a regular basis. The lower enzyme activities in muscle may even aid the remarkable diving capabilities of the Weddell by helping to keep its metabolic rate low, thereby conserving oxygen for the vital organs (ie. brain and heart) (see Chapter 1).

The low enzyme activities in the leopard seal (relative to the crab-eater and harbor seals) is more difficult to understand. As an active predator of fish, cephalopods, and larger animals (penguins and even other seals) the leopard seal might be expected to have higher metabolic potential than is indicated by these enzyme activities. Although, apparently, able to "run down" swimming penguins, cephalopods, and fish -- these seals are just as likely to catch their prey by stealth, while entering or leaving the water (Ridgway 1972; Martin 1977). This type of feeding would not require a particularly highly-geared metabolism. Visible evidence of this low metabolic potential in their skeletal muscle may be seen in the general helplessness of these seals out of the water. Although this is probably due, in part, to inefficient biomechanical aspects of their mode of terrestrial locomotion, since they are agile swimmers (Martin 1977).

The 3 antarctic seals exhibit a normal relative capacity for utilizing fats as fuel. The HOAD activity is slightly higher than the CS activity in all 3 species to about the same degree as observed in both harbor seals (Chapter 2) and fin whales (Chapter 4). Redox balance appears to be maintained by the malate-aspartate shuttle (high GOT activity) moreso than the α-glycerophosphate cycle (as represented by the α-GPDH activities). This is also the case in both fin whale and harbor seal (Chapters 2 and 4).

Muscle relationships. The relation of the enzymatic profile to the specific function of each muscle in the 3 antarctic seal species is undertaken below. In general, only relative (intraspecific), rather than absolute (interspecific/comparative) enzyme activities are referred to. That is, a

"high" or "low" activity is only "high" or "low" in relation to the other muscles of that seal species examined -- not necessarily with regard to the other seal species unless specifically stated.

The m. masseter (MAS) of the crab-eater seal has the lowest activities of LDH, PK, CS, and HOAD of any of the 3 muscles examined. This generally low catabolic potential in the MAS of crab-eater seals is due to its unusual (for a seal) feeding habits (Martin 1977). Since it feeds like a "gulping" baleen whale, siphoning krill through its specially adapted teeth, rather than biting and chewing larger prey, it has little need for high catabolic enzyme activities in this muscle. Leopard seals, Weddell seals (Table 12), and harbor seals (Chapter 2), on the other hand, all bite and chew their food. As a result, each of these seals has a MAS that has consistently higher LDH and PK activities than their respective DIA muscle (Table 12; Chapter 2), and sometimes higher than m. psoas (PSO) or m. longissimus (LD). Fin whale, another "krill" feeder, has LDH and PK relationships between its MAS and the other muscles (LDH and DIA) identical to the crab-eater.

The DIA's of all 3 antarctic phocids appear to be very aerobic muscles. They all have relatively low activities of glycolytic enzymes (LDH, PK, and α-GPDH) and the highest activities of aerobic pathway enzymes (HOAD, CS, and GOT). They each seem to be particularly prone to fat utilization (high HOAD activity), as well. This same pattern is very evident in harbor seal (Chapter 2), but less so in fin whale (Chapter 4). This especially aerobic nature of DIA in all these species is, obviously, due to this muscles contraction only during respiration.

The relationship of the LD to the other muscles examined is a bit more difficult to assess in these 3 seal species. But it appears that the LD, in all 3 cases, tends to have relatively high glycolytic potential, coupled with average levels of aerobic enzymes in comparison to the other muscles. Although "average" has little meaning when so few muscles are involved in the analysis. This mixed aerobic/anaerobic nature of swimming muscles is very evident in harbor seal (Chapter 2).

The m. psoas (PSO) of Weddell seal is consistently lower in glycolytic enzymes (LDH, PK, and α-GPDH), and higher in the levels of aerobic enzymes (HOAD, CS, and GOT) than the LD. This more aerobic nature of PSO indicates 2 things: 1) it is useful, by its action as a hip flexor (Travill 1962; Kendall et al. 1971; Gray 1989; MacConaill and Basmajian 1977; Basmajian 1978; McMahon 1984), during the swimming stroke on a regular basis, but is not as great a force generating muscle as LD, and 2) it confirms that the m. psoas magnus (from which these PSO samples were taken) is more regularly involved in the swimming stroke than the "PSO" from harbor seals, which is a sample of the m. psoas minor (Chapter 2).

Enzyme ratios. The advantage of utilizing enzyme ratios for interspecies comparisons is very apparent with the antarctic seal muscles. The potential problem with a systematic error (see results), and the majority of the differences between the phocid seals disappear when viewing the enzyme activity ratios.

The LDH/PK activity ratios in all 3 species are virtually identical (Table 14). The 0.7+ values are much closer to the harbor seal value for these 3 muscles (DIA, MAS, LDD) of 0.80 (Chapter 3) than the fin whales' 0.27 (Chapter 5). These high PK/LDH values are also fairly high compared with other animal species (Hochachka 1985), although not unusually so (possibly due to the particular muscles sampled). Two much more effective ratios in discriminating between muscles and species are LDH/CS and LDH/HOAD. Both of these ratios (in all 3 seal species) have values very close to the mean harbor seal muscle values of about 50, but much lower than the 200+ ratios evident in fin whale. This striking dissimilarity between the phocid seal species and the fin whale may, however, be due to a scaling phenomenon involving the aerobic enzymes and LDH in the fin whale (Chapter 4). But comparison of 2 enzymes that do not appear to "scale upwards" in fin whale muscle also exhibits similarity between phocids and a dissimilarity to the fin whale. The CPK/PK ratios of all 4 seal species are clearly lower than in fin whale. This relationship even extends to the CPK/LDH ratio, although it is not as striking a difference. And the differences apparent in the LDH/aerobic enzyme ratios are also evident with PK/CS and PK/HOAD. These ratios in fin whale are clearly higher than the very nearly identical ratios found in the phocid seal species. The remainder of the ratios are quite similar in all 5 species of marine mammal (Table 14).

So, even though the 4 phocids have very different habits, they appear to have metabolic pathway relationships that are virtually identical to one another. This similarity may be due to the one common thread running through each of their lifestyles -- diving, in search of prey, escape from

predation, or exploration. The fin whale, although also a diving mammal, has much less reason to extend its dives up to or beyond the aerobic limit. Its food supply (krill) is very near the surface, and encounters with killer whales or man are rare -- so little "escape diving" is necessary in these huge cetaceans (Martin 1977). This may account for its different enzyme relationships (Chapter 5).

Perhaps more important is the similarity between the phocids. Weddell seals are clearly the marathon divers of these 4 species. Yet, the enzymatic machinery (other than absolute activities) seems to be virtually identical in all 4 species (Table 14). Even the maximum enzyme activities are about the same in Weddell and leopard seals (Table 12). This similarity between seals of such widely varying lifestyles and diving capabilities seems to confirm what is suggested by the detailed harbor seal muscle analysis (Chapter 3). The muscles of these diving seals appear to be extremely well insulated from any hypoxic stress resulting from their diving activities. If this were not the case, some adaptation in the enzymatic machinery would be expected. So the remarkable physiological adaptations to diving in marine mammals (see Chapter 1), potentially coupled to other types of metabolic adjustments (ie. metabolic depression, increased efficiency), seem to allow the muscles of these animals sufficient oxygen to meet metabolic requirements.

Summary. The aerobic nature of phocid seal skeletal muscle, as indicated by enzyme data on harbor seal muscle (Chapters 2 and 3), is confirmed by analysis of the skeletal muscle from 3 antarctic species. Weddell, crab-

eater and leopard seals all exhibit maximal enzyme activities and enzyme ratio patterns in muscle very similar to one another and to the harbor seal. Clear differences are evident with metabolic pathway relationships between these 4 phocids and fin whale skeletal muscle (Table 14). The enzyme activities in the antarctic seal species is discussed in terms of lifestyle differences. The activities in individual muscles are assessed as to their specific function. The general patterns observed are consistent with the idea that phocid seal muscle is largely insulated from hypoxic stress regardless of the diving habits of the seal.

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APPENDIX 1. STATISTICALLY SIGNIFICANT DIFFERENCES OF MAXIMUM ENZYME ACTIVITIES AND ENZYME ACTIVITY RATIOS BETWEEN MUSCLES OF THE HARBOR SEAL (18 PAGES).

Muscles are listed in descending order of the mean.
Means with the same letter are not significantly different (95% confidence level).
Differences are based on Student-Newman-Keuls (SNK) multiple range tests.
Abbreviations are listed in Materials and Methods.

LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	1081.00	4	GMS
	A			
	A	1080.80	4	PMS
	A			
	A	1062.78	4	SEM
	A			
B	A	1021.25	4	LDS
B	A			
B	A	967.88	4	ILD
B	A			
B	A	958.28	4	GMD
B	A			
B	A C	943.68	4	ILS
B	A C			
B	A C	939.51	4	PMD
B	A C			
B	A C	927.20	4	LDD
B	A C			
B	A C	917.11	4	OBL
B	A C			
B	A C	911.50	4	TLT
B	A C			
B	A C	909.72	4	TLG
B	A C			
B D	A C	886.22	4	PAL
B D	A C			
B D	A C	870.50	4	PSO
B D	C			
B D	E C	843.74	4	DLT
B D	E C			
B D	E C	826.64	4	DEP
D	E C			
D	E C	733.19	4	BFM
D	E			
D	E	709.45	4	INT
D	E			
D	E	696.65	4	EDC
	E			
	E	670.83	4	LAT
	E			
	E	659.55	4	SPT
		638.47	4	ATL
		636.79	4	EXT
	G	450.30	4	MAS
	G			
	G	433.57	4	DIA

PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	1061.34	4	GMS
	A			
B	A	1020.26	4	PMS
B	A			
B	A	946.66	4	PSO
B	A			
B	A C	917.40	4	TLT
B	A C			
B	A C	895.38	4	LDS
B	A C			
B	A C	887.86	4	OBL
B	A C			
B	A C	886.08	4	ILS
B	A C			
B D	A C	838.42	4	PMD
B D	A C			
B D	A C	828.60	4	SEM
B D	C			
B D	E C	775.62	4	BFM
B D	E C			
B D	E C	766.32	4	DLT
B D	E C			
B D	E C	765.97	4	TLG
B D	E C			
B D	E C	765.29	4	LDD
B D	E C			
B D	E C	760.18	4	GMD
B D	E C			
B D	E C	754.37	4	PAL
B D	E C			
B D	E C	752.54	4	ILD
B D	E C			
B D	E C	744.82	4	DEP
D	E C			
D	E C	655.20	4	LAT
D	E C			
D	E C	650.06	4	SPT
D	E C			
D	E C	637.65	4	EDC
D	E			
D	E	565.21	4	INT
	E			
	E	538.97	4	EXT
	E			
	E	514.62	4	ATL
	G	395.51	4	MAS
	G			
	G	282.35	4	DIA

a-GPDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	34.457	4	TLG
	A A			
	B A	32.000	4	PMD
	B A			
	B A C	31.237	4	PMS
	B A C			
	B D A C	30.075	4	LDD
	B D A C			
	B D A C	29.805	4	LDS
	B D A C			
	B D A C	29.410	4	ILS
	B D A C			
	B D A C	28.955	4	ILD
	B D A C			
	B D A C	28.932	4	SEM
	B D A C			
	B D A C	28.725	4	OBL
	B D A C			
	B D A C	27.855	4	PSO
	B D A C			
	B D A C	27.835	4	DLT
	B D A C			
	B D A C	26.987	4	GMD
	B D A C			
E	B D A C	26.747	4	GMS
E	B D A C			
E	B D A C	26.627	4	TLT
E	B D A C			
E	B D A C	26.570	4	DEP
E	B D A C			
E	B D A C	26.082	4	PAL
E	B D A C			
E	B D A C	24.375	4	MAS
E	B D C			
E	B D C	22.625	4	INT
E	B D C			
E	B D C	21.572	4	SPT
E	B D C			
E	B D C	21.507	4	ATL
E	D C			
E	D C	20.542	4	EXT
E	D			
E	D	19.857	4	BFM
E	D			
E	D	19.192	4	LAT
E				
E	F	16.477	4	EDC
	F			
	F	10.782	4	DIA

