Inhibition of shivering in hypothermic seals during diving

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Kvadsheim, Petter H., Lars P. Folkow, Arnoldus Schytte Blix. Inhibition of shivering in hypothermic seals during diving. *Am J Physiol Regulatory Integrative Comp Physiol* 000: R 000 – R 000, 2004. – The mammalian response to hypothermia is increased metabolic heat production, usually by way of muscular activity, such as shivering. Seals, however, have been reported to respond to diving with a hypothermia, which in other mammals under other circumstances, would have elicited vigorous shivering. In the diving situation shivering could be counter productive, since it obviously would increase oxygen consumption and therefore reduce diving capacity. We have measured the electromyographic (EMG) activity of three different muscles and rectal and brain temperature of hooded seals (*Cystophora cristata*) when exposed to low ambient temperatures in a climatic chamber, and when performing series of experimental dives in cold water. In air the seals had a normal mammalian shivering response to cold. Muscles were recruited in a sequential manner until body temperature stopped dropping. Shivering was initiated when rectal temperature fell below $35.3 \pm 0.6^\circ C$ (n=6). In the hypothermic diving seal, however, the EMG activity in all the muscles that had been shivering vigorously prior to submergence was much reduced, or stopped altogether, while it increased again upon emergence, but was again reduced if diving was repeated. It is concluded that shivering is inhibited during diving to allow a decrease in body temperature, whereby oxygen consumption is decreased and diving capacity is extended.

hooded seal, (*Cystophora cristata*), shivering EMG, body core temperature, thermoregulation
SCHOLANDER et al. (23) first reported that body core temperature decreases during experimental diving in seals, and some forty years later Kooym an et al. (17) showed that central arterial temperature of a freely diving Weddell seal (*Leptonychotes weddelli*) decreased by about 3°C during a 53 min dive. Hill et al. (12), moreover found that the aortic temperature of freely diving Weddell seals was reduced by about 2°C in periods of active diving, compared to resting periods without diving. Finally, Odden et al. (21) reported that brain temperature in hooded seals (*Cystophora cristata*) and harp seals (*Pagophilus groenlandicus*) may be reduced by as much as 3°C during relatively short (9-15 min) experimental dives. Scholander et al. (23) suggested that the reduction in body core temperature is caused by a reduction in metabolic heat production, which typically occurs in diving seals (*i.e.*, 6, 15, 24). Odden et al. (21), on the other hand, suggested that body cooling during diving is instead caused by a physiologically controlled increase in heat loss, that in turn will cause a metabolic depression due to Q_{10}-effects. In any case, a reduction of deep body temperature, and in particular brain temperature, by as much as 3°C within minutes, will normally lead to vigorous shivering in mammals (25). Now, if body cooling is an effect of reduced metabolism, as suggested by Scholander et al. (23), shivering would compromise metabolic depression, and hence, the body cooling. If, instead, any cooling of the body is caused by a controlled increase in heat loss to enhance the metabolic depression through Q_{10}-effects, as suggested by Odden et al. (21), shivering would again be counter productive.

In the present study we have tested the hypothesis that shivering is inhibited in hypothermic diving seals. We have first exposed two seals to low ambient temperatures in a climatic chamber, while we recorded rectal temperature and
muscular activity in three different muscles, to determine if they responded in the proper mammalian way to a drop in deep body temperature when they were not diving. Second, we made three seals perform a series of experimental dives, while we recorded muscular activity, brain and rectal temperature and heart rate.
METHODS

Animals. Three 1-2 year old hooded seals (*Cystophora cristata*) (K-3/00: 1 year, male, 62 kg; K-5/99: 2 years, male, 81 kg; K-6/99: 2 years, male, 92 kg) were caught as pups in the pack ice of the Greenland Sea and raised in captivity at Tromsø, in 45 000 l sea water pools with wooden ledges, where they were offered capelin (*Mallotus villosus*), or herring (*Clupea harangus*), supplemented with a vitamin complex (4). The animals were collected under permits issued by The Royal Norwegian Ministry of Fisheries, and experiments carried out under permit from the Norwegian Animal Research Authority.

