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REVIEW

Role of Histidine-Related Compounds as Intracellular Proton Buffering Constituents in Vertebrate Muscle

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Abstract—The intracellular non-bicarbonate buffering capacity of vertebrate muscle is mainly supported by the imidazole groups of histidine residues in proteins, free L-histidine in some fish species, and histidine-containing dipeptides such as carnosine, anserine, and balenine (ophidine). The proton buffering capacity markedly differs between muscle types and animal species depending on the ability for anaerobic exercise. The capacity is typically high in fast-twitch glycolytic muscles of vertebrates adapted for anaerobic performance such as burst swimming in fishes, prolonged anoxic diving in marine mammals, flight in birds, sprint running in mammalian sprinters, and hopping locomotion in some terrestrial mammals. A high correlation between buffering capacity, concentration of histidine-related compounds in muscle, and percentage of fast-twitch fibers in all vertebrates adapted for intense anaerobic performance clearly supports the idea that proton buffering is the main physiological function of histidine-related compounds.[1]

Key words: histidine, carnosine, anserine, balenine, buffering capacity, vertebrate, muscle, anaerobic exercise

During high-intensity anaerobic exercise, a large number of protons accumulate in vertebrate muscle as ATP is hydrolyzed to ADP [1]. The proton accumulation causes a decrease in intracellular pH (pH_i) which, in turn, causes the inactivation of glycolytic enzymes such as phosphofructokinase and hence a decrease in glycolytic flux [2]. The decrease in glycolytic flux due to low pH_i is also found during prolonged anoxia of perfused rat heart [2]. The changes of pH_i are known to affect the rate of other various metabolic functions [2]. As a defense mechanism against changes in pH_i, proton buffering systems may evolve in the cell. Thus, a high buffering capacity in muscle can stabilize intramuscular pH and enhance the capability for anaerobic exercise performance or anoxia tolerance [2, 3].

The intracellular non-bicarbonate buffering of vertebrate muscle is dominated by the imidazole group which exists in histidine residues of proteins, in free L-histidine, and in histidine-containing dipeptides such as carnosine, anserine, and balenine (also known as ophidine) [1-4]. Because the p*K* values of these imidazole groups are close to pH_i, one of the two nitrogens of the imidazole ring can be protonated in the physiological range of pH. Thus, imidazole groups are utilized as potent proton buffering constituents. The regulatory process keeping pH_i close to the p*K* values of imidazole groups is called "alphastat regulation". Its role is to maintain α-imidazole relatively constant (α-imidazole being defined as non-protonated imidazole/(non-protonated imidazole + protonated imidazole)). Typical α_{Imid} is conserved at a value of about 0.55 in intracellular fluid [3]. Inorganic orthophosphate also serves as a typical inorganic buffer component in addition to imidazole compounds (Table 1).

The proton buffering capacity markedly differs between muscle types and animal species depending on the ability for anaerobic exercise [3]. This review mainly focuses on the relationship between the buffering capacities of histidine-related compounds (HRC) and the anaerobic capabilities of vertebrate skeletal muscle.

[1] Editor's note: Carnosine is now well known to be an efficient intracellular pH buffer (V. Skulachev, H. Abe), hydrophilic antioxidant (A. Boldyrev, E. Decker), heavy metal chelator (P. Trombley, E. Baran), potent anti-glycating agent (A. Hipkiss), and regulator of many specific receptors (F. Margolis, A. Fasolo, D. Miller) and enzymes (I. Severina, S. Stvolinsky). These features make this compound useful to treat ischemic brain and heart (D. Dobrota, P. Roberts, G. Zaloga) and to decelerate some senescence processes (A. Wang, S. Gallant, R. Holliday, G. McFarland). Many of these properties of carnosine are discussed in this volume.

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Table 1. Apparent p*K* values of imidazole groups (according to [3-5])

Substance	p*K*
Typical histidyl-imidazole in proteins	6.5 (25°C)
adjacent to acidic (–) group	7-8 (25°C)
adjacent to basic (+) group	5-6 (25°C)
L-Histidine	6.21 (20°C)
Carnosine	7.01 (20°C)
Anserine	7.15 (20°C)
Balenine	6.93 (20°C)
Inorganic orthophosphate	6.88 (20°C)

Note: p*K* measurements were performed at temperatures specified in parentheses.