G6PDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.095000	4	DIA
	B	0.000000	4	BFM
	B			
	B	0.000000	4	DEP
	B			
	B	0.000000	4	ATL
	B			
	B	0.000000	4	DLT
	B			
	B	0.000000	4	EDC
	B			
	B	0.000000	4	EXT
	B			
	B	0.000000	4	GMD
	B			
	B	0.000000	4	GMS
	B			
	B	0.000000	4	ILD
	B			
	B	0.000000	4	ILS
	B			
	B	0.000000	4	INT
	B			
	B	0.000000	4	LAT
	B			
	B	0.000000	4	LDD
	B			
	B	0.000000	4	LDS
	B			
	B	0.000000	4	MAS
	B			
	B	0.000000	4	OBL
	B			
	B	0.000000	4	PAL
	B			
	B	0.000000	4	PMD
	B			
	B	0.000000	4	PMS
	B			
	B	0.000000	4	PSO
	B			
	B	0.000000	4	SEM
	B			
	B	0.000000	4	SPT
	B			
	B	0.000000	4	TLG
	B			
	B	0.000000	4	TLT

CPK

SNK	GROUPING	MEAN	N	MUSCLE
	A	3381.8	4	TLG
	A			
B	A	3153.4	4	DLT
B	A			
B	A	3120.8	4	PMS
B	A			
B	A C	3023.6	4	TLT
B	A C			
B	A C	2917.0	4	PSO
B	A C			
B	A C	2863.5	4	BFM
B	A C			
B	A C	2840.8	4	PMD
B	A C			
B	A C	2825.2	4	SEM
B	A C			
B	A C	2746.7	4	GMS
B	A C			
B	A C	2713.5	4	MAS
B	A C			
B	A C	2635.2	4	ILS
B	A C			
B	A C	2622.1	4	OBL
B	A C			
B	A C	2600.2	4	LDD
B	C			
B	C	2565.9	4	DEP
B	C			
B	C	2535.9	4	ILD
B	C			
B	C	2519.6	4	LAT
B	C			
B	C	2506.3	4	GMD
B	C			
B	C	2505.5	4	PAL
B	C			
B	C	2426.4	4	SPT
B	C			
B	C	2421.0	4	LDS
B	C			
B	C	2381.8	4	EXT
	C			
	C	2244.0	4	ATL
	C			
	C	2211.7	4	INT
	C			
	C	2203.5	4	EDC
	D	1302.1	4	DIA

PFK

SNK	GROUPING	MEAN	N	MUSCLE
	A	75.875	4	PMS
	A			
B	A	71.412	4	GMS
B	A			
B	A	70.197	4	TLG
B	A			
B	A C	68.460	4	PMD
B	A C			
B	A C	67.725	4	GMD
B	A C			
B	A C	66.012	4	SEM
B	A C			
B	A C	64.672	4	ILS
B	A C			
B	A C	64.190	4	LDD
B	A C			
B D	A C	63.185	4	LDS
B D	A C			
B D	A C	62.912	4	PSO
B D	A C			
B D	A C	60.557	4	OBL
B D	A C			
B D	A C	58.717	4	BFM
B D	A C			
B D	A C	58.582	4	DLT
B D	A C			
B D	A C	57.945	4	ILD
B D	A C			
B D	A C	55.797	4	TLT
B D	C			
B D	E C	49.532	4	PAL
B D	E C			
F B D	E C	47.605	4	EDC
F B D	E C			
F B D	E C	47.560	4	DEP
F D	E C			
F D	E C	44.080	4	LAT
F D	E			
F G D	E	39.317	4	EXT
F G	E			
F G	E H	31.020	4	SPT
F G	E H			
F G	E H	29.487	4	MAS
F G	H			
F G	H	25.965	4	INT
G	H			
G	H	21.582	4	ATL
	H			
	H	16.037	4	DIA

HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	25.225	4	GMD
	A			
B	A	22.777	4	ILD
B	A			
B	A	22.610	4	LDS
B	A			
B	A C	21.577	4	LDD
B	A C			
B D	A C	19.482	4	DIA
B D	A C			
B D	A C	19.122	4	PMS
B D	A C			
B D	A C	19.105	4	GMS
B D	A C			
B D	A C	19.047	4	PMD
B D	A C			
B D	A C	18.890	4	ILS
B D	A C			
B D	A C	18.807	4	DLT
B D	A C			
B D	A C	18.632	4	TLT
B D	A C			
B D	A C	18.327	4	EXT
B D	C			
B D	E C	17.520	4	OBL
B D	E C			
B D	E C	16.815	4	MAS
B D	E C			
B D	E C	16.387	4	PSO
B D	E C			
B D	E C	16.265	4	DEP
B D	E C			
B D	E C	16.005	4	LAT
B D	E C			
B D	E C	15.767	4	INT
B D	E C			
B D	E C	15.660	4	PAL
B D	E C			
B D	E C	15.552	4	EDC
B D	E C			
B D	E C	15.465	4	SEM
D	E C			
D	E C	14.462	4	SPT
D	E			
D	E	12.802	4	TLG
D	E			
D	E	12.297	4	BFM
	E			
	E	10.760	4	ATL

CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	35.435	4	DIA
	A			
B	A	32.527	4	GMD
B	A			
B	A C	31.512	4	LDS
B	A C			
B D	A C	27.480	4	EXT
B D	A C			
B D	A C	27.410	4	ILD
B D	A C			
B D	A C	26.557	4	GMS
B D	A C			
B D	A C	26.195	4	LDD
B D	A C			
B D	A C	26.050	4	INT
B D	A C			
B D	A C	24.330	4	ILS
B D	A C			
E B D	A C	23.322	4	OBL
E B D	A C			
E B D	A C	23.235	4	PMD
E B D	C			
E B D	C	20.307	4	LAT
E B D	C			
E B D	C	19.937	4	DEP
E B D	C			
E B D	C	19.760	4	PAL
E B D	C			
E B D	C	19.552	4	BFM
E B D	C			
E B D	C	19.365	4	SPT
E D	C			
E D	C	18.395	4	EDC
E D	C			
E D	C	18.200	4	PMS
E D	C			
E D	C	18.162	4	PSO
E D	C			
E D	C	17.840	4	SEM
E D	C			
E D	C	17.647	4	TLT
E D				
E D		16.887	4	DLT
E D				
E D		15.155	4	ATL
E				
E		10.150	4	TLG
E				
E		10.045	4	MAS

CPT

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.46250	4	DIA
	A			
B	A	0.40750	4	GMD
B	A			
B	A C	0.37250	4	LDD
B	A C			
B D	A C	0.35000	4	ILD
B D	A C			
B D	A C	0.34250	4	PMD
B D	A C			
B D	A C	0.31500	4	DEP
B D	A C			
B D	A C	0.30500	4	TLT
B D	A C			
B D	A C	0.30000	4	ILS
B D	A C			
B D	A C	0.29500	4	GMS
B D	A C			
B D	A C	0.29500	4	LDS
B D	A C			
B D	A C	0.29250	4	EXT
B D	A C			
B D	A C	0.27750	4	LAT
B D	A C			
B D	A C	0.26000	4	DLT
B D	A C			
B D	A C	0.26000	4	INT
B D	A C			
B D	A C	0.25500	4	PAL
B D	A C			
B D	A C	0.25250	4	PSO
B D	A C			
B D	A C	0.25250	4	EDC
B D	A C			
B D	A C	0.24500	4	MAS
B D	A C			
B D	A C	0.24500	4	PMS
B D	C			
B D	C	0.22750	4	OBL
B D	C			
B D	C	0.22500	4	ATL
B D	C			
B D	C	0.22000	4	SPT
B D	C			
B D	C	0.21750	4	SEM
D	C			
D	C	0.16250	4	BFM
D				
D		0.13000	4	TLG

CAT

SNK	GROUPING	MEAN	N	MUSCLE
	A	3.9575	4	GMD
	A			
B	A	3.6050	4	ILD
B	A			
B	A	3.4850	4	LDD
B	A			
B	A C	3.2100	4	LDS
B	A C			
B	A C	3.1850	4	PMD
B	A C			
B D	A C	2.9500	4	MAS
B D	A C			
B D	A C	2.8650	4	OBL
B D	A C			
E B D	A C	2.8425	4	GMS
E B D	A C			
E B D	A C	2.8225	4	PMS
E B D	A C			
E B D	A C	2.7975	4	DEP
E B D	A C			
E B D	A C	2.7800	4	ILS
E B D	A C			
E B D	A C	2.6550	4	EXT
E B D	C			
E B D F	C	2.3800	4	TLT
E B D F	C			
E B D F	C	2.3425	4	DLT
E B D F	C			
E B D F	C	2.2575	4	TLG
E B D F	C			
E B D F	C	2.2275	4	SEM
E B D F	C			
E B D F	C	2.1550	4	PAL
E B D F	C			
E B D F	C	2.1550	4	INT
E B D F	C			
E B D F	C	2.0850	4	DIA
E B D F	C			
E B D F	C	2.0575	4	PSO
E D F	C			
E D F	C	1.5475	4	LAT
E D F	C			
E D F	C	1.5425	4	ATL
E D F				
E D F		1.4550	4	EDC
E F				
E F		1.2050	4	SPT
F				
F		0.9700	4	BFM

GPT

SNK	GROUPING	MEAN	N	MUSCLE
	A	9.6400	4	LDD
	A			
	B A	8.6775	4	DEP
	B A			
	B A	8.6475	4	PMS
	B A			
	B A C	8.3150	4	PMD
	B A C			
	B A C	8.2025	4	ILD
	B A C			
	B A C	7.9200	4	LDS
	B A C			
	B A C	7.3575	4	SEM
	B A C			
	B D A C	7.1025	4	ILS
	B D A C			
E	B D A C	6.7475	4	EXT
E	B D A C			
E	B D A C	6.6550	4	TLT
E	B D A C			
E	B D A C	6.6075	4	ATL
E	B D A C			
E	B D A C	6.5775	4	DLT
E	B D C			
E	B D C	6.4625	4	GMD
E	B D C			
E	B D C	6.2675	4	INT
E	B D C			
E	B D C	6.2325	4	PSO
E	B D C			
E	B D C	6.2150	4	DIA
E	B D C			
E	B D F C	5.8675	4	OBL
E	B D F C			
E	B D F C	5.5975	4	GMS
E	B D F C			
E	B D F C	5.4125	4	TLG
E	B D F C			
E	B D F C	5.3500	4	MAS
E	D F C			
E	D F C	5.1325	4	EDC
E	D F C			
E	D F C	5.0200	4	PAL
E	D F			
E	D F	4.0200	4	SPT
E	F			
E	F	3.5125	4	LAT
	F			
	F	2.9725	4	BFM

