Magnetic resonance imaging and its applications in morphological studies of pinnipeds

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The Saimaa ringed seal (*Phoca hispida saimensis*) is an endangered endemic subspecies of Lake Saimaa, Finland. Due to its threatened status, the morphology and physiology of the subspecies cannot be studied using live individuals. Magnetic resonance imaging (MRI) is an accurate research method to study normal anatomy and pathology of the subspecies. A female seal cadaver was examined with MRI in three orientations. Morphological structures were identified and verified by reference slice sectioning and dissection. The results provide a morphological MRI reference database for ringed seals and for other pinnipeds. Moreover, pathological and possibly infectious lesions were visible in the blubber and in the apex of the lung. In addition to morphological studies of pinnipeds, MRI can supplement conventional autopsies.

Introduction

The first complete morphological studies of pinnipeds were carried out by Murie (1868, 1870). Later studies concentrated on selected anatomical regions of particular interest, such as the circulatory system (Munkacsi & Newstead 1985, Newstead & Munkacsi 1986, MacDonald *et al.* 1995), the sensory anatomy (Pardue *et al.* 1993), and the musculoskeletal system (Koster *et al.* 1990). Computer tomography (CT) and magnetic resonance imaging (MRI) have been used extensively to study the morphology of diverse species, such as the Caspian seal *Phoca caspica* (Endo *et al.* 1999), the Northern elephant seal *Mirounga angustirostris* (Thornton *et al.* 2001, 2005) and odontocetes (Cranford *et al.* 1996, Marino *et al.* 2001a, 2001b, 2002, 2004, Ridgway *et al.* 2002).

The Saimaa ringed seal (*Phoca hispida saimensis* Nordquist, 1899) is an endangered subspecies (Sipilä *et al.* 1990, Sipilä 1991, 2003, Reijnders *et al.* 1993, Ranta *et al.* 1996) endemic to Lake Saimaa (61°05′–62°36′N, 27°15′–30°00′E) in Finland. Its diet consists of small fish species (Kunnasranta *et al.* 1999, Kunnasranta 2001). The subspecies differentiated approximately 8000 years ago (Hyvärinen &



Fig. 1. (a) The axial, (b) sagittal and (c) coronal orientations used in the magnetic resonance imaging.

Nieminen 1990) and is morphologically different from the Baltic (*P. h. botnica*) and the Ladoga (*P. h. ladogensis*) subspecies. Ringed seals are among the smallest seals with a body mass (BM) of 90–100 kg and a body length of 105–145 cm (Helle 1992); the Saimaa ringed seal is the smallest subspecies. Due to its endangered status, it is impossible to study its anatomy using live individuals and thus previous studies relied on the examination of carcasses. The aims of this study were to create a practical application of MRI to study post-mortem morphology of the Saimaa ringed seal, to describe its macroscopic MRI anatomy, and to gather information from aspects not readily accessible in routine autopsies.

Materials and methods

The carcass of a juvenile female seal used in the present study was located on 16 February 2002 in Pihlajavesi (a basin of Lake Saimaa, 61°51.984'N, 28°51.278'E) trapped in a fishing net under ice. The seal had probably died within the last few days, and due to the cold water, the carcass was in good condition without any visible signs of autolysis or external trauma. The seal was delivered to authorities and kept frozen at -20 °C. The BM of the seal was 38 kg and the standard body length (nose-to-tail in a straight line, Ryg et al. 1990) was 125 cm. The epiphyses were visible in the tibiae indicating immaturity. Based on this, the seal was estimated to be approximately one year old, as whelping of the Saimaa ringed seal takes place between late February and early March (Hyvärinen et al. 1999). The carcass was used with permission of the Finnish Ministry of Agriculture and Forestry.

MRI was performed in Jyväskylä, Finland using the 1.5 T (Signa CV/i, General Electric Medical Systems, Milwaukee, WI, USA) MRI equipment. The specimen was placed into the equipment in a prone and head first position. A head coil was used for the head region and a body coil for the rest of the seal. Both T1 and T2 MR-contrasts in axial, sagittal and coronal planes (Fig. 1) were initially obtained. As the T1 relaxation time is temperature-sensitive, the carcass was thawed and warmed to +18 °C in order to use existing sequences designed for human imaging. The sequences were optimised to cover both normal anatomy and pathological findings. The available T1 sequences were originally planned for imaging of living tissues. Because the carcass used in this study was of room temperature, the T1 contrast in these images was weak. Therefore, the T2 images proved to be more informative.