TELEOST FISHES

Proton buffering capacity (termed β value and measured as the "slyke" unit) is defined as the μmoles of sodium hydroxide or hydrogen chloride required to change the pH of one gram of tissue by one unit, i.e., from 6 to 7 or from 6.5 to 7.5 [4, 6-8]. Table 2 shows β values, total HRC contents, and lactate dehydrogenase (LDH) activities in the white and red muscles of teleost fishes having a wide variety of anaerobic performance capabilities. Fish myotomal muscle comprises two functionally different muscle fiber types, red and white, as it is the case in other vertebrates. In fish, however, the two muscles are separated spatially. Fish red muscle exists as a thin triangular strip running longitudinally beneath the lateral line (superficial red muscle) or located more axially, extending to the vertebrae (deep-seated red muscle as in tuna fishes). Fish red muscle is an aerobic slow-twitch oxidative tissue and it is recruited during sustained, steady state swimming. In contrast, fish white muscle mainly consists of fast-twitch glycolytic fibers

Table 2. Buffering capacity (β), concentration of histidine-related compounds (HRC), and lactate dehydrogenase (LDH) activity in fish muscle (according to [4, 6, 8-13])

Species	β		HRC		LDH
	WM	RM	WM	RM	WM
Endothermic scombrids					
Katsuwonus pelamis (skipjack tuna)	122 ± 6	81 ± 34	109-148	21-42	2056
Thunnus alalunga (albacore tuna)	115 ± 13	58 ± 17	71-121	20-23	3451 ± 518
Auxis thazard (frigate mackerel)	109	—	110*	—	1186
Thunnus albacares (yellowfin tuna)	108 ± 11	82	74-91	10	2185 ± 285
Euthynnus lineatus (black skipjack tuna)	105 ± 8	83 ± 15	73-105**	11**	1572 ± 448
Active pelagic ectotherms					
scombrids					
mean for 4 species	99 ± 14	67 ± 7	16-31***	7-13***	1574 ± 267
non-scombrids					
mean for 11 species	63.3	—	<40****	<12****	455 ± 189
Deep-sea and demersal fishes					
mean for 9 species	46 ± 3	—	trace	—	59 ± 53

Note: Values (means ± SD) are expressed as μmole NaOH per pH unit per gram of wet weight of muscle (pH 6-7) in case of β, as μmole per gram of wet weight of muscle in case of HRC, and as activity units per gram of wet weight of muscle in case of LDH. Abbreviations: WM, white muscle; RM, red muscle; –, not determined.

* Values for *Auxis tapeinocephalus*.

** Values for *Euthynnus affinis*.

*** Values for chub mackerel *Scomber japonicus*.

**** Values for sardine *Sardinops melanostictus*.

and is recruited during anaerobic burst locomotion only lasting for short times.

The white muscle shows much higher β values and HRC contents than red muscle in a given species. HRC contents in red muscle correspond generally to one-tenth to one-quarter of the contents in the white muscle of various fish species [4]. The β values and HRC contents are typically high in endothermic scombrids such as tuna fishes. Tunas have a large mass of deep-seated red muscle surrounded by the "rete mirabile" (dense capillary network) used as a vascular countercurrent heat exchanger that maintains muscle temperature above ambient temperature by up to 10°C [3]. Tunas contain 60-100 μmol/g muscle wet weight of free L-histidine and 15-60 μmol/g muscle wet weight of anserine in their white muscle [4], and show over 100 slykes of proton buffering capacity (Table 2). In the white muscle of skipjack tuna, for instance, total HRC content reaches about 150 mM. A large amount of free L-histidine is also found in salmonids and cyprinids and is thought to have the same physiological function as histidine-containing dipeptides [4, 9]. Tunas also have the highest LDH activity (Table 2) found in any animals including mammals and they show very high capacities for anaerobic glycolysis. These observations clearly support the idea that high muscle buffering capacity is necessary to support the high speed, long duration, and increasing frequency of burst swimming typically seen in tuna fishes.