GOT

SNK	GROUPING	MEAN	N	MUSCLE
	A	70.970	4	PMD
	A			
	B A	70.160	4	LDD
	B A			
	B A C	69.762	4	GMD
	B A C			
	B A C	68.440	4	ILD
	B A C			
	B A C	68.117	4	PMS
	B A C			
	B A C	63.985	4	DEP
	B A C			
	B A C	63.640	4	LDS
	B A C			
	B A C	62.957	4	SEM
	B A C			
	B D A C	61.660	4	TLT
	B D A C			
	B D A C	61.630	4	ILS
	B D A C			
	B D A C	61.282	4	GMS
	B D A C			
	B D A C	61.155	4	DLT
	B D A C			
	B D A C	60.345	4	OBL
	B D A C			
	B D A C	57.892	4	PSO
	B D A C			
	B D A C	55.935	4	EDC
	B D A C			
	B D A C	54.027	4	PAL
	B D A C			
	B D A C	53.812	4	MAS
	B D A C			
	B D A C	52.382	4	EXT
	B D A C			
	B D A C	51.915	4	DIA
	B D A C			
	B D A C	47.812	4	INT
	B D A C			
	B D A C	47.670	4	LAT
	B D C			
	B D C	47.190	4	TLG
	B D C			
	B D C	46.865	4	ATL
	D C			
	D C	46.475	4	SPT
	D			
	D	40.012	4	BFM

PK/LDH

SNK GROUPING	MEAN	N	MUSCLE
A	1.11459	4	PSO
A	1.06398	4	BFM
A	1.01535	4	TLT
A	1.00341	4	SPT
A	1.00076	4	GMS
B A	0.98263	4	LAT
B A	0.96011	4	OBL
B A	0.94595	4	PMS
B A	0.93728	4	ILS
B A	0.91275	4	EDC
B A	0.90096	4	DEP
B A	0.90046	4	DLT
B A	0.89100	4	PMD
B A	0.87494	4	LDS
B A	0.87409	4	MAS
B A	0.85650	4	PAL
B A	0.84683	4	TLG
B A	0.84665	4	EXT
B A	0.83130	4	LDD
B A	0.81224	4	ATL
B A	0.79519	4	INT
B A	0.79511	4	GMD
B A	0.79005	4	SEM
B A	0.78649	4	ILD
B	0.65364	4	DIA

PK/CS

SNK GROUPING	MEAN	N	MUSCLE
A	71.764	4	TLG
B A	67.837	4	BFM
B A C	62.526	4	PSO
B A C	57.990	4	SEM
B A C	56.656	4	GMS
B D A C	53.979	4	PMS
B D A C	51.633	4	TLT
B D A C	50.361	4	OBL
B D A C	50.090	4	PAL
B D A C	49.127	4	DEP
B D A C	48.080	4	ATL
B D A C	47.024	4	ILS
B D A C	46.885	4	SPT
B D A C	45.479	4	PMD
E B D A C	43.530	4	LAT
E B D A C	41.071	4	EDC
E B D A C	40.609	4	DLT
E B D C	39.792	4	LDS
E B D C	36.162	4	INT
E B D C	36.145	4	LDD
E D C	33.091	4	ILD
E D C	30.816	4	GMD
E D C	30.247	4	EXT
E D	23.651	4	MAS
E	15.300	4	DIA

PK/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	90.595	4	TLG
	B	66.091	4	PMS
	C B	61.385	4	TLT
	C B D	58.666	4	SEM
	C E B D	56.843	4	PSO
F	C E B D	51.430	4	GMS
F	C E B D	49.861	4	BFM
F	C E B D	49.008	4	DLT
F	C E B D	47.525	4	PAL
F	C E B D	46.968	4	MAS
F	C E B D	45.660	4	DEP
F	C E B D	45.019	4	PMD
F	C E B D	44.422	4	ILS
F	C E B D	43.367	4	ATL
F	C E B D	42.980	4	OBL
F	C E B D	40.819	4	SPT
F	C E B D G	37.714	4	EDC
F	C E B D G	36.640	4	LAT
F	C E D G	33.469	4	LDD
F	C E D G	33.375	4	ILD
F	C E D G	32.158	4	LDS
F	E D G	27.437	4	GMD
F	E G	27.051	4	INT
F	G	23.753	4	EXT
	G	10.382	4	DIA

LDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	86.98	4	TLG
B	A	72.25	4	SEM
B	A C	63.29	4	BFM
B D	A C	59.97	4	ATL
B D	A C	59.21	4	PAL
B D	A C	57.88	4	PMS
B D	A C	57.20	4	GMS
B D	A C	56.15	4	PSO
B D	A C	55.94	4	DEP
B D	C	53.01	4	OBL
B D	C	51.36	4	TLT
B D	C	50.57	4	PMD
B D	C	50.07	4	ILS
B D	C	47.45	4	SPT
B D	C	46.22	4	INT
B D	C	45.46	4	DLT
B D	C	45.30	4	LDS
B D	C	44.99	4	EDC
B D	C	44.48	4	LAT
B D	C	43.53	4	LDD
B D	C	42.74	4	ILD
B D	C	38.36	4	GMD
B D	C	35.63	4	EXT
D	C	27.58	4	MAS
D		23.01	4	DIA

LDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	104.604	4	TLG
	B	73.669	4	SEM
C	B	71.875	4	PMS
C	B D	59.874	4	TLT
C E	B D	56.551	4	DLT
C E	B D	55.111	4	PAL
C E	B D	52.996	4	GMS
C E	B D	52.893	4	PSO
C E	B D	52.624	4	DEP
C E	B D	52.482	4	MAS
C E	B D	51.877	4	ATL
C E	B D	50.526	4	PMD
C E	B D	48.881	4	BFM
C E	B D	48.423	4	ILS
C E	B D	48.172	4	OBL
C E	B D	44.449	4	ILD
C E	D	43.400	4	LDD
C E	F D	42.303	4	SPT
E	F D	40.937	4	EDC
E	F D	38.207	4	LAT
E	F D	37.315	4	LDS
E	F D	36.217	4	GMD
E	F D	33.189	4	INT
E	F	27.935	4	EXT
	F	16.528	4	DIA

HOAD/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	1.5468	4	DIA
B	A	1.2957	4	ILD
B	A	1.2837	4	SEM
B	A	1.2807	4	GMD
B	A	1.2723	4	INT
B	A	1.2560	4	ATL
B	A	1.2333	4	LDD
B	A	1.1885	4	TLG
B	A	1.1881	4	EXT
B	A	1.1716	4	PAL
B	A	1.1648	4	MAS
B	A	1.1558	4	LDS
B	A	1.1348	4	DLT
B	A	1.1263	4	PMD
B	A	1.1212	4	DEP
B	A	1.0973	4	EDC
B	A	1.0781	4	OBL
B	A	1.0713	4	ILS
B	A	1.0674	4	PMS
B	A	1.0577	4	GMS
B	A	1.0247	4	LAT
B	A	1.0177	4	SPT
B	A	0.9960	4	TLT
B	A	0.9457	4	BFM
B	A	0.9357	4	PSO

a-GPDH/LDH

SNK GROUPING	MEAN	N	MUSCLE
A	0.084257	4	DIA
B	0.043399	4	EXT
C B	0.037218	4	INT
C B	0.034784	4	GMD
C B	0.030892	4	LAT
C B	0.030595	4	LDS
C B	0.030351	4	SPT
C B	0.029056	4	ILD
C B	0.028857	4	LDD
C B	0.028317	4	EDC
C B	0.027767	4	BFM
C B	0.025189	4	GMS
C B	0.025031	4	ILS
C B	0.025009	4	OBL
C B	0.024797	4	PMD
C B	0.024356	4	DEP
C B	0.024283	4	ATL
C B	0.023016	4	PAL
C B	0.022642	4	MAS
C B	0.021994	4	PSO
C B	0.019778	4	DLT
C B	0.019404	4	TLT
C B	0.017253	4	SEM
C B	0.016889	4	PMS
C	0.011166	4	TLG

CPK/LDH

SNK GROUPING	MEAN	N	MUSCLE
A	2.0402	4	DIA
B A	1.7868	4	INT
B A	1.7687	4	BFM
B A C	1.5940	4	EXT
B A C	1.4731	4	SPT
B A C	1.4607	4	LAT
B A C	1.4131	4	LDS
B A C	1.4004	4	ATL
B A C	1.3746	4	PAL
B A C	1.3499	4	GMD
B A C	1.3438	4	GMS
B A C	1.3035	4	ILS
B A C	1.2821	4	OBL
B A C	1.2532	4	PMD
B A C	1.2481	4	LDD
B A C	1.2323	4	ILD
B A C	1.2289	4	DEP
B A C	1.2281	4	SEM
B A C	1.2117	4	PSO
B A C	1.2006	4	EDC
B C	0.9665	4	PMS
B C	0.9613	4	TLT
B C	0.9498	4	TLG
B C	0.9022	4	DLT
C	0.6559	4	MAS

CPK/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	7.0914	4	MAS
	B	4.6963	4	DIA
C	B	4.4477	4	EXT
C	B	4.4033	4	TLG
C	B	4.3810	4	ATL
C	B D	4.3000	4	DLT
C	B D	3.8847	4	INT
C	B D	3.8480	4	LAT
C	B D	3.7303	4	SPT
C	B D	3.6804	4	BFM
C	B D	3.5456	4	DEP
C	B D	3.4981	4	EDC
C	B D	3.4454	4	LDD
C	B D	3.4209	4	SEM
C	B D	3.4019	4	ILD
C	B D	3.3844	4	PMD
C	B D	3.3241	4	GMD
C	B D	3.3162	4	PAL
C	B D	3.2933	4	TLT
C	B D	3.1631	4	PSO
C	B D	3.1448	4	OBL
C	B D	3.1196	4	PMS
C	B D	3.0344	4	ILS
C	D	2.7650	4	LDS
	D	2.6522	4	GMS

a-GPDH/PFK

SNK	GROUPING	MEAN	N	MUSCLE
	A	1.5730	4	ATL
B	A	1.2245	4	INT
B		0.8921	4	DIA
B		0.8309	4	MAS
B		0.7181	4	SPT
B		0.6013	4	PAL
B		0.5593	4	DEP
B		0.5334	4	EXT
B		0.5136	4	ILD
B		0.5038	4	TLT
B		0.5035	4	DLT
B		0.4984	4	TLG
B		0.4769	4	LDS
B		0.4757	4	OBL
B		0.4732	4	LDD
B		0.4732	4	PMD
B		0.4649	4	LAT
B		0.4555	4	ILS
B		0.4508	4	PSO
B		0.4417	4	SEM
B		0.4171	4	PMS
B		0.3974	4	GMD
B		0.3897	4	GMS
B		0.3529	4	BFM
B		0.3469	4	EDC

