9 Diving metabolism

The regulation of diving metabolism has been the subject of much investigation, discussion, and debate. Since the experiments of Irving and Scholander (Irving, 1939, Irving *et al.*, 1941b, Scholander, 1940, Scholander *et al.*, 1942a, 1942b), the significance of a low rate of oxygen consumption and slow depletion of oxygen stores has long been emphasized. It is the rate of metabolism during the breath-hold period that is the critical determinant of breath-hold capacity, the duration of aerobic metabolism, and any need for anaerobic metabolism. Despite many investigations and experimental approaches, however, the metabolic rate during a dive has remained an elusive parameter to measure or even estimate.

There do not appear to be any unique molecular mechanisms of hypometabolism in marine mammals and seabirds (see review below). Low metabolic rate during a dive is most likely secondary to (a) regulation of heart rate and tissue perfusion; (b) regional hypothermia, and (c) a low cost of locomotion during the dive. Actual measurement of that metabolic rate during the dive has been difficult. This chapter will focus on the measurement and interpretation of whole-body metabolic rates in diving animals. Scholander's findings during forced submersions will be summarized first, followed by a review of potential mechanisms of metabolic suppression. Next, the terms *basal metabolic rate* and *resting metabolic rate* will be defined as these are essential control reference values from which to evaluate the level of hypometabolism. Fourth, metabolic rate determination techniques and the difficulty of assigning a value for metabolic rate during a breath hold in intermittently breathing animals will be reviewed. Lastly, estimates of diving metabolic rate from the literature will be summarized.

9.1 Forced submersions and metabolic rate

Diving metabolism is the end result of the many physiological processes and biomechanical adaptations reviewed in previous chapters. Heart rate, organ perfusion, body temperature, muscle workload, buoyancy, hydrodynamics, and the costs of hunting and prey capture/digestion all potentially contribute to the rate of metabolism and oxygen store depletion during a dive. In his 1940 monograph, Scholander reported that the pre-submersion oxygen consumption of the seals in his study was elevated and that the seals incurred an oxygen debt during submersion. That oxygen deficit was not fully accounted for by oxygen store depletion, glycolysis, or excess post-submersion oxygen

consumption. He concluded that the submerged seal reduced its metabolism relative to the elevated metabolic rate of the pre-submersion period, i.e., it was hypometabolic. As reviewed in Chapters 5, 7, and 8, that decreased metabolic rate was most probably due to the observed decline in heart rate/organ perfusion and possibly also to decreased body temperatures with hypothermia-related declines in tissue metabolic rate.

In relation to muscle tissue metabolic rate during forced submersion, it should also be noted that rate of decline of muscle O_2 content during the forced submersion of seals was elevated, at five times the typical resting rate of oxygen consumption in mammalian muscle (Blei *et al.*, 1993, Kooyman and Ponganis, 1998, Scholander, 1940). During the anaerobic phase of the forced submersion, the measured muscle lactate accumulation and presumed creatine phosphate depletion could account for maintenance of that elevated muscle metabolic rate (Kooyman and Ponganis, 1998). Consequently, there was no evidence of hypometabolism at the muscle-tissue level during the forced submersion of seals.

Scholander also found that ducks did not repay their oxygen debts, but again, changes in heart rate and possibly body temperature probably contributed to the lower metabolic rate. Notably, in a study 40 years later, there was no evidence for a decrease in ATP turnover rates at the tissue level in muscle (Stephenson and Jones, 1992b). Interestingly, in contrast to seals and ducks, high ventilatory rates in Scholander's forcibly submerged penguins more than accounted for the oxygen shortfall during their submersions. Thus, there was no indirect evidence of a decline in metabolism in the forcibly submerged penguin.