As seen in Table 2, ectothermic scombrids such as chub mackerel or Pacific bonito have lower β values and HRC contents than warm-bodied tuna [11]. Of the actively foraging pelagic ectotherms, non-scombrids such as sardine, rainbow trout, and several bass species have much lower β and HRC in their white muscle. Of these fish species, sardine *Sardinops* spp. contains 40 μmol/g of free L-histidine and rainbow trout contains about 20 μmol/g of anserine in white muscle, but other white-fleshed fishes have only trace amounts of HRC, even in their white muscle. Deep-sea and demersal fishes, with more sluggish locomotor activities, are found to have the lowest buffering capacities, HRC levels, and LDH activities [3, 8, 11].

The correlation between β values and LDH activities is shown in Fig. 1 for various fish species listed in Table 2. These two parameters are highly correlated (r = 0.83), indicating that buffering capacity is high in the white muscles having high glycolytic capacities and hence in species with high burst exercise capability [8, 11].

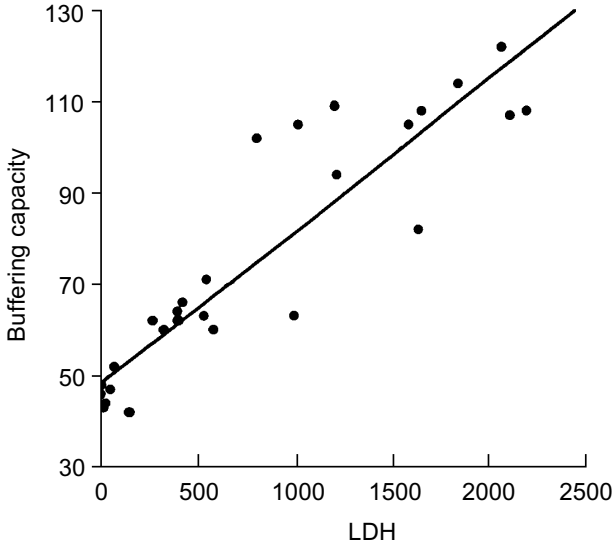

Fig. 1. Correlation between lactate dehydrogenase activity (units/g muscle) and buffering capacity (μmol NaOH per pH unit per g muscle, pH 6-7) in fish white muscle (data compiled from references [8, 11, 16]).

MARINE MAMMALS

Table 3 shows muscle β values, HRC contents, and LDH activities in several diving mammals and birds, as well as terrestrial animals for comparison. The muscles of these animals contain less than 1 μmol/g of free L-histidine but a rather large amount of one of the dipeptides. Whale muscle is unique because it contains a large amount of balenine, ranging from 20 to 80 μmol/g [14]. Whale typically shows β values as high as warm-bodied fishes. Marine diving mammals, which contain a rather large amount of carnosine in their skeletal muscle, have higher average β than terrestrial mammals [8]. LDH activity does not differ significantly between marine and terrestrial mammals, but correlates closely with β [8]. The β value also correlates strongly with muscle myoglobin concentration in these diving mammals [8]. Thus, HRC buffering may prevent a decrease in muscle pH during prolonged breath-hold diving, when oxygen stores are depleted and large amounts of lactate and protons produced.

TERRESTRIAL ANIMALS

Interspecies differences in β values and HRC contents are very large among terrestrial animals. As shown in Table 4, terrestrial mammals and birds have large amounts of carnosine or anserine, or even a small amount of balenine. Birds typically have a larger amount of anserine than carnosine, and total HRC contents are higher in white breast muscle of chicken and turkey than in red leg muscle. This distribution pattern of HRC is also found in deer muscle, which contains the highest amount of balenine in the terrestrial mammals thus far examined. Mammals containing higher anserine than carnosine are rather limited to some special species such

Table 3. Buffering capacity (β), concentration of histidine-related compounds (HRC), and lactate dehydrogenase (LDH) activity in the muscle of marine and terrestrial animals (according to [8, 15, 16])