PFK/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.080361	4	BFM
	B A	0.078114	4	TLG
	B A	0.073306	4	PSO
	B A	0.072600	4	PMD
	B A	0.071304	4	GMD
	B A	0.070433	4	PMS
	B A	0.069797	4	LDD
	B A	0.069429	4	EDC
	B A	0.068993	4	DLT
	B A	0.068894	4	ILS
	B A C	0.067052	4	MAS
	B A C	0.066454	4	GMS
	B D A C	0.066309	4	LAT
	B D A C	0.065628	4	OBL
E	B D A C	0.062389	4	SEM
E	B D A C	0.061928	4	EXT
E	B D A C	0.061753	4	LDS
E	B D A C	0.060871	4	TLT
E	B D A C	0.059889	4	ILD
E	B D A C	0.057815	4	PAL
E	B D A C	0.057706	4	DEP
E	B D C	0.046832	4	SPT
E	D C	0.036965	4	DIA
E	D	0.036195	4	INT
E		0.033964	4	ATL

a-GPDH/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.064271	4	MAS
	B	0.045155	4	TLG
	B	0.043615	4	ATL
	B	0.040948	4	INT
	B	0.039267	4	LDD
	B	0.038784	4	ILD
	B	0.038359	4	DIA
	B	0.038259	4	PMD
	B	0.038162	4	EXT
	B	0.037647	4	DLT
	B	0.035605	4	GMD
	B	0.035566	4	SEM
	B	0.035331	4	DEP
	B	0.034748	4	PAL
	B	0.033854	4	LDS
	B	0.033096	4	SPT
	B	0.032970	4	ILS
	B	0.032656	4	OBL
	B	0.031087	4	PMS
	B	0.030050	4	PSO
	B	0.029296	4	LAT
	B	0.029058	4	TLT
	B	0.026039	4	EDC
	B	0.025814	4	BFM
	B	0.025726	4	GMS

a-GPDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	3.9488	4	TLG
	B	2.7095	4	MAS
	B			
C	B	2.0915	4	PMS
C	B			
C	B	1.8751	4	SEM
C	B			
C	B	1.8315	4	DLT
C	B			
C	B	1.7915	4	TLT
C				
C		1.6184	4	PAL
C				
C	D	1.6013	4	PMD
C	D			
C	D	1.5511	4	PSO
C	D			
C	D	1.5222	4	ATL
C	D			
C	D	1.4794	4	SPT
C	D			
C	D	1.4728	4	DEP
C	D			
C	D	1.4333	4	ILS
C	D			
C	D	1.4182	4	OBL
C	D			
C	D	1.3042	4	LDD
C	D			
C	D	1.2789	4	GMS
C	D			
C	D	1.2482	4	ILD
C	D			
C	D	1.2447	4	BFM
C	D			
C	D	1.1215	4	LDS
C	D			
C	D	1.0341	4	LAT
C	D			
C	D	0.9982	4	INT
C	D			
C	D	0.9483	4	EDC
C	D			
C	D	0.9425	4	GMD
C	D			
C	D	0.8538	4	EXT
	D			
	D	0.3688	4	DIA

a-GPDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	3.3188	4	TLG
	B	2.0727	4	SEM
	B			
C	B	1.9953	4	ATL
C	B			
C	B	1.8842	4	PSO
C	B			
C	B	1.7547	4	BFM
C	B			
C	B	1.7537	4	DEP
C	B			
C	B	1.7436	4	PMD
C	B			
C	B	1.7336	4	PAL
C	B			
C	B	1.6846	4	PMS
C	B			
C	B	1.6374	4	OBL
C	B			
C	B	1.5618	4	ILS
C	B			
C	B	1.5270	4	MAS
C	B			
C	B	1.5218	4	DLT
C	B			
C	B	1.4953	4	INT
C	B			
C	B	1.4934	4	TLT
C	B			
C	B	1.4857	4	SPT
C	B			
C	B	1.4330	4	LDD
C	B			
C	B	1.4159	4	GMS
C	B			
C	B	1.3196	4	LAT
C	B			
C	B	1.3189	4	LDS
C	B			
C	B	1.2827	4	ILD
C	B			
C	B	1.1689	4	EXT
C	B			
C	B	1.1050	4	GMD
C	B			
C	B	1.0727	4	EDC
C				
C		0.5944	4	DIA

PFK/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	6.2698	4	TLG
	A			
B	A	5.1666	4	BFM
B				
B	C	4.5101	4	SEM
B	C			
B	C D	4.2122	4	PSO
B	C D			
B	C D	4.0206	4	PMS
B	C D			
B	C D	3.7100	4	GMS
B	C D			
B	C D	3.7025	4	PMD
B	C D			
B E	C D	3.4754	4	OBL
B E	C D			
B E	C D	3.4239	4	ILS
B E	C D			
B E	C D	3.2736	4	TLT
B E	C D			
B E	C D	3.2150	4	DEP
B E	C D			
B E	C D	3.0958	4	PAL
B E	C D			
B E	C D	3.0908	4	DLT
B E	C D			
B E	C D	3.0650	4	EDC
B E	C D			
B E	C D	3.0096	4	LDD
B E	C D			
B E	C D	2.8465	4	LAT
B E	C D			
B E	C D	2.8076	4	LDS
B E	C D			
B E	C D	2.7642	4	GMD
B E	C D			
B E	C D	2.5892	4	ILD
E	C D			
E	C D	2.1689	4	EXT
E	C D			
E	C D	2.1354	4	SPT
E	C D			
E	C D	2.0260	4	ATL
E	C D			
E	C D	1.8328	4	MAS
E	D			
E		1.5694	4	INT
E				
E		0.8211	4	DIA

GOT/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.18386	4	DIA
	B	0.13825	4	MAS
	C	0.09799	4	EXT
	C			
	C	0.09561	4	GMD
	C			
	C	0.09250	4	ATL
	C			
D	C	0.09196	4	LDD
D	C			
D	C	0.09133	4	ILD
D	C			
D	C	0.09018	4	EDC
D	C			
D	C	0.08596	4	PMD
D	C			
D	C	0.08594	4	DEP
D	C			
D	C	0.08424	4	INT
D	C			
D	C	0.08298	4	DLT
D	C			
D	C	0.07611	4	SEM
D	C			
D	C	0.07281	4	PAL
D	C			
D	C	0.07271	4	LAT
D	C			
D	C	0.07266	4	LDS
D	C			
D	C	0.07152	4	SPT
D	C			
D	C	0.07002	4	OBL
D	C			
D	C	0.06979	4	ILS
D	C			
D	C	0.06728	4	TLT
D	C			
D	C	0.06700	4	PMS
D	C			
D	C	0.06272	4	PSO
D	C			
D	C	0.06259	4	TLG
D	C			
D	C	0.05952	4	GMS
D				
D		0.05166	4	BFM

PK/PFK

SNK	GROUPING	MEAN	N	MUSCLE
	A	33.433	4	ATL
	A			
B	A	27.315	4	INT
B	A			
B	A	22.975	4	SPT
B	A			
B	A	22.829	4	DIA
B				
B		17.397	4	TLT
B				
B		17.395	4	PAL
B				
B		15.825	4	GMS
B				
B		15.608	4	DEP
B				
B		15.439	4	LAT
B				
B		15.206	4	PSO
B				
B		14.643	4	OBL
B				
B		14.139	4	LDS
B				
B		13.892	4	EXT
B				
B		13.719	4	ILS
B				
B		13.706	4	MAS
B				
B		13.579	4	BFM
B				
B		13.468	4	ILD
B				
B		13.446	4	PMS
B				
B		13.316	4	EDC
B				
B		13.261	4	DLT
B				
B		12.720	4	SEM
B				
B		12.452	4	PMD
B				
B		11.977	4	LDD
B				
B		11.267	4	GMD
B				
B		11.031	4	TLG

GOT/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	4.3477	4	SEM
	A			
	A	4.3270	4	ATL
	A			
	A	4.1828	4	DEP
	A			
	A	4.1250	4	TLG
	A			
	A	3.7587	4	PMD
	A			
	A	3.6977	4	PSO
	A			
	A	3.5894	4	PMS
	A			
	A	3.5817	4	EDC
	A			
	A	3.4942	4	PAL
	A			
	A	3.4730	4	BFM
	A			
	A	3.4446	4	TLT
	A			
	A	3.4426	4	OBL
	A			
	A	3.3671	4	SPT
	A			
	A	3.3162	4	LDD
	A			
	A	3.2990	4	DLT
	A			
	A	3.2635	4	ILS
	A			
	A	3.2358	4	MAS
	A			
	A	3.2161	4	GMS
	A			
	A	3.0889	4	LAT
	A			
	A	3.0377	4	INT
	A			
	A	3.0167	4	ILD
	A			
	A	2.8927	4	EXT
	A			
	A	2.8160	4	LDS
	A			
	A	2.7991	4	GMD
	A			
	A	2.7842	4	DIA

GOT/PFK

SNK	GROUPING	MEAN	N	MUSCLE
	A	4.0400	4	DIA
	B	2.8991	4	ATL
C	B	2.2508	4	INT
C	B	1.8497	4	MAS
C	B	1.6451	4	SPT
C		1.3479	4	DEP
C		1.3384	4	EXT
C		1.2273	4	ILD
C		1.1841	4	EDC
C		1.1787	4	TLT
C		1.1759	4	PAL
C		1.1206	4	LAT
C		1.1043	4	LDD
C		1.0813	4	DLT
C		1.0785	4	GMD
C		1.0578	4	PMD
C		1.0223	4	LDS
C		1.0124	4	OBL
C		0.9630	4	PSO
C		0.9589	4	SEM
C		0.9524	4	ILS
C		0.9006	4	PMS
C		0.8780	4	GMS
C		0.7065	4	BFM
C		0.6677	4	TLG

GOT/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.119940	4	MAS
	A	0.119720	4	DIA
	B	0.082515	4	EXT
	B	0.082323	4	EDC
	B	0.077915	4	DEP
	B	0.075931	4	LDD
	B	0.075778	4	PMD
	B	0.075776	4	ATL
	B	0.073425	4	GMD
	B	0.072724	4	DLT
	B	0.071939	4	ILD
	B	0.071872	4	SPT
	B	0.071245	4	LAT
	B	0.069398	4	PSO
	B	0.068281	4	TLT
	B	0.067169	4	INT
	B	0.065759	4	OBL
	B	0.065431	4	ILS
	B	0.063480	4	PMS
	B	0.062730	4	PAL
	B	0.062485	4	LDS
	B	0.059599	4	SEM
	B	0.057251	4	GMS
	B	0.054653	4	BFM
	B	0.052661	4	TLG

PFK/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	8.1371	4	TLG
	B	4.8900	4	PMS
C	B	4.4649	4	SEM
C	B D	3.9209	4	DLT
C	B D	3.8251	4	TLT
C	B D	3.7225	4	PSO
C	B D	3.6854	4	PMD
C	B D	3.6177	4	BFM
C	B D	3.4934	4	MAS
C	B D	3.4346	4	ILS
C	B D	3.3056	4	PAL
C	B D	3.2916	4	GMS
C	B D	3.0623	4	OBL
C	B D	3.0350	4	DEP
C E	B D	2.8390	4	EDC
C E	B D	2.8227	4	LDD
C E	B D	2.5241	4	ILD
C E	B D	2.4862	4	LAT
C E	B D	2.3834	4	GMD
C E	D	2.2683	4	LDS
C E	D	2.1750	4	SPT
C E	D	2.0810	4	ATL
E	D	1.7589	4	EXT
E	D	1.4175	4	INT
E		0.6557	4	DIA

HOAD/CPT

SNK	GROUPING	MEAN	N	MUSCLE
	A	351.74	4	TLG
	A	203.26	4	INT
	A	152.45	4	BFM
	A	145.77	4	DIA
	A	109.05	4	LDS
	A	107.27	4	EXT
	A	103.60	4	SPT
	A	101.91	4	PAL
	A	100.98	4	ATL
	A	100.92	4	SEM
	A	100.77	4	LAT
	A	98.33	4	OBL
	A	89.26	4	EDC
	A	88.87	4	GMS
	A	84.64	4	PMS
	A	83.86	4	GMD
	A	83.11	4	ILS
	A	80.45	4	DEP
	A	80.21	4	ILD
	A	79.01	4	LDD
	A	76.75	4	PSO
	A	74.98	4	PMD
	A	66.79	4	DLT
	A	60.39	4	TLT
	A	58.14	4	MAS

HOAD/CAT

SNK	GROUPING	MEAN	N	MUSCLE
	A	22.191	4	BFM
	A			
B	A	19.286	4	SPT
B	A			
B	A C	17.893	4	LAT
B	A C			
B D	A C	16.702	4	DIA
B D	A C			
B D	A C	15.347	4	INT
B D	A C			
B D	A C	13.466	4	EDC
B D	A C			
B D	A C	12.086	4	EXT
B D	C			
B D	C	10.776	4	PAL
B D	C			
B D	C	10.244	4	LDS
B D	C			
B D	C	10.102	4	ATL
B D	C			
B D	C	9.239	4	GMD
B D	C			
B D	C	9.158	4	GMS
B D	C			
B D	C	8.806	4	ILS
B D	C			
B D	C	8.784	4	PSO
B D	C			
B D	C	8.462	4	SEM
B D	C			
B D	C	7.985	4	TLT
B D	C			
B D	C	7.760	4	OBL
B D	C			
B D	C	7.663	4	DLT
B D	C			
B D	C	7.538	4	PMD
B D	C			
B D	C	7.455	4	ILD
B D	C			
B D	C	7.364	4	LDD
B D	C			
B D	C	7.298	4	DEP
B D	C			
B D	C	6.912	4	PMS
D	C			
D	C	6.088	4	TLG
D				
D		4.345	4	MAS

APPENDIX 2. CORRELATION MATRIX BETWEEN MAXIMUM ENZYME ACTIVITES AND ENZYME ACTIVITY RATIOS OF HARBOR SEAL MUSCLE.