9.2 Biochemical mechanisms of metabolic suppression

At this point, it is worth reviewing the potential role of metabolic suppression in limiting the rate of metabolism during diving. As mentioned briefly in Chapter 7, there is no evidence that the decrease in metabolic rate during the forced submersions of seals is secondary to any unique underlying mechanism at the tissue level. Hypoxia-linked metabolic suppression was proposed to account for low diving metabolic rates and less-than-expected increases in post-dive blood lactate concentrations of Weddell seals (*Leptonychotes weddellii*) in the mid-1980s (Guppy *et al.*, 1986, Hochachka, 1988). This hypothesis of downregulation of metabolism in diving seals was based on the lack of a compensatory increase in lactate concentration (a reverse Pasteur effect) in anoxia-tolerant lower vertebrates (Hochachka, 1986a, 1986b, 1986c, Hochachka and Guppy, 1987, Hochachka and Somero, 1984). In fish, amphibians, and reptiles that tolerate prolonged dormant periods of hypoxia/anoxia, the decrease in metabolic rate is primarily based upon ion channel arrest (decreased Na-K ATPase activity, and density) and arrest of protein synthesis at the level of translation (Bickler and Buck, 2007, Hochachka and Lutz, 2001, Hochachka *et al.*, 1996). Metabolic rate is also decreased in hibernating mammals (Malan, 2014, Quinones *et al.*, 2014), but again, these animals are not travelling and foraging during hibernation.

In mammalian tissues, hypoxic metabolic suppression has been demonstrated, usually in cell cultures, but only after exposures to extremely low concentration of

oxygen (i.e., P_{O2}: 10 mm Hg or 1.33 kPa) for prolonged periods of time (30 min to hours) (Wheaton and Chandel, 2011). Cellular mechanisms of such metabolic suppression in mammalian cells include: (a) decreased Na-K-ATPase activity (probably through endocytosis of the enzyme); (b) decreased sensitivity of contractile myofilaments in "hibernating" heart muscle; (c) stabilization of hypoxia-inducible factor 1 (HIF 1), which (1) limits electron transport chain activity through several mechanisms including alteration of the expression of cytochrome c oxidase subunits, and (2) upregulates expression of glycolytic enzymes and also expression of pyruvate dehydrogenase kinase which decreases the activity of pyruvate dehydrogenase, decreasing conversion of pyruvate to acetyl-coA; (d) inhibition of protein synthesis at the level of mRNA translation via activation of the mTOR (mammalian target of rapamycin) pathway; and (e) activation of adenosine monophosphate protein kinase (AMPK) (promotes endocytosis of Na-K-ATPase and activates mTOR pathway) (Arai *et al.*, 1991, 1995, Arsham *et al.*, 2003, Comellas *et al.*, 2006, Emerling *et al.*, 2009, Hudson *et al.*, 2002, Iyer *et al.*, 1998, Liu *et al.*, 2006, Semenza, 2004, 2007).

Such metabolic suppression may also occur in the cells of seals on exposure of cell cultures to prolonged, severe hypoxia, but whether this explains the low metabolic rate, hypoxic tolerance, and diving ability of seals is another question for several reasons. First, the tissues of most mammals are not tolerant of extreme hypoxia, and, therefore, despite the observation of these mechanisms of hypoxic metabolic suppression in mammalian cell cultures, they do not afford additional protection to severe ischemia and hypoxemia in the typical mammal, and, therefore, do not yet account for the hypoxic tolerance of seals. Second, most of these responses require hours of exposure to extreme hypoxemia (P_{O2} of 10 mm Hg or 1.33 kPa), far longer and lower than the hypoxemia in dives of most seals, even elephant seals. Third, metabolic suppression, especially as demonstrated in lower vertebrates, occurs during periods of dormancy or hibernation, not during diving and hunting. Although various physiological indices of renal, hepatic, and neural function are better preserved in seals than terrestrial controls during hypoxic/anoxic exposure (Folkow *et al.*, 2008, Halasz *et al.*, 1974, Hochachka *et al.*, 1988, Hong *et al.*, 1982, Koschier *et al.*, 1978), the underlying mechanisms of such protection and their contribution to metabolic rate reduction are still unknown.