Protocol parameters for the head were as follows: slice thickness 4.5 mm, slice interval 0.5 mm, time to repetition 3400 ms, time to echo 83 ms, field of view 260 mm, matrix 512×384 pixels, number of averages 3. Protocol parameters for the rest of the body were as follows: slice thickness 8.0 mm, slice interval 2.0 mm, time to repetition 4700 ms, time to echo 98 ms, field of view 400 mm, matrix 512×384 pixels, number of averages 3.

After obtaining the conventional MR images 0.5% gadodiamide contrast agent (OmniscanTM, GE Healthcare, Chalfont St. Giles, UK) was injected into the lungs via the airways to enhance the visibility of the bronchi. The volumes of organs and tissues were determined by marking tissue margins onto MRI slices, calculating the surface area in mm² and multiplying it by the sum of the slice thickness and the slice interval. The visual appearance of air and various tissues in MRI is given in Table 1.

After imaging, the seal was refrozen for reference sectioning performed with a log saw. This was carried out to establish reference standards for tissue sizes and positions for the subspecies as described previously with abdominal CT examinations for the ringed seal (Phoca hispida; Smodlaka et al. 2004). The head was cut into 1-cm sections and the rest of the body into 2-cm sections. The sections were photographed and refrozen to allow further dissection performed later with thawed slices to compare findings with the MRI images and to confirm structures found with the MRI technique when needed. The studies of Murie (1868, 1870), Koster et al. (1990), Endo et al. (1999, 2002) and Smodlaka et al. (2004) were used as references to identify structures and to compare them with earlier findings. The anatomical nomenclature is based on Nomina Anatomica Veterinaria (International Committee on Veterinary Gross Anatomical Nomenclature ICVGAN 2003).

Results

A 4–5 cm thick layer of blubber covered the trunk. The body volume of the carcass without flippers was approximately 37 140 cm³ and the volume of the blubber 18 530 cm³, 50% of the

body volume. In the deep layers of the blubber, there was a membranous structure recognized as subcutaneous fascia, as it did not contain muscular fibers in the dissection. In the sagittal and coronal planes an accurate topographical view of the internal organs, principal arteries and veins as well as the skeletal and muscular systems was visualised (Figs. 2–4). The epiphyseal lines were open in the tibiae (Fig. 3).

Behind the snout the vibrissae were embedded in lines in adipose and connective tissues. Inside the nasal cavity the conchas formed a folded structure (Fig. 5). Mm. masseter and temporalis were the most prominent muscles in the head region (Figs. 6 and 7). The strongest single muscles of the tongue, m. genioglossus, m. geniohyoideus and m. pterygoideus medialis were also identified (Fig. 8). The auditory canal was 7-cm long with multiple curves. Cavitas tympanica with the senses of hearing and equilibrium was prominent (Fig. 6). The gray and white matters of the brain were clearly distinguished and the cerebral cortex was densely convoluted (Figs. 7 and 8). The volume of the brain was 190 cm³, approximately 0.5% of the body volume. Medulla oblongata was sharply curved towards medulla spinalis. The epiglottis covered aditus laryngis tightly. The esophagus was situated dorsally to the trachea in this region. At the level of the first cervical vertebra it migrated to the right side of the trachea before bending to the dorsal region of cavum thoracis (Fig. 9). Glandula thyroidea was situated caudally from

Table 1. The visual appearance of air and various tissues in T2 and T1 images.

Air Black	Black
BlubberLight gBoneBlackBrain: gray matterLight gBrain: white matterDark gCoagulated bloodLight gUncoagulated bloodBlackFree fluidBrightLiverBlackMuscleDark gPancreasLight gSpleenLight g	jray Bright Dark gray jray Light gray jray Dark gray jray Light gray Dark gray Dark gray Light gray jray Dark gray gray Light gray gray Bright





Fig. 2. T2 coronal image of the thoracic region of the ringed seal. lu = lung, as = atrium sinistrum, vs = ventriculus sinister, vd = ventriculus dexter, ad = atrium dextrum, tp = truncus pulmonalis, ap = arteria pulmonalis, a = aorta, div = discus intervertebralis, cv = columna vertebralis, gh = articulatio glenohumerale, h = humerus, sh = sinushepaticus, bl = blubber. The arrow indicates the abnormal cavity in the blubber.