* = Statistically significant correlation at the 95% confidence level. All correlations are between data ranks (Spearman correlations). See Materials and Methods for abbreviations.

	LDH	PK	a-GPDH	G6PDH	CPK	PFK	HOAD	CS	CPT	CAT	GPT	GOT
PK/LDH	-0.04	*0.51	0.13	*-0.31	*0.23	*0.22	0.05	-0.09	-0.12	-0.08	-0.15	0.04
PK/CS	*0.36	*0.59	*0.29	*-0.34	*0.37	*0.36	-0.06	*-0.56	*-0.55	*-0.42	-0.09	-0.16
PK/HOAD	*0.29	*0.32	0.01	*-0.33	*0.63	*0.26	*-0.87	-0.03	-0.15	-0.11	-0.14	0.04
LDH/CS	*0.45	*0.41	*0.31	*-0.33	*0.35	*0.33	-0.13	*-0.61	*-0.58	*-0.42	-0.04	-0.19
LDH/HOAD	*0.34	*0.21	-0.01	*-0.28	*0.58	*0.24	*-0.91	0.01	-0.12	-0.06	-0.09	0.05
HOAD/CS	-0.08	0.01	0.19	0.11	*-0.39	-0.05	*0.86	*-0.34	-0.19	-0.15	0.08	-0.16
a-GPDH/LDH	-0.19	-0.05	*0.65	-0.17	0.15	-0.05	*0.22	-0.19	*-0.25	0.17	*0.23	0.07
CPK/LDH	*-0.65	*-0.39	*-0.21	-0.05	*0.36	*-0.35	*-0.42	*-0.36	-0.18	*-0.31	*-0.33	*-0.34
PFK/LDH	*0.20	*0.39	*0.31	*-0.29	*0.37	*0.74	0.01	0.11	0.05	*0.22	0.01	0.17
a-GPDH/PK	-0.17	*-0.39	*0.49	0.09	-0.01	*-0.21	0.17	-0.13	-0.14	0.17	*0.28	-0.01
a-GPDH/HOAD	*0.22	0.15	*0.26	*-0.34	*0.68	0.18	*-0.85	-0.09	*-0.21	0.01	-0.02	0.07
a-GPDH/CS	0.16	*0.23	*0.65	*-0.33	*0.38	0.16	0.04	*-0.62	*-0.61	*-0.26	0.11	-0.15
PK/PFK	*-0.24	-0.04	-0.14	0.11	*-0.25	*-0.61	0.09	*-0.23	*-0.22	*-0.31	-0.08	-0.17
GOT/CS	0.06	0.15	*0.29	-0.13	*0.27	0.12	-0.04	*-0.62	*-0.51	*-0.31	*0.24	0.12
CPK/PK	*-0.56	*-0.72	*-0.27	0.17	*0.21	*-0.46	*-0.45	*-0.25	-0.06	*-0.22	*-0.23	*-0.35
a-GPDH/PFK	*-0.34	*-0.34	*0.26	0.13	-0.15	*-0.62	0.18	*-0.28	*-0.31	-0.08	0.12	-0.16
PFK/CS	*0.51	*0.61	*0.41	*-0.33	*0.52	*0.76	-0.11	*-0.28	*-0.28	-0.11	0.03	0.05
GOT/PK	*-0.44	*-0.67	*-0.24	*0.34	*-0.31	*-0.42	0.01	*0.26	*0.34	*0.32	*0.33	*0.32
GOT/PFK	*-0.57	*-0.62	*-0.35	*0.32	*-0.46	*-0.85	0.07	-0.01	0.07	-0.03	0.11	0.02
GOT/LDH	*-0.56	*-0.43	*-0.21	*0.32	*-0.25	*-0.38	0.11	*0.25	*0.33	*0.31	*0.28	*0.39
PFK/HOAD	*0.34	*0.28	0.05	*-0.31	*0.66	*0.44	*-0.83	0.04	-0.07	0.01	-0.09	0.09
HOAD/CPT	0.03	0.13	*0.25	-0.01	*-0.21	-0.01	*0.66	*-0.39	*-0.58	*-0.25	0.03	-0.18
HOAD/CAT	-0.18	-0.06	-0.07	0.17	*-0.44	-0.19	*0.71	*-0.41	*-0.26	*-0.47	-0.17	*-0.35

APPENDIX 3. STATISTICALLY SIGNIFICANT DIFFERENCES OF MAXIMUM ENZYME ACTIVITIES AND ENZYME ACTIVITY RATIOS BETWEEN MUSCLES OF THE FIN WHALE (11 PAGES).

Muscles are listed in descending order of the mean.
Means with the same letter are not significantly different (95% confidence level).
Differences are based on Student-Newman-Keuls (SNK) multiple range tests.
Abbreviations are listed in Materials and Methods.

LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	2062.6	4	LD
	A			
B	A	1738.3	4	SPD
B	A			
B	A	1713.2	4	RAM
B	A			
B	A	1696.6	4	RAP
B	A			
B	A	1630.8	4	ECM
B				
B	C	1267.2	4	ICV
B	C			
B	C D	1089.5	4	IL
B	C D			
B	C D	1027.3	4	ICD
	C D			
E	C D	945.0	4	HYP
E	C D			
E	C D	785.2	4	OBL
E	C D			
E	C D	701.7	4	LAT
E	C D			
E	C D	646.9	4	EXT
E	D			
E	D	380.5	4	DLT
E	D			
E	D	362.0	4	INT
E	D			
E	D	340.0	4	IS
E	D			
E	D	337.5	4	PM
E	D			
E	D	310.6	4	TRI
E	D			
E	D	305.8	4	DIA
E				
E		215.5	4	EDC
E				
E		212.6	4	PNC
E				
E		203.6	4	BH
E				
E		195.6	4	MYL
E				
E		167.7	4	MAS

PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	492.48	4	LD
	A			
B	A	442.13	4	RAM
B				
B	C	351.86	4	SPD
B	C			
B	C D	330.11	4	RAP
B	C D			
B	C D	329.22	4	ECM
B	C D			
B	C D	328.92	4	IL
B	C D			
B	C D	298.63	4	ICV
	C D			
E	C D	242.51	4	HYP
E	C D			
E	C D	192.55	4	ICD
E	C D			
E	C D	190.63	4	OBL
E	C D			
E	C D	190.39	4	LAT
E	D			
E	D	175.27	4	EXT
E				
E		137.58	4	DLT
E				
E		127.81	4	IS
E				
E		120.40	4	PM
E				
E		119.62	4	DIA
E				
E		117.64	4	TRI
E				
E		114.78	4	PNC
E				
E		105.53	4	MYL
E				
E		91.53	4	BH
E				
E		88.79	4	INT
E				
E		85.66	4	EDC
E				
E		74.80	4	MAS

AGPDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	13.097	4	LD
	A			
B	A	11.735	4	IL
B	A			
B	A C	11.190	4	ICD
B	A C			
B D	A C	7.497	4	HYP
B D	A C			
B D	A C	7.445	4	RAM
B D	A C			
B D	A C	7.382	4	ECM
B D	A C			
B D	A C	6.572	4	SPD
B D	C			
B D	C	5.360	4	EXT
B D	C			
B D	C	4.585	4	DIA
B D	C			
B D	C	4.572	4	TRI
D	C			
D	C	4.160	4	ICV
D	C			
D	C	3.810	4	OBL
D	C			
D	C	3.807	4	INT
D	C			
D	C	3.615	4	PM
D		3.447	4	MAS
D		3.320	4	LAT
D		3.245	4	MYL
D		3.132	4	EDC
D		3.117	4	DLT
D		2.940	4	RAP
D		2.852	4	PNC
D		2.835	4	IS
D		2.315	4	BH

CPK

SNK	GROUPING	MEAN	N	MUSCLE
	A	2723.6	4	RAM
	A			
B	A	2441.7	4	SPD
B	A			
B	A	2399.8	4	RAP
B	A			
B	A C	2314.3	4	IL
B	A C			
B	A C	2305.6	4	DIA
B	A C			
B D	A C	2218.8	4	LAT
B D	A C			
B D	A C	2207.6	4	LD
B D	A C			
B D	A C	2196.8	4	ECM
B D	A C			
B D	A C	2192.5	4	MAS
B D	C			
B D	E C	2062.7	4	EXT
B D	E C			
B D	E C	2040.2	4	BH
B D	E C			
B D	E C	1952.6	4	PM
B D	E C			
F B D	E C	1868.3	4	INT
F B D	E C			
F B D	E C	1844.5	4	DLT
F D	E C			
F G D	E C	1706.0	4	TRI
F G D	E C			
F G D	E C	1669.6	4	IS
F G D	E			
F G D	E	1599.3	4	OBL
F G D	E			
F G D	E	1570.2	4	ICD
F G D	E			
F G D	E	1556.9	4	HYP
F G	E			
F G	E	1522.8	4	ICV
F G				
F G		1290.3	4	EDC
G				
G		1183.7	4	MYL
G				
G		1122.6	4	PNC

HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	7.852	4	SPD
	A	7.017	4	ICV
	A	6.362	4	RAM
	A	5.265	4	DIA
	A	5.110	4	ICD
	A	5.007	4	OBL
	A	4.975	4	TRI
	A	4.975	4	EDC
	A	4.950	4	MAS
	A	4.345	4	PM
	A	4.285	4	MYL
	A	4.285	4	EXT
	A	4.230	4	HYP
	A	4.085	4	LD
	A	3.937	4	IL
	A	3.920	4	INT
	A	3.845	4	BH
	A	3.700	4	LAT
	A	3.520	4	PNC
	A	3.492	4	IS
	A	3.455	4	DLT
	A	3.447	4	ECM
	A	2.357	4	RAP

CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	6.5900	4	SPD
B	A	6.1750	4	RAM
B	A	5.7825	4	ICV
B	D A	4.7275	4	LD
B	D	4.1775	4	HYP
B	D	4.0700	4	OBL
B	D	3.6950	4	EXT
B	D	3.6075	4	EDC
	D	3.1925	4	MAS
	D	3.1675	4	ECM
	D	3.0975	4	TRI
	D	3.0250	4	ICD
	D	2.9575	4	IL
	D	2.9475	4	DIA
	D	2.6050	4	LAT
	D	2.5275	4	RAP
	D	2.2725	4	DLT
	D	2.2700	4	MYL
	D	2.2375	4	BH
	D	2.1675	4	PM
	D	2.1325	4	PNC
	D	1.8000	4	INT
	D	1.6525	4	IS

GOT

SNK	GROUPING	MEAN	N	MUSCLE
	A	22.562	4	ICV
	A			
B	A	18.572	4	RAM
B	A			
B	A	18.500	4	DIA
B	A			
B	A,	17.300	4	TRI
B	A			
B	A C	17.102	4	SPD
B	A C			
B D	A C	15.822	4	OBL
B D	A C			
B D	A C	15.595	4	HYP
B D	A C			
B D	A C	15.185	4	BH
B D	A C			
B D	A C	14.560	4	EDC
B D	C			
B D	C	13.577	4	MAS
B D	C			
B D	C	12.882	4	IL
B D	C			
B D	C	12.685	4	ECM
B D	C			
B D	C	12.445	4	LAT
B D	C			
B D	C	12.230	4	DLT
B D	C			
B D	C	11.787	4	LD
B D	C			
B D	C	11.780	4	PM
B D	C			
B D	C	10.902	4	INT
B D	C			
B D	C	10.775	4	ICD
B D	C			
B D	C	10.445	4	PNC
B D	C			
B D	C	10.350	4	EXT
B D	C			
B D	C	8.845	4	MYL
D	C			
D	C	7.425	4	IS
D				
D		7.120	4	RAP