EXPERIMENTAL PROTOCOL

Shivering in cold air. Two of the experimental animals (K-3/00 and K-6/99) were gradually trained to accept being restrained on a specially designed board and left for about 4 hours inside a climatic chamber (type 24/50 DU; Weiss Technik, Giessen, Germany). Prior to experiments, the animals were instrumented with EMG electrodes, in *musculus humerotrapezius, musculus spinotrapezius* and *musculus latissimus dorsi* (14) and a thermocouple to record rectal temperature. The temperature inside the climatic chamber was then gradually reduced from +20°C to -10°C in 30 min, whereafter it was further reduced to a stable –35°C in 70 min. The experiment was terminated after about 3 hours of exposure to –35°C, or earlier, if rectal temperature fell below 33°C. At all times the animal was observed by use of a video camera to enable us to distinguish between EMG activity caused by shivering and that caused by movements of head and flippers. This experiment was repeated four times in each animal.
Shivering during diving. In preparation for the experiments, the animal was placed on a specially designed restraining board of classical design, and all three animals gradually accustomed to experimental dives of 10-15 min duration in water of 3-4°C. Prior to each experiment, the animal was instrumented with EMG-electrodes, in *m. humerotrapezius*, *m. spinotrapezius* and *m. latissimus dorsi*, as well as with electrodes for measurements of heart rate and a thermocouple for measurements of rectal temperature, and in some of the experiments brain temperature was measured as well (see below). Prior to diving the animals were allowed to equilibrate in the water for at least 1 h, whereafter they were submerged three times for 10 or 15 min, separated by recovery periods of 40-60 min. If, after the third dive the animal was shivering, then it was submerged again for 3-5 min after 3-5 min of recovery. During these experiments the animals were always directly observed, so that EMG activity caused by shivering could be distinguished from that caused by movements of head and flippers. This series of experiments was repeated 3 times in animals K-3/00 and K-6/00, the latter subsequently being instrumented with a probe for measurements of brain temperature, whereafter the series of experiments were repeated three more times. K-5/99 was instrumented with a brain probe from the very beginning and the series of dive-experiments were carried out twice in this animal.

EMG. Shivering was recorded as EMG-activity in three large skeletal muscles along the dorsal side of the animal. We used the anatomical description of Howell (14) in combination with information obtained from dissection of a hooded seal of the same age as our experimental animals to determine the correct placement of the EMG-electrodes in *m. humerotrapezius*, *m. spinotrapezius* and *m. latissimus dorsi*. The EMG electrodes were home made intramuscular electrodes similar to those described by
Hohtola et al. (13): Two 0.55 mm thick needle electrodes were mounted 3mm apart on a PVC-disc, and insulated, except for a 3 mm tip, by 0.1 mm of Plastic Spray (PRF 202, Taerosol OY, Kangasala, Finland). The other ends of the needles were soldered to a cable and connected to a differential amplifier and a band pass filter with high pass and low pass cut-off frequencies at 10 Hz and 500 Hz, respectively (Myosystem 2000, Noraxon OY, Oulu, Finland). The length of the needles was adjusted so that the tips penetrated the blubber layer and reached 1-5 mm into the underlying muscle. Blubber thickness was measured prior to instrumentation using an ultrasound apparatus (SDR 1200, Philips Ultrasound Inc, Santa Ana, CA, USA). A subcutaneous electrode was placed mid laterally on the animal as a grounding electrode. The needles were inserted under local anaesthesia (Subcutaneous injection of 2-3 ml Xylocain (10 mg/ml) AstraZeneca, Södertälje, Sweden). The amplified and filtered EMG signal was then rectified and averaged using a RC-filter with a time constant of 10 s before it was conveyed to a A/D-converter and data acquisition system which stored the data every 20 s (Lab-Acq Pro. and Insta–Trend Pro., Dianachart Inc., Oak Ridge, NJ, USA). Un-rectified signals were also recorded using a universal amplifier with a second band pass filter (high and low pass cut-off frequencies at 10 Hz and 100 Hz, respectively) and a printer sampling at 250 kHz (TA 4000, Gould Inc, Valley View, OH, USA). In other words we recorded the mean rectified EMG values every 20s, in addition to continuous recording of the un-rectified (raw-data) signals. Baseline un-biological noise levels for the electrodes were determined after each experiment by submerging them in physiological saline. Baseline noise was subsequently subtracted from the mean rectified values.