Fig. 3. T2 coronal image of the abdominal region of the ringed seal. co = costae, vpp = ventriculus, pars pylorica, d = duodenum, sh = sinus hepaticus, fv = gastric ventricle filled with fish, it = intestinum tenue, li = liver, ki = kidney, iaf = intraabdominal fluid, u = vesica urinaria, f = femur, t = tibia, ag = articulatio genus, mqf = m. quadriceps femoris, bl = blubber. The white arrow indicates the abnormal cavity in the blubber of the seal and the black arrow indicates the open epiphyseal line of the right tibia.



Fig. 4. T2 sagittal image of the abdominal region of the ringed seal. The black area marked with * inside v. cava posterior is uncoagulated blood and the area marked with ** is coagulated blood, sh = sinus hepaticus, hs = hepatic sinusoids, vcp = v. cava posterior, int = intestinum tenue, li = liver, d = duodenum, pa = pancreas, cf = caput ossis femoris, mes = m. erector spinae, bl = blubber.

the larynx on both sides of the trachea. *A. carotis communis* could be identified bilaterally until its bifurcation.

Several *lymphonodi* were detected in the axillary region (Fig. 10a). *Atrium dextrum* of the heart was large as compared with *atrium sinis*-*trum*, stretched and filled with coagulated blood (Figs. 2 and 11). The lungs (Figs. 11 and 12), *cavum pleurae* and *cavum pericardii*, were filled with fluid but it was not blood as verified by dissection (Fig. 11a). In the apex of the right lung there was a deficiency of the contrast medium (Fig. 12). *Mm. pectorales superficiales* were identified (Figs. 10a and 11a). Inside the spinal canal in the cervical area there were two thick extradural intravertebral veins, which combined caudally (Fig. 10a).



Fig. 5. T2 axial image of the rostrum of the ringed seal. sm = sinus maxillaris, nc = nasal cavity, oc = oral cavity, to = tongue, ma = mandibula, e = eye, I = lens.



Fig. 6. T2 coronal image of the head of the ringed seal. bl = blubber, ce = cerebrum, e = eye, l = lens, i = iris, cab = camera anterior bulbi, no = nervus opticus, mm = m. masseter, mt = m. temporalis, mac = meatus acusticus externus, ct = cavum tympani, co = cochlea.

The liver appeared as dark, lobulated tissue and the reniculate kidneys consisted of numerous small units (Figs. 3, 4, 13 and 15). The pancreas was situated retroperitoneally between the kidneys dorsally to *pars pylorica* and *intestinum tenue*. The stomach was filled with fish. *Fundus ventriculi* was 9.4 cm wide in the axial direction as compared with the 22.7 cm width of *cavum abdominis* at the corresponding level. *Corpus ventriculi* and *pars pylorica* curved



Fig. 7. (a) T2 axial image of the head of the ringed seal and (b) a photograph of the corresponding reference section. ce = cerebrum, fv = fourth ventricle, mt = m. temporalis, coc = cochlea, dse = ductus semicircularis, la = larynx, po = pons.



Fig. 8. T2 sagittal image of the head of the ringed seal. vi = vibrissae, e = eye, oc = oral cavity, mg = m. genioglossus, es = esophagus, t = trachea, ce = cerebrum, cc = corpus callosum, cr = cerebellum, po = pons, mo =medulla oblongata, ds = dorsum sellae, gp = glandulapituitaria, ms = medulla spinalis, cv = columna vertebralis, div = discus intervertebralis, mlc = m. longus capitis, la = larynx, ep = epiglottis, bl = blubber.

sharply towards the pylorus and duodenum. Pars pylorica was situated by the ventral wall of cavum abdominis. The spleen was located at the dorsal side of the ventriculus and caudally to fundus ventriculi. The duodenum and multiple loops of the small intestine were detected in the images, but the colon was clearly distinguished only in its descending part. Colon transversum and colon descendens had larger diameters than the small intestine and contained fecal mass. The largest structure of the upper abdomen was sinus hepaticus. It was filled with coagulated blood and its volume was 710 cm³ or 1.9% of the body volume (Figs. 2-4). The same mass filled also the hepatic sinusoids (Figs. 4 and 13a). The sinus was constricted at the level of the diaphragm. Urethra, vagina (Fig. 16) and cervix uteri were visible. Vesica urinaria contained urine, which appeared bright in T2 images. The two ducti lactiferi were visible in the blubber (Fig. 14).