PK/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.66165	4	MYL
	A			
B	A	0.55813	4	PNC
B				
B	C	0.47240	4	BH
B	C			
B	C	0.44983	4	MAS
B	C			
B	C	0.41354	4	EDC
B	C			
B	C	0.40180	4	DLT
B	C			
B	C	0.39465	4	PM
B	C			
B	C	0.38232	4	TRI
B	C			
B	C	0.38163	4	DIA
B	C			
B	C	0.37427	4	IS
B	C			
B	C	0.32300	4	OBL
B	C			
B	C	0.31908	4	IL
	C			
	C	0.28385	4	EXT
	C			
	C	0.27264	4	LAT
	C			
	C	0.26444	4	INT
	C			
	C	0.25898	4	HYP
	C			
	C	0.25824	4	RAM
	C			
	C	0.25323	4	ECM
	C			
	C	0.23781	4	LD
	C			
	C	0.23517	4	ICV
	C			
	C	0.23470	4	ICD
	C			
	C	0.20084	4	SPD
	C			
	C	0.19666	4	RAP

LDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	667.09	4	RAP
	A			
B	A	581.78	4	ECM
B				
B	C	445.51	4	LD
B	C			
B	C D	376.24	4	ICD
B	C D			
B	C D	365.83	4	IL
	C D			
	C D	291.15	4	SPD
	C D			
	C D	283.36	4	RAM
	C D			
	C D	271.95	4	LAT
	C D			
	C D	260.36	4	HYP
	C D			
	C D	245.32	4	ICV
	C D			
	C D	211.74	4	INT
	C D			
	C D	210.55	4	IS
	C D			
	C D	206.49	4	EXT
	C D			
	C D	177.58	4	OBL
	C D			
	C D	167.18	4	DLT
	C D			
	C D	159.66	4	PM
	D			
	D	109.96	4	DIA
	D			
	D	106.12	4	TRI
	D			
	D	102.19	4	PNC
	D			
	D	99.91	4	MYL
	D			
	D	94.23	4	BH
	D			
	D	60.60	4	EDC
	D			
	D	60.13	4	MAS

LDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	938.8	4	ECM
	A			
B	A	730.9	4	RAP
B	A			
B	A	541.9	4	LD
B				
B		330.7	4	HYP
B				
B		310.4	4	IL
B				
B		299.8	4	RAM
B				
B		286.5	4	SPD
B				
B		267.3	4	ICD
B				
B		224.8	4	ICV
B				
B		206.8	4	LAT
B				
B		188.4	4	EXT
B				
B		146.3	4	OBL
B				
B		112.1	4	INT
B				
B		111.4	4	DLT
B				
B		103.2	4	IS
B				
B		85.5	4	PM
B				
B		69.5	4	TRI
B				
B		64.5	4	PNC
B				
B		61.0	4	BH
B				
B		58.3	4	DIA
B				
B		50.0	4	MYL
B				
B		45.0	4	EDC
B				
B		37.1	4	MAS

PK/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	130.24	4	RAP
	A			
B	A	112.32	4	IL
B	A			
B	A C	109.32	4	ECM
B	A C			
B	A C	106.67	4	LD
B	C			
B	D C	78.33	4	IS
B	D C			
B	D C	73.52	4	LAT
B	D C			
B	D C	73.07	4	RAM
B	D C			
B	D C	68.13	4	ICD
B	D C			
B	D C	66.45	4	HYP
B	D C			
B	D C	62.68	4	SPD
B	D C			
B	D C	61.94	4	DLT
B	D C			
B	D C	59.32	4	ICV
B	D C			
B	D C	55.71	4	PM
B	D C			
B	D C	54.83	4	PNC
B	D C			
B	D C	53.38	4	OBL
	D C			
	D C	52.49	4	EXT
	D C			
	D C	50.24	4	INT
	D C			
	D C	49.24	4	MYL
	D			
	D	43.05	4	DIA
	D			
	D	40.59	4	TRI
	D			
	D	40.50	4	BH
	D			
	D	27.28	4	MAS
	D			
	D	24.98	4	EDC

PK/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	145.18	4	ECM
	A			
B	A	140.48	4	RAP
B	A			
B	A C	129.82	4	LD
B	A C			
B	D A C	109.78	4	IL
B	D A C			
B	D A C	82.98	4	HYP
B	D A C			
B	D A C	76.70	4	RAM
B	D A C			
B	D A C	61.80	4	SPD
B	D C			
B	D C	55.30	4	LAT
B	D C			
B	D C	52.92	4	ICV
	D C			
	D C	46.60	4	EXT
	D C			
	D C	46.48	4	ICD
	D C			
	D C	41.11	4	DLT
	D C			
	D C	39.01	4	IS
	D C			
	D C	38.27	4	OBL
	D C			
	D C	34.31	4	PNC
	D			
	D	28.80	4	PM
	D			
	D	25.91	4	INT
	D			
	D	25.37	4	BH
	D			
	D	25.30	4	MYL
	D			
	D	25.10	4	TRI
	D			
	D	22.29	4	DIA
	D			
	D	18.00	4	EDC
	D			
	D	16.61	4	MAS

HOAD/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	2.1403	4	INT
	A	2.1164	4	IS
	A	1.9902	4	PM
	A	1.9672	4	TRI
	A	1.8988	4	MYL
	A	1.8637	4	DIA
	A	1.7900	4	ICD
	A	1.7050	4	BH
	A	1.6712	4	MAS
	A	1.6467	4	PNC
	A	1.5226	4	DLT
	A	1.4799	4	OBL
	A	1.4416	4	EDC
	A	1.3903	4	LAT
	A	1.3540	4	IL
	A	1.1691	4	ICV
	A	1.1601	4	EXT
	A	1.1375	4	SPD
	A	1.0197	4	RAM
	A	1.0195	4	ECM
	A	0.9551	4	RAP
	A	0.9206	4	HYP
	A	0.8523	4	LD

a-GPDH/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.020261	4	MYL
	A	0.019834	4	MAS
B	A	0.015104	4	TRI
B	A	0.015053	4	EDC
B	A	0.014701	4	DIA
B	A	0.014381	4	PNC
B	A	0.012956	4	BH
B	A	0.012860	4	PM
B	A	0.012757	4	ICD
B	A	0.011920	4	IL
B	A	0.011514	4	INT
B	A	0.008513	4	IS
B	A	0.008506	4	DLT
B	A	0.008441	4	HYP
B	A	0.007641	4	EXT
B	A	0.007416	4	OBL
B		0.006217	4	ECM
B		0.006196	4	LD
B		0.004564	4	LAT
B		0.004313	4	RAM
B		0.004133	4	SPD
B		0.003217	4	ICV
B		0.001963	4	RAP

a-GPDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	3.896	4	IL
	A	3.442	4	LD
	A	3.189	4	ECM
	A	3.054	4	ICD
	A	2.934	4	HYP
	A	1.725	4	EXT
	A	1.331	4	RAM
	A	1.200	4	RAP
	A	1.140	4	INT
	A	1.086	4	SPD
	A	0.944	4	TRI
	A	0.912	4	LAT
	A	0.908	4	DLT
	A	0.858	4	DIA
	A	0.827	4	PM
	A	0.817	4	PNC
	A	0.813	4	IS
	A	0.810	4	OBL
	A	0.795	4	MYL
	A	0.776	4	ICV
	A	0.741	4	MAS
	A	0.678	4	EDC
	A	0.557	4	BH

a-GPDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	4.0031	4	IL
	A	3.9175	4	ICD
	A	2.8617	4	LD
	A	2.4752	4	ECM
	A	2.3128	4	HYP
	A	2.2216	4	INT
	A	1.8158	4	EXT
	A	1.7554	4	IS
	A	1.6409	4	PM
	A	1.6088	4	DIA
	A	1.6047	4	TRI
	A	1.5615	4	MYL
	A	1.3667	4	PNC
	A	1.3564	4	DLT
	A	1.2574	4	RAP
	A	1.2510	4	RAM
	A	1.2409	4	MAS
	A	1.2406	4	LAT
	A	1.1979	4	OBL
	A	1.1670	4	SPD
	A	1.0401	4	BH
	A	0.9000	4	EDC
	A	0.8624	4	ICV

CPK/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	13.686	4	MAS
	A			
B	A	11.229	4	BH
B	A			
B	A C	10.786	4	MYL
B	C			
B	D C	7.676	4	DIA
	D C			
E	D C	8.299	4	PM
E	D C			
E	D C	6.055	4	EDC
E	D C			
E	D C	5.892	4	PNC
E	D C			
E	D C	5.723	4	TRI
E	D C			
E	D C	5.721	4	INT
E	D C			
E	D C	5.381	4	DLT
E	D			
E	D	5.139	4	IS
E	D			
E	D	3.479	4	OBL
E	D			
E	D	3.459	4	EXT
E	D			
E	D	3.278	4	LAT
E	D			
E	D	2.214	4	IL
E	D			
E	D	2.063	4	ICD
E	D			
E	D	1.717	4	SPD
E	D			
E	D	1.658	4	HYP
E	D			
E	D	1.639	4	ECM
E	D			
E	D	1.592	4	RAM
E	D			
E	D	1.460	4	RAP
E	D			
E	D	1.361	4	ICV
E				
E		1.084	4	LD

a-GPDH/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.05493	4	ICD
	A			
B	A	0.04430	4	MAS
B	A			
B	A	0.04364	4	INT
B	A			
B	A	0.04317	4	IL
B	A			
B	A	0.04210	4	DIA
B	A			
B	A	0.03862	4	TRI
B	A			
B	A	0.03639	4	EDC
B	A			
B	A	0.03204	4	MYL
B	A			
B	A	0.03176	4	HYP
B	A			
B	A	0.03065	4	EXT
B	A			
B	A	0.03063	4	PM
B	A			
B	A	0.02654	4	BH
B	A			
B	A	0.02566	4	LD
B	A			
B	A	0.02535	4	PNC
B	A			
B	A	0.02415	4	IS
B	A			
B	A	0.02281	4	ECM
B	A			
B	A	0.02266	4	DLT
B	A			
B	A	0.02266	4	SPD
B	A			
B	A	0.02188	4	OBL
B	A			
B	A	0.01678	4	LAT
B	A			
B	A	0.01664	4	RAM
B				
B		0.01346	4	ICV
B				
B		0.00939	4	RAP

GOT/CS

SNK GROUPING	MEAN	N	MUSCLE
A	6.7323	4	BH
A			
B A	6.4600	4	DIA
B A			
B A	6.4126	4	TRI
B A			
B A	6.3638	4	INT
B A			
B A C	5.4745	4	DLT
B A C			
B A C	5.4433	4	PM
B A C			
B A C	4.9300	4	PNC
B A C			
B A C	4.8579	4	LAT
B A C			
B A C	4.6671	4	OBL
B A C			
B A C	4.5668	4	MAS
B A C			
B A C	4.5282	4	IL
B A C			
B A C	4.4921	4	IS
B A C			
B A C	4.3973	4	MYL
B A C			
B A C	4.2333	4	EDC
B A C			
B A C	4.1981	4	ECM
B A C			
B A C	4.1661	4	ICV
B A C			
B A C	3.7419	4	HYP
B A C			
B A C	3.5227	4	ICD
B C			
B C	3.0262	4	RAM
B C			
B C	3.0118	4	EXT
B C			
B C	2.8734	4	SPD
B C			
B C	2.8422	4	RAP
C			
C	2.5157	4	LD