*Brain temperature.* Brain temperature was measured as described by Odden et al. (21): A 30 mm long and 1.5 mm thick blind-ended stainless steel pipe was surgically
implanted into the brain of the animals, under full isofluorane anaesthesia. The probe was placed 10 mm laterally to the midline into the left cerebral hemisphere, near the third ventricle (as verified *post mortem*). Instrumented animals were not used in any experiments until at least 48 h after implantation of the probe. Before each experiment a copper-constantan thermocouple was introduced into the probe under a light sedation (0.6 mg/kg *i.m.* injection of Zoletil forte vet. (tiletamin-zolazepam), Virbac SA, Carros cedex, France). The thermocouple was connected through a thermocouple amplifier with internal temperature reference (AD 595 CD, Analog Devices Inc., Norwood, MA, USA) to an A/D- converter and data acquisition system, as described for the EMG-signals. After sedation the animals were monitored in air for at least 1 h to allow complete recovery before the series of diving experiments commenced.

*Rectal temperature.* Rectal temperature was recorded with a copper-constantan thermocouple that was inserted 20 cm into the rectum of the animal. The thermocouple was connected through a thermocouple amplifier with internal temperature reference to an A/D-converter and data acquisition system as described for brain temperature. All thermocouples were calibrated at 0°C and 40°C, and the measured temperatures were linearized according to Tøien (26). All temperatures were recorded every 20 s as the mean value of the last 20 s.

*Heart rate.* During the diving experiments heart rate was recorded by use of two subcutaneous electrodes placed anterior and posterior to the heart along the dorsal midline, and a third electrode was placed laterally on the animal. The electrodes were connected to a monitoring unit (CM-4008, Medi-Stim AS, Oslo, Norway) and recorded on a printer (TA 4000, Gould Inc, Valley View, OH, USA).

*Statistics:* In Figures 1, 3 and 4, EMG data are presented as medians of the sampled mean rectified values for extended periods (minutes). Medians better represent
shivering activity than means, since the muscle contractions involved in even small movements of the animal generates larger EMG-signals than the signals generated by shivering. Bouts of EMG activity, due to animal movements, would have an unproportional influence on EMG data if means were used instead of medians. Moreover, given the dual source of EMG signals (shivering or physical movements), EMG data are hardly normally distributed, which further warrants the use of median instead of mean. When using median values interquartile range is used as measure of variance (Fig. 4). Because the absolute magnitude of an EMG-signal picked up by an electrode will vary greatly from muscle to muscle and from experiment to experiment (26), a relative change in EMG-signal (relative to the starting point of each experiment) was used when comparing different muscles and different experiments (Fig. 5), and the results are presented as means ± SEM. Body temperature data are presented as means ± SD. Comparisons of body core temperatures of shivering and non-shivering animals were made using two-tailed Students t-test for unpaired samples. Differences in relative mean rectified EMG levels recorded under various experimental conditions were compared using a one sample t-test. A p-value of <0.05 was taken to indicate a statistically significant difference.
RESULTS

Shivering in cold air. When the seals were exposed to ambient air temperatures of –35°C for extended periods, rectal temperature decreased, and shivering ensued when rectal temperature dropped below 35.3 ± 0.6°C (n=6) (Fig. 1). The three large muscles, from which EMG-signals were recorded, were typically recruited in a sequential manner if rectal temperature continued to decrease. The sequence of recruitment was consistent within, but not between animals. In animal K-3/00 shivering always started in m. humerotrapezius, and if needed, spread to m. latissimus dorsi and m. spinotrapezius (Fig. 1), while in animal K-6/99 it usually started in m. latissimus dorsi and only occasionally spread to the other two (Fig. 1).

FIGURE 1 ABOUT HERE

Shivering during diving. Of a total of 33 dives in our three animals, 20 were discarded since the animal did not shiver prior to the dive. These could therefore not be used to address the hypothesis. On average rectal temperature dropped 0.5 ± 0.3°C (n=33) and brain temperature 0.7 ± 0.5°C (n=13) in response to the 10-15 min dives. The average rectal temperature in animals that shivered before a dive was 35.7 ± 0.6°C (n=19), compared to 36.3 ± 0.5°C (n=19) in animals that did not shiver prior to a dive. The average brain temperature in animals that shivered prior to a dive was 37.3 ± 0.6°C (n=7), compared to 37.6 ± 0.6°C (n=9) in animals that did not shiver. These differences in body temperature were statistically significant for rectal temperature (p=0.004, Two tailed Students t-test, unpaired samples), but not for brain temperature (p=0.24). In water, like in cold air, shivering was not necessarily activated in all
muscles simultaneously. Fig. 2 shows unrectified (raw-data) EMG signals from one muscle, and heart rate, before, during and after a 10 min dive, followed by a recovery period of 3.5 min, before a second dive of 3 min duration in animal K-5/99. In this case heart rate dropped from about 130 beats·min\(^{-1}\) before the dives to about 20 beats·min\(^{-1}\) during the dives, and increased again to 130 beats·min\(^{-1}\) upon emergence. This was more or less the pattern in all dives in all three animals. In the experiment illustrated in Fig. 2, only *m. latissimus dorsi* was activated. The shivering activity of this muscle before the series of dives was clearly suppressed during the dive. After the dive, shivering was quickly reactivated, but was again suppressed upon re-submergence, whereafter it was reactivated upon re-emergence. This pattern of inhibition and activation of shivering during and after dives, respectively, is also illustrated in Fig. 3 and 4, which shows the median values of mean rectified EMG after transformation of the un-rectified EMG-signal from Fig. 2 before, during and after dives.