Two additional possible mechanisms of metabolic suppression under hypoxic conditions involve the gasotransmitters hydrogen sulfide (H_2S) and nitric oxide (NO). H_2S is known to bind to complex IV (cytochrome c) of the mitochondrial respiratory transport chain, and such inhibition may represent a mechanism of metabolic suppression (Blackstone *et al.*, 2005, Kolluru *et al.*, 2013). In a similar fashion, the inhibition of cytochrome c by NO may also be a mechanism of metabolic suppression under hypoxic conditions (Fago *et al.*, 2012, Helbo *et al.*, 2013, Shiva *et al.*, 2007).

Inhalational administration of H_2S to mice can suppress metabolic rate and induce suspended animation (Blackstone *et al.*, 2005). Recently, changes in H_2S metabolism have been postulated to underlie the metabolic suppression found in hibernating bears (Revsbech *et al.*, 2014). H_2S production also increases during hypoxia (Kolluru *et al.*, 2013, Olson *et al.*, 2006). Consequently, although there is no evidence available in diving mammals and birds, H_2S could potentially contribute to a decrease in metabolic rate during hypoxia in these animals. Presumably, H_2S accumulation in the brain under such conditions would be minimal as suspended animation is not compatible with active diving.

The generation of NO from nitrite by the nitrite reductase activity of deoxyhemoglobin and deoxymyoglobin has been postulated to represent a mechanism whereby NO's inhibition of cytochrome oxidase may suppress metabolic rate during hypoxia as well as limit the generation of oxygen free radicals after ischemia-reperfusion events (Fago *et al.*, 2012, Flogel *et al.*, 2010, Jensen *et al.*, 2014, Shiva *et al.*, 2007, Soegaard *et al.*, 2012). Although the nitrite reductase activities of different whale myoglobins are not elevated, the high concentration of myoglobin in whale muscle creates the potential for significant NO formation by dexoymyoglobin from nitrite under hypoxic conditions (Helbo and Fago, 2012). Elevated blood nitrite levels in porpoises also support the potential for the use of this pathway to titrate metabolic rate during hypoxia (Soegaard *et al.*, 2012). The full significance of this potential pathway of metabolic regulation in diving animals remains to be investigated.

Metabolic suppression in diving marine mammals may also be partly secondary to ischemic preconditioning, a process in which a brief period of tissue ischemia can decrease ATP depletion rates and improve tissue survival during subsequent ischemic episodes (see Chapter 13). In addition, other mechanisms of suppression may involve the development of hypercarbia and intracellular acidosis during apnea (Elsner, 2015, Malan, 2014) and, at least in some marine mammals, elevated levels of endogenous carbon monoxide (Kajimura *et al.*, 2010, Pugh, 1959, Tift *et al.*, 2014). Hypercarbia has been associated with reduced metabolic rates during hibernation, and carbon monoxide binds to cytochrome oxidase, decreasing oxygen flux.

9.3 Basal and resting metabolic rates

Basal metabolic rate is the oxygen consumption of an awake, adult, post-absorptive animal at rest in a thermoneutral environment; it is typically calculated on the basis of classic allometric equations for birds ($L\ O_2\ min^{-1} = 0.0132$ (body $mass^{0.729}$) with mass in kilograms) or mammals ($L\ O_2\ min^{-1} = 0.0101$(body $mass^{0.75}$) with mass in kilograms) (Aschoff and Pohl, 1970, Kleiber, 1975). The term *standard metabolic rate* has been considered equivalent to the basal metabolic rate, but it has also been used to distinguish the metabolic rate measured under the above conditions as opposed to basal metabolic rate predicted by allometric equations (Hurley and Costa, 2001).