CPK/PK

SNK GROUPING	MEAN	N	MUSCLE
A	30.232	4	MAS
B			
B	23.218	4	BH
B			
C B	21.393	4	INT
C B			
C B	21.367	4	DIA
C B			
C B D	17.100	4	PM
C B D			
C E B D	15.625	4	EDC
C E B D			
C E B D	15.430	4	TRI
C E B D			
C E B D	15.046	4	MYL
C E B D			
C E B D	14.534	4	IS
C E B D			
C E B D	13.500	4	DLT
C E D			
C E D	12.165	4	EXT
C E D			
C E D	12.073	4	LAT
E D			
E D	10.222	4	PNC
E D			
E D	9.665	4	OBL
E D			
E D	9.651	4	SPD
E D			
E D	8.226	4	ICD
E D			
E D	8.169	4	RAP
E D			
E D	7.941	4	IL
E D			
E D	7.254	4	ECM
E D			
E D	6.303	4	HYP
E D			
E D	6.132	4	ICV
E D			
E D	6.131	4	RAM
E			
E	4.605	4	LD

GOT/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.18912	4	DIA
	A	0.18546	4	MAS
	A	0.17632	4	EDC
B	A	0.16801	4	BH
B	A	C 0.15963	4	TRI
B	D A	C 0.12462	4	INT
B	D A	C 0.10312	4	MYL
B	D A	C 0.10258	4	PM
B	D A	C 0.09416	4	PNC
B	D A	C 0.09202	4	ICV
B	D A	C 0.09076	4	DLT
B	D A	C 0.08826	4	OBL
B	D	C 0.06811	4	LAT
B	D	C 0.06407	4	IS
B	D	C 0.06272	4	HYP
B	D	C 0.05977	4	ICD
B	D	C 0.05916	4	EXT
	D	C 0.05502	4	SPD
	D	0.04366	4	IL
	D	0.04245	4	RAM
	D	0.04096	4	ECM
	D	0.02461	4	LD
	D	0.02367	4	RAP

GOT/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.08332	4	MAS
	A	0.08033	4	BH
B	A	0.07006	4	EDC
B	A	C 0.06485	4	MYL
B	A	C 0.06203	4	DIA
B	D A	C 0.05880	4	TRI
E B	D A	C 0.05393	4	PNC
E B	D F	C 0.03907	4	PM
E B	D F	C 0.03541	4	DLT
E B	D F	C 0.03266	4	INT
E	D F	C 0.02894	4	OBL
E	D F	0.02321	4	IS
E	F	0.02018	4	ICV
E	F	0.01853	4	LAT
E	F	0.01684	4	EXT
E	F	0.01614	4	HYP
E	F	0.01463	4	ICD
E	F	0.01312	4	IL
	F	0.01095	4	RAM
	F	0.01029	4	SPD
	F	0.00958	4	ECM
	F	0.00578	4	LD
	F	0.00427	4	RAP

APPENDIX 4. CORRELATION MATRIX BETWEEN MAXIMUM ENZYME ACTIVITIES AND ENZYME ACTIVIY RATIOS OF FIN WHALE MUSCLE.

* = Statistically significant correlation at the 95% confidence level.
All correlations are between data ranks (Spearman correlations).
See Materials and Methods for abbreviations.

	LDH	PK	a-GPDH	CPK	HOAD	CS	GOT
PK/LDH	*-0.77	*-0.44	*-0.25	-0.18	0.04	*-0.32	-0.05
PK/CS	*0.62	*0.73	*0.35	*0.21	*-0.45	-0.15	*-0.31
PK/HOAD	*0.77	*0.83	*0.39	*0.25	*-0.49	0.13	-0.15
LDH/CS	*0.86	*0.76	*0.41	*0.28	*-0.33	0.05	-0.18
LDH/HOAD	*0.91	*0.81	*0.42	*0.27	*-0.41	*0.23	-0.09
HOAD/CS	*-0.64	*-0.61	*-0.29	-0.17	*0.32	*-0.53	-0.11
a-GPDH/LDH	*-0.72	*-0.57	0.14	*-0.21	0.08	*-0.31	-0.11
CPK/LDH	*-0.94	*-0.85	*-0.48	-0.06	0.05	*-0.44	-0.04
a-GPDH/PK	*-0.39	*-0.45	*0.41	-0.11	0.07	-0.19	-0.07
a-GPDH/HOAD	*0.42	*0.45	*0.76	0.18	*-0.41	-0.01	-0.15
a-GPDH/CS	0.11	0.15	*0.66	0.08	*-0.21	*-0.34	*-0.21
GOT/CS	*-0.54	*-0.53	*-0.27	-0.14	-0.14	*-0.62	*0.27
CPK/PK	*-0.75	*-0.88	*-0.49	0.09	0.01	*-0.41	-0.06
GOT/PK	*-0.77	*-0.86	*-0.45	*-0.26	*0.23	*-0.21	*0.34
GOT/LDH	*-0.93	*-0.83	*-0.46	*-0.29	0.19	*-0.29	*0.23

APPENDIX 5. STATISTICALLY SIGNIFICANT DIFFERENCES OF MAXIMUM ENZYME ACTIVITIES AND ENZYME ACTIVITY RATIOS BETWEEN MUSCLES AND BETWEEN SPECIES OF THE ANTARCTIC PHOCID SEALS (22 PAGES).

Muscles are listed in descending order of the mean.
Means with the same letter are not significantly different (95% confidence level).
Differences are based on Student-Newman-Keuls (SNK) multiple range tests.
Abbreviations are listed in Materials and Methods.

LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	812.5	4	LD Crab-eater
	B	564.2	4	DIA Crab-eater
	C	466.5	4	LD Leopard
	D	442.1	4	MAS Crab-eater
	E	387.6	4	MAS Leopard
	F	371.1	4	MAS Weddell
	G	351.7	4	LD Weddell
	H	334.9	3	PSO Weddell
	I	308.3	4	DIA Leopard
	J	281.3	2	DIA Weddell

PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	536.8	4	LD Crab-eater
	B	379.3	4	DIA Crab-eater
	C	373.0	4	MAS Crab-eater
	D	308.3	4	MAS Leopard
	E	300.7	4	LD Leopard
	F	280.6	4	LD Weddell
	G	250.6	4	MAS Weddell
	H	247.9	3	PSO Weddell
	I	221.1	2	DIA Weddell
	J	205.3	4	DIA Leopard

CPK

SNK	GROUPING	MEAN	N	MUSCLE
	A	1448.6	4	MAS Weddell
	A			
	A	1378.0	4	MAS Crab-eater
	A			
	A	1369.9	4	LD Leopard
	A			
	A	1308.6	4	DIA Leopard
	A			
	A	1297.9	4	MAS Leopard
	A			
	A	1295.6	4	LD Crab-eater
	A			
	A	1284.7	2	DIA Weddell
	A			
	A	1269.6	3	PSO Weddell
	A			
	A	1210.8	4	LD Weddell
	A			
	A	1028.2	4	DIA Crab-eater

CS

SNK	GROUPING		MEAN	N	MUSCLE
	A		14.245	4	DIA Crab-eater
	A				
B	A		12.870	2	DIA Weddell
B	A				
B	A	C	9.237	4	LD Crab-eater
B	A	C			
B	A	C	9.197	4	DIA Leopard
B		C			
B		C	7.867	4	MAS Crab-eater
		C			
		C	6.460	4	MAS Weddell
		C			
		C	5.987	4	LD Leopard
		C			
		C	5.770	4	MAS Leopard
		C			
		C	4.977	3	PSO Weddell
		C			
		C	4.645	4	LD Weddell

HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	19.812	4	DIA Crab-eater
	A			
	A	19.700	4	DIA Leopard
	A			
B	A	16.085	2	DIA Weddell
B				
B	C	12.395	4	LD Leopard
B	C			
B	C	12.060	3	PSO Weddell
B	C			
B	C	11.152	4	LD Crab-eater
	C			
D	C	8.170	4	LD Weddell
D	C			
D	C	5.805	4	MAS Crab-eater
D				
D		4.110	4	MAS Weddell
D				
D		3.752	4	MAS Leopard

AGPDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	23.15	4	MAS Crab-eater
	B	18.84	4	MAS Weddell
	C	13.00	4	DIA Crab-eater
	D	9.69	4	LD Crab-eater
	E	8.53	4	MAS Leopard
	F	7.41	4	LD Weddell
	G	6.59	4	LD Leopard
	H	4.77	3	PSO Weddell
	I	3.36	4	DIA Leopard
	J	3.11	2	DIA Weddell

GOT

SNK	GROUPING	MEAN	N	MUSCLE
	A	55.89	4	DIA Crab-eater
	B	54.26	4	MAS Crab-eater
	C	42.27	4	DIA Leopard
	D	35.04	4	LD Crab-eater
	E	32.15	4	LD Leopard
	F	31.92	4	MAS Leopard
	G	21.05	4	MAS Weddell
	H	18.63	2	DIA Weddell
	I	17.15	3	PSO Weddell
	J	16.18	4	LD Weddell

PK/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.84468	4	MAS Crab-eater
	A	0.79830	4	MAS Leopard
	A	0.79345	4	LD Weddell
	A	0.79166	2	DIA Weddell
	A	0.73888	3	PSO Weddell
	A	0.69182	4	LD Crab-eater
	A	0.67975	4	MAS Weddell
	A	0.67828	4	DIA Crab-eater
	A	0.67499	4	DIA Leopard
	A	0.65694	4	LD Leopard

LDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	86.94	4	LD Crab-eater
	A			
	A	85.72	4	LD Weddell
	A			
	A	78.76	4	LD Leopard
	A			
	A	68.67	3	PSO Weddell
	A			
	A	68.08	4	MAS Leopard
	A			
	A	59.08	4	MAS Weddell
	A			
	A	56.45	4	MAS Crab-eater
	A			
	A	49.15	2	DIA Weddell
	A			
	A	41.93	4	DIA Crab-eater
	A			
	A	36.69	4	DIA Leopard

PK/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	67.62	4	LD Weddell
	A			
B	A	58.47	4	LD Crab-eater
B	A			
B	A	54.10	4	MAS Leopard
B	A			
B	A	50.99	3	PSO Weddell
B	A			
B	A	50.03	4	LD Leopard
B	A			
B	A	47.74	4	MAS Crab-eater
B	A			
B	A	40.75	4	MAS Weddell
B	A			
B	A	37.32	2	DIA Weddell
B	A			
B	A	28.84	4	DIA Crab-eater
B				
B		25.00	4	DIA Leopard

LDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	115.54	4	MAS Leopard
B	A	97.75	4	MAS Weddell
B	A	86.24	4	MAS Crab-eater
B	A C	72.66	4	LD Crab-eater
B D	C	55.77	4	LD Weddell
D	C	37.93	4	LD Leopard
D	C	29.34	4	DIA Crab-eater
D	C	27.77	3	PSO Weddell
D		19.33	2	DIA Weddell
D		17.08	4	DIA Leopard