Animals	β	HRC	LDH
Marine animals			
little-piked whale	111	68.5	—
spotter porpoise	84.1	—	1222
northern fur seal	79.1	—	1120
harbor seal	76.2	43.9	1379
Weddell seal	72.1	—	1270
sea lion	61.5	—	707
sea otter	70.6	—	801
Adelie penguin	70.0	—	2076
Terrestrial animals			
chicken (*pectoralis minor*)	82.8	43.5	—
pig (*psoas muscle*)	78.6	23.3	—
(*biceps femoris*)	63.2	15.4	615
ox (*biceps femoris*)	69.0	18.3	1016
rabbit	66.9	—	1887
dog	50.2	—	772

Note: See the note of Table 2 for units of β, HRC, and LDH activity; –, not determined.

as kangaroo, goat and sheep, rabbit, and deer which show hopping locomotion [4]. In contrast, carnosine content is higher in ox, pig, horse, and many other mammalian muscles. As seen in Table 3, buffering capacities of these terrestrial mammals and birds are as high as those of the active pelagic fishes shown in Table 2.

Rao and Gault [17] quantified the characteristics of bovine muscle for five white muscles abundant in fast fibers and seven red muscles abundant in slow fibers (Table 5). Carnosine content is significantly higher in white than in red muscle whereas no significant difference is seen in anserine content. In white muscle, inor-

Table 4. Concentration of histidine-related compounds in the muscle of terrestrial mammals and birds (according to [15])

Animals	Muscles	*n*	Carnosine	Anserine	Balenine
Ox	leg	5*	26.1 ± 3.7	5.94 ± 1.75	0.103 ± 0.026
Pig	leg	6*	29.5 ± 9.6	1.42 ± 0.29	1.77 ± 0.71
Horse	leg	3**	42.6 ± 12.6	0.176 ± 0.030	0.019 ± 0.004
Deer	leg	5**	3.35 ± 0.68	13.9 ± 1.93	3.91 ± 0.74
Chicken	leg	3*	5.70 ± 1.7	17.1 ± 3.7	0.055 ± 0.028
	breast	4*	10.4 ± 1.3	32.0 ± 1.4	0.197 ± 0.031
Turkey	leg	3**	4.53 ± 0.68	20.5 ± 1.9	0.077 ± 0.009
	breast	2**	11.2 ± 1.3	46.0 ± 0.8	0.810 ± 0.023

Note: Values are expressed as μmol/g wet weight (means ± SD).
* Average from the same muscle from different animals.
** Average from different muscles of one animal.

ganic phosphate is also significantly higher than in red muscle. After 48 h post-mortem, these muscles show an intracellular pH below 6, lower in white than in red. They exhibit no significant difference in buffering capacity from pH_i to 5 (Table 5), but significantly differ from each other in β from pH_i to more acidic pH values [17]. Thus, a strong correlation exists between carnosine and inorganic phosphate contents, and buffering capacity in bovine muscle.

Mammalian muscle is a mixture of three fiber types, slow-twitch oxidative red fiber (type I), fast-twitch oxidative-glycolytic white fiber (type IIA), and fast-twitch glycolytic white fiber (type IIB). Table 6 shows the HRC and taurine contents in the middle gluteal muscle of camel [18]. The carnosine and anserine contents of both type IIA and IIB fibers are significantly higher than those of type I fiber but there is no significant difference between type IIA and IIB. In contrast, taurine is much more concentrated in type I fiber in camel, as is the case in fish muscle; taurine content is much higher in fish red muscle than white and is thought to contribute to red muscle buffering [6].