In the front limbs, the musculature surrounding the scapula was stronger on the ventral than on the dorsal side (Fig. 10). *Musculus supraspinatus* and *m. infraspinatus* constructed a muscular mass with insertions on *tuberculum majus* on the dorsal side. Under *m. deltoideus* there was a muscle mass consisting of *m. infraspinatus* and *m. teres minor*. *Musculus teres major* and *m. latissimum dorsi* formed a unified mass. *Musculus brachialis* and *m. biceps brachii* were



Fig. 9. (a) T2 axial image of the neck region of the ringed seal and (b) a photograph of the corresponding reference section. bl = blubber, mes = m. erector spinae, tr = trachea with fluid, es = esophagus, ms = medulla spinalis, pt = processus transversus.



Fig. 10. (a) T2 axial image of the thoracic region of the ringed seal and (b) a photograph of the corresponding reference section. Lymph nodes (ln) are seen in the axillary region. The two extradural intravertebral veins are pointed by an arrow on the dorsal surface of medulla spinalis (ms). lu = lungs, b = bronchus, ba = bulbus aortae, vca = vena cava anterior, cv = columna vertebralis, st = sternum, mps = m. pectorales superficiales, ex = extensors of forearm, fl = flexors of forearm, ra = radius, w = wrist joint, di = digits, bl = blubber.



Fig. 11. (a) T2 axial image of the thoracic region of the ringed seal and (b) a photograph of the corresponding reference section. ad = atrium dextrum, vd = ventriculus dexter, scf = subcutaneous fascia, lu = lungs, plf = pleural fluid, b = bronchus, pe = pericardial space, vs = ventriculus sinister, mpp = musculus papillaris, a = aorta, cv = columna vertebralis, ms = medulla spinalis, st = sternum, msa = m. serratus anterior, mps = m. pectorales superficiales, mes = m. erector spinae, bl = blubber.



Fig. 12. 3D reconstruction of the lungs of the ringed seal filled with gadolinium contrast medium. The arrow indicates a pathological area in the apex of the right lung not filled with the contrast medium.

the most prominent muscles of the flexor side of the brachial region and *m. triceps brachii* of the extensor side. In the forearms the flexors and extensors could be identified only as muscle groups, not as individual muscles. The musculature of the trunk was the strongest and the most prominent caudally. In the dorsal part of the neck *m. sternocleidomastoideus* was identified. *Musculus semispinalis capitis* formed a considerable mass of longitudinal musculature. The principal transversal muscles were *m. obliquus capitis inferior* and *m. rectus capitis posterior major*. The largest muscle group of the trunk was *m. erector spinae* consisting of multiple parts (Figs. 4, 9, 11 and 14). The three muscle layers consisting of *m. obliquus externus abdominis*, *m. obliquus internus abdominis* and *m. transversus abdominis* were visible in the ventral wall of the abdomen (Fig. 13).

Typical for pinnipeds, the pelvis and the hind limbs were small as compared with the body size and the front limbs (Figs. 15 and 16). The femoral bones were attached to the pelvis nearly perpendicularly and, in a similar manner, the tibia and the fibula were attached perpendicularly to the femur (Figs. 3 and 15). In this position, *caput ossis femoris* was situated more dorsally than the distal end of the bone. The tibia was situated ventrally and the fibula dorsally. The individual muscles of the hind legs could be identified only as extensor and flexor groups.

Discussion

As compared with anatomical dissections, MRI is a non-invasive method to study normal anatomy and pathological findings with advantages, as it can be performed fast and easily and it enables studying both alive and dead tissues *in situ*.