PK/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	90.86	4	MAS Leopard
	A	75.74	4	MAS Crab-eater
B	A	68.14	4	MAS Weddell
B	A C	48.94	4	LD Crab-eater
B	A C	44.66	4	LD Weddell
B	C	24.48	4	LD Leopard
B	C	20.55	3	PSO Weddell
B	C	20.07	4	DIA Crab-eater
B	C	14.99	2	DIA Weddell
	C	11.74	4	DIA Leopard

a-GPDH/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.053311	4	MAS Crab-eater
	A			
	A	0.051783	4	MAS Weddell
	B	0.023027	4	DIA Crab-eater
	B			
	B	0.021966	4	MAS Leopard
	B			
	B	0.020623	4	LD Weddell
	B			
	B	0.014502	3	PSO Weddell
	B			
	B	0.014431	4	LD Leopard
	B			
	B	0.013403	4	LD Crab-eater
	B			
	B	0.012372	4	DIA Leopard
	B			
	B	0.012290	2	DIA Weddell

a-GPDH/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	2.9574	4	MAS Crab-eater
	A			
	A	2.9090	4	MAS Weddell
	B	1.9531	4	LD Weddell
	B			
	B	1.4818	4	MAS Leopard
	B			
C	B	1.1149	4	LD Crab-eater
C	B			
C	B	1.0889	4	LD Leopard
C	B			
C	B	0.9639	4	DIA Crab-eater
C	B			
C	B	0.9633	3	PSO Weddell
C				
C		0.3725	4	DIA Leopard
C				
C		0.2782	2	DIA Weddell

a-GPDH/HOAD

SNK	GROUPING	MEAN	N	MUSCLE
	A	4.7973	4	MAS Weddell
	A			
	A	4.3806	4	MAS Crab-eater
	B	2.4456	4	MAS Leopard
	C	1.3204	4	LD Weddell
	C			
	C	0.8923	4	LD Crab-eater
	C			
	C	0.6787	4	DIA Crab-eater
	C			
	C	0.5292	4	LD Leopard
	C			
	C	0.3958	3	PSO Weddell
	C			
	C	0.1756	4	DIA Leopard
	C			
	C	0.1750	2	DIA Weddell

a-GPDH/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.078269	4	MAS Weddell
	A			
	A	0.065498	4	MAS Crab-eater
	B	0.034171	4	DIA Crab-eater
	B			
	B	0.027733	4	MAS Leopard
	B			
	B	0.025761	4	LD Weddell
	B			
	B	0.021722	4	LD Leopard
	B			
	B	0.019976	3	PSO Weddell
	B			
	B	0.018856	4	LD Crab-eater
	B			
	B	0.018578	4	DIA Leopard
	B			
	B	0.014999	2	DIA Weddell

CPK/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	7.2360	4	DIA Leopard
	A			
B	A	6.0139	4	MAS Weddell
B	A			
B	A	5.8692	2	DIA Weddell
B	A			
B	A C	5.2858	3	PSO Weddell
B	A C			
B	A C	4.7805	4	LD Leopard
B	C			
B	C	4.5306	4	LD Weddell
B	C			
B	C	4.2130	4	MAS Leopard
B	C			
B	C	3.7697	4	MAS Crab-eater
	C			
	C	2.7509	4	DIA Crab-eater
	C			
	C	2.5059	4	LD Crab-eater

CPK/LDH

SNK	GROUPING	MEAN	N	MUSCLE
	A	4.8332	4	DIA Leopard
	A			
	A	4.6727	2	DIA Weddell
	A			
	A	4.0902	4	MAS Weddell
	A			
	A	3.8625	3	PSO Weddell
	A			
B	A	3.5334	4	LD Weddell
B	A			
B	A C	3.3647	4	MAS Leopard
B	A C			
B	A C	3.1565	4	LD Leopard
B	A C			
B	A C	3.1279	4	MAS Crab-eater
B	C			
B	C	1.8436	4	DIA Crab-eater
	C			
	C	1.7399	4	LD Crab-eater

GOT/PK

SNK	GROUPING	MEAN	N	MUSCLE
	A	0.21878	4	DIA Leopard
	B	0.15654	4	MAS Crab-eater
C	B	0.14618	4	DIA Crab-eater
C				
C	D	0.10916	4	LD Leopard
C	D			
C	D	0.10390	4	MAS Leopard
	D			
	D	0.08728	2	DIA Weddell
	D			
	D	0.08697	4	MAS Weddell
	D			
	D	0.07200	3	PSO Weddell
	D			
	D	0.06956	4	LD Crab-eater
	D			
	D	0.06295	4	LD Weddell

GOT/LDH

SNK	GROUPING		MEAN	N	MUSCLE
	A		0.14475	4	DIA Leopard
	A				
	A		0.12500	4	MAS Crab-eater
	B		0.09821	4	DIA Crab-eater
	B				
C	B		0.08276	4	MAS Leopard
C	B				
C	B	D	0.07208	4	LD Leopard
C	B	D			
C	B	D	0.07046	2	DIA Weddell
C		D			
C		D	0.05862	4	MAS Weddell
C		D			
C		D	0.05245	3	PSO Weddell
		D			
		D	0.04894	4	LD Crab-eater
		D			
		D	0.04792	4	LD Weddell

GOT/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	6.9792	4	MAS Crab-eater
	B	5.5578	4	MAS Leopard
	B			
	B	5.3521	4	LD Leopard
	B			
C	B	4.7942	4	DIA Leopard
C				
C	D	4.1072	4	LD Crab-eater
C	D			
C	D	4.0780	4	DIA Crab-eater
	D			
	D	3.5233	4	LD Weddell
	D			
	D	3.4522	3	PSO Weddell
	D			
	D	3.3980	4	MAS Weddell
	E	2.3334	2	DIA Weddell

HOAD/CS

SNK	GROUPING	MEAN	N	MUSCLE
	A	2.4435	3	PSO Weddell
	A			
B	A	2.1473	4	DIA Leopard
B	A			
B	A	2.0944	2	DIA Weddell
B	A			
B	A	2.0785	4	LD Leopard
B	A			
B	A C	1.7227	4	LD Weddell
B	C			
B	D C	1.4590	4	DIA Crab-eater
	D C			
	D C	1.2244	4	LD Crab-eater
	D			
	D	0.7301	4	MAS Crab-eater
	D			
	D	0.6401	4	MAS Leopard
	D			
	D	0.6203	4	MAS Weddell

LDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	606.3	12	Crab-eater Seal
	B	387.5	12	Leopard Seal
	C	343.0	13	Weddell Seal

PK

SNK	GROUPING	MEAN	N	ANIMAL
	A	429.7	12	Crab-eater Seal
	B	271.4	12	Leopard Seal
	C	254.7	13	Weddell Seal

CPK

SNK	GROUPING	MEAN	N	ANIMAL
	A	1325.49	12	Leopard Seal
	A			
	A	1308.92	13	Weddell Seal
	A			
	A	1233.96	12	Crab-eater Seal

CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	10.450	12	Crab-eater Seal
	B	6.985	12	Leopard Seal
	B			
	B	6.545	13	Weddell Seal

HOAD

SNK	GROUPING	MEAN	N	ANIMAL
	A	12.257	12	Crab-eater Seal
	A			
	A	11.949	12	Leopard Seal
	B	9.036	13	Weddell Seal

a-GPDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	15.28	12	Crab-eater Seal
	B	9.66	13	Weddell Seal
	C	6.16	12	Leopard Seal

GOT

SNK	GROUPING	MEAN	N	ANIMAL
	A	48.40	12	Crab-eater Seal
	B	35.45	12	Leopard Seal
	C	18.28	13	Weddell Seal

a-GPDH/LDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	0.029914	12	Crab-eater Seal
	A			
	A	0.027516	13	Weddell Seal
	B	0.016256	12	Leopard Seal

PK/LDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	0.74560	13	Weddell Seal
	A			
	A	0.73826	12	Crab-eater Seal
	A			
	A	0.71008	12	Leopard Seal

PK/CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	50.854	13	Weddell Seal
	A			
	A	45.015	12	Crab-eater Seal
	A			
	A	43.046	12	Leopard Seal

LDH/CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	67.963	13	Weddell Seal
	A			
	A	61.773	12	Crab-eater Seal
	A			
	A	61.176	12	Leopard Seal

LDH/HOAD

SNK	GROUPING	MEAN	N	ANIMAL
	A	62.75	12	Crab-eater Seal
	A			
	A	56.85	12	Leopard Seal
	A			
	A	56.62	13	Weddell Seal

PK/HOAD

SNK	GROUPING	MEAN	N	ANIMAL
	A	48.249	12	Crab-eater Seal
	A			
	A	42.360	12	Leopard Seal
	A			
	A	41.758	13	Weddell Seal

HOAD/CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	1.6220	12	Leopard Seal
	A			
	A	1.6070	13	Weddell Seal
	B	1.1378	12	Crab-eater Seal

a-GPDH/CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	1.7611	13	Weddell Seal
	A			
	A	1.6787	12	Crab-eater Seal
	B	0.9811	12	Leopard Seal

a-GPDH/HOAD

SNK	GROUPING	MEAN	N	ANIMAL
	A	2.0006	13	Weddell Seal
	A			
	A	1.9839	12	Crab-eater Seal
	B	1.0501	12	Leopard Seal

a-GPDH/PK

SNK	GROUPING	MEAN	N	ANIMAL
	A	0.039508	12	Crab-eater Seal
	A			
	A	0.038926	13	Weddell Seal
	B	0.022678	12	Leopard Seal

CPK/PK

SNK	GROUPING	MEAN	N	ANIMAL
	A	5.4099	12	Leopard Seal
	A			
	A	5.3672	13	Weddell Seal
	B	3.0088	12	Crab-eater Seal

CPK/LDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	3.9559	13	Weddell Seal
	A			
	A	3.7848	12	Leopard Seal
	B	2.2371	12	Crab-eater Seal

GOT/PK

SNK	GROUPING	MEAN	N	ANIMAL
	A	0.14395	12	Leopard Seal
	A			
	A	0.12410	12	Crab-eater Seal
	B	0.07617	13	Weddell Seal

GOT/LDH

SNK	GROUPING	MEAN	N	ANIMAL
	A	0.099863	12	Leopard Seal
	A			
	A	0.090716	12	Crab-eater Seal
	B	0.055726	13	Weddell Seal

GOT/CS

SNK	GROUPING	MEAN	N	ANIMAL
	A	5.2347	12	Leopard Seal
	A			
	A	5.0548	12	Crab-eater Seal
	B	3.2853	13	Weddell Seal

APPENDIX 6. CORRELATION MATRIX BETWEEN MAXIMUM ENZYME ACTIVITIES AND ENZYME ACTIVITY RATIOS OF THE 3 ANTARCTIC PHOCID SEAL SPECIES.

* = statistically significant correlation at the 95% confidence level. All correlations are between data ranks (Spearman correlations). See Materials and Methods for abbreviations.

	LDH	PK	CPK	CS	HOAD	a-GPDH	GOT
PK/LDH	-0.17	0.26	0.14	-0.21	*-0.42	0.11	-0.12
PK/CS	0.24	*0.44	0.01	*-0.64	*-0.56	0.01	*-0.39
PK/HOAD	*0.33	*0.52	0.21	-0.31	*-0.91	*0.57	-0.12
LDH/CS	0.31	*0.34	-0.08	*-0.64	*-0.45	-0.08	*-0.49
LDH/HOAD	*0.38	*0.51	0.21	-0.28	*-0.88	*0.59	-0.11
HOAD/CS	-0.21	*-0.36	-0.32	-0.17	*0.69	*-0.71	-0.14
a-GPDH/LDH	-0.02	0.11	0.18	0.16	*-0.45	*0.85	0.17
a-GPDH/CS	0.21	0.31	0.12	-0.26	*-0.75	*0.81	-0.03
a-GPDH/HOAD	0.27	*0.41	0.19	-0.08	*-0.79	*0.87	0.06
a-GPDH/PK	0.04	0.08	0.19	0.18	*-0.38	*0.85	0.23
CPK/PK	*-0.86	*-0.94	*0.45	-0.31	0.01	*-0.42	*-0.35
CPK/LDH	*-0.92	*-0.77	*0.59	*-0.33	-0.17	-0.31	*-0.36
GOT/PK	-0.13	-0.27	0.04	*0.55	*0.49	0.02	*0.75
GOT/LDH	-0.17	-0.17	0.14	*0.52	*0.34	0.09	*0.78
GOT/CS	0.16	0.21	0.09	-0.15	-0.22	0.15	*0.58