Figure 2 represents muscle homogenate buffering and the major compounds contributing to buffering in the pH range of 6.5-7.5 [4, 16]. The relative contribution of contractile proteins to total buffering is a few percents, while soluble proteins contribute from 9 to 38%. The contribution of soluble proteins is rather high for dark-fleshed fishes containing a lot of myoglobin such as tuna and mackerel. The contributions of HRC in whale skeletal muscle, skipjack tuna white muscle, and marlin white muscle are as high as 25, 40, and 60%, respectively. Blue marlin, *Makaira nigricans*, contains 120 μmol per g of anserine in white muscle on average [4, 6]. The contribution of HRC is also high for bovine, porcine, and chicken muscle, ranging from 12 to 23%, but it is only 1 to 6% for carp and flounder white muscle that contain only a small amount of HRC. In contrast, the contribution of inorganic phosphate to total muscle β is high in the white muscle of trout, carp, and flounder, ranging from 50 to 80%. The concentration of inorganic phosphate is rather species independent and includes phosphate liberated from organic phosphates such as phosphocreatine and ATP. In the case of mammalian muscle, a rather high contribution is attributed to unknown compounds, which may include some nucleotides, organic acids, and taurine. These data indicate that the large variation in muscle β is, therefore, mainly due to changing levels of HRC. Thus, the accumulation of HRC in muscle increases the muscle β and, hence, the muscle anaerobic capability. This strategy is used by animals such as tunas, billfishes such as marlin, and marine mammals that show an elevated capacity for burst anaerobic swimming or for anoxia tolerance.

Table 5. Characteristics of bovine white and red muscle (according to [17])

Parameter	White muscles	Red muscles
White fiber**, %	58.0 ± 2.7	37.5 ± 3.6
Red fiber**, %	41.9 ± 2.7	59.8 ± 4.3
Carnosine*, g per 100 g wet weight	0.41 ± 0.06	0.29 ± 0.04
Anserine, g per 100 g wet weight	0.052 ± 0.018	0.043 ± 0.010
Inorganic phosphate*, g per 100 g wet weight	0.18 ± 0.02	0.15 ± 0.01
pH_i*	5.48 ± 0.06	5.89 ± 0.19
β (pH_i 5.0), μmol H^+ per g wet weight	46.4 ± 4.8	41.8 ± 5.1

Note: Fiber types were based on myosin ATPase staining method. pH_i means intracellular pH. Value of β (pH_i 5.0) means the buffering capacity (milliequivalents HCl per 100 g muscle required to lower the muscle pH from pH_i to 5.0). Values are means ± SD for five different white muscles and for seven different red muscles.
* $p < 0.01$.
** $p < 0.001$.

Table 6. Concentration of histidine-related compounds and taurine in the *middle gluteal* muscle of camel (according to [18])

Compound	Type I	Type IIA	Type IIB
Carnosine	23.6 ± 5.3	37.2 ± 8.6*	45.6 ± 8.0*
Anserine	28.4 ± 5.4	38.4 ± 9.8**	35.6 ± 6.4*
Total HRC	52.0 ± 8.6	75.5 ± 12.7*	81.2 ± 10.8*
Taurine	41.9 ± 10.1	24.1 ± 7.9*	23.3 ± 11.5*

Note: Values are means ± SD for four post-mortem muscles as mmol per kg dry weight. Type IIA and IIB are significantly different compared with type I muscle.
* $p < 0.05$.
** $p < 0.01$.