The conditions specified for basal metabolic rate determinations are difficult to achieve in experimental situations, and rarely occur in the wild. Indeed, elevations of oxygen consumption in animals "at rest" during experiments have been attributed to anticipatory increases in metabolic rate under these conditions (Taylor *et al.*, 1987, Williams *et al.*, 1993). Thus, resting metabolic rate of an animal in a study may be different from predicted basal metabolic rate. Furthermore, whether the standard conditions have been satisfied during measurements of metabolic rate of marine mammals at rest has been controversial (Hurley and Costa, 2001, Lavigne *et al.*,

1986, Williams *et al.*, 2004). Suffice it to say that metabolic rate measured at rest in marine mammals is often 1.4–3.0 times the allometrically predicted basal metabolic rate (see previous references). This elevation in metabolic rate at rest in marine mammals has also been attributed to thermoregulatory requirements, body composition, and high-protein diets, and has even been found to be associated with large alimentary tracts (specifically, the length of the small intestine) (Lavigne *et al.*, 1986, Williams *et al.*, 2001b).

9.4 Metabolic rate measurements

Oxygen consumption measurements in mammals and birds have most commonly been measured with respirometry techniques, doubly labeled water techniques, and heart rate measurements. Another approach, the Fick method, can also provide estimation of oxygen consumption, but it requires accurate measurements of cardiac output and the arterio-mixed venous oxygen content difference. Despite the value of this approach, the required arterial and pulmonary artery catheterizations have made it impractical in marine mammals and seabirds. In terrestrial mammals, the Fick method is more often used to calculate cardiac output on the basis of measured oxygen consumption and the arterio-venous oxygen difference (Taylor *et al.*, 1987). Consequently, respirometry, doubly labeled water, and heart rate have been the three basic approaches used to assess oxygen consumption in diving mammals and birds.

Open flow respirometry requires the animal to breathe into a chamber or mask that allows for accurate measurement of (a) the rate of airflow through the system; and (b) the fractional oxygen concentration exiting the system (Fedak *et al.*, 1981, Withers, 2001). While this approach has been applied to many marine mammals and seabirds, its disadvantage is that the animal must return to the breathing chamber in order for the analysis to be conducted. Potentially, this technique is applicable to animals in laboratory tanks and swim flumes, to animals trained to breathe beneath a dome at sea, and to wild animals freely diving under sea ice at an isolated dive hole to which they must return. However, it is not applicable to free-diving animals at sea. In addition, even at an isolated dive hole on the sea ice of McMurdo Sound, although this technique has been used with great success in Weddell seals (Castellini *et al.*, 1992b, Kooyman *et al.*, 1973a, Ponganis *et al.*, 1993a, Williams *et al.*, 2004), this approach is not necessarily feasible for other species. Emperor penguins (*Aptenodytes forsteri*), for example, prefer to dive in groups and will not tolerate a dome over the dive hole as they usually exit the water after each dive.

Another limitation of respirometry in estimation of oxygen consumption during diving is that the measurements are only made during the surface interval. The oxygen consumed during the surface interval is a function of the metabolic rate and duration of the dive as well as the metabolic rate and duration of the surface interval. Thus, this measurement does not directly represent measurement of the actual oxygen consumption rate during the dive. Various approaches have been used to calculate "diving metabolic rate" with respirometry. In a method based on Scholander's original study

in 1940, oxygen consumption during the submersion period was calculated from the volume of oxygen consumed above the baseline level during the surface interval until the post-submersion oxygen consumption returned to the baseline level. That oxygen volume was then divided by the duration of the submersion and was considered to represent the oxygen consumption rate during the submersion (Hurley and Costa, 2001, Williams *et al.*, 2004). The limitation to this method is that, on a theoretical basis, oxygen consumption during the hyperventilation and tachycardia of the surface interval is not necessarily equivalent to that consumed at rest when the heart rate is lower, organ perfusion is lower, and the cost of breathing is less.

In another approach used by Castellini and co-workers, the entire amount of oxygen consumed during a surface interval between dives (or until oxygen consumption returned to baseline levels during prolonged surface intervals) was divided by the time of the dive and surface interval (or the time of the dive and the surface interval time until baseline levels were reached) (Castellini *et al.*, 1992b). This provided a metabolic rate of the dive event (dive plus surface interval). The limitation of this method is that it does not represent the actual rate of oxygen consumption during the dive. In ducks and Humboldt penguins (*Spheniscus humbodti*), multiple linear regressions of volume of oxygen consumed against surface interval duration and dive duration have provided estimation of the oxygen consumed during the dive (Bevan *et al.*, 1992, Butler and Woakes, 1984, Stephenson, 1994, Woakes and Butler, 1983). The limitation here is that the regression coefficients only represent the mean oxygen consumption rates of dive and surface intervals at their mean durations.