When all the data from all muscles in all experiments where shivering occurred prior to a dive was to be combined (Fig. 5), relative changes in median values of mean rectified EMG was employed. Thus, 100% was chosen as the value prior to dives, and the values obtained during and after the dive were converted to percentages relative to that value in each experiment (Fig. 5). In so doing we found that there was a noticeable reduction in EMG activity during the dive in all muscles that were shivering prior to the dive. After the dives EMG activity always increased, but was reduced again if diving was repeated (Fig. 5). The reduction in relative EMG activity
during dives was statistically significant (p<0.05; using One sample t-test, hypothesized mean=100) in all muscles, except for *m. spinotrapezius* during dives and *m. humerotrapezius* during re-dives (Fig. 5). When data from all muscles were pooled, however, the reduction in EMG-activity during diving was significant during both dives and re-dives (Fig. 5).

FIGURE 5 ABOUT HERE
DISCUSSION

Shivering in cold air. This study has shown that seals exposed to ambient temperatures below their lower critical temperature start shivering to increase heat production in the normal mammalian way (Fig. 1). The two largest muscles from which EMGs were recorded, *m. humerotrapezius* and *m. latissimus dorsi*, were usually the first to be called upon to shiver, while the smallest, *m. spinotrapezius*, was usually the last. Previous studies have shown an increase in oxygen consumption when ambient air temperature drops below –11°C in subadult gray seals (*Halichoerus grypus*) (7) and -13°C in subadult harbour seals (*Phoca vitulina*) (11). In the present study subadult hooded seals were exposed to an air temperature of –35°C, and although we did not exactly determine the lower critical temperature of our animals, we can safely assume that –35°C is way outside their thermoneutral zone. This is also supported by the fact that even though the largest animal (K-6/99) seemed to tolerate a greater hypothermia before shivering was activated than the smaller one (K-3/00, Fig. 1), they both invariably shivered when exposed to that temperature. The mean rectal temperature when shivering first started was 35.3°C, which is between 1-2°C below a normal rectal temperature for these animals. Rectal temperature therefore seems to be an acceptable indication of thermal status of seals in air. This also seems to be true in water, because rectal temperature of seals that were shivering just prior to a dive was significantly lower than in seals that did not shiver. However, rectal temperature in seals that were shivering in water just prior to a dive was higher, but not significantly higher (p = 0.1), than the rectal temperature at onset of shivering in air. This apparent difference in the thermal sensitivity of seals in air and water may be misleading because body temperatures are represented by rectal temperature, which is not always a good, albeit commonly used, indicator of thermal status (e.g. 20). If authentic, it is
probably caused by differences in the input from central thermoreceptors. Seals have a high hypothalamic thermosensitivity similar to terrestrial mammals of similar size (25; based on data from 10), but they have the lowest sensitivity to thermal stimulation of the skin of all mammals studied (25; based on data from 10). It is therefore likely that differences in brain temperature more than differences in skin temperature, explain why the seals wore shivering in cold water, already when rectal temperature was 35.7°C, while rectal temperature fell below 35.3°C before they start to shiver at an ambient temperature of -35°C in air.

The reason why the shivering response in the non-diving situation was studied in air instead of in water, is that we knew from previous studies of similar-sized arctic seals (8, 18), that water temperatures of 2-4°C would not be below the lower critical temperatures of our seals, and hence that they would not shiver in such water, unless they were diving.