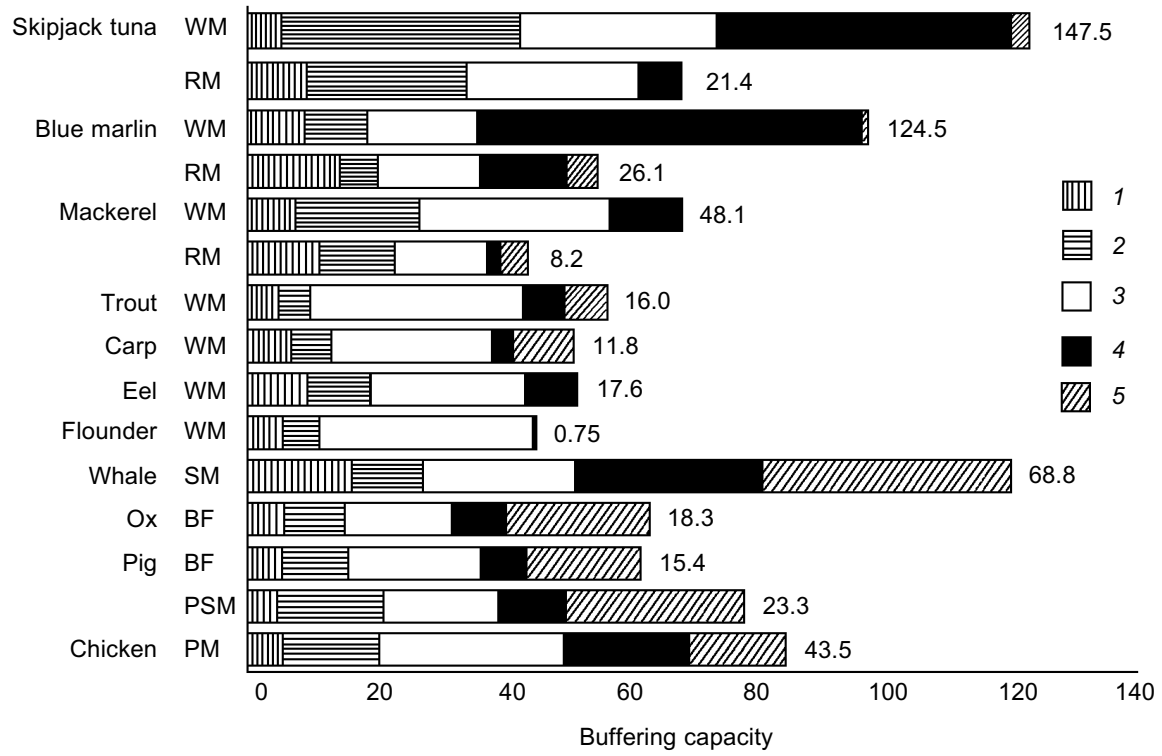

Fig. 2. Contribution of proteins (contractile (*1*) and soluble (*2*)), inorganic orthophosphate (*3*), histidine-related compounds (*4*), and unknown compounds (*5*) to the buffering capacity of muscle homogenate from several vertebrates. Buffering capacity is expressed as μmol NaOH per pH unit per g muscle over pH 6.5-7.5. Values indicated on the bars represent the concentration of total histidine-related compounds (μmol/g muscle). WM, white muscle; RM, red muscle; SM, skeletal muscle; BF, *biceps femoris*; PSM, psoas muscle; PM, *pectoralis minor* (from reference [4]).

MAMMALIAN SPRINTERS

The white muscle fibers (type IIA and IIB) are especially abundant in the muscle of outstanding mammalian sprinters such as thoroughbred horse (Table 7). The superficial portion of the middle gluteal muscle contains many type IIB fibers and the deep portion many type IIA and I fibers [19]. Estimated carnosine levels are over two times higher in type IIB than in IIA (Table 7). The contribution of carnosine to the total buffering

Table 7. Fiber types, carnosine content, and buffering capacity of the *middle gluteal* muscle of thoroughbred horse (according to [19])

Parameter	Type I	Type IIA	Type IIB
Fiber section area, %			
superficial (<4 cm, n = 10)	5.1 ± 4.2	28.9 ± 6.1	66.0 ± 7.8
deep (<9 cm, n = 10)	21.3 ± 6.0	46.0 ± 5.5	32.7 ± 7.9
Estimated carnosine, μmol per g dry weight	54 ± 15	85 ± 15	180 ± 15
β_{total}	88	98	130
$\beta_{carnosine}$	18	28	60
$\beta_{carnosine}/\beta_{total}$, %	20	29	46

Note: Buffering capacity (β) is microequivalent of H^+ per g muscle dry weight required to lower the pH from 7.1 to 6.5.

Table 8. Effects of breed of horse on muscle fiber types and carnosine content (according to [20])

Parameter	Quarter Horses (n = 6)	Thoroughbreds (n = 6)	Standardbreds (n = 5)
Type I fiber, %	12.2 ± 1.1[a]	15.0 ± 1.8[b]	16.2 ± 0.6[b]
Type IIA fiber, %	49.0 ± 1.8[a]	61.0 ± 2.8[b]	60.0 ± 1.2[b]
Type IIB fiber, %	38.8 ± 1.0[a]	24.0 ± 1.8[b]	23.8 ± 1.2[b]
Carnosine, μmol per g wet weight	39.2 ± 1.8[a]	31.3 ± 2.9[b]	27.6 ± 0.6[b]

Note: Percentages of fibers are based on the staining negative for succinate dehydrogenase for fast twitch glycolytic fiber and for myosin ATPase for slow twitch oxidative fiber.
[a, b] Values in the same horizontal line with different superscript letters are significantly different ($p < 0.05$).

capacity in muscle is estimated to be 46% in type IIB fiber.