Another approach to estimate metabolic rate of marine mammals and seabirds is the doubly labeled water (DLW) technique (Nagy, 1980, Speakman, 1998). It is based on the facts that labeled oxygen is removed from the body by both carbon dioxide and water, while labeled hydrogen is removed only via water. Hence, the rate of carbon dioxide production (and indirectly, oxygen consumption) can be calculated from the difference between the elimination of the two labels (Nagy, 1980, Speakman, 1998). These techniques have been applied extensively to many species of marine mammals and seabirds to measure field metabolic rates in the wild (Nagy *et al.*, 1999, Shaffer, 2011). While valuable in ecological modeling, application of DLW field metabolic rates to estimation of diving metabolic rate is limited because the DLW field metabolic rate is determined over a period of many days, and, thus, is not directly applicable to individual dives, groups of dives, or different types of dives. For example, use of either the field metabolic rate or the estimated foraging metabolic rate determined with DLW in emperor penguins significantly underestimated the duration of aerobic metabolism during dives of these birds (Nagy *et al.*, 2001). Furthermore, many validation studies of the DLW technique in comparison to respirometry have revealed a wide range of individual error (i.e., –40 to +80% differences), although differences between mean values of groups are usually much closer and acceptable (Bevan *et al.*, 1995a, 1995b, Boyd *et al.*, 1995, Butler *et al.*, 2004, Sparling *et al.*, 2008). Although field metabolic rates may be useful to begin to assess the potential range for diving metabolic rate (Costa *et al.*, 2001), these limitations in use of the DLW data should be remembered.

The third approach to estimate metabolic rate in marine mammals and seabirds is the heart rate method, in which the linear relationship between oxygen consumption and heart rate in an exercising animal (Bevan *et al.*, 1995b, Boyd *et al.*, 1995, Fedak, 1986, Kooyman and Ponganis, 1994, Williams *et al.*, 1991, 1992c) is used to predict metabolic rate (Butler, 1994, Butler *et al.*, 2004). This technique is based on the assumption that stroke volume and the arterio-venous difference in oxygen content is constant (or at least the average values over the exercise period are constant). However, stroke volume in seals is not necessarily constant between eupnea and apnea, or surface and submerged swimming (Ponganis *et al.*, 1990). In addition, the arterio-venous difference in oxygen content can also change throughout a breath hold (Kerem and Elsner, 1973). Therefore, use of a heart rate–oxygen consumption relationship determined in a swim flume may not be accurate to predict the oxygen consumption during or throughout a dive. However, given that values will average out over long periods of time, such an approach has been used to estimate a field metabolic rate (Bevan *et al.*, 1995a, 2002, Boyd *et al.*, 1999, Butler *et al.*, 2004, Froget *et al.*, 2004, Green *et al.*, 2003).

9.5 Diving metabolic rates: marine mammals

In consideration of diving metabolism and hypometabolism, it is important to define the resting value to which "hypometabolism" is referred. For example, mean oxygen consumption values of prolonged submergences of voluntarily submerged seals and sea lions, although less than values "at rest," were still not less than the allometrically predicted basal metabolic rates (Hurley and Costa, 2001, Webb *et al.*, 1998). The values "at rest" were 1.3 to 3.0 times the allometrically predicted rate. Therefore, it is not surprising that metabolic rates associated with longer submergences were less than values measured "at rest" (Hurley and Costa, 2001, Scholander, 1940, Webb *et al.*, 1998) or that metabolic rate declined as breath-hold time or percent of time submerged increased in other studies (Castellini *et al.*, 1992b, Fedak *et al.*, 1988, Gallivan, 1981, Thorson, 1993, Tift *et al.*, 2013, Webb *et al.*, 1998). As discussed in prior chapters, these changes in metabolic rate are probably secondary to perfusion-related declines in tissue oxygen consumption associated with slower heart rates during submersion as well as to possible changes in thermoregulatory requirements. However, in consideration of the need for further "downregulation" of metabolism at the cellular level, it is notable that the metabolic rates associated with submergences in almost all these studies were not below the allometrically predicted basal metabolic rate. Most of these studies were in laboratory situations. Field metabolic rates of young, translocated elephant seals at sea were, on average, about 1.5 times greater than allometrically predicted resting rates. (Maresh *et al.*, 2014). In contrast, estimations of the metabolic rate required to maintain aerobic metabolism during the repetitive, long-duration dives of elephant seals at sea predict significant "hypometabolism" with estimated metabolic rates as much as 60% below the allometrically predicted resting metabolic rate (Hindell *et al.*, 1992, Le Boeuf *et al.*, 1988).