Fig. 13. (a) T2 axial image of the abdominal region of the ringed seal and (b) a photograph of the corresponding reference section. scf = subcutaneous fascia, v = ventriculus, vcp = vena cava posterior expanded as sinus hepaticus, hs = hepatic sinusoid, li = liver, vh = vena hepatica, sp = spleen, iaf = intraabdominal fluid, mta = m. transversus abdominis, moi = m. obliquus internus, moe = m. obliquus externus, bl = blubber. M. erector spinae consists in this level of mst = m. spinalis thoracis, mlt = m. longissimus thoracis and mit = m. iliocostalis thoracis.

The detection limit of MRI is 0.1 cm as compared with 0.5 cm of dissections. MR images can be scanned in multiple directions to follow the course of specific structures and three-dimensional reconstructions and magnifications are easy to perform. Soft tissue margins can be identified more clearly than in dissections and highresolution sequences can be scanned from small details. In the present study MRI was applied to a rare subspecies in order not only to perform a study on a particular anatomic region but also to create a reference database on the MRI anatomy of the whole pinniped body for comparative analyses on morphology and pathology.

The thickness of the blubber in the Saimaa ringed seal varies seasonally being the highest in winter, when insulation is needed the most, and the lowest in summer. In the Baltic ringed seal the blubber constitutes approximately 44%–48% of BM in winter (Määttänen 1991), which is quite similar to the results of this study with 50% of the body volume consisting of blubber in February. The estimation based on the MR



Fig. 14. T2 axial image of the abdominal region of the ringed seal. dl = ductus lactiferi, int = intestinum tenue, cv = columna vertebrae, mes = m. erector spinae, mp = m. psoas, bl = blubber, iaf = intraabdominal fluid.

images confirmed the formula developed by Ryg *et al.* (1990) to calculate the blubber content of pinnipeds using body length (m), BM (kg) and blubber thickness (m) in the equation: blubber% = $4.44 + (5693 \times \sqrt{\text{length/BM} \times \text{blubber thickness}}) = 50.9\%$.



Fig. 15. T2 coronal image of the abdominal region of the ringed seal. ki = kidneys, sh = sinus hepaticus, li = liver, v = ventriculus, ar = a. renalis, iaf = intraabdominal fluid, int = intestinum tenue, mp = m. psoas, r = rectum, p = pelvis, ax = articulatio coxae, f = femur, mqf = m. quadriceps femori, ag = articulatio genus, bl = blubber.



Fig. 16. (a) T2 axial image of the ringed seal pelvis, and (b) a photograph of the corresponding reference section . u = vesica urinaria, sa = sacrum, bl = blubber, gl = gluteal muscle group, r = rectum, v = vagina, fi = fibula, eg = extensor group of muscles, t = tibia, fg = flexor group, mra = m. rectus abdominis, moi = m. obliquus internus.

The cerebral volume of the seal (190 cm³) or 0.5% of the body volume) was on the same scale as determined for the Saimaa ringed seal by Määttänen (1991) with a brain volume of about 200 cm³ for a 40-kg seal. The Saimaa ringed seal has a large relative brain volume and encephalisation quotient (1.6) as compared with those of other subspecies. This is supported by the results of Bininda-Emonds (2000), who measured a smaller brain volume, 0.32%-0.33% of the body volume, from 29 marine ringed seal individuals. The larger relative brain volume of the Saimaa ringed seal is hypothesised to be due to the challenging environment of Lake Saimaa with labyrinthine islands and poor visibility due to the opaque water (Määttänen 1991). Amano et al. (2002) studied skull variations in seven subspecies of ringed seals. They discovered that the skull of the Saimaa ringed seal was larger in the region of the mandible, zygomatic width and jugal length, while the length of the row of teeth was shorter as compared with the other ringed seal subspecies. They presumed this to be a result of the diet of the subspecies - small schooling fish instead of crustaceans.

The requirements for three-dimensional underwater predation demand exact sensory data of body positions and the direction of the surface. This was observed in the large volume of the sense of balance in the inner ears. Ringed seals generate sounds both in water and on land and thus require auditory capability in both environments (Supin et al. 2001). Furthermore, the importance of the vibrissal apparatus in the natural history of seals is emphasised by the volume of the vibrissae and their follicles in the snout. In addition to tactile stimuli, the vibrissae can detect swimming velocity and discriminate objects by size (Hyvärinen 1989, Dehnhardt et al. 1998). The main muscles of mastication and other muscles in the facial and nuchal regions conformed to previous findings of Endo et al. (2002). The esophagus was situated at the dorsal side of the trachea in the upper part of the neck but migrated to the right side of the trachea at the level of the first cervical vertebra. This phenomenon has not been previously described but it may be a consequence of the position the carcass was frozen into and should be reconfirmed in the future.