The muscle fiber type distribution and carnosine levels also differ between breeds of horses (Table 8). Of the middle gluteal muscle of Quarter Horses (sprinter), thoroughbreds, and standardbreds, type IIA fibers are lowest and type IIB are highest in Quarter Horses. Carnosine content is also the highest in Quarter Horses. From these results, we can conclude that horses selected for sprinting have a higher percentage of fast-twitch glycolytic fibers and require more muscle buffering capacity [20].

As shown in Table 9, the β value is also high in the muscles of another sprinter, the greyhound dog, compared with human muscle [21]. Significant differences in β_{total} exist between man, horse, and dog ($p < 0.001$). Total HRC is also high in greyhound as well as thoroughbred horse. The contribution of HRC to β_{total} reaches the high value of 25% in greyhound dogs.

HUMANS

As seen in Table 9, human muscle contains a rather small amount of HRC, which contributes little to β_{total}. The carnosine level in the *quadriceps femoris* muscle of human volunteers is significantly higher ($p < 0.05$) in male than in female subjects of similar age and training status (Table 10), although large inter-individual differences are found in both sexes [22]. As shown in Table 11, the carnosine level is twice as high in type II fibers of human *vastus lateralis* muscle compared with type I [23]. In contrast, the taurine level is four times higher in type I than type II muscle fibers as described earlier in camel and fish. This is also true in thoroughbred horses [24], where the contribution of carnosine to muscle β_{total} is 9.4% in type II but only 4.5% in type I fibers.

Parkhouse et al. [25] characterized the effects of exercise training on muscle fiber types, HRC levels, and β values in human *vastus lateralis* muscle (Table 12). Four groups of five subjects were compared: highly trained 800-m sprinters, rowers (varsity oarsmen), endurance trained marathon runners, and untrained controls. The marathon runners were significantly ($p < 0.05$) older than the other groups. Body weight and fat

Table 9. Dipeptide content and buffering capacity in the muscle of thoroughbred horse, greyhound dog, and human (according to [21])

Parameter	Horse*	Dog**	Human***
Carnosine, μmol per g dry weight	108.3 ± 15.9	33.0 ± 19.1	16.0 ± 7.2
Anserine, μmol per g dry weight	n.d.	48.6 ± 18.4	n.d.
β_{total}	117.7 ± 8.5	105.2 ± 9.1	79.5 ± 8.0
$\beta_{dipeptide}$	36.0 ± 5.3	26.0 ± 10.1	5.3 ± 2.4
$\beta_{dipeptide}/\beta_{total}$, %	30.6	24.7	6.7

Note: See the legend of Table 7 for buffering capacity; n.d., not detected.
* *Middle gluteal* muscle (n = 20).
** Mean ± SD for five different muscles in four dogs.
*** *Vastus lateralis* muscle (n = 20).

Table 10. Carnosine content in the *quadriceps femoris* muscle of male and female humans (according to [22])

Parameter	Male (n = 33)	Female (n = 17)	All subjects (n = 50)
Carnosine, μmol per g dry weight			
means ± SD	21.3 ± 4.2	17.5 ± 4.8	20.0 ± 4.7
range	12.5-30.7	7.2-27.7	7.2-30.7
Age, years	22.5 ± 3.4	21.7 ± 4.0	22.4 ± 3.8
Body mass, kg	78.0 ± 11.0	65.1 ± 7.7	73.6 ± 11.7

Note: Means ± SD of carnosine content are significantly different between male and female ($p < 0.05$).

