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Intestinal lactase activity in southern right whale calves (*Eubalaena australis*)

KEVIN D. KOHL,¹ Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112, U.S.A.; CARINA F. MARÓN, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112, U.S.A. and Instituto de Conservación de Ballenas, CC 39, Buenos Aires, 1623, Argentina; ANDREA CHIRIFE and MATÍAS DI MARTINO, Southern Right Whale Health Monitoring Program, Los Alerces 3376, Puerto Madryn, 9120, Chubut, Argentina; M. DENISE DEARING, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112, U.S.A.; VICTORIA J. ROWN-TREE, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112, U.S.A. and Whale Conservation Institute/Ocean Alliance, Gloucester, Massachusetts 01930, U.S.A.

Suckling mammals receive nutrients and energy from milk produced by their mothers. Lactose is a common carbohydrate found in the milk of most mammals and is an important energy source for mammalian offspring (Karasov and Martinez del Rio 2007). However, the milk composition and nutrient utilization of baleen whales is poorly understood compared to other mammalian groups (Ofstedal 1997). Several recent studies have measured carbohydrate composition in the milk of some baleen whales (Bryde's whale, *Balaenoptera edeni*; sei whale, *Balaenoptera borealis* Lesson; and the minke whale, *Balaenoptera acutorostrata*), and found them to produce milk with considerable lactose content (Urashima *et al.* 2002, 2007). Thus, it has been suggested that baleen whale calves may utilize lactose as an energy source (Urashima *et al.* 2002, 2007). However, it is unknown whether baleen whales have the ability to digest lactose. Measurements of the lactase activity in the intestines of suckling baleen whales could indicate their potential to utilize lactose sugar as an energy source.

As lactose enters the small intestine, a lactase enzyme in the brush border hydrolyzes the majority of this sugar, producing monosaccharides that can be transported across the gut lining (Stevens and Hume 1995). The young of mammals that produce lactose-rich milk (such as cattle, horses, and primates) tend to have high lactase

¹Corresponding author (e-mail: kevin.kohl@utah.edu).

activity in their intestines (Auricchio *et al.* 1965, Siddons 1968, Roberts *et al.* 1973, Urashima *et al.* 2012). Conversely, the young of some mammalian groups (such as monotremes, marsupials, and pinnipeds) that produce milk lower in lactose content tend to have low or absent intestinal lactase activity. (Messer and Kerry 1973; Stewart *et al.* 1983; Crisp *et al.* 1987, 1988, 1989a, 1989b; Messer *et al.* 1987). This pattern is congruent with the adaptive modulation hypothesis, which states that activities of digestive enzymes should match levels of substrates in the diet to avoid wasting biosynthetic energy or available membrane space (Diamond and Hammond 1992). However, lactase activity has never been measured in the gut of a suckling baleen whale.

We investigated the presence of lactase activity in baleen whales by measuring enzyme activity in the small intestines of southern right whale calves (*Eubalaena australis*) that stranded at Península Valdés, Argentina, in 2010 and 2011. Small samples of intestinal tissues were collected from seven dead southern right whale calves, of ages one day to three months old by the Southern Right Whale Health Monitoring Program (SRWHMP) research team. For consistency, samples were collected from the proximal small intestine (duodenum), adjacent to the stomach. Total body lengths and state of decomposition were recorded for each calf (Uhart *et al.* 2008, 2009). Condition code 2 included fresh dead animals with normal appearance, not bloated, little scavenger damage, fresh smell, minimal drying or wrinkling of skin, eyes and mucous membranes, clear eyes and tongue, and penis not protruded (Geraci and Lounsbury 2005). Condition code 3 included dead animals with an intact carcass but bloating evident (tongue and penis protruded), with skin cracked and sloughing, scavenger damage possible, characteristic mild odor, dry mucous membranes, and sunken or missing eyes (Geraci and Lounsbury 2005). Samples were not collected from calves in more advanced states of decomposition since most organs were liquefied. Tissues were packed in ice in the field, stored in -20°C freezers and transported to the University of Utah for biochemical assays.

The presence of lactase activity was measured following a previously developed colorimetric method (Dahlqvist 1984). Roughly 1 g of intestinal tissue was thawed on ice. The mucosa was cut away from muscle layers and homogenized in 350 mMol mannitol in 1 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes)-KOH buffer, pH 7.0. To isolate the brush border, an aliquot of homogenate (1,400 μL) was then centrifuged at $8,200 \times g$ for 60 min at 4°C . The supernatant was removed and the pellet was reconstituted in 1,400 μL of Hepes-KOH buffer. Lactase activity was measured by incubating 30 μL of tissue with 30 μL of 56 mM lactose in a 0.1 M maleate and NaOH buffer at 37°C for 20 min. The assay was conducted multiple times for each individual with maleate-NaOH buffers of pH values ranging from 5.0 to 7.5 in 0.5 increments. Next, 400 μL of a stop-develop reagent (GAGO-20 glucose assay kit; Sigma Aldrich, St. Louis, MO) was added to each tube, which was then vortexed and incubated at 37°C for 30 min. Finally, 400 μL of 12 NH_2SO_4 was added to each tube. Several 200 μL aliquots were transferred to a 96-well plate and the absorbance was read at 540 nm using a BioTek PowerWave HT microplate spectrophotometer (Broadview, IL). Protein content was measured using a Bradford assay and a standard curve generated using bovine serum albumin. Activity (in $\mu\text{mol}/\text{mg}$ protein/min) was determined by comparing absorbance values to a standard curve using the above assay and known amounts of glucose. For each sample, the relative enzyme activity was calculated as a function of pH, expressed as activity at a particular pH divided by maximal activity at the pH that was found to be optimal in the specific sample.