Diving metabolic rate has been determined with respirometry in two phocid pinnipeds, the Weddell seal and the gray seal (*Halichoerus grypus*). In Weddell seals diving at an isolated dive hole beneath the sea ice of McMurdo Sound, diving metabolic rates were variable and decreased with increasing dive duration (Castellini *et al.*, 1992b, Kooyman *et al.*, 1973a, Ponganis *et al.*, 1993a). Average diving metabolic rate was 4.5 ml O_2 kg^{-1} min^{-1}, about 1.6 times the predicted basal metabolic rate, but not significantly different from values measured from seals at rest (Castellini *et al.*, 1992b). In the gray seal study, which involved captive animals "diving" in an underwater maze, diving metabolic rate was also variable and decreased with slower swim speeds and longer dive durations. Diving metabolic rate again averaged 1.7 times the predicted basal metabolic rate and was less than the measured resting metabolic rate at the surface (Sparling and Fedak, 2004).

A later study of Weddell seals equipped with a video camera and accelerometer further analyzed the contribution of feeding and stroke rate to the variability in diving metabolic rate (Williams *et al.*, 2004). In general, diving metabolic rates were similar to those of the previous Weddell seal studies. Feeding during dives resulted in a 45% increase in diving metabolic rate in comparison to that of fasting dives of similar duration and distance traveled. The elevation in diving metabolic rate could continue as long as five hours after fish ingestion. Locomotor activity increased diving metabolic rate 1.3–3.5 times above resting rates dependent on dive duration. Oxygen consumption increased linearly with the number of strokes during a dive, and the average cost per stroke was 0.044 ml O_2 kg^{-1} min^{-1}. Thus, diving metabolic rate was found to be a function of dive duration, number of strokes, and feeding.

However, in other species, prey ingestion may not always immediately result in digestion and an increase in metabolic rate. In gray seals, the elevation in metabolic rate associated with digestion (specific dynamic action) can be delayed for periods of many hours after foraging behavior (Sparling *et al.*, 2007). In summary, although average diving metabolic rates of seals are above allometrically predicted basal metabolic rates, they are usually near the metabolic rate of an animal at rest. Overall, the cost of diving is quite low.

The cost of diving also appears to be low in trained Steller sea lions (*Eumetopias jubatus*) in that diving metabolic rates determined by respirometry were near resting metabolic rates at the surface (Fahlman *et al.*, 2008, Hastie *et al.*, 2007). Resting rates were 55–85% above the allometrically predicted resting metabolic rate. Diving metabolic rate did decrease with dive duration; metabolic rate during short-duration dives could be as high as 25 ml O_2 kg^{-1} min^{-1}, while most longer-duration dives (4–7 min) had metabolic rates of 6–10 ml O_2 kg^{-1} min^{-1}.

In sea otters (*Enhydra lutris*), mean diving metabolic rate of single dives was 17.6 ml O_2 kg^{-1} min^{-1}, and that of foraging dives was 22 ml O_2 kg^{-1} min^{-1} (Yeates *et al.*, 2007). The single-dive value, equivalent to that of a sea otter swimming horizontally underwater (Williams, 1989), was 1.3 times measured resting metabolic rate and 3.7 times the allometrically predicted metabolic rate. The foraging dive value was 1.6 and 4.8 times those respective values. Once again, diving metabolic rate was relatively close to the measured resting metabolic rate. The relatively high diving and resting metabolic rates of sea otters were attributed to high maintenance costs (thermoregulation, digestion) and high locomotory costs.