The diving response of pinnipeds includes dramatic modifications of the circulatory system characteristic to air-breathing species that stay submerged for long periods of time (Wilson 1979, Mottishaw *et al.* 1999, Hochachka 2000, Thornton *et al.* 2005). The enlargement of *v. cava posterior* together with *sinus hepaticus* is one of the key elements providing a large capacitance vessel (Burow 1838, Harrison *et al.* 1954, Ronald *et al.* 1977). As the seal descends into water, blood from superficial tissues and non-vital organs enters the core of the body and is stored in *sinus hepaticus*. Blood flow into the thoracic portion of *v. cava posterior* and to *atrium dextrum* is prevented by a sphincter at the level of the diaphragm (Woodward 1910, Harrison *et al.* 1954). While the sphincter itself was not visible, the narrowing of *v. cava posterior* at the level of the diaphragm was obvious. The presence of the diving response evidenced that the seal died submerged by asphyxiation. The large volume of the veins as compared with that of the arteries probably derived partly from the diving response and partly from the post-mortem constriction of the arteries. In some seals, *v. cava posterior* is a paired vessel in the abdominal region (Berta *et al.* 1999) but only one enlarged vein was visible in the Saimaa ringed seal.

The pressure exerted on the circulatory system was transmitted to atrium dextrum and ventriculus dexter evidenced by their expanded volumes. This could be partly caused by the lethal entanglement in the net, but the elasticity of the right cardiac walls indicates that they evolved to withstand this expansion. This has been previously described, as Murie (1870) and Blix and Hol (1973) also recorded the large volume capacity of the right side of the heart of pinnipeds. Bulbus aortae was visible as an enlargement of the aorta, typical for pinnipeds that have the capacity to dive deep (Drabek 1975). Inside the spinal canal two large extradural intravertebral veins were visible. These veins are connected to cranial venous sinuses with multiple anastomoses to vv. cavae anterior and *posterior* and serve as important routes for venous drainage from the brain. During diving this system participates in the control of intracranial pressure (Ronald et al. 1977).

The skeletal and muscular structures of the seal reflected its natural history. The strongest muscles for swimming were located in the lower back. The muscles of the front limbs were the largest on the ventral side indicating their extensive use when moving on land and on ice as indicated by English (1976). In the case of the ringed seal, also the need to scrape breathing holes through the ice requires strength from these muscles. The long anterior extension of *manubrium sterni* and the enlarged *tuberculi* of the humerus provide large areas of attachment for the muscles. As detected earlier in pinnipeds (Hojo 1975), the Saimaa ringed seal did not have claviculae. This increases flexibility, which is typical for the pinniped thorax. In Phocidae, a common feature is the lateral aversion of the ileum and the deep lateral excavation of the iliac wing. They offer attachment surface for *mm. erector spinae*, which are also important muscles for swimming (Berta *et al.* 1999).

Pathological findings that would have probably been ignored in dissection were detected with MRI. Inside the blubber on the right side of the carcass there was a channel or a cavity, the presence of which was verified in dissection. The cavity was 2-cm deep and formed a curving channel with fat degeneration. The probable explanations for this are post-traumatic or inflammatory processes. In the apex of the right lung there was a deficiency in the airway contrast medium. Unfortunately this part of the lung was too decomposed to allow for taking of reliable histological samples, which further emphasizes the practicality of MRI in the study of pathological findings. This type of signal defect in MRI is usually caused by scarred tissue, which may result from e.g. infections.

The results of the present study can be applied to other ringed seal subspecies and other pinnipeds, many of which are threatened and cannot be studied *in vivo*. Furthermore, morphological structures and findings accessible by MRI are often easily disturbed or destroyed in dissection or too decomposed to allow accurate dissections to begin with. In addition, MRI can supplement data gathered by conventional necropsies with accurate *in situ* findings as evidenced by the pathological lesions detected with MRI.

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