Table 11. Buffering capacity and carnosine and taurine contents in the human *vastus lateralis* muscle (n = 4) (according to [23])

Parameter	Type I	Type II
Taurine, μmol per g dry weight	39.2 ± 17.8	9.6 ± 2.6
Carnosine, μmol per g dry weight	10.5 ± 7.6	23.2 ± 8.1
β_{total}	77.5	81.7
$\beta_{carnosine}$	3.5	7.7
$\beta_{carnosine}/\beta_{total}$, %	4.5	9.4

Note: See the legend of Table 7 for buffering capacity.

reserves were also significantly higher in untrained controls than in the other groups (data not shown). With respect to anaerobic speed test (high-intensity running performance), the sprinters performed significantly better than the rowers who also performed significantly better than the marathoners and untrained controls. Post-exercise blood lactate differed significantly between the sprinters, rowers, and the other two groups. Significant differences in the fast-twitch percentage were found between the groups ($p < 0.05$). Muscle buffering capacity was significantly higher in the sprinters and rowers than in the marathoners and untrained subjects. Results show no significant difference in histidine levels, but carnosine levels are significantly elevated in the sprinters and rowers. Significant ($p < 0.05$) correlations are found between β and carnosine levels ($r = 0.69$), and β and fast-twitch percentage ($r = 0.51$). Overall, these data suggest that repetitive high-intensity anaerobic exercise causes muscle adaptations that include an increase in percent fast-twitch fibers and muscle β. The observed increase in carnosine levels may contribute, at least partly, to the increase of muscle β values.

Since the early works of Bate-Smith [7] and Davey [26], the importance of non-bicarbonate intracellular buffering of vertebrate muscle has been demonstrated. In this review, I show that the buffering capacity is typically high in the fast-twitch glycolytic and anaerobic white muscle of vertebrates adapted for anaerobic performance such as burst swimming, prolonged breath-hold diving, flight, sprint running, and intense hopping locomotion. These activities are especially necessary for animals dwelling in the open oceans, grassy plains, or in the sky where they allow them to escape predators or to catch their prey. Imidazole buffer systems appear to have evolved in the muscles of vertebrates requiring high burst exercise capabilities or high anoxia tolerance for their survival. The fact that buffering capacity, HRC content, and percent fast-twitch fibers are highly correlated for a wide variety of vertebrate muscles supports the idea that the main physiological function of HRC is proton buffering. Inorganic phosphate, histidine residues in proteins, and organic phosphate compounds also contribute to proton buffering. However, the cellular concentration of these other buffering components

Table 12. Buffering capacity and histidine-related compounds in human *vastus lateralis* muscle with special reference to different exercise training (according to [25])

Groups	Age, years	Anaerobic speed test, sec	Post-load blood lactate, mM	Fast twitch, %	β, μmol per g per pH unit	Histidine, μmol per g	Carnosine, μmol per g
Sprinters (n = 5)	20.6 ± 2.3	115 ± 18**	21.9 ± 1.5**	56.6 ± 7.0	30.03 ± 5.6*	0.64 ± 0.06	4.93 ± 0.76*
Rowers (n = 5)	20.6 ± 1.8	76 ± 9*	13.9 ± 0.9*	50.4 ± 12.3	31.74 ± 7.2*	0.71 ± 0.10	5.04 ± 0.72*
Marathoners (n = 5)	37.8 ± 9.3	53 ± 15	10.1 ± 3.1	33.0 ± 12.2	20.83 ± 4.4	0.63 ± 0.14	2.80 ± 0.74
Untrained (n = 5)	22.6 ± 0.9	38 ± 9	10.1 ± 2.6	50.6 ± 9.9	21.25 ± 5.0	0.89 ± 0.29	3.75 ± 0.86

Note: Values are means ± SD. Buffering capacity (β) is μmol H^+ per g muscle wet weight required to change the pH by one unit over the pH range 7.0-6.0.

* Significantly higher ($p < 0.01$) than in marathoners and untrained.

** Significantly higher ($p < 0.01$) than in all other groups.

may be constrained because their primary physiological roles have nothing to do with buffering. In contrast, HRC, and especially the metabolically inert dipeptides among them, can be stored in large amounts without harmful side effect to the cell. Finally, combining varying amounts of different HRC constituents, each with their own p*K* values (ranging from 6.21 to 7.15, see Table 1) may provide the necessary flexibility to cope with the particular physiological challenges of each vertebrate species.

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