9.6 Diving metabolic rates: birds

Oxygen consumption rates in voluntarily diving birds were above metabolic rates measured at rest. In tufted ducks and lesser scaups (*Aythya fuligula*, *A. affinis*), oxygen consumption measurements from surface intervals revealed metabolic rates approximately 3.5 times that measured at rest (Bevan and Butler, 1992b, Bevan *et al.*, 1992, Stephenson, 1994, Woakes, 1988, Woakes and Butler, 1983). Oxygen consumption in Humboldt penguins voluntarily diving in a tank was less elevated, about 26% above resting levels (Butler and Woakes, 1984). In all these species, heart rate during dives was also elevated above resting rate (see Chapter 5) in agreement with the oxygen consumption data. Thus, in these voluntarily diving birds in tanks, there was no evidence of a reduction in oxygen consumption relative to rates at rest.

However, as reviewed in Chapter 6, the longer dive durations of deep-diving species at sea have raised the question of potential metabolic rate reductions by other mechanisms such as hypothermia and severe bradycardia (Bevan *et al.*, 1997, 2002, Boyd and Croxall, 1996, Butler, 2000, 2004, Handrich *et al.*, 1997, Wright *et al.*, 2014). Although foraging metabolic rates of penguins range from four to nine times basal metabolic rate (for a review, see Nagy *et al.*, 2001), oxygen consumption measurements during dives of penguins at sea have yet to be determined.

Due to the difficulty and theoretical limitations of respirometry, DLW, and heart rate techniques in estimating diving metabolic rate, the actual oxygen store depletion rate during dives of emperor penguins at an isolated dive hole was assessed by measurement of changes in air sac P_{O2}, arterial and venous hemoglobin saturation, and muscle myoglobin saturation (Meir and Ponganis, 2009, Stockard *et al.*, 2005, Williams *et al.*, 2011a). Based on the changes in these oxygen stores for dives of about 6-min duration (such dives are assumed to be aerobic as they are not associated with increases in post-dive lactate concentrations (Ponganis *et al.*, 1997c)), the average actual oxygen store depletion rate during such dives was 6.8 ml O_2 kg^{-1} min^{-1}, slightly greater than the allometrically predicted basal metabolic rate (Williams *et al.*, 2011a). That value is also about one-third the field metabolic rate determined with DLW in penguins at the isolated dive hole, and equivalent to the oxygen consumption rates of emperor penguins resting in water in a flume, or standing in their thermoneutral zone (Williams *et al.*, 2011a). Thus, although oxygen store depletion rates are probably variable secondary to the physiological responses associated with the nature and circumstances of a given dive, this finding confirmed that the cost of diving was indeed quite low in emperor penguins, just as in seals.

9.7 Diving metabolic rates: summary

In conclusion, available diving metabolic rates in both seals and penguins indicate that the cost of diving is quite low. However, as yet, there is no evidence of unique molecular mechanisms of metabolic suppression in actively diving birds and mammals. In seals, mean diving metabolic rates are typically above the allometrically predicted basal metabolic rate, but often near or below metabolic rate measured in the animal at

rest. In the emperor penguin, the actual oxygen store depletion rate is near both the rate measured at rest and also that predicted by allometric equations. In contrast, in other avian species, diving metabolic rates are typically above the metabolic rate measured at rest. The variability in diving metabolic rate is secondary to multiple factors, including dive duration, the degree of bradycardia and peripheral vasoconstriction, regional hypothermia, locomotory effort, and digestion. The role of other mechanisms such as hypoxic metabolic suppression in reduction of diving metabolic rate is not yet established, but has often been invoked to account for the routine long-duration dives of "surfacers" such as elephant seals.