Shivering during diving. It is well documented that seals have the ability to cool their body core during prolonged diving (3, 12, 17, 21, 22). The present study has shown that diving also inhibits shivering in seals. This may well be rather important, since, if shivering was not inhibited, the animals ability to cool during diving, and hence its ability to save oxygen, and thereby its ability to extend the duration of the dives, would be compromised. We suggest that inhibition of shivering is an integral part of the complex "package" of reflexes, which in common often are referred to as the "diving responses" of seals (2). Previous studies have shown that shivering in mammals is inhibited by hypoxia (e.g. 9, 16). However, arctic seals are well equipped with both blood and muscle oxygen stores (e.g. 5, 19), and are therefore fairly well oxygenated for several minutes into a dive, and since shivering in our animals was
inhibited instantly upon submersion, the inhibition of shivering was hardly caused by hypoxia.

Our finding that diving overrides thermoregulatory induced shivering, might at first seem contradictory to the findings of Hammel et al. (10), showing that during experimental hypothalamic warming in harbour seals, the diving response did not override thermoregulatory induced vasodilatation in the flippers. However, if we accept the proposal by Blix et al. (3) that body temperature is actively down-regulated by means of controlled perfusion of primarily the front-flippers, this all makes much sense. During diving it is simply advantageous for the animals to get cold. In the experiments of Hammel et al. (10) the animal had its brain heated when it already were striving to get cold, and hence the lack of vasoconstriction in the flipper during diving, while in our experiments inhibition of shivering was necessary to ensure that body cooling persisted.

Oxygen consumption during diving can at present not be measured directly, and instead the indirect approach to use respirometry to determine oxygen extraction rates before and after a dive has been used (6, 15, 22, 24). During a dive the extraction of atmospheric oxygen is of course zero, since the animal is not breathing, while oxygen consumption by the tissues is still going on. After a dive oxygen extraction rate is therefore, for a while, well above the pre-dive level, because the animal is "repaying" its oxygen debt. Such studies clearly show that seals that dive for prolonged periods lower their oxygen consumption during diving. This may be a result of the widespread peripheral vasoconstriction which renders most internal organs uncirculated, and hence, partly anaerobic, during long dives and/or a Q_{10}-effect caused by body core cooling (3). In estimating the amount of oxygen consumed during the dive, it is therefore not correct to assume that the extra consumption after the dive equals
consumption during the dive (15, 22). In fact, our finding of a reduced body core
temperature together with inhibition of shivering during the dives implies that a
presently unknown fraction of the "apparent oxygen debt" after the dive will consist of
oxygen spent on shivering in order to bring body core temperature back to normal.
The dive consumption rates for oxygen obtained so far may therefore be
overestimated.

In conclusion; this study has shown that seals have a normal thermoregulatory
shivering response to hypothermia in air, while diving inhibits shivering and thereby
allows body temperature and oxygen consumption to drop. This adaptation may
contribute to extend the diving capability of the animal and explain how seals
repeatedly are reported to exceed their calculated aerobic dive limit.
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FIGURE LEGENDS

Fig. 1. Mean ambient temperature (♦), rectal temperature (◇), and median values of mean rectified EMG (m.r. EMG) in 5 min periods in three muscles, *m. humerotrapezius* (●), *m. spinotrapezius* (▲) and *m. latissimus dorsi* (■), of two hooded seals (K-3/00 and K-6/99) resting in a climatic chamber.

Fig. 2. Typical un-rectified EMG recordings from *m. latissimus dorsi* and ECG of a hooded seal (K-5/99); 1 min before a dive, 3 min into a 10 min dive, 2 min after the dive, 1 min into a second 3 min re-dive and 2 min into the recovery from the second re-dive. The recovery period between dives lasted 3.5 min.

Fig. 3. Mean brain (●) and rectal temperature (◇), and median values of mean rectified EMG (m.r. EMG) in 2 min periods in *m. latissimus dorsi* (■) during a 6.5 min period before a dive, during a 10 min dive, a 3.5 min period between dives, a 3 min re-dive and a 6.5 min period after the re-dive in a hooded seal (K-5/99).

Fig. 4. Median values of mean rectified EMG from *m. latissimus dorsi* during a 6.5 min period before a dive, during a 10 min dive, a 3.5 min period between dives, a 3 min re-dive and a 6.5 min period after the re-dive in a hooded seal (K-5/99).

Fig. 5. Relative EMG-activity (median values of mean rectified EMG in per cent of pre-dive values), with SEM error bars, of three muscles before, during 10-15 min dives, during the short 3-5 min period between dives, during short 3-5 min re-dives and after both dives. Small numbers above columns denote number of trials, while the
asterices indicate a significant reduction during dives from the 100% pre-dive level, and n.s. a not significant reduction from this level.
Figure 2
Figure 3
Figure 5

![Graph showing muscle activity during different phases of diving.](image-url)