To ensure that we had isolated brush-border lactase and were not measuring intracellular β -galactosidase activity, lactase activity was measured in the presence of 1 mM 4-(hydroxymercuri) benzoic acid, which inhibits β -galactosidase activity. We found that this compound did not significantly alter measured lactase activity ($P = 0.58$) and thus, concluded that our measurements were of brush-border lactase activity. Maltase activity was also measured at pH 6.5 in four randomly chosen samples using the above protocol with maltose as the substrate for comparison. Given that various digestive enzymes exhibit different rates of inactivation when exposed to high temperature (Dahlqvist 1959), we cannot be certain that our study was not affected by differential postmortem changes. Despite this uncertainty, our analyses should be informative regarding the presence of lactase activity, especially given the difficulty of acquiring samples from live baleen whale calves.

The seven calves from which small intestinal lactase activities were assayed ranged in size from 4.1 to 7.3 m in length (mean 5.19 ± 0.42 m) and were between one day and three months old. All calves in our study were likely still nursing given that lactation lasts a year in most right whales (Thomas and Taber 1984, Hamilton 1995) and that milk was found in the esophagus and stomach of three of the seven dead calves (SRWHMP, unpublished data). Six of the sampled calves were in body condition 2. One whale that was in body condition 3 had the highest lactase and maltase activities, and so was included in the analysis.

The lactase enzyme exhibited a pH optimum of 6.5 (Fig. 1), which has been demonstrated in other mammals (Sall and Férard 2003). Whale calves exhibited an average brush-border lactase activity of 6.69 ± 2.18 $\mu\text{mol/g}$ protein/min. Maltase activity was higher, with an average activity of 20.82 ± 8.35 $\mu\text{mol/g}$ protein/min. Interestingly, these values are similar to activity measured in a variety of other mammals that consume milk low in lactose (Table 1).

In most mammals, lactase activity decreases over the course of development and after weaning (Henning 1985). We were unable to find any differences in lactase activity between calves of different lengths; however, our sample size was extremely limited and all calves were still likely to be in the early stages of nursing (Thomas and Taber 1984, Hamilton 1995).

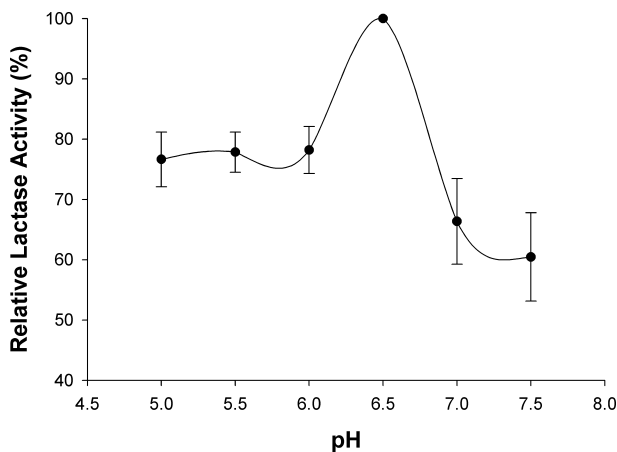


Figure 1. pH optimum of brush-border lactase from southern right whale calves.

Table 1. Intestinal lactase and maltase activities of suckling mammals that consume low-lactose or high-lactose milk.

	Lactase Activity ($\mu\text{mol/g}$ protein/min)	Maltase Activity ($\mu\text{mol/g}$ protein/min)	Reference
Southern right whale (<i>Eubalaena australis</i>)	6.7	20.8	This study
Low-lactose milk			
Echidna (<i>Tachyglossus aculeatus</i>)	14.5	18	Stewart <i>et al.</i> (1983)
Brush-tail possum (<i>Trichosurus vulpecula</i>)	6.0	Not measured	Crisp <i>et al.</i> (1989b)
Crabeater seal (<i>Lobodon carcinophagus</i>)	12	16	Crisp <i>et al.</i> (1988)
High-lactose milk			
Human (<i>Homo sapiens</i>)	56	94	Auricchio <i>et al.</i> (1965)
Cow (<i>Bos taurus</i>)	58.6 ^a	4.8	Siddons (1968)

^aDetermined by averaging the proximal intestinal sections of animals 4–43 d old.

This study is the first to measure intestinal lactase and maltase activities in suckling cetaceans. Many aquatic mammals supply carbohydrates to their young in the form of oligosaccharides, which may be important for providing antimicrobial activity, serving as prebiotics, or fueling the growth of large brains (Urashima *et al.* 2009; Eisert *et al.* 2013, 2014). However, recent studies have suggested that baleen whales may utilize other saccharides such as lactose as an energy source (Urashima *et al.* 2002, 2007). Indeed, we found detectable lactase activity in the intestines of southern right whale calves, suggesting that they could utilize lactose as an energy source. This is in contrast to some animals, such as macropod marsupials, where lactase activity is completely absent (Crisp *et al.* 1987). However, the measured lactase activity was quite low, and similar to that of other mammals that produce low-lactose milk (Table 1). One explanation (stated earlier) for the low lactase activity could be the postmortem degradation of digestive enzymes. However, it is also possible that the milk of southern right whales has low-lactose content, causing the calves to maintain low levels of lactase activity as predicted by the adaptive modulation hypothesis. Additional measurements of lactase activities in other whale species and milk composition studies will help to elucidate the relative importance of lactose and other saccharides in the nutrition of baleen whale